Protein Kinases: Docking and Homology Modeling Reliability

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Received April 28, 2010

A database of about 700 high-resolution kinase structures was used to test the reliability of 17 docking procedures (using six docking software packages) by means of self- and cross-docking studies. The analysis of about 80 000 docking calculations suggests that the docking of an unknown ligand into a kinase has a probability of only 30−37% to be a correct ligand pose. However, based on the hypothesis that docking calculations are more reliable if the ligand to be docked is similar to the ligand present in the complex from which the target docking protein has been extracted, we propose an automated procedure that is able to improve the docking accuracy, suggest the best protein for docking studies, and assess the statistical reliability of docking calculations. The results were also transferred to the homology modeling field and led us to propose an alternative strategy based on ligand similarity for the development of kinase models whose experimental structure was not known. Our results suggest that in many cases this approach can give better results than the classical homology modeling procedure based exclusively on the sequence homology.

INTRODUCTION

Protein kinases represent one of the largest groups of proteins in the human genome with over 900 kinases recognized to date.[1] These enzymes play a major role in eukaryotic signal transduction via regulation of the phosphorylation states and thus the cellular functions of substrate proteins. The similarity of the mechanism and structure of their catalytic domains remains a major obstacle to the rational development of specific inhibitors for the treatment of human diseases ranging from cancer to autoimmunity.[2] The approval of imatinib (Gleevec) for chronic myeloid leukemia (CML) as well as gefitinib (Iressa) and erlotinib (Tarceva) for nonsmall cell lung cancer (NSCLC) has provided proof of principle that small molecule kinase inhibitors can be effective drugs.[3−5]

To design highly selective or particular multitarget kinase ligands, computational chemistry is a very helpful tool for predicting the geometry of kinase−ligand complexes (by means of docking software) and for providing atomic-resolution models of kinases without an experimentally known 3D structure (by means of homology modeling techniques).

One of the key questions regarding prediction of the geometry of kinase−ligand complexes is whether it is possible to measure the level of reliability of docking software for predicting the binding pose of ATP binding site ligands. In recent years, many studies have been carried out to address this question, and it is also interesting to note that many companies were involved in these projects.

In 2004, Perola and co-workers from Vertex Pharmaceuticals Inc. examined the ability of GOLD, GLIDE, and ICM to reproduce the native ligand conformation (self-docking) for a database of 200 high-resolution protein−ligand complexes that also includes kinases. Then, in conjunction with three different scoring functions, the four docking software packages were also used to carry out virtual screening simulations using enriched libraries.[6] In 2007, Sutherland and co-workers from Eli Lilly and Co. performed a cross-docking study with CDocker and FRED using multiple X-ray structures for eight proteins that also included kinases CDK2 and MAPK14.[7]

In 2008, Verdonk and co-workers from Astex Therapeutics Ltd. reported a cross-docking study for GOLD using the Astex Diverse Set database that included high-resolution structures of CDK2, p38, Chk1, and c-Abl tyrosine kinases.[8] Voigt and co-workers from the Schering-Plough Research Institute reported a cross-docking study using the GOLD and GLIDE software packages for a data set of 150 high-resolution in-house crystal structures of CDK2 complexes. On the basis of the obtained results, the authors combined the docking results from multiple protein conformations in a consensus fashion, enhancing the docking accuracy for GLIDE.[9] In 2009, Gleeson and Gleeson from GlaxoSmith-Kline applied the QM/MM method to optimize and rescore GOLD-derived cross-docking results obtained for 15 X-ray crystal structures of six kinase types,[10] and finally, in the same year, Armen and co-workers reported an evaluation of explicit receptor flexibility for p38a mitogen-activated kinase using molecular dynamics and torsion angle molecular dynamics.[11]

Therefore, considering the importance of the molecular docking studies in the field of kinase inhibitor design, we carried out an exhaustive analysis of several docking

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10.1021/ci100161z
Published on Web 07/27/2010

procedures using a large number of kinase structures. We used a database of about 700 high-resolution kinase structures, which was the largest kinase database ever used for self- and cross-docking studies.

The goal of these studies was to define rules that can help computational chemists determine the expected percentage of success of a docking study for a specific kinase target. Beyond simple analysis of the self- and cross-docking results, we also tried to correlate them with factors such as the similarity among ligands and binding sites.

The obtained results also led us to investigate and propose alternative strategies for the homology modeling (HM) of kinase proteins. In the literature, a large number of kinase 3D structures, developed by means of HM techniques, has been reported. Most of them were obtained using the 3D structure possessing the highest sequence similarity with the target kinase as a template. In this paper, we developed and tested an alternative HM strategy in which the template choice was based on the ligand similarity rather than the sequence similarity between the template and the kinase protein to be built. Our preliminary results suggest that because the influence of the ligand on the kinase shape has a key role, use of the template with the highest sequence similarity cannot always be the best choice.

RESULTS AND DISCUSSION

A total of 711 X-ray structures of kinases complexed with ligands were retrieved from the RCSB protein data bank[12] following the procedure described in the Experimental Section at the end of this paper. All ligands were extracted from the X-ray complexes, subjected to conformational search (CS), and then used for the calculations.

The reliability of various docking software packages was assessed in two steps. The first step consisted of a self-docking approach in which each ligand was docked back into its native protein structure. The second one was a cross-docking study carried out for the kinases for which the experimental structures of a series of several complexes were known. For each kinase type, each ligand was docked in all the available structures of that kinase type.

Self-Docking Step. All ligands were extracted from the selected 711 X-ray complexes and subjected to CS. They were then redocked into their corresponding proteins. The docking results were evaluated through a comparison of the found docked position of the ligand with the experimental one. For this purpose, the root-mean-square deviation (rmsd) between the positions of the heavy atoms of the ligand in the calculated and experimental structures was calculated.

Six different docking software packages with a total of 17 different docking procedures (see Experimental Section for details) were tested for their ability to self-dock kinase ligands, resulting in 12 087 docking results.

Two parameters were taken into account when comparing the reliability of the results obtained from the different docking procedures: (a) the average root-mean-square deviation (armsd) of the position of the ligand resulting from the docking with respect to the experimental disposition and (b) the number of ligands with a reliable docking pose (i.e., the number of ligands with a rmsd smaller than 2.0 Å).

As shown in Figure 1A, the use of GOLD[13] using the ChemPLP fitness function (GOLD_PLP), GLIDE[9] with the extra precision mode (GLIDE_XP) and AUTODOCK 4[14] (AD4) resulted in an armsd of about 2.5 Å followed by FRED[15] using a combination of the six available scoring functions (FRED_ALL, see the Experimental Section for details) that resulted in an armsd of 2.9 Å. Analysis of the compounds with a rmsd smaller than 2.0 Å confirmed the results of the armsd analysis. As shown in Figure 1B, about 60% of docked compounds showed a rmsd smaller than 2.0 Å using GOLD_PLP and GLIDE_XP and about 55% of compounds showed a rmsd smaller than 2.0 Å using AD4 and FRED_ALL.

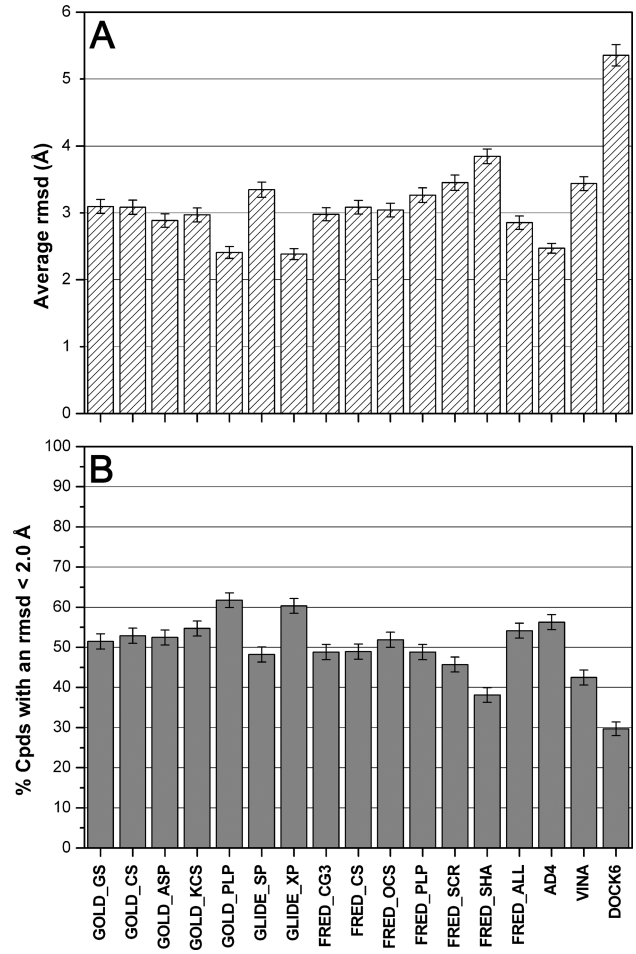

Figure 1. Results of the self-docking study. For each procedure, the armsd (A) and the number of poses with a rmsd less than 2 Å (B) is reported. Error bars denote the standard error of the analysis.

From the self-docking studies it appeared that four docking procedures (GOLD_PLP, GLIDE_XP, AD4, and FRED_ALL), each one corresponding to a different software package, were the most reliable.

The ability of a docking software package to find good poses is usually influenced by the structural complexity of the ligands, which can make an exhaustive conformational analysis difficult.

Therefore, to test the presence of a correlation between the ligand properties and the docking results, the latter were correlated with the molecular weight (MW) and the number of rotatable bonds of the ligands. Surprisingly, as shown in Figure 2, there was no prominent correlation between the number of rotatable bonds of the ligands and the docking results. Moreover, the only noticeable conclusion with regard

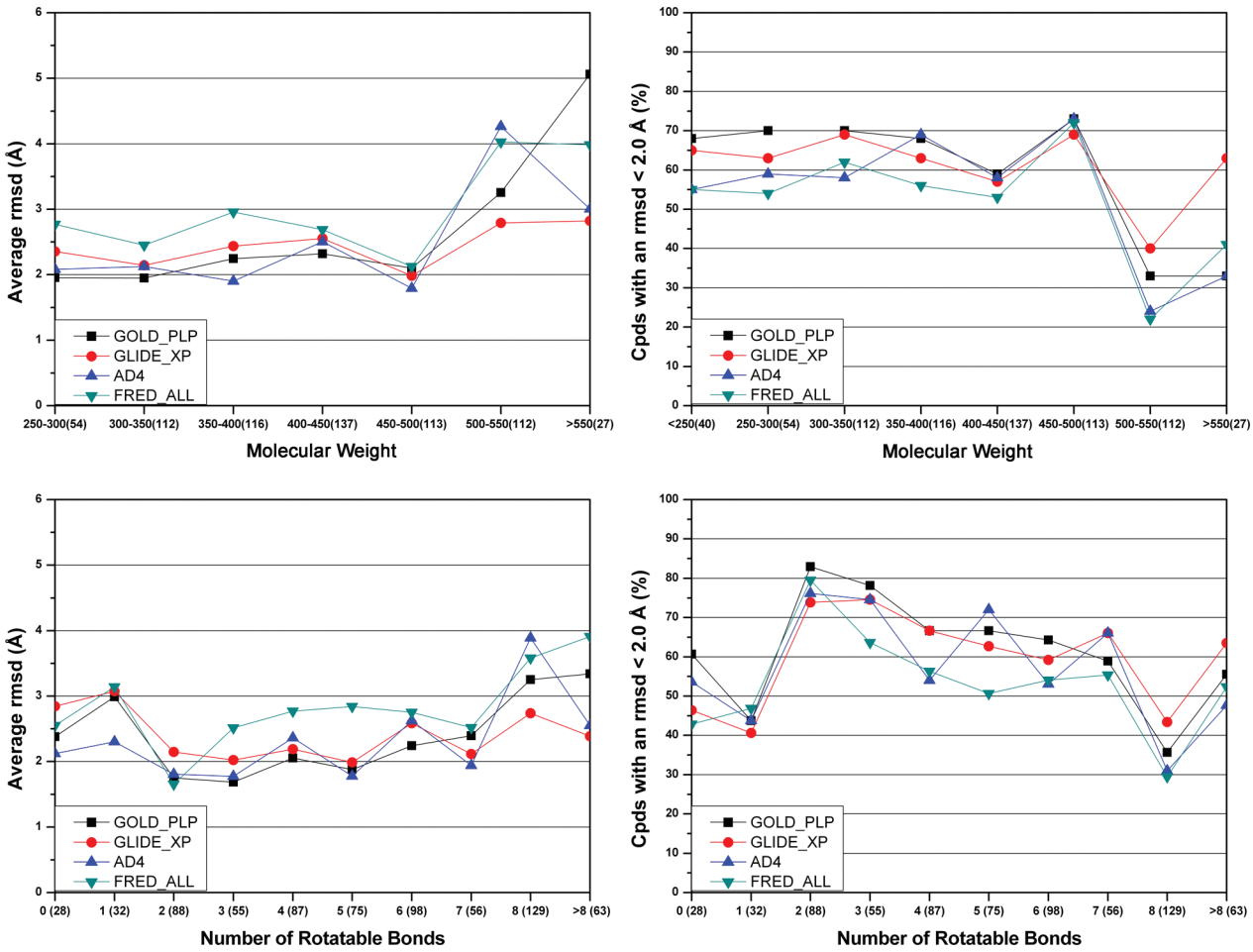

Figure 2. Results of the self-docking study. For the four best procedures, the armsd (left) and number of poses with a rmsd less than 2 Å (right) is reported against the MW (up) and the number of rotatable bonds (down) of the ligands.

to the MW of the ligands was that ligands with a MW greater than 500 Da had worse docking results.

Cross-Docking. Cross-docking involves the docking of a ligand into the protein structure obtained from a different complex. Compared to self-docking studies, cross-docking analysis better verifies how efficiently docking studies can support lead identification and structure−activity relationship studies where no structural information are experimentally available for the ligands of interest. In this case, the ligand of interest is docked into an available protein structure that is usually complexed with a different ligand. Cross-docking analysis essentially simulates this situation because it evaluates how efficiently a docking software package is able to reproduce the experimentally determined binding pose of a ligand by docking it into a protein whose 3D structure was determined in complex with a different ligand.

All kinase types with at least six structures deposited in the RCSB protein data bank[12] were studied (see Table 1 for the list of examined kinases). For all complexes of each kinase type, the ligands were extracted and docked in all structures of the same kinase type and the obtained docking results were compared with the experimentally determined ligand dispositions (see the Experimental Section for details). We carried out the cross-docking studies using the GOLD_PLP, GLIDE_XP, AD4, and FRED_ALL docking procedures because they showed the best self-docking results and corresponded to four different docking software packages. As a result, 22 kinase types with a total of 421 kinase structures and 17 285 docking calculations for each docking procedure were taken into account (see Table 1).

Table 1. Kinases Taken into Account for the Cross-Docking Studies

kinase name	species	no. of structures
tyrosine-protein kinase ABL1	human	11
tyrosine-protein kinase ABL 1	mouse	8
cell division protein kinase 2	human	104
serine/threonine-protein kinase Chk1	human	31
casein kinase II subunit alpha	*Zea mays*	22
death-associated protein kinase 1	human	10
epidermal growth factor receptor	human	10
ephrin type-B receptor 4	human	8
glycogen synthase kinase-3 beta	human	15
tyrosine-protein kinase Lck	human	17
MAP kinase-activated protein kinase 2	human	8
hepatocyte growth factor receptor	human	11
mitogen-activated protein kinase 10	human	23
mitogen-activated protein kinase 14	human	44
mitogen-activated protein kinase 14	mouse	8
3-phosphoinositide-dependent protein kinase 1	human	14
serine/threonine-protein kinase pim-1	human	22
tyrosine-protein kinase Src	chicken	11
serine/threonine-protein kinase 6	human	17
vascular endothelial growth factor receptor 2	human	7
vascular endothelial growth factor receptor 2 (V916T)	human	12
wee1-like protein kinase	human	8

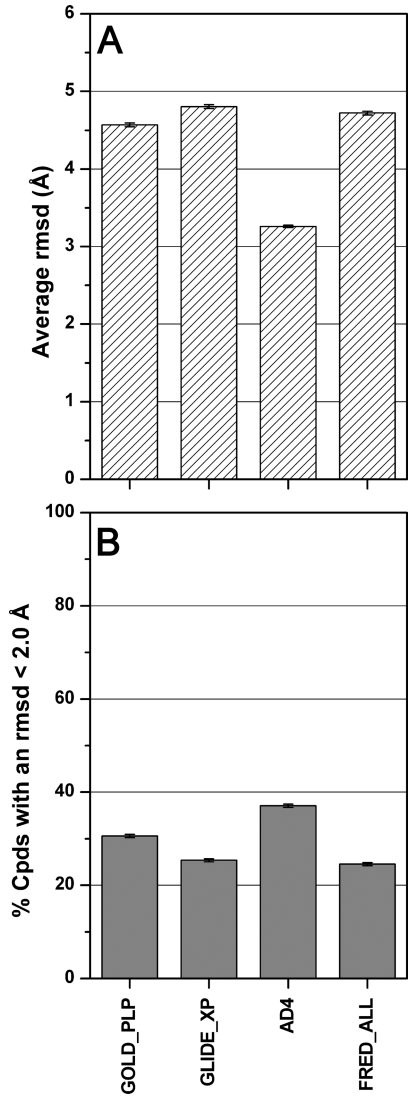

Figure 3. Results of the cross-docking study. For the four best procedures, the armsd (A) and the number of poses with a rmsd less than 2 Å (B) is reported. Error bars denote the standard error of the analysis.

Figure 3 summarizes the main results obtained from the cross-docking studies. AD4 showed the best armsd (3.3 Å), whereas the other three docking procedures resulted in an armsd of 4.6−4.8 Å. The percentage of compounds demonstrating a good disposition, defined as having a rmsd smaller than 2.0 Å with respect to the experimental disposition inside the binding site, was about 37% using AD4, about 31% using GOLD_PLP, and about 25% using GLIDE_XP and FRED_ALL (see Figure 3B). These data consider all structures together giving overall results; however, as shown in the Supporting Information, Table S1, evaluating the armsd for the single kinase types and then averaging these values, the obtained results were similar to those obtained considering all the docking results together, with an armsd of 3.7 Å for AD4 and 5.0−5.2 Å for the other three docking procedures. Furthermore, as shown in Table S2, Supporting Information, averaging the percentage of compounds with a good disposition, evaluated for each kinase type, we obtained similar results to that obtained considering all the docking results together, with 35% of good docking pose using AD4, about 29% using GOLD_PLP and GLIDE_XP, and 28% using FRED_ALL.

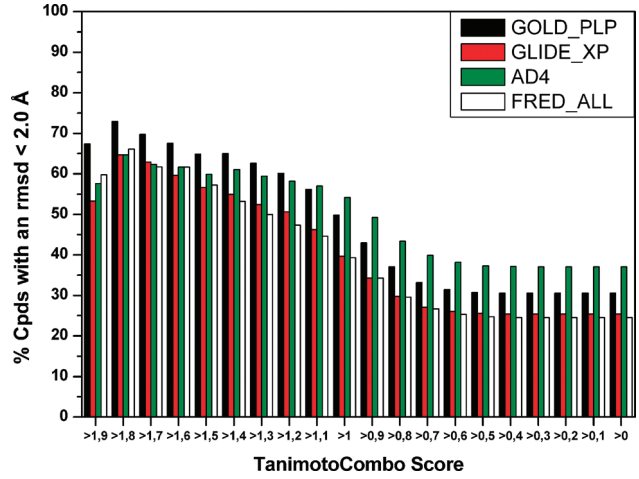

Figure 4. For the four best docking procedures, the number of poses with a rmsd less than 2 Å is reported against various threshold values of the TanimotoCombo score.

In going from the self-docking to the cross-docking calculations, the statistical results worsened dramatically. This behavior was probably due to the fact that kinases are very flexible proteins. To test this hypothesis, all binding sites of the same kinase type were aligned to each other to determine the level of flexibility of the binding sites.

The alignment of the heavy atoms of the binding site resulted in an armsd of 1.2 Å with 70% of the superimpositions showing a rmsd greater than 1.0 Å, thus confirming a high level of flexibility.

In conclusion, due to the kinase flexibility, the standard docking of a ligand in a protein structure whose 3D structure has been determined in complex with a different ligand had only about 37% of a chance of giving a reliable pose. This percentage can be considered as a reliable approximation of the probability that a docking software package will give a good prediction of the real pose of a ligand into a kinase whose structure has been experimentally determined in complex with a different ligand.

Ligand Similarity. Probably, the most important weakness of docking software is the fact that it is unable to take into account the protein flexibility in a complete manner. This is particularly evident in the case of kinases, for which the binding site can adopt different interacting conformations depending on the ligand structure. For this reason, it could be interesting to analyze how the similarity of the ligands is related to the docking results.

Using the OpenEye software, all ligands were extracted from the original complexes, subjected to CS (through Macromodel[16] and OMEGA2 program[17]), and aligned to the starting reference ligand structures (through the ROCS program[18]). OMEGA2 takes into account the flexibility of a molecule by generating all representative conformers, while ROCS compares the shapes of the molecules using a smooth Gaussian function and takes into account the chemistry alignment (see the Experimental Section for details). The ligand alignment score (TanimotoCombo score) was then correlated with the docking results of the cross-docking studies. Figure 4 and Table 2 summarize the obtained results.

This analysis took into account all ligands complexed with the same kinase: each of these ligands was then cross-docked in all other experimental structures of the same kinase. For example, consider an experimental kinase structure A com-

Table 2. Correlation between the Ligand Similarity (expressed as the TanimotoCombo score) and the Docking Results (expressed as the percentage of compounds with an rmsd smaller than 2.0 Å with respect to the experimentally determined ligand pose) for the Four Best Procedures[a]

TanCombo Score	docking calculations[b]	% compounds with an rmsd <2.0 Å			
		GOLD_PLP	GLIDE_XP	AD4	FRED_ALL
>1.90	92	67.4	53.3	57.6	59.8
>1.80	218	72.9	64.7	64.7	66.1
>1.70	337	69.7	62.9	62.3	61.7
>1.60	490	67.6	59.6	61.6	61.6
>1.50	671	64.8	56.6	59.9	57.2
>1.40	894	65.0	54.9	61.1	53.2
>1.30	1155	62.6	52.4	59.4	50.0
>1.20	1504	60.1	50.6	58.2	47.3
>1.10	2113	56.2	46.2	57.0	44.6
>1.00	3520	49.8	39.6	54.1	39.3
>0.90	6321	43.0	34.3	49.2	34.3
>0.80	10 513	37.1	29.7	43.4	29.6
>0.70	14 409	33.1	27.1	39.9	26.6
>0.60	16 466	31.4	26.0	38.2	25.3
>0.50	17 165	30.7	25.5	37.3	24.7
>0.40	17 276	30.6	25.4	37.1	24.6
>0.30	17 285	30.6	25.4	37.1	24.6
>0.20	17 285	30.6	25.4	37.1	24.6
>0.10	17 285	30.6	25.4	37.1	24.6
>0.00	17 285	30.6	25.4	37.1	24.6

[a] A TanimotoCombo score of 2 means that two ligands are identical. [b] Number of analyzed docking results that satisfy the TanimotoCombo score threshold reported in the first column.

plexed with ligand A and an experimental kinase structure B complexed with ligand B. To cross-dock ligand B into protein A, complex B was first aligned to A; then the first docking pose disposition of ligand B into protein A was compared with the experimental disposition of ligand B into its protein B (resulting in a rmsd value). Ligand B was then subjected to a conformational search using OMEGA2, and its similarity with reference ligand A was evaluated using ROCS (resulting in a TanimotoCombo score value).

At this point, we searched for a correlation between the rmsd cross-docking values and the TanimotoCombo scores. As shown in Figure 4 and Table 2, the docking results seemed to be highly correlated with the ligand similarity. This correlation indicates that the probability of obtaining a good docking pose was higher if the ligand to be docked was similar to the ligand present in the complex from which the target docking protein was extracted. For example, when similar ligands (TanimotoCombo score > 1.2) were considered, about 60% of the poses found through the GOLD_PLP docking method were good, whereas when all compounds were considered (TanimotoCombo score > 0.0), only about 31% of the poses found through the same method were good (see Figure 4).

A comparison of the four docking procedures suggests that for compounds with a TanimotoCombo score greater than 1.2, GOLD_PLP was the best procedure, guaranteeing a reliable result in more than 60% of the cases. For compounds with a similarity score lower than 1.2, AD4 was the best approach, guaranteeing a reliable result in 37−57% of the cases, where the percentage depended strictly on the TanimotoCombo score (see Table 2). The data associated with a very high TanimotoCombo score are not statistically significant because they take into account only a small

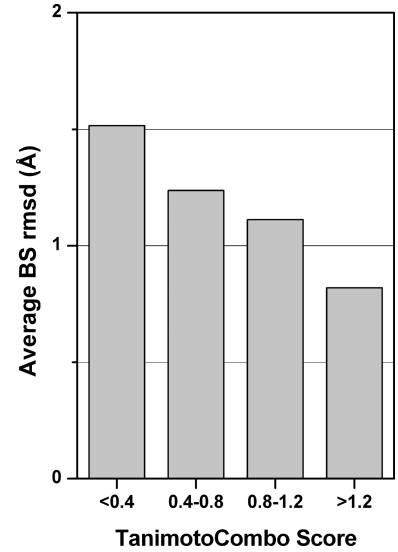

Figure 5. Average rmsd among the binding sites of the structures of the same kinase type against various ranges of the TanimotoCombo score of the corresponding ligands.

number of ligands. Therefore, it is preferable to consider the data corresponding to TanimotoCombo scores smaller than 1.5. That means that, even for very similar compounds (i.e., with a TanimotoCombo score of 1.9−1.6), it is preferable to consider a TanimotoCombo score threshold of 1.5, with about a 65% probability of obtaining a reliable docking pose using the GOLD_PLP method.

These data clearly demonstrate that prior to docking a ligand into a protein kinase, analysis of the similarity between the ligand to be docked and the ligand extracted from the original complex of the kinase of interest is very useful because the analysis can tell us which of the other reported structures of that kinase is the best protein candidate and provide a statistical parameter directly connected with the reliability of our docking studies.

Binding Site Similarity. The relationship found between ligand similarity and reliability of the docking poses should be due to the ability of the ligands to influence the conformation of the kinase binding sites.[19] To evaluate this influence, the similarity of the binding sites of a kinase complexed with different ligands was correlated with the similarity of the ligands. The parameter for evaluating the similarity of the binding site was the rmsd of the heavy atoms of the binding site residues in the various complexes superimposed through the ProFit program[20] (see the Experimental Section for details).

As shown in Figure 5, the complexes whose ligands were characterized by a similarity TanimotoCombo score value greater than 1.2 resulted in an average binding site rmsd of 0.8 Å, and this rmsd value worsened for the ligands that were characterized by a TanimotoCombo score smaller than 1.2.

Homology Modeling Studies. A large number of kinase 3D structures, developed by means of HM techniques, have been reported in the literature. Most of them have been obtained using the 3D structure possessing the highest sequence similarity with the target kinase (HM sequence similarity-based technique, SSB) as a template. However, our data suggest that kinases adapt their binding site for interaction with the ligands, and this adaptation appears to be fundamental for docking prediction. On the basis of this

Table 3. Binding Site (BS) and Docking Comparison between the Experimental X-ray Kinase Structures (Exp) and the Homology Models Obtained by Means of the Ligand Similarity-Based (LSB) and Sequence Similarity-Based (SSB) Techniques

gene name	PDB code	docking analysis (rmsd, Å)			BS analysis (rmsd, Å)	
		exp	LSB	SSB	LSB	SSB
DAPK2	2CKE	1.9	2.0	1.9	2.4	1.4
MAP3K5	2CLQ	1.3	1.9	5.1	1.8	3.8
Fyn	2DQ7	1.2	1.2	5.1	1.2	2.1
CAMK2G	2V7O	6.6	5.3	5.8	1.4	1.5
CAMK2A	2VZ6	1.3	5.5	8.3	1.7	2.1
CAMK4	2W4O	9.6	5.0	6.8	2.1	2.5
CAMK2B	3BHH	6.4	8.1	6.2	2.3	1.1
EPHA7	3DKO	0.8	2.5	10.6	1.8	2.2
FGFR-1	1FGI	2.0	1.6	5.8	2.9	2.6
TNK2	1U4D	1.1	1.1	4.7	2	2.9
CHEK2	2CN8	0.3	1.0	1.8	1.3	2.5
cki1	1EH4	5.9	5.3	4.7	2	2
CLK1	2VAG	0.7	5.2	4.3	1.8	1.4
CSNK2A1	2ZJW	2.3	3.1	2.4	1.4	1.8
IGF1R	2ZM3	1.0	9.7	9.5	1.9	1.2

result, we tested the possibility of developing kinase homology models created from a kinase template cocrystallized with a ligand similar to the one of interest (HM ligand similarity-based technique, LSB). To compare the docking reliability between classical SSB homology models and that obtained using the LSB approach, a test set of 15 kinase structures corresponding to 15 different kinase types was selected from the starting database of 711 kinases (see Table 3) and homology models of these kinases were built using both SSB and LSB methods (see the Experimental Section for details). The obtained models were compared with the experimentally determined structure of that kinase by measuring both the rmsd of alignment of the heavy atoms of the binding site and the rmsd between the found docked position of the ligand in the model and the experimental one.

Table 3 shows the comparison of the binding sites disposition. Analysis of the LSB models reveals that 11 out of 15 models showed a rmsd value smaller than 2.0 Å with respect to the experimental structures, whereas only 7 SSB models showed a rmsd value smaller than 2.0 Å. An analysis of the docking calculations for the 15 ligands also showed a result in favor of the LSB technique. The self-docking studies into the 15 experimentally determined kinase structures gave 10 results with a rmsd smaller than 2.0 Å (using the GOLD_PLP methods). The docking of these ligands into the LSB models gave six results with a rmsd smaller than 2.0 Å, whereas the docking of these ligands into the SSB models gave only two results with a rmsd smaller than 2.0 Å (see Table 3), resulting in a P value of 0.05.

These data strongly suggest that for analysis of kinase−ligand interactions where there are no experimentally determined kinase structures, a homology model of the protein built using a kinase complexed with a ligand similar to the one of interest as a template could be reliable for docking studies. If there is good ligand similarity, this approach should be more reliable than a classical homology model built using the kinase with the best amino acid sequence similarity as a template.

The docking calculations in the homology models can also be used for analyzing the different results obtained by applying a "hard scoring function" (such as GOLDScore) and a "softer scoring function" (such as ChemPLP). In particular, the use of a soft scoring, by reducing the steepness of the repulsion terms, should produce better results with low-quality structures like homology models.[21,22] For this reason we carried out the self-docking studies into the 15 experimentally determined kinase structures and in the LSB homology models using also the GOLDScore fitness function (GOLD_GS). As reported above using the GOLD_PLP method, the self-docking studies into the 15 experimentally determined kinase structures gave 10 results with a rmsd smaller than 2.0 Å and 60% of these compounds showed a reliable docking pose in the LSB models. As shown in the Supporting Information, Table S3, using the GOLD_GS method, the self-docking studies into the 15 X-ray structures gave 7 results with a rmsd smaller than 2.0 Å and only about 29% of these compounds showed a reliable docking pose in the LSB models. Therefore, in the field of the HM ligand docking, use of the GOLD_PLP approach significantly improves the results with respect to a "hard score" method such as GOLD_GS.

CONCLUSIONS

In this study we investigated the performance of six docking software packages (with a total of 17 docking procedures) to predict the binding disposition of kinase ligands. We used a very large kinase database, and the self-docking results highlight that four procedures, each one corresponding to a different software package, were the most reliable (i.e., GOLD_PLP, GLIDE_XP, AD4, and FRED_ALL) with about 55−60% of docked compounds showing a rmsd smaller than 2.0 Å. Compared to self-docking, the cross-docking study better simulates the difficulties of predicting the binding disposition of new kinase ligands because it evaluates how efficiently a docking method is able to reproduce the binding pose of a ligand by docking it into a protein whose 3D structure was determined in complex with a different ligand. By analysis of about 70 000 docking studies, our cross-docking results show that AD4 was the best docking method with about 37% of compounds showing a rmsd smaller than 2.0 Å. These results are not encouraging because they suggest that without taking into account any other information, only about 37% of the results of ligand docking into a kinase protein were reliable. However, careful analysis of the cross-docking studies suggests that the docking success was correlated with the binding site similarity, and the latter is strongly affected by the similarity of the ligands. Therefore, the current opinion that the probability of obtaining a good docking pose is greater if the ligand to be docked is similar to the ligand present in the complex from which the target docking protein has been extracted appears to be verified also for kinases. This observation was already reported by Verdonk and co-workers that in 2008, analyzing 1112 non-native structures for 65 drug targets, verified that docking against non-native structures of complexes containing ligands that are similar to the ligand that is being docked results in significantly higher docking performance and sampling performance.[8] Beyond this confirmation, we also tried to define a procedure that can improve the docking results and assess the statistical reliability of the docking calculations. This procedure is based

on the ligand similarity. If there are more complexes of the kinase of interest, the procedure suggests the best protein for docking studies and assess, using the data of Table 2, the probability of obtaining a reliable result before the docking calculations are done. These results were transferred to the HM field and led us to propose an alternative strategy for the development of kinase models whose experimental structures were not known. Our preliminary studies suggest that if the RCSB protein databank contained at least one kinase binder with a certain degree of similarity with the ligands of interest, then the LSB procedure gave better results than the classical SSB technique.

This study opened up the way to a lot of further improvements and new developments. In particular, in-house software was developed with an updatable internal database that allowed the testing of new docking software in a semiautomatic way. Therefore, we could enlarge the docking software panel and test the reliability of other software packages. The large amount of data obtained could also be used to statistically modify the scoring functions of docking software to further improve the kinase docking accuracy. In the presence of a series of compounds with a known activity, the top docking pose analysis can be seen as a preliminary step for examination of different binding modes relative to the known SAR. In these cases analysis of a larger number of docking poses is preferred;[23] therefore, analysis of the docking software taking into account more docking poses is another interesting goal. We are carrying out preliminary studies using AD4, and the analyzed data show that taking into account the docking pose with the best rmsd among the first 20 docking poses, the redocking calculations resulted in an armsd of 2.0 Å with about 64% of docked compounds that showed a rmsd smaller than 2.0 Å (considering only the first docking pose, the self-docking calculation resulted in a armsd of 2.5 with about 60% of compounds that showed a rmsd smaller than 2.0 Å). Finally, the LSB homology modeling technique could be further refined and automated to provide an efficient alternative tool to the SSB technique.

EXPERIMENTAL SECTION

Kinase−Ligand Complex Structures. In the SWISS-PROT protein sequence database,[24] we retrieved all proteins belonging to the protein kinase superfamily that catalyze a chemical reaction in which ATP is involved. As a result, 6182 kinase proteins were found (database updated on 07/20/2009) and 1730 PDB structures (derived from X-ray and NMR studies) were downloaded from RCSB protein databank.[12] Only the X-ray structures were retained, and then using in-house software we removed all the pdb files of kinases that (a) were not complexed with ligands, (b) had peptide ligands, or (c) contained allosteric ligands. Moreover, all solvent molecules and ions were removed, and if the pdb file contained more than one protein unit, only a monomer was retained. This analysis resulted in the selection of 711 kinase structures. Hydrogen atoms were added by means of the OpenEye Babel software[25] for the proteins and Szybki[26] software for the ligands. The ligands were extracted from the X-ray complexes and then subjected, after a minimization step, to a conformational search of 1000 steps in a water environment (using the generalized-Born/surface-area model) by means of the Macromodel software.[16] The algorithm used was the Monte Carlo method with the MMFFs force field and a distance-dependent dielectric constant of 1.0. In order to verify if the ligand preparation method reported above could lead to bias in docking calculations, a test set of 25 ligands was extracted from the X-ray complexes, converted to Smiles strings (detecting chirality from the 3D input structures), and converted back to 3D structure using Accelrys DS Visualizer.[27] After a minimization step, the obtained structures were then subjected to the conformational protocol described above and the geometry of the best conformations were compared with that obtained starting from the ligand X-ray coordinates. The results confirmed that the conformational search protocol was tight enough to remove the influence of the starting ligand geometry since using the two methods we obtained very similar results. Prior to the conformational search, the protonation state of the ligands was evaluated using the Epik software.[28] All compounds were then visually checked focusing attention on the common groups that bind to the hinge region of the binding site. In particular, we fixed the staurosporine structures, replacing the 1*H*-isoindol-1-one with the isoindolin-1-one system (see, for example, 1AQ1[29]) and the pyrrole−indolinone structures, replacing the 2*H*-indol-2-one with the indolin-2-one group (see, for example, 2JAV[30]).

Self-Docking Analysis. The ligands optimized in the way described above were docked into their corresponding proteins by means of the AUTODOCK 4.0,[14] DOCK 6.0,[31] FRED 2.1,[15] GLIDE 5.0,[9] GOLD 4.1,[13] and AUTODOCK VINA 1.0[32] software packages.

The docking results were evaluated through a comparison of the found docked positions of the ligands with the experimental ones. As a measure of docking reliability, the rmsd between the positions of the heavy atoms of the ligand in the calculated and experimental structures was taken into account. The rmsd analysis could be generated automatically using an internal algorithm for AUTODOCK, DOCK, and GLIDE. For all other docking calculations, the rmsd analysis was developed using the rms_analysis software of the GOLD suite.

Cross-Docking Analysis. Among the starting 711 kinase structures, we selected the kinase types with at least six X-ray structures. As a result, 22 kinase types were selected for a total of 421 structures. Using the Mustang software,[33] the backbone atoms of the structures for each kinase type were aligned with each other using a reference kinase structure. Then each ligand was docked into all the available structures of the kinase type from which the ligand was extracted, and the docking reliability was evaluated by calculating for each ligand and each kinase structure the rmsd (heavy atoms) between the reference position of the ligand in the experimental kinase−ligand complex and that predicted by the docking software in the various kinase structures. The rmsd analysis was carried out as described above for the self-docking studies.

Binding Site Comparison. For each kinase type, comparison of the binding site disposition was developed using ProFit.[20] Starting from the complexes previously aligned on the basis of the backbone atoms (see above), all residues possessing at least an atom within a radius of 5 Å from the ligands, including gap residues (for a maximum of 2 consecutive gap residues), were considered as belonging to the binding site. At this point, all residues that were present

in at least one of the determined binding sites were taken into account, generating a list of residues common to all binding sites of a kinase. Then an alignment of these residues for all binding sites of a kinase type was performed, and for each comparison, the rmsd (heavy atoms) value was calculated.

Docking Procedures. For all docking analyses, only the best pose was taken into account.

AUTODOCK 4.0. The Autodock Tools utilities[34] were used in order to identify the torsion angles in the ligands, add the solvent model, and assign the Gasteiger atomic charges to the protein. The ligand Gasteiger charge was calculated using Szybki.[26] The regions of interest used by Autodock were defined by considering the reference ligand as the central group of a grid box of 15 Å in the *x*, *y*, and *z* directions. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations. By use of the Lamarckian genetic algorithm, the docked compounds were subjected to 20 runs of the Autodock search using 2 500 000 steps of energy evaluation and the default values of the other parameters. The software has a semiempirical free energy scoring function that is based on a linear regression analysis, the AMBER force field, and a large set of diverse protein−ligand complexes with known inhibition constants.

DOCK 6.0. The molecular surface of the binding site was calculated by means of the MS program,[31] generating the Connolly surface with a probe with a radius of 1.4 Å; the points of the surface and the vectors normal to it were used by the Sphgen program[31] to build a set of spheres, with radii varying from 1.4 to 4 Å that describe, from a stereoelectronic point of view, the negative image of the site. The spheres within a radius of 10 Å from the reference ligand were used to represent the site. For each ligand, the DOCK 6.0 calculated 500 orientations; of these, the best grid scored was taken into consideration. The grid-based score is based on the nonbonded terms of the molecular mechanic force field. The ligand charge was calculated using the AM1-BCC method, as implemented in the Antechamber suite of Amber 10.[35]

FRED 2.1. FRED[15] requires a set of input conformers for each ligand. The conformers were generated by OMEGA2;[17] in order to avoid bias the ligands obtained from the CS using Macromodel (see above) were used as starting structures. We applied the following modifications to the default settings of OMEGA2: the energy window was set to 50.0, the maximum number of output conformers was set to 10 000, the time limit was set to 1200, and the rmsd value below which two conformations were considered to be similar was set at 0.3 Å. The region of interest for the docking studies was defined in such a manner that it contained all residues which stayed within 10 Å from the ligand in the X-ray structures. The FRED docking method roughly consisted of two steps: shape fitting and optimization. During shape fitting, the ligand was placed into the binding site using a smooth Gaussian potential. A series of three optimization filters was then processed, which consisted of refinement of the positions of the hydroxyl hydrogen atoms of the ligand, rigid body optimization, and optimization of the ligand pose in the dihedral angle space. In the last optimization step, the Chemgauss2 scoring function was used. After the docking calculation, the poses were scored independently by the six available scoring functions, i.e., Chemgauss3 (CG3), Chemscore (CS), Plp (PLP), OEChemscore (OCS), Screenscore (SCR), Shapegauss (SHA), and by a combination of them (FRED_ALL). CG3 is the third version of the Chemgauss scoring function, which uses smooth Gaussian functions to represent the shape and chemistry of molecules. CS was derived empirically from a set of 82 protein−ligand complexes for which measured binding affinities were available, and it was trained by regression against measured affinity data. PLP is a knowledge-based simplified energetic model that includes intramolecular energy terms for the ligand, given by torsional and nonbonded functions, and intermolecular ligand−protein steric and hydrogen-bond interaction terms calculated from a piecewise linear potential summed over all protein and ligand heavy atoms. OCS is an OpenEye variant of the Chemscore. SCR derives from a combination of the PLP and FlexX score. SHA is a shape-based scoring function that uses smooth Gaussian functions to represent the shapes of molecules.

GLIDE 5.0. The binding site was defined by a rectangular box of 15 Å in the *x*, *y*, and *z* directions centered on the ligand. The possibility of docking compounds with more than 120 atoms was activated, whereas the GLIDE[9] defaults were used for all other parameters. The GlideScore fitness function is based on Chemscore but includes a steric-clash term, adds buried polar terms to penalize electrostatic mismatches has modifications on other secondary terms. Two docking analyses were carried out using the standard precision (SP) and extra precision (XP) methods. The XP mode is a refinement tool designated for use only on good ligand poses, the sampling is based on an anchor and refined growth strategy, and the scoring function includes a more complete treatment of some of the SP terms, such as the solvation and hydrophobic terms.

GOLD 4.1.1. The region of interest for the docking studies was defined in such a manner that it contained all residues which stayed within 10 Å from the ligand in the R structures; the "allow early termination" command was deactivated, while the possibility for the ligand to flip ring corners was activated. For all other parameters, the GOLD[13] defaults were used and the ligands were subjected to 30 genetic algorithm runs. Five docking analyses were carried out. In the first four cases, the four fitness functions implemented in GOLD, i.e., GOLDScore (GS), ChemScore (CS), ASP, and ChemPLP (PLP), were used. In the fifth case, we used the kinase scoring function (KCS) implemented in GOLD (applicable using ChemScore as a fitness function), which calculates a contribution for weak CH···O interactions. The GS fitness function is made up of four components: the protein−ligand hydrogen-bond energy, the protein−ligand van der Waals energy, the ligand internal van der Waals energy, and the ligand torsional strain energy. The CS fitness function is the same scoring function used in the FRED calculations (see above). ASP is an atom−atom potential derived from a database of protein−ligand complexes; this fitness scoring function was developed using information about the frequency of interaction between ligand and protein atoms of the existing ligand−protein structures in the PDB. Finally, the ChemPLP is an empirical fitness function in which the Piecewise Linear Potential is used to model the steric complementarity between protein and ligand and the distance- and angle-dependent hydrogen and metal bonding terms from CS are considered.

AUTODOCK VINA 1.0. The input files for the protein and ligands originated from the Autodock Tools utilities[34] for the AUTODOCK 4.0 calculations were also used for the AUTODOCK VINA[32] calculations, including the grid box

Table 4. CPU Time Required for the Docking of a Single Ligand

docking method	CPU time[a]
GLIDE_SP	1
GLIDE_XP	23
VINA	13
DOCK6	25
FRED_ALL	22
FRED_CG3	20
FRED_CS	20
FRED_OECS	20
FRED_PLP	20
FRED_SCR	20
FRED_SHAPE	22
GOLD_GS	69
GOLD_CS	23
GOLD_KCS	23
GOLD_PLP	19
GOLD_ASP	23
AD4	246

[a] The CPU time values are reported with respect to the time required by the GLIDE_SP docking calculation.

dimensions. The exhaustiveness parameter was set to 10 and the Energy_range to 1, whereas for all other parameters, the AUTODOCK VINA defaults were used. The VINA scoring function combines certain advantages of knowledge-based potentials and empirical scoring functions, extracting information from both the conformational preferences of the receptor−ligand complexes and the experimental affinity measurements.

Calculation of Standard Errors. For values with continuous distributions, standard errors were calculated as follows

$$\text{standard error} = \text{standard deviation}/\sqrt{N}$$

For reliable/unreliable docking results (i.e., docking within 2.0 Å rmsd or not) standard errors were calculated using the standard deviation from Bernoulli trials

$$\text{standard error} = \sqrt{(P(1 - P)/N)}$$

where P is the probability of success and N is the number of samples.[7]

CPU Time. Table 4 shows the CPU time required for running the docking calculation of a single ligand. The use of GLIDE with the standard precision mode resulted in the faster docking approach, and the time necessary for running the docking calculation with the other docking methods is reported as a relative value of the GLIDE standard precision CPU time. These data highlight that most of the docking methods require a very similar CPU time, with the exception of GLIDE with the standard precision mode that is the faster approach and AD4 that requires a CPU time about 10-fold greater than the other docking methods.

Ligands Similarity Analysis. Comparison of the compounds belonging to the same kinase type was done using the ROCS 3.0 software,[18] which is a shape-similarity method based on the Tanimoto-like overlap of volumes. The alignment was assessed using the TanimotoCombo score, which combines the Tanimoto shape score with the color score for the appropriate overlap of groups with similar properties (donor, acceptor, hydrophobe, cation, anion, and ring). The ROCS default values were used for all other parameters. The maximum number of conformations per molecule was 10 000, which were already generated using OMEGA2 for the FRED docking calculations. The experimental structures of the ligands extracted from the complexes were used as reference structures. Then all the OMEGA2-generated conformers of the ligands complexed with proteins belonging to the same kinase type were aligned in turn to all reference structures. As a result, each ligand of one kinase type was aligned to all other ligands complexed with the same kinase type, and each alignment resulted in a TanimotoCombo score that measures the degree of similarity between the two compounds. The TanimotoCombo score can assume values from 0.0 to 2.0; when two compounds are perfectly aligned (and, therefore, are identical both structurally and conformationally), the TanimotoCombo score is 2.0.

Homology Modeling Procedure. For the test set, 15 structures corresponding to 15 different kinase types were selected from the starting database of 711 kinases. Each of the 15 ligands was then extracted from the corresponding structure and subjected to an extensive conformational search using OMEGA2 (these calculations were already carried out for all ligands for the self-docking analysis using the FRED software). A database of the 711 ligands extracted from their corresponding kinase structures was generated, and each of the 15 ligands was subjected to a shape similarity-based analysis using the TanimotoCombo score of the ROCS 3.0 software and using each of the 711 ligands of the database as a reference structure. As a result, each of the 15 ligands was aligned with the 711 ligands. To select the template for the development of the ligand similarity-based homology model for each of the 15 ligands, the ligand belonging to the database of 711 ligands (excluding the ligand object of the analysis) with the best TanimotoCombo score was taken into account together with the ligands that had TanimotoCombo scores that differed less than 0.2 from the best score. Among these ligands and their corresponding kinases, the template with the highest homology sequence was then chosen as a template. Kinase−ligands complexes whose ligands did not show at least one TanimotoCombo score greater than 1.2 were not included in the test set.

All primary sequences were obtained from the SWISS-PROT protein sequence database.[24] For the SSB homology modeling procedure, sequence similarity searches were carried out using Blast and, excluding the kinase object of the study, the X-ray structure of the kinase with the highest degree of sequence similarity with the target was chosen. Then, for both LSB and SSB procedures, the sequence alignment of the chosen kinases was performed using the CLUSTAL W software[36] with a gap open penalty of 10 and a gap extension penalty of 0.05. Table 5 shows the ligand similarity (TanimoCombo score) and sequence similarity (Blosum weighted matrix from CLUSTAL W) for the template used for making the homology models using the two procedures.

The HM models were constructed using Modeler.[37] Five structures were optimized using the default variable target function method (VTFM) with conjugate gradients and then refined using molecular dynamics with simulated annealing. Among the five resulting structures, the best one was chosen on the basis of the Discrete Optimized Protein Energy (DOPE) and the GA341 assessment methods. All models were built keeping the template ligands in the binding site during construction of the homology models. The docking

Table 5. TanimotoCombo Score and Alignment Score between the Kinases of the Test Set and the Templates Selected for the Development of the Ligand-Based and Sequence-Based Homology Models[a]

	ligand-based HM			sequence-based HM	
exp. struct.	LSB Templ.	TanCombo Score	alignment score	SSB Templ.	alignment score
2CKE	2CSN	1.6	19	3F5U	77
2CLQ	1BYG	1.9	21	3COM	30
2DQ7	1QPJ	1.9	69	3D7U	83
2V7O	1UVR	1.6	26	2WEL	91
2VZ6	2BHE	1.7	27	2WEL	92
2W4O	2J51	1.8	29	1A06	46
3BHH	3GGF	1.5	26	2V7O	89
3DKO	2OFV	1.5	41	3FXX	81
1FGI	3G0F	1.5	50	3CLY	88
1U4D	1DM2	1.7	24	2HZI	39
2CN8	1ZLT	1.7	30	2JAM	43
1EH4	3FUP	1.2	18	2CHL	56
2VAG	2VTH	1.2	25	2WU6	60
2ZJW	1M2R	1.4	74	3E3B	86
2ZM3	1AGW	1.5	38	1I44	79

[a] The TanimotoCombo Score only refers to the ligand-based homology models. The PDB code of the kinase templates (LSB and SSB Templ.) used for the development of the LSB and SSB model is also reported.

studies were then carried out using the GOLD_PLP method and following the docking procedure described above. The choice of GOLD_PLP as the docking method was due to the fact that in the cross-docking studies this procedure was the most reliable for compounds with a similarity score greater than 1.2.

Supporting Information Available: Additional statistical cross-docking statistical results (Table S1 and S2); comparison between the GOLD_PLP and GOLD_GS docking results evaluated for the experimental X-ray kinase structures and the homology models obtained by means of the ligand similarity-based technique (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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CI100161Z