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Identifying the binding mode of a molecular scaffold

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Abstract

We describe a method for docking of a scaffold-based series and present its advantages over docking of individual ligands, for determining the binding mode of a molecular scaffold in a binding site. The method has been applied to eight different scaffolds of protein kinase inhibitors (PKI). A single analog of each of these eight scaffolds was previously crystallized with different protein kinases. We have used FlexX to dock a set of molecules that share the same scaffold, rather than docking a single molecule. The main mode of binding is determined by the mode of binding of the largest cluster among the docked molecules that share a scaffold. Clustering is based on our 'nearest single neighbor' method [J. Chem. Inf. Comput. Sci., 43 (2003) 208–217]. Additional criteria are applied in those cases in which more than one significant binding mode is found. Using the proposed method, most of the crystallographic binding modes of these scaffolds were reconstructed. Alternative modes, that have not been detected yet by experiments, could also be identified. The method was applied to predict the binding mode of an additional molecular scaffold that was not yet reported and the predicted binding mode has been found to be very similar to experimental results for a closely related scaffold. We suggest that this approach be used as a virtual screening tool for scaffold-based design processes.

Introduction

Many studies in the field of structure based drug design (SBDD) are based on a crystal structure of a complex between a ligand and a biological target [1, 2]. Details of the binding mode are essential for any attempt to improve the binding affinity by some extension or modification of that ligand. But, altering parts of that ligand's structure could damage the activity and so, based on chemical analysis and knowledge, it is common to regard some parts of the ligand as its 'scaffold' which should not be modified. This scaffold serves as the common denominator for constructing a series of 'substitutions', which amount to molecular manipulations of the scaffold by some synthetic methods. The construction of a scaffold is often based upon synthetic considerations that determine which positions can be varied. Several programs have been produced to aid in designing high-affinity molecular candidates based on a given scaffold [3, 4] and they operate best if such a structure of a protein-ligand complex is available and if a scaffold can be identified. Of course, it is assumed in such cases, that modifications that do not affect the scaffold will not affect the binding mode that was detected in the crystal. This is certainly an overly-optimistic outlook. A tougher situation is encountered in those cases where the binding affinities of a series of molecules, with a common scaffold, are known from experiment but a binding mode or binding modes are yet unknown due to the lack of any crystal complex or nuclear magnetic resonance structures. A more challenging case is when no data exists for the binding affinity of a known mo-

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lecular series, while the worst case is if the substituted scaffold has not yet been synthesized and should be planned or filtered by virtual means.

The Protein Data Bank [5] currently contains many native structures and structures of protein-ligand complexes. The possibilities for protein-ligand complex formation are infinite, and only a small fraction of such complexes have been crystallized and analyzed. Only in a few cases it is possible to find crystal structures of complexes of a few ligands with the same protein. Thus, having computer-assisted methods that are likely to indicate the binding mode(s) of a new inhibitor in a given binding site and which are based on minimal knowledge of the binding site characteristics, is an advantage. Virtual docking approaches were pioneered by Kuntz et al. [6], and were applied for predicting the structure of many protein-ligand complexes. Since then, the number of virtual docking methods has been growing rapidly. Nevertheless, the success of most of these docking programs (see [7] for an updated list) for 'rigid protein' docking techniques of single ligands is still about 30%–50%. However, computer docking experiments on a relatively small number of molecules can be extremely helpful for lead generation, in comparison to high throughput screening [9]. The design of new drugs involves, in many cases, modifications of a single scaffold [10, 11]. Therefore, the mediocre rates of successful docking might restrict its usage for drug design purposes.

We propose an alternative strategy to the common approach of docking a single or a few representative ligands from the same family [12] into a specific binding site. Instead, we recommend docking a large set of different ligands that share the same molecular scaffold, to the same target site, in order to identify the preferred binding mode/s of that scaffold. The method or, rather, combination of methods, may be named 'scaffold driven docking'. We demonstrate this method with protein members of the protein kinase (PK) family. This family [13] includes hundreds of proteins that are involved in many functions in the cell cycle [14]. Their association with disease is due to excess activity, and thus it is desired to inhibit their activity through competitive binding to the catalytic site. The main target in this site has been the ATP binding domain and in the last two decades, a large number of ATP analog inhibitors of this family have been examined [14, 15]. Most of them contain a planar, conjugated component. There is wide interest in predicting the bound structures of these molecules as part of a substantial effort to produce PK inhibitors with improved specificity. Therefore, a reliable prediction of a scaffold's binding mode to several PKs could assist in designing more specific and potent inhibitors for subgroups of this large protein family.

We applied the scaffold-driven docking to predict the binding mode(s) of candidate complexes taken from three different families of protein kinases: transmembrane spanning tyrosine kinases (FGFr), tyrosine kinases (src family) and cyclin dependent kinase (CDK). Eight tyrosine kinase-inhibitor complexes (out of about 50 in the Protein Data Bank [5]) were 'decomposed' into protein and ligand and reconstructed via FlexX [16]. The selected complexes represent three major families of protein kinases, Src, FGFr, and Map kinase. Out of these eight complexes, only three were reconstructed with distance root-mean-square deviation (dRMSD, without superimposing) values below 2 Å and therefore are considered to be successful. With the scaffold-driven docking, which uses the same force field, only one of this set of eight complexes was incorrectly docked to some extent. Moreover, an additional predicted complex suggests that this method may be able to predict alternative scaffold binding modes that could be an aid to experimentalists.

Scaffold-driven docking

The following procedure was applied:

1. **Select –** (a) The structure of the target protein binding site. In the cases described here it is taken from known protein-ligand complexes. (b) A series of ligands for docking by considering their affinities to the protein target, which should preferably be in the micromolar or lower range.

2. **Dock** the molecular series into the active site using the same force field or default parameters for all the molecules in the series. The FlexX scoring function used in this work is described in ref. 16. Briefly, it includes the following terms:

$$\begin{aligned}\Delta G = \Delta G_0 \; & + \Delta G_{rot}{}^{\bullet} N_{rot} \\ & + \Delta G_{hb}{}^{\bullet} \sum_{\text{H}-\text{bonds}} f(\Delta R, \Delta\alpha) \\ & + \Delta G_{io}{}^{\bullet} \sum_{\text{Ionic int.}} f(\Delta R, \Delta\alpha) \\ & + \Delta G_{aro}{}^{\bullet} \sum_{\text{Aro int.}} f(\Delta R, \Delta\alpha) \\ & + \Delta G_{lipo}{}^{\bullet} \sum_{\text{Lipo cont.}} f(\Delta R)\end{aligned}$$

where N_{rot} is the number of rotatable bonds that are immobilized in the complex. ΔG_0, ΔG_{rot}, ΔG_{hb},

ΔG_{io} are adjustable parameters. ΔG_{aro} accounts for the interactions of aromatic groups, ΔG_{lipo} accounts for pairwise sums of atom-atom contacts and $f(\Delta R, \Delta\alpha)$ is a scaling function penalizing deviations from the ideal geometry.

3. **Cluster** the conformations into 'families' (clusters) based on the 'nearest single neighbor' (NSN) method [17]. This clustering method, which is described in detail elsewhere, is based on the proximity of the cRMSD (with superimposing) between the scaffolds' heavy atoms of the ligands series. The cRMSD matrix between all conformations of the docked structures is initially calculated. Then, starting with the lowest energy conformer (or any other conformation), the matrix is reordered by searching for that conformation which has the lowest cRMSD to the current one, and positioning it to be the next along the matrix's rows/columns. The reordered cRMSD matrix serves for computing the value of a specific cutoff that determines the boundaries of clusters. The process of reordering and assigning clusters is fully automatic.

4. **Score –** Each of the clusters presents a scaffold mode of binding, and is scored according to the number of conformations in that cluster. The cluster with the largest number of conformations is the preferred binding mode, and was found in most cases to comprise more than a third of the total number of docked ligands, and about double the size of any other cluster for that scaffold. Other criteria should be applied to distinguish between clusters if this main criterion of cluster size is not fulfilled: (1) proximity to the active site, measured as the distance between the center of mass (CM) of the scaffolds' atoms from the CM of pre-defined atoms in the binding/catalytic site, and (2) the number of H-bonds between the partners (it is constant for a mode of binding of a cluster, because the range of fluctuations of its molecules is quite small due to these H-bonds). Additional criteria such as the energy of binding can be useful in certain cases. In this work, it was required to apply additional criteria in a single case out of eight.

Experimental method

(a) **Single molecule docking –** The molecules PD173074, SU5402, and SU4984 (Figure 1a) were docked into fibroblast growth factor receptor-1 (FGFr-1) ATP binding domain (PDB codes: 2FGI, 1FGI, 1AGW). PP1 and PP2 molecules were docked into Hck (1QCF) and Lck (1QPE) src-family protein ATP binding domains, respectively. Purvalanol B, Olomoucine, and Hymenialdisine molecules were docked into CDK-2, Map kinase-2, and CDK-2 ATP binding domains (PDB codes: 1CKP, 4ERK and 1DM2), respectively. In addition, multiple modes of each of these molecules were collected and clusterd into families, for comparison to the method of scaffold docking presented below. These complexes, and the final results for single molecule docking by FlexX, are given in Table 1.

(b) **Scaffold docking –** Eight series of analogs, including the few [8] crystallized analogs (a total of 210 ligands) were docked into the ATP binding domains of the original crystals. Table 1 presents also the ligand series for each of the original complexes. The lists of the ligands series are as follows: 38 analogs based on scaffold PD-1 (Table 2) were docked into 2FGI. Twenty ligands based on scaffold SU-1 and 21 analogs based on scaffold SU-2 were docked into 1FGI and 1AGW, respectively (Table 3). Thirty-one ligands based on scaffold PD-2 (Table 4) were docked into 1AGW. Twenty-five analogs based on scaffold PP (Table 5) were docked into the ATP binding domains of Hck and Lck (PDB codes: 1QCF, and 1QPE, respectively). Twenty-five analogs based on scaffolds PU (Table 6) and 30 analogs based on scaffolds Olo (Table 7) were docked into the ATP binding domain of CDK-2 and Map kinase 2 (PDB codes: 1CKP, 4ERK). Finally, 20 analogs based on scaffold Hy (Table 8) were docked into the ATP binding domain of CDK-2 (PDB code: 1DM2).

Docking was performed by the automated flexible docking program FlexX [16] as implemented within SYBYL 6.7 [18]. All FlexX parameters were set to their default standard values, and only the best scored pose was used for the scaffold-based docking. For docking single ligands in order to compare them to the 'scaffold docking', we retained a 100 poses per ligand. After removing all water molecules, the active sites were dissected from the crystal structure of the enzyme complexes by including all residues that have at least a single (heavy) atom at a distance of 7 Å from any of the ligand's heavy atoms. Residues were kept fixed in their crystallographic positions in all docking experiments. Average dRMSD values were calculated for each cluster by averaging the dRMSD (without superimposing) of the docked ligands' scaffold (heavy atoms) with respect to the active site X-ray mode.

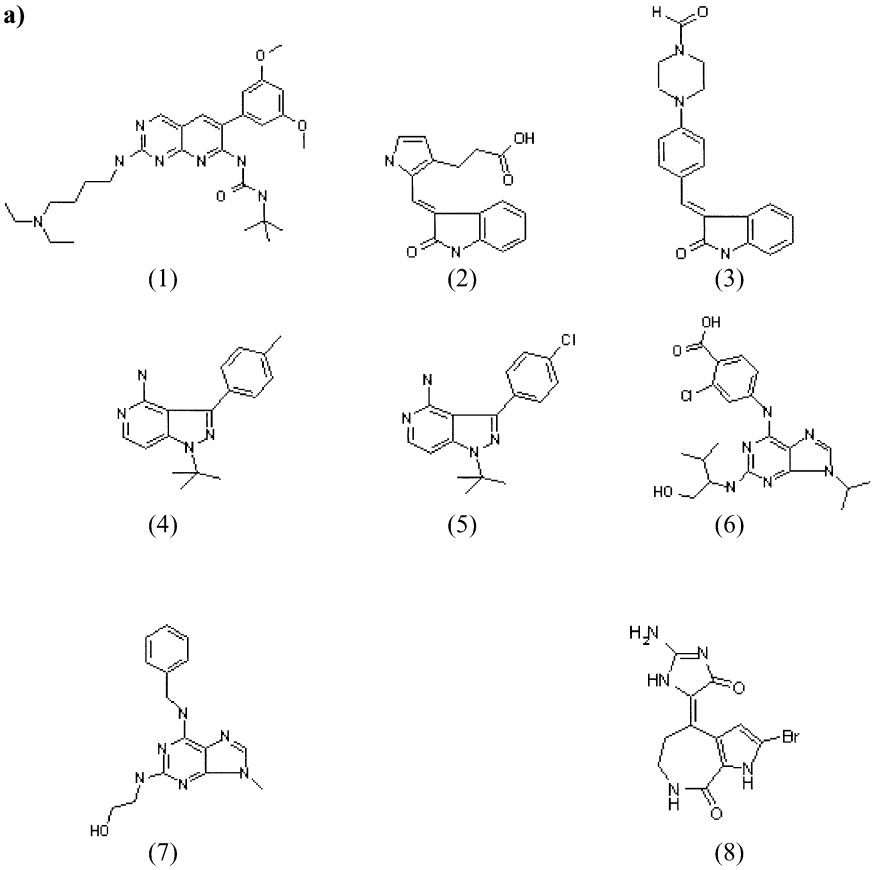


Figure 1. (a) The structures of: (1) PD173074, (2) SU5402, (3) SU9804, (4) PP1, (5) PP2, (6) Purvalanol B, (7) Olomoucine, and (8) Hymenialdisine. (b) The molecular scaffolds: (I) PD-1 (derived from PD173074), (II) PD-2, (III) SU-1 (derived from SU5402), (IV) SU-2 (derived from SU9804), (V) PP (derived from PP1 and PP2), (VI) Pu (derived from Purvalanol B), (VII) Olo (derived from Olomoucine), and (VIII) Hy (derived from Hymenialdisine).

Table 1. Protein kinases used in this study.

Analog no.	PDB code	Protein name	Ligand name	Best dRMSD to experimental mode* in single docking	No. of ligands in scaffold driven docking
1	2FGI	Fgfr-1	PD173074	1.1	38
2	**	Fgfr-1	PD-2		31
3	1FGI	Fgfr-1	SU5402	3.2	20
4	1AGW	Fgfr-1	SU4984	3.7	21
5	1QCF	Hck	PP1	0.3	25
6	1QPE	Lck	PP2	0.5	25
7	1CKP	CDK2	Purvalanol B	7.2	25
8	4ERK	Map Kinase 2	Olomoucine	5.4	30
9	1DM2	CDK2	Hymenialdisine	***	20

*Only the scaffold heavy atoms were taken into account.
**No member of this series was crystallized (see text).
***The ligand completely failed to dock.

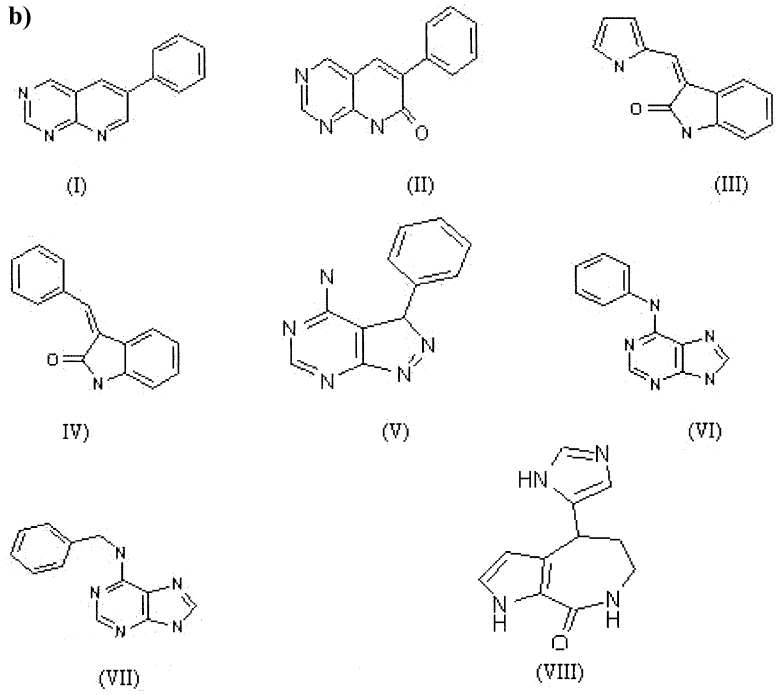


Figure 1. (Continued).

Results

In general, one can consider most of the PKI scaffolds as being composed of two main fragments, i.e., a heterocyclic fragment (mimicking the adenine moiety in ATP), and an attached ring system with, at least, one ring. While the adenine-like fragment is non-specific by its nature, the additional rings increase the specificity of different scaffolds to different PKs. Tables 2–8 contain the details of the input data, while Tables 9–18 present the docking results.

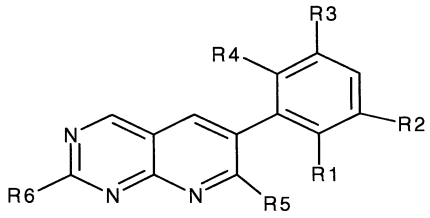
I. Transmembrane spanning tyrosine kinase

PD173074, SU5402 and SU4984 (Figure 1a, 1–3, respectively) are tyrosine kinase inhibitors (TKI), whose binding modes to Fibroblast Growth Factor (FGFr-1) were resolved by crystallography [19, 20]. Both SU5402 and SU4984 scaffolds include a 1,3-dihydro-indol-2-one fragment, which is attached to different types of rings in each. FlexX was used to dock the molecules into their binding sites. Analysis of the top 30 solutions revealed that, in those single molecule docking experiments, the best dRMSD of SU5402 and SU4984 molecules solutions was more than 3 Å from the experimental mode, while the dRMSD of PD173074 was found to be 1.1 Å.

Micromolar or stronger affinity to FGFr-1 kinase domain was found for analogs of PD173074 and of SU5402 [21–25], and several of those were used in our docking experiments of scaffolds series PD-1 and SU-1 (Tables 2 and 3). To the best of our knowledge, no additional molecules (except SU4984) with the SU-2 scaffold were tested for their FGFr-1 kinase affinity. Thus, in order to study this scaffold, the substituent R-groups for scaffold SU-1 were used for this other scaffold (SU-2) and should thus be regarded as a virtual set of candidates.

The analyses of the docking results for the above three series are detailed in Tables 9–11. Once the three molecular series had been docked into the FGFr-1 ATP binding site, each set of ligands was clustered, and a representative conformation (the best scored by FlexX) from each cluster was further analyzed. For the PD-1 scaffold (Table 9), four clusters with more than a single conformation were obtained. The largest cluster includes 45% of the ligands, and is 2.4-fold larger than the next cluster. Its average distance from the binding site atoms CM is also the shortest among these four clusters. The binding mode of this scaffold is close to the crystallographic binding mode with an average dRMSD of 1.2 Å for all of the cluster ligands, while three of them were found at a dRMSD of 0.5 Å. Some

Table 2. The R-groups attached to scaffold PD-1, taken from ref. [21, 22, 24, 25].

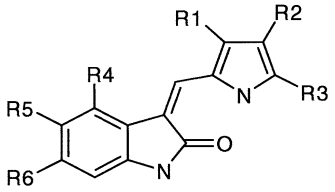


PD-1

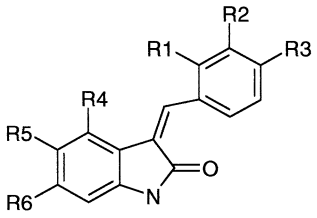
Analog no.	R1	R2	R3	R4	R5	R6
1	Cl	-	-	Cl	NH_2	NH_2
2	Br	-	-	Br	NH_2	NH_2
3	Cl	-	-	Cl	$NHCONC(Me)_3$	$NH(CH_2)_3\ N(Et)_2$
4	Me	-	-	Me	NH_2	$NH(CH_2)_3\ N(Et)_2$
5	Br	-	-	Br	$NHCONC(Me)_3$	$NH(CH_2)_3\ N(Et)_2$
6	Me	Me	Me	Me	$NHCONC(Me)_3$	NH_2
7	-	OMe	OMe	-	$NHCONC(Me)_3$	NH_2
8	Cl	-	-	Cl	NHCON-Ph	$NH(CH_2)_4\ N(Et)_2$
9	Cl	-	-	Cl	$NHCONC(Me)_3$	$NH(CH_2)_3\ N(Me)_2$
10	Cl	-	-	Cl	$NHCONC(Me)_3$	$NMe(CH_2)_3\ N(Me)_2$
11	Cl	-	-	Cl	$NHCONC(Me)_3$	$NH(CH_2)_3$-Npiperazine
12	Cl	-	-	Cl	$NHCON(CH_2)_2CH_2$	$NH(CH_2)_3$-Npiperazine
13	Cl	-	-	Cl	NHCON-CycloHexane	$NH(CH_2)_3$-Npiperazine
14	Cl	-	-	Cl	$NHCON(CH_2)_2NMe_2$	$NH(CH_2)_3$-Npiperazine
15	Cl	-	-	Cl	$NHCONEt_2$	$NH(CH_2)_3$-Npiperazine
16	Cl	-	-	Cl	NHCON-Phe	$NH(CH_2)_3$-Npiperazine
17	Cl	-	-	Cl	NHCON-Phe-1-Ome	$NH(CH_2)_3$-Npiperazine
18	Cl	-	-	Cl	NHCON-Phe-2-Ome	$NH(CH_2)_3$-Npiperazine
19	Cl	-	-	Cl	NHCON-Phe-3-Ome	$NH(CH_2)_3$-Npiperazine
20	Cl	-	-	Cl	$NHCON\ Me_2$	$NH(CH_2)_3$-Npiperazine
21	Cl	-	-	Cl	$NHCON\ CH_2C(Me)_3$	$NH(CH_2)_4\ N(Et)_2$
22	Cl	-	-	Cl	$NHCON\ CHN(Me)_2$	$NH(CH_2)_4\ N(Et)_2$
23	Cl	-	-	Cl	NHCON-Ph	$NH(CH_2)_3$-Npiperazine
24	Cl	-	-	Cl	$NHCONC(Me)_3$	$NH(CH_2)_4\ N(Et)_2$
25	Cl	-	-	Cl	NHCON-CycloHexane	$NH(CH_2)_3$-Npiperazine
26	Cl	-	-	Cl	NHCON- Phe – 3- OCH_3	$NH(CH_2)_3$-Npiperazine
27	Cl	-	-	Cl	NHCON- Phe – 3-Me	$NH(CH_2)_3$-Npiperazine
28	Cl	-	-	Cl	$NHCON\ C(Me)_3$	$O(CH_2)_2N(Et)_2$
29	-	$N(Me)_2$	$N(Me)_2$	-	$NHCON\ C(Me)_3$	NH_2
30	-	CF_3	CF_3	-	$NHCON\ C(Me)_3$	NH_2
31	-	OEt	OEt	-	$NHCON\ C(Me)_3$	NH_2
32	Cl	-	-	Cl	NH_2	CH_3
33	Cl	-	-	Cl	NHCOH	CH_3
34	Cl	-	-	Cl	NHCOMe	CH_3
35	Cl	-	-	Cl	$NHCOCH_2Me$	CH_3
36	Cl	-	-	Cl	$NHCOCH_2Me$	CH_3
37	Cl	-	-	Cl	$NHCOCH_2Me$	CH_3
38*		OMe	OMe		$NHCON\ C(Me)_3$	$NH(CH_2)_4\ N(Et)_2$

- Stands for hydrogen atoms.
*PD173074 molecule.

Table 3. The R-groups attached to scaffolds SU-1 and SU-2, taken from ref. [23, 49]. In the case of scaffold SU-2, the attached R-groups should be considered as a virtual set.



SU-1



SU-2

Analog no.	R1	R2	R3	R4	R5	R6
1**	$(CH_2)_2COOH$	CH_3	-	-	-	-
2	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	-
3	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	Br	-
4	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	SO_2NH_2	-
5	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	CO_2H	-
6	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	OCH_3
7	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	Ph
8	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	Ph-2-OCH_3
9	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	Ph-1-OCH_3
10	$(CH_2)_2COOH$	$(CH_2)_4^*$		-	-	Ph-3-OCH_3
11	-	$(CH_2)_4^*$		-	-	-
12	-	$(CH_2)_4^*$		-	Br	-
13	-	$(CH_2)_4^*$		-	SO_2NH_2	-
14	-	$(CH_2)_4^*$		-	-	OCH_3
15	-	$(CH_2)_4^*$		-	-	Ph
16	-	$(CH_2)_4^*$		-	-	Ph-2-OCH_3
17	-	$(CH_2)_4^*$		-	-	Ph-3-OCH_3
18	Me	-	Me	-	-	
19	$(CH_2)_2COOH$	Me		Me		
20	$(CH_2)_2COOH$				Br	
21***	-	-	Piperazine–4-COH	-	-	-

*This R-group connects R2 with R3.
**SU5402 molecule attached only to SU-1 series.
***SU4984 molecule attached only to SU-2 series.
- Stands for hydrogen atoms.

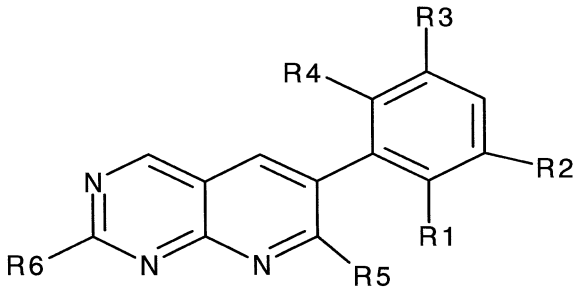
ligands that represent the main clusters are shown in Figure 2.

A similar scenario is observed for scaffold SU-1 (Table 10). The largest cluster includes 65% of the ligands and is 4.3-fold greater than the next cluster. This scaffold's CM is also the closest to the binding site atoms. This scaffold's position is similar to the experimental binding mode by an average dRMSD of 1.1 Å, while three of them were found at a dRMSD of 0.8 Å. In the case of scaffold SU-2, two larger clusters of similar size were found (clusters 1 and 2 in Table 11). In this case, two additional criteria were applied to choose the 'best' cluster, (1) the average distance of the scaffolds' CM from the binding site atoms, and (2) the number of H-bonds that each scaffold can generate (an H-bond X-H- - - -Y is considered to have a X——Y distance cutoff of $\leq$ 4 Å and an angle X-H- - - -Y of $180 \pm 30°$). Cluster 1 is superior in both, being closer to the CM of the binding site, and forming two H-bonds with the binding site atoms, while the scaffold of cluster 2 is further away from the CM and can form only a single H-bond.

In both of these cases (scaffolds SU-1, SU-2), the second most populated cluster has been found to be in a 'trans' configuration to the attached ring (Figure 3).The prevailing mode of both was 'cis' for the

a)

b)



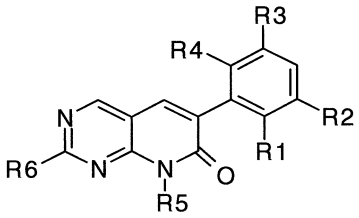
PD-1

Analog No.	R1	R2	R3	R4	R5	R6
3	Cl	-	-	Cl	NHCONC(Me) $_3$	NH$(CH_2)_3$ N$(Et)_2$
11	Cl	-	-	Cl	NHCONC(Me) $_3$	NH$(CH_2)_3$-Npiperazine
15	Cl	-	-	Cl	NHCONEt_2	NH$(CH_2)_3$-Npiperazine
17	Cl	-	-	Cl	NHCON-Phe-1-Ome	NH$(CH_2)_3$-Npiperazine
19	Cl	-	-	Cl	NHCON-Phe-3-Ome	NH$(CH_2)_3$-Npiperazine
21	Cl	-	-	Cl	NHCON $CH_2C(Me)_3$	NH$(CH_2)_4$ N$(Et)_2$
22	Cl	-	-	Cl	NHCON CHN$(Me)_2$	NH$(CH_2)_4$ N$(Et)_2$
23	Cl	-	-	Cl	NHCON-Ph	NH$(CH_2)_3$-Npiperazine
24	Cl	-	-	Cl	NHCONC(Me) $_3$	NH$(CH_2)_4$ N$(Et)_2$
29	-	N$(Me)_2$	N$(Me)_2$	-	NHCON C(Me) $_3$	NH_2
30	-	CF_3	CF_3	-	NHCON C(Me) $_3$	NH_2
33	Cl	-	-	Cl	NHCOH	CH_3
34	Cl	-	-	Cl	NHCOMe	CH_3
36	Cl	-	-	Cl	NHCOCH_2 Me	CH_3
37	Cl	-	-	Cl	NHCOCH_2 Me	CH_3
38*		OMe	OMe		NHCON C(Me) $_3$	NH$(CH_2)_4$ N$(Et)_2$
39	Cl	-	-	Cl	NHCOCH_2 Me	CH_3

(-) - Stands for Hydrogen atoms.

Figure 2. (a) Stereoscopic view of ligands that were included in the major PD-1 series' cluster. (b) A list of the R-groups present in the cluster.

Table 4. Scaffold PD-2 attached R-groups, taken from ref. [22, 50].



PD-2

Analog no.	R1	R2	R3	R4	R5	R6
1	-	OMe	OMe	-	Me	NH_2
2	Cl	-	-	Cl	Me	NH_2
3	Cl	-	-	Cl	Me	-
4	Cl	-	-	Cl	Me	Me
5	Cl	-	-	Cl	Me	NMe_2
6	Cl	-	-	Cl	Me	NHPh
7	Cl	-	-	Cl	Me	$N(CH_2)_3$-Npiperazine
8	Cl	-	-	Cl	Me	$N(CH_2)_3$NMorpholine
9	Cl	-	-	Cl	Me	$O(CH_2)_3NEt_2$
10	Cl	-	-	Cl	Me	$O(CH_2)_2NEt_2$
11	Cl	-	-	Cl	Me	$NHPhO(CH_2)_2NEt_2$
12	Cl	-	-	Cl	Me	$N(CH_2)_3$-Ph-3-CH_2CO_2H
13	Cl	-	-	Cl	Me	$N(CH_2)_3$-Npiperazine
14	Cl	-	-	Cl	Et	NH_2
15	Cl	-	-	Cl	Pr	NH_2
16	Cl	-	-	Cl	Bu	NH_2
17	Cl	-	-	Cl	i-Bu	NH_2
18	Cl	-	-	Cl	CH_2COOMe	NH_2
19	Cl	-	-	Cl	CH_2COO-t-Bu	NH_2
20	Cl	-	-	Cl	CH_2COOH	NH_2
21	Cl	-	-	Cl	CH_2Ph	NH_2
22	Cl	-	-	Cl	$(CH_2)_3NMe_2$	NH_2
23	Cl	-	-	Cl	$(CH_2)_3OCH_2Ph$	NH_2
24	Cl	-	-	Cl	$(CH_2)_3OH$	NH_2
25	-	-	-	-	Me	NHPh
26	Cl	-	-	Cl	Me	$NPh(CH_2)_2NEt_2$
27	-	-	-	-	Et	NHPh
28	-	-	-	-	Et	NH-4-Pyridine
29	-	-	-	-	Me	NHPh
30	-	-	-	-	Me	NH-4-Pyridine
31	-	-	-	-	Me	$O(CH_2)_2NEt_2$

- Stands for hydrogen atoms.

pyrolle or benzene ring. The 1,3-dihydro-indol-2-one part of the two SU scaffolds was found to be in the same mode as in the crystal structure.

An additional analog series, which is based on scaffold PD-2 (Figure 1b-II), was studied (Table 4). In this case, there is no crystallized ligand-protein complex to compare to. PD-2 differs from PD-1 by a single carbonyl that replaces an aromatic CH, transforming the character of this pyridine based ring to an amide and changing its basicity. Applying the same docking procedure to this series, we find a very similar binding mode to that of PD-1 (PDB code: 2FGI) favoured

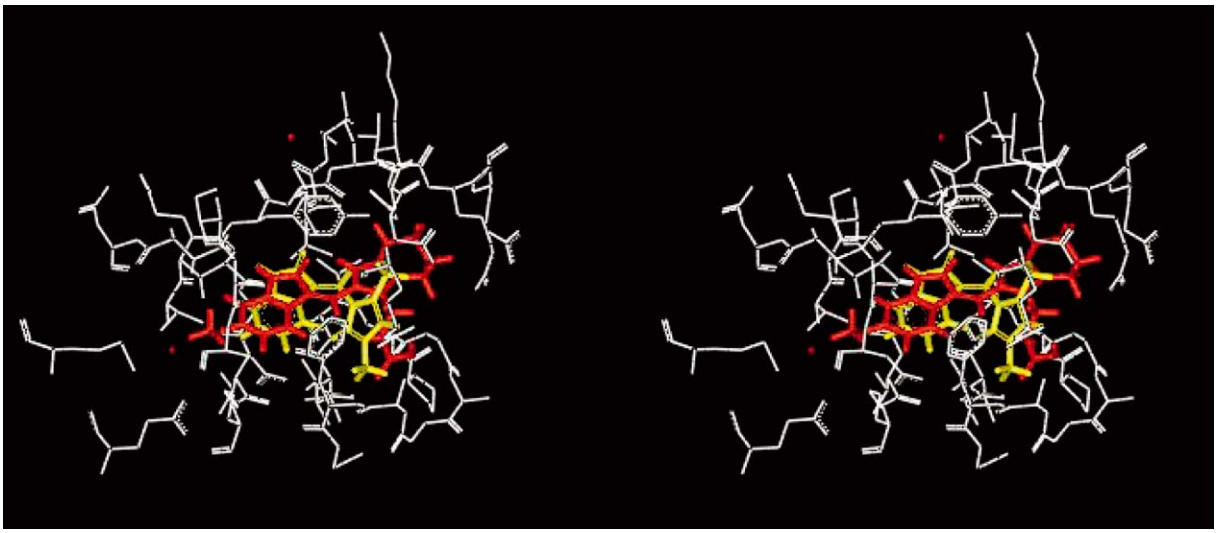


Figure 3. Stereoscopic view of the phenyl ring conformation of scaffold SU-2, in two alternative positions. The cis conformation is colored yellow and the trans conformation is colored red. FGFr-1 is colored white.

by the largest cluster of PD-2, that includes 45% of the total conformations and is 2-fold larger than the second cluster. This cluster also has the shortest average distance from the binding site atoms (Table 12). The binding mode compared to PD-1 is displayed in Figure 4.

II. Tyrosine kinase

Crystal structures are available for complexes of Hck and Lck with ligands that are specific kinase inhibitors. These crystals contain, respectively, PP1 and PP2, that share the same molecular scaffold that is named PP (Figure 1b–V). The series of molecules for our docking were constructed on the basis of experimental and patent data [26, 27]. FlexX was able to reconstruct, in a single docking experiment, the binding mode of both PP1 and PP2 within Hck and Lck, respectively.

Applying the scaffold driven docking method to these receptors, the same representative set of ligands that is based on the PP scaffold (Table 5) was used for both Hck and Lck. The largest clusters were found to be close to the experimental binding mode, with average dRMSD values of 0.1 Å and 0.3 Å, respectively (Tables 13 and 14).

III. Cyclin dependent kinase

Three specific cyclin dependent kinase (CDK) inhibitors, Purvalanol-B, Olomoucine, and Hymenialdisine, whose crystal structures with CDK-2 (Purvalanol-B and Hymenialdisine) and Map Kinase-2 (Olomoucine) are available, were used. The scaffolds of these molecules are named PU, Olo, and Hy, respectively (Figure 1b(VI–VIII)). The attached R-groups used in these cases (Tables 6–8) were taken from a general classification of groups [28–31] while only a small number of ligands were presented explicitly in these references. As previosuly, the results obtained for the Pu and Hy scaffold based series (Tables 15 and 17, respectively) suggest that the largest clusters do find the correct geometry of the scaffold within the protein.

Olo analog series is the only exception among our tests that docked correctly only its adenosine-like moiety into Map Kinase-2, in the major cluster. The attached ring conformation that was incorrectly docked in this cluster was docked correctly in the second sized cluster (Figure 5, Table 16).

IV. Clustering multiple poses of a single ligand

In order to more clearly distinguish between the method proposed in this paper, to dock N different molecules sharing a scaffold, rather than docking a single molecule N times, we tested the ability to achieve similar results by using the 100 best scored poses for single ligands, those of the original protein-ligand that were studied by us (Table 1). The results of these multiple poses are summarized in Table 18. For three ligands (PD173074, Purvalanol B, and SU5402), the correct binding mode was easily identifed by cluster size. In an additional case (Olomoucine), a partially correct binding mode was found for this ligand by

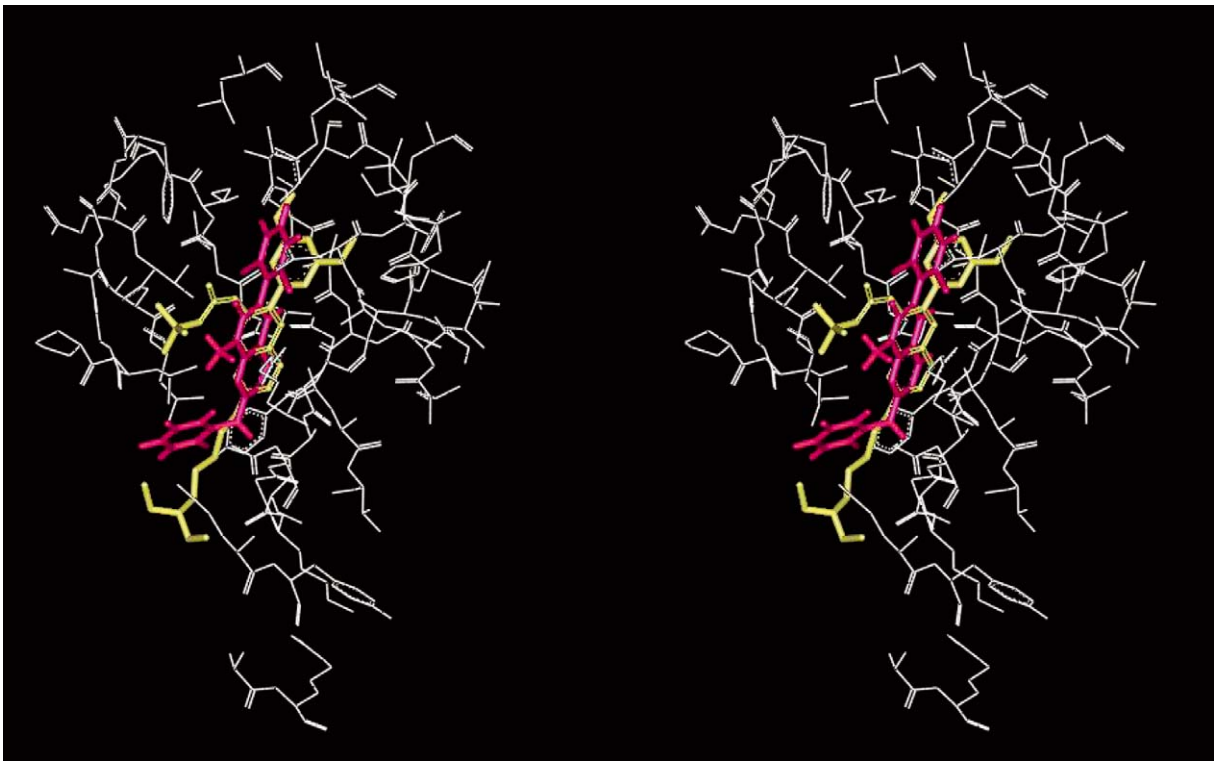


Figure 4. Stereoscopic view of the crystallographic binding mode of PD173074 (yellow) versus that of PD-2 (red), within the active site of FGFr-1.

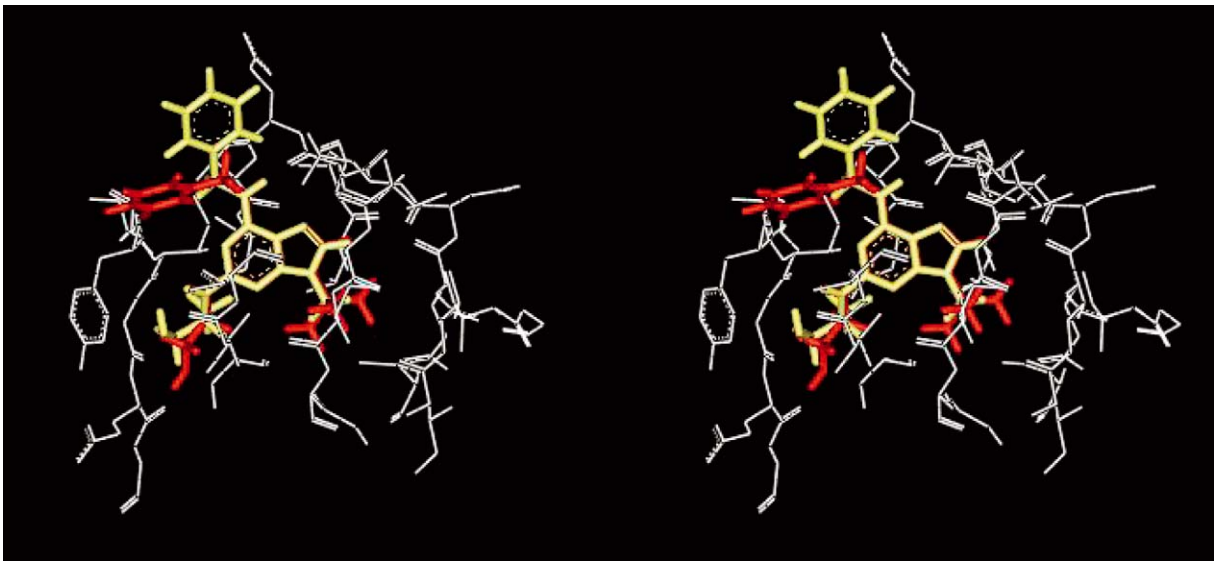
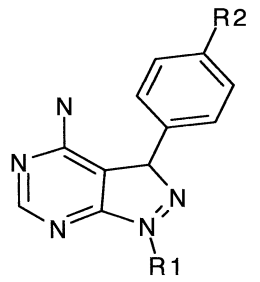


Figure 5. Two alternative positions of the phenyl ring conformation of scaffold Olo, within the binding site of Map kinase-2 (white). The cis conformation is colored red, and the trans conformation is colored yellow (stereoscopic view).

Table 5. Scaffold PP attached R-groups. R-group types were chosen according to ref. [26, 27].

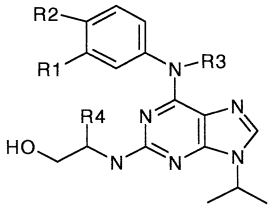


PP

Analog no.	R1	R2
1*	t-Bu	Me
2**	t-Bu	Cl
3	i-Pr	Me
4	i-Pr	Cl
5	Pr	Me
6	Pr	Cl
7	Et	Me
8	Et	Cl
9	Me	Me
10	Me	Cl
11	t-Bu	Br
12	i-Pr	Br
13	Pr	Br
14	Et	Br
15	Me	Br
16	Me	i-Pr
17	Et	i-Pr
18	i-Pr	i-Pr
19	Pr	i-Pr
20	Bu	i-Pr
21	t-Bu	i-Pr
22	H	i-Pr
23	H	Et
24	H	Me
25	H	H

*PP1 molecule.
**PP2 molecule.

Table 6. Scaffold Pu with attached R-groups. The R-groups selected were chosen according to ref. [30, 31].



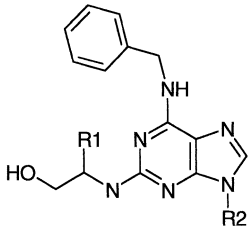
Pu

Analog no.	R1	R2	R3	R4
1	Cl	-	-	i-Pr
2*	Cl	CO_2H	-	i-Pr
3	Cl	-	-	-
4	Cl	-	Me	-
5	-	-	-	-
6	-	-	-	Et
7	Cl	-	-	Pro
8	Cl	-	-	Me
9	Br	CO_2H	-	t-Bu
10	-	-	Et	t-Bu
11	Br	Br	-	Me
12	CO_2H	Me	-	-
13	CO_2H	Me	-	i-Pr
14	Br	Br	-	-
15	OCH_3	Cl	-	-
16	Cl	OCH_3	-	Et
17	OCH_3	CO_2H	-	Me
18	-	-	Me	t-Bu
19	-	-	Et	Et
20	Cl	Cl	-	t-Bu
21	Cl	Br	-	i-Pr
22	OH	-	-	Pro
23	OH	Cl	-	Et
24	OH	Me	-	i-Pr
25	Cl	Cl	-	t-Bu

- Stands for hydrogen atoms.
*This molecule is Purvalanol.

both the single ligand multiple poses (Table 18) and by the scaffold-based method (Table 16). In the other four cases (PP1, PP2, SU4984, and Hymenialdisine) the results were less satisfactory in the single ligand multiple posing. For Hymenialdisine no binding mode was found. For SU4984, none of the suggested binding modes resembles the experimental binding mode. In the case of PP-1 and PP-2 the correct binding mode cluster was not among the two most populated clusters. The proximity to the binding site, being in favor of much less populated binding modes, cannot be a reliable criterion on its own. In the scaffold-based method, such ambiguities were not detected, as at least one of the most populated clusters was found to be in close proximity to the binding site (see Table 11 for the SU-2 scaffold (which includes SU4984)).

Table 7. Scaffold Olo attached R-groups. The R-groups selected were chosen according to ref. [28].

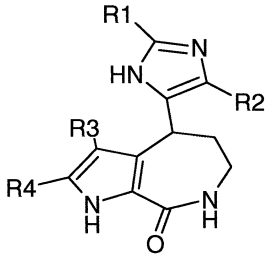


Olo

Analog no.	R1	R2
1	-	-
2	Me	-
3	Et	-
4	Pr	-
5	i-Pr	-
6	t-Bu	-
7*	-	Me
8	Me	Me
9	Et	Me
10	Pr	Me
11	i-Pr	Me
12	t-Bu	Me
13	-	Et
14	Me	Et
15	Et	Et
16	Pr	Et
17	i-Pr	Et
18	t-Bu	Et
19	-	i-Pr
20	Me	i-Pr
21	Et	i-Pr
22	Pr	i-Pr
23	i-Pr	i-Pr
24	t-Bu	i-Pr
25	-	t-Bu
26	Me	t-Bu
27	Et	t-Bu
28	Pr	t-Bu
29	i-Pr	t-Bu
30	t-Bu	t-Bu

- Stands for hydrogen atoms.
*This molecule is Olomucine.

Table 8. Scaffold Hy attached R-groups. The R-groups selected were chosen according to ref. [29].



Hy

Analog no.	R1	R2	R3	R4
1*	NH_2	=O	-	Br
2	NH_2	=O	-	-
3	NH_2	=O	Br	Br
4	=O	=O	Br	Br
5	NH_2	NH_2	-	Cl
6	NH_2	NH_2	Cl	Cl
7	NH_2	-	-	-
8	NH_2	-	-	Cl
9	-	-	Cl	-
10	-	-	Br	Br
11	-	NH_2	NH_2	-
12	-	NH_2	-	NH_2
13	NH_2	-	Br	-
14	NH_2	=O	-	NH_2
15	NH_2	=O	Br	Cl
16	=O	=O	Cl	Cl
17	NH_2	=O	NH_2	NH_2
18	=O	=O	NH_2	-
19	-	=O	Cl	NH_2
20	NH_2	-	Br	Br

- Stands for hydrogen atoms.
*Hymenialdisine molecule.

Discussion

Despite the enormous effort in this field, the rate of success in structural predictions of molecular complexes of proteins is not high [8]. More recently, in addition to the on-going effort to increase the reliability of existing methods, several strategies were introduced for analysis. One such trend is the effort to increase the scoring reliability, via combinations of scoring functions [32, 33]. Docking ligands into a few protein conformational states taken from experiment [34] or from simulation [35, 38] is another approach used to increase the rate of success in docking. A similarity driven method [36] was introduced as a tool for re-scoring docking results. A family docking approach was devised for increasing diversity in docking screens [37] while all family members are assumed to bind in a mode similar to that of the highest scored one. As part of this wide interest in increasing the reliability of docking methods as well as improving the process of lead optimization, we

Table 9. Results of docking the scaffold PD-1 based ligands (Table 2) into FGFr-1, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	17	1.0	-
2	7	4.9	-
3	6	5.8	-
4	3	6.6	-
5	1	2.1	-
6	1	7.8	-
7	1	8.7	-
8	1	3.9	-
9	1	19.0	-

*The experimental binding mode is within an average dRMSD of 1.2 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.5 Å was obtained for 3 ligands.

Table 10. Results of docking the scaffold SU-1 based ligands (Table 3) into FGFr-1, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	13	1.5	2
2	3	7.5	2
3	1	4.7	2
4	1	9.3	1
5	1	4.9	1
6	1	13.4	-

*The experimental binding mode is within an average dRMSD of 1.1 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.8 Å was obtained for 3 ligands.

describe herein a methodology for improving the determination of binding modes. This method assumes that molecules sharing a common scaffold also share binding characteristics.

We present a method that is useful in order to find binding modes of sets of molecules that are related to each other through a 'scaffold'. This variation on existing docking methods was provoked by the quality of results that we encountered by applying FlexX in single docking experiments with the PK series. Despite the ability to retain many docking alternatives for a single ligand in such an experiment, we could not reliably reconstruct the correct binding mode as the

Table 11. Results of docking the scaffold SU-2 based ligands (Table 3) into FGFr-1, ATP binding site*.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1**	5	2.1	2
2	4	5.2	1
3	2	3.9	2
4	2	5.5	1
5	2	9.3	1
6	1	10.4	1
7	1	10.1	1
8	1	10.1	1

*Three ligands failed to dock.
**The experimental binding mode is within an average dRMSD of 1.4 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.9 Å was obtained for a single ligand.

Table 12. Results of docking the scaffold PD-2 based ligands (Table 4) into FGFr-1, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	14	0.9	-
2	7	3.4	-
3	6	6.3	-
4	1	8.9	-
5	1	7.0	-
6	1	13.9	-
7	1	15.0	-

*The experimental binding mode is within an average dRMSD of 0.6 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.1 Å was obtained for a single ligand.

Table 13. Results of docking the scaffold PP based ligands (Table 5) into Hck, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	21	0.8	3
2	4	2.1	2

*The experimental binding mode is within an average dRMSD of 0.3 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.1 Å was obtained for a single ligand.

Table 14. Results of docking the scaffold PP based ligands (Table 5) into Lck, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	20	1.2	2
2	5	2.1	2

*The experimental binding mode is within an average dRMSD of 0.5 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.3 Å was obtained for a single ligand.

Table 15. Results of docking the scaffold Pu based ligands (Table 6) into CDK-2, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1*	21	1.8	1
2	2	0.7	0
3	2	5.1	1

*The experimental binding mode is within an average dRMSD of 0.8 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.1 Å was obtained for a single ligand.

Table 16. Results of docking the scaffold Olo based ligands (Table 6) into Map kinase-2, ATP binding site.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1**	20	3.7	-
2*	6	3.9	-
3	2	6.3	1
4	1	4.1	-
5	1	3.8	-

*The experimental binding mode is within an average dRMSD of 1.2 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.9 Å was obtained for a single ligand.
**The binding mode of the adenosine-like moiety is similar to the experimental binding mode (an average dRMSD of 1.4 Å), while the phenyl is oriented differently compared to that mode.

Table 17. Results of docking the scaffold Hy based ligands (Table 7) into CDK-2, ATP binding site*.

Cluster no.	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
1**	8	1.2	2
2	2	2.4	2

*Ten ligands failed to dock.
**The experimental binding mode is within an average dRMSD of 1.2 Å from the scaffold position (only the scaffold heavy atoms were taken into account). The minimum dRMSD of 0.6 Å was obtained for a single ligand.

Table 18. Major clusters taken from the best 100 poses of the experimentally determined ligands.

Complex	Cluster size	Scaffold-binding site center of masses distances (Å)	Number of H-bonds (scaffold-binding site)
PD173074	100*	1.0	-
SU5402	99*	1.3	2
	1	2.1	2
SU4984	26	6.5	-
	14	8.5	-
	10	8.1	-
	10	9.4	-
	4	4.0	-
	2	2.4	1
PP1	13	12.6	-
	13	6.6	-
	6*	1.0	3
PP2	27	7.1	1
	23	16.0	-
	16*	1.1	2
	6	6.2	1
Olomoucine	99**	3.7	-
	1	1.8	-
Purvalanol B	88*	1.8	1
	12	2.7	-
Hymenialdisine	0	-	-

*The docked scaffold position is within an average dRMSD of 1.5 Å from the experimental binding mode (only the scaffold heavy atoms were taken into account).
**The binding mode of the adenosine-like moiety is similar to the experimental binding mode (an average dRMSD of 1.4 Å), while the phenyl is oriented differently compared to that mode.

best scored pose (see Table 1). By using the scaffold of such molecules and running the docking experiments for a series of ligands based on that scaffold, keeping in each case only the best scored pose for each, we obtain a set of results which is capable of detecting the correct binding mode better than the 'single structure' experiments. We have shown that this method can predict nearly all of the binding modes resolved by experiments of the examined PK, while most of them were not detected by single docking experiments. For one of these scaffolds (SU-2), the correct binding mode was achieved by attaching several R-groups to it, that were not examined previously but were adapted from similar scaffolds (Table 3), while in some other cases (PP, Pu, Hy, Olo scaffolds), most of the R-groups were chosen from 'general types' of groups, which were suggested by the literature. In addition to the testing of existing binding modes, we have also predicted the binding mode for scaffold PD-2 (which is quite similar to that of PD-1) in its interaction with FGFr-1. We found that PD-2 should bind in a similar binding mode to PD-1. This prediction will hopefully be tested experimentally in the future. These results of binding to FGFr, compared to the unsuccessful isolated docking results of PD173074, SU5402, and SU4984, emphasize the advantages of using a guided approach such as the one presented here, which is based on statistical success rather than on the chance of a single ligand docking experiment.

The cases of PP1/Lck and PP2/Hck complexes demonstrate the potential contribution of our proposed docking procedure to specificity design. A similar scaffold has been found to be correctly positioned within different proteins. Therefore, using such complex structures of a single scaffold with a series of proteins from the same family, may introduce modifications which could improve the molecular affinity to one of these proteins and not to the others.

From the results of Tables 9–17 it may be seen that, in each set of ligands (scaffold), the chances for correct docking are reasonably large. In Table 9, the chances are 17/38 or 45%. In Table 10, they are 65%. They are much higher, in the 80–85% range, in Tables 13–15. These are, of course, results of the scaffold driven docking experiments. A single docking experiment might end up with a wrong result even if its chance to be correct is 80% and higher. Therefore, a single experiment is not helpful for identifying the binding mode. The variations of success between the different scaffolds are too large and so, they do not supply us with any predictive ability for the outcome of a new experiment. In a recent comparison of docking methods by single docking experiments, a dRMSD of 2.0 Å increased the success rate to 66%–76% [39]. All of our best results have a much lower dRMSD, between 0.3 Å and 1.4 Å.

This method could be useful for scaffold-based design in appropriate cases. Examples of its potential use vary from constructing a series following lead molecule generation [40], to simply enhancing the predictive ability of the existing docking tools. There are analogous methods for searching the space of ligand binding by 'multiple choices', such as extensively exploring the conformational space by increasing the ligand's conformational sampling [41], or simultaneous docking and scoring the ligand via different methods and generating a consensus scoring between these methods [33, 42–44]. Our current strategy suggests an alternative by exploring a series of ligands which share a common scaffold. It has the advantage of being fast (about 1 minute per ligand) as the length of the most time-consuming process, the docking, grows linearly with the size of the ligand sample. It has an additional benefit of discovering alternative binding modes that may be further explored. Finally, it does not require the application of a few docking methods to form a concensus. This concensus may be formed within a single method, for a wide series with similar basic backbone, the 'scaffold'. Thus, it could be applied within other docking techniques that could fail with a single ligand.

FlexX, like other docking methods, predicts correctly the binding mode in a significant population for a series of molecules, therefore one may expect that the main experimental binding mode of a molecular series could be discovered by docking 'different' molecules that share the same scaffold. This suggestion was shown to be successful in nearly all of the examples presented in this paper. It is successful in situations of low diversity between ligands, such as in the case of the PP series (Table 5), as well as in other cases of greater diversity within the series such as PD-1 (Table 2). It operates well also in cases where only a single or very few ligands were experimentally studied previously (scaffolds SU-2, PU, Olo, Hy and PP). Relevant R-groups were taken either from patents (PU, Olo, Hy and PP) or extracted from very similar scaffolds (SU-2). For most of these scaffolds our method was able to identify the correct binding mode.

Therefore, we believe that this protocol could be useful also for studying the binding modes of ligands in other, non-PK, protein families. It is important to

mention that the contribution of enzyme flexibility to successful docking has not been considered at all. It is quite clear that, in many cases, even molecules with a similar scaffold but with different 'substitutions' will give rise at least to variations in the local conformations of an active site. This is even more pronounced in clear cases of 'induced fit' along ligand binding, such as HIV-1 proteinase (1). Such variations could be included by docking the ligands into multiple conformations of the protein [35, 45, 46]. Moreover, the current discussion may be restricted to those cases where a reference protein complex structure exists.

Flexibility plays an important role in the interactions of protein kinases with ligands [47]. Thus, it is not expected that a ligand that bears no similarity to ligands that were extracted from crystal structures would 'dock' properly even in a scaffold-based docking procedure as the one that we propose here. This should not deter one from using this method: the need for flexibility on the protein side could be introduced by several approaches [48].

To summarize, the strategy that was demonstrated here was shown to be succesful for a set of inhibitors of protein kinases. We believe that it can be applied in many more cases and that it enables the discovery of binding modes for scaffolds, known or not, real or virtual, to respective protein sites. This would be especially the case if the scaffold dominates the structure while substituents are an 'add on', though important, feature. The limits of size for such 'add ons', and a more exact definition of scaffolds require further probing.

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