Improved FlexX Docking Using FlexS-Determined Base Fragment Placement

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We report on a novel hybrid FlexX/FlexS docking approach, whereby the base fragment of the test ligand is chosen by FlexS superposition onto a cocrystallized template ligand and then fed into FlexX for the incremental construction of the final solution. The new approach is tested on the diverse 200 protein−ligand complex dataset that has been previously described (Kramer et al. *Proteins: Struct., Funct., Genet.* **1999**, *37*, 228−241) for FlexX validation. In total, 62.9% of the complexes can be reproduced at rank 1 by our approach, which compares favorably with 46.9% when using FlexX alone. In addition, we report "cross-docking" experiments in which several receptor structures of complexes with identical proteins have been used for docking all cocrystallized ligands of these complexes. The results show that, in almost all cases, the hybrid approach can acceptably dock a ligand into a foreign receptor structure using a different ligand template, can give solutions where FlexX alone fails, and tends to give solutions that are more accurately positioned.

I. INTRODUCTION

Structure-based drug design efforts (those which use three-dimensional structural information from the protein target) have increased over recent years in parallel with the quantity of structural information (particularly the X-ray crystallographic information) available for the protein targets. Protein−ligand docking is a popular structure-based design technique, and a wide range of algorithms are currently employed in the pharmaceutical and biotechnology industries.[1−5] Recently, it has been commented that "none of these methods can be viewed as offering a robust and accurate solution to the docking problem";[4] no algorithm is perfect, and each performs better for certain classes of receptor than others.[6] One problem not addressed until fairly recently is that docking algorithms usually treat the protein target as a rigid entity, hardly representative of in vivo behavior, although this is now beginning to be addressed.[7−10] Various other techniques have also been applied in an attempt to improve the ligand-docking accuracy, both in terms of the ligand-positioning[11,12] and the ligand-scoring parts of the algorithms. With respect to the latter, efforts in improving the best ligand solution can be divided into the use of the scoring function as a fitness function that drives the ligand placement[13−15] and a screening function that filters poor solutions after the docking has taken place.[13,16] Ligand-based structure-guided docking approaches make use of cocrystallized ligands to guide the docking of other molecules toward a binding mode that is compatible with the one observed experimentally. To date, these implementations use a Gaussian-based molecular field similarity of a target ligand to a reference structure; this similarity is then used in addition to the docking score.[17−19]

Here, we describe a new automated hybrid approach for guided docking that is based upon the incremental construction algorithm of FlexX[1,20,21] and essentially uses the information not only from the protein target but also from the cocrystallized ligand via the small molecule alignment method of FlexS,[22] which (as described above) is increasingly common to obtain. Klebe et al.[23] have applied this philosophy by using a more linear workflow; after pharmacophore filtering using UNITY, hit compounds were ranked by FlexS superposition onto a known reference, followed by docking the best compounds using FlexX. With this approach, the information from the active cocrystallized ligand is used by FlexS to select hits; this is followed by using information from the protein target via FlexX to select hits; in our approach, we are directly linking these two methods together for each individual compound. In this study, the dataset used has been described previously, is distributed with the FlexX software, and consists of 200 diverse protein−ligand complexes.[24,25]

II. OVERVIEW OF THE APPROACH

The FlexX method[1] consists of fragmenting the ligand into components by severing it at all acyclic single bonds, placing a selection of base fragments into the active site (on the basis of interactions with the active site), and then incrementally constructing the remainder of the ligand by linking components in compliance with a torsional database. At each construction stage, the best partial solutions are selected and taken forward to the following construction step. This method is, thus, dependent on the base fragment selection and placement and assumes that the best base fragments interact with the active site to give a good score.

The scoring function used by FlexX is based on that originally developed by Böhm[26] and has been described in detail previously.[1] Briefly, the function contains contributions dependent on the number of rotatable bonds and surface areas of hydrogen-bonding, ionic, aromatic, and lipophilic interactions.

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The FlexS method[22] follows a similar strategy to that of FlexX: ligand fragmentation, base fragment selection and placement, and incremental construction. Various pharmacophoric interaction geometries are defined for the ligand; rather than scoring an interaction with a protein active site, the overlay of these features between the reference and target ligands is scored and volume overlap terms for Gaussian functions describing a number of physicochemical properties (partial charge, hydrophobicity, hydrogen-bonding donor and acceptor potential) are included. Again, at each construction stage, the best partial solutions are selected and taken forward to the following construction step.[27]

Our strategy focuses on improving the FlexX base fragment placement by using the information from the known cocrystallized reference ligand and, thus, improves the overall docked solution placement. It is intuitive that selecting a base fragment from the reference ligand and using the coordinates of this fragment for the incremental construction step would improve the redocking results for FlexX; the search space for FlexX would be narrowed considerably to those solutions that overlapped with the reference ligand, and hence, the resulting docked solutions would naturally have lower root-mean-square (rms) deviations from this reference. In practice, this approach is fairly redundant as the aim of docking is not to reproduce the coordinates of the reference ligand (this is purely validation of the docking algorithm) but to investigate binding modes of potential lead compounds and help prioritize them for synthesis (although it could be more effective for combinatorial series that share the base fragment).

With this in mind, we decided to investigate the FlexS algorithm, which structurally superimposes pairs of ligands, and to use this for base fragment selection and placement. As the correspondence between atoms is not a prerequisite for FlexS, the cocrystallized template ligand can be different from the ligand being docked. The recent versions of FlexX and FlexS include extensions for the PYTHON programming language[28] (PyFlexX, PyFlexS), and as such, all commands are available to facilitate automation of the approach. Using this, the ligand reference was used as the template for FlexS; the test ligand was then fragmented and the base fragment selected and placed using FlexS. The best scoring base placement from FlexS was then used in FlexX, and the remaining fragments were added using the standard incremental construction algorithm. The solution is thus positioned in the active site such that there is overlap with the known bound ligand; it should also maximize any possible favorable interactions with the active site within this constraint.

III. MATERIALS AND METHODS

The FlexX and FlexS methods have been described in detail elsewhere.[1,22] Below is a brief description of the steps used to perform the docking. All experiments were performed on a 3.2 GHz Pentium 4 Linux workstation with 1 GB of RAM running RedHat Enterprise WS 3.0. Figures illustrating the results were produced using LITHIUM.[29] The hybrid approach was scripted using PYTHON 2.2.3, with the PyFlexX and PyFlexS modules distributed with FlexX 1.13.2 and FlexS 1.11.1, respectively, as part of the SYBYL 6.92 suite.[30]

1. The Dataset. The dataset of 200 protein−ligand complexes previously described by the authors of FlexX[24] and now distributed with the software was used to validate our approach. A brief description of this, taken from the original paper, follows.

Preparation of the ligand input files was performed using SYBYL.[30] The receptor description files provided were used to describe each protein, the ligands from each PDB were used as the reference coordinate set, and the test ligands were produced by extracting them from each PDB, atom typing them and then adding hydrogens, followed by energy minimization. All carboxylic acid and phosphoric acid groups were ionized, and all amino, amidino, and guanidine groups (but no amide groups) were protonated. Assignments of hydrogen positions for the receptor are made on the basis of default rules, except for the definition of the torsion angles at the hydroxyl groups of the amino acids serine, threonine, and tyrosine and the hydrogen position inside the histidine side chain. Suitable torsion angles and the optimal tautomeric histidine state, respectively, were selected by visual inspection of the protein. The residues of lysine and arginine are protonated, and the acid groups of aspartic and glutamic acid are ionized. To define the active site of the protein, all atoms were selected that are located no farther than 6.5 Å apart from a ligand atom at its crystalline position. Generally, all water molecules of the PDB file were removed, apart from 1aaq, 4phv, 1lna, and 1xie where key water molecules were retained.

This validation mimics that described in the original paper, namely, using the approach to reproduce the protein−ligand complexes. The results were judged by measuring the rms deviation from the coordinates of the original ligands. Several experiments with a variety of parameters were performed and are described below.

2. Docking with the Combined FlexS/FlexX Approach. (a) The test and reference ligands were read into FlexS and the placebas algorithm used to select and superimpose the base fragment. The standard triangle placement algorithm was used; if this failed, then the more rigorous optimization algorithm was attempted (RigFit).[31] This allows rigid-body superpositioning of molecules and molecular fragments onto the reference molecule by optimizing the overlap of several Gaussian functions representing various chemical properties (steric occupancy, partial atomic charge, hydrophobicity, and hydrogen-bonding potential). The base placement ranked 1 was then saved to a file for the following steps.

(b) The receptor description file was read into FlexX along with the test ligand. The base placement produced by step a was then loaded as a reference placement using the mapref routine. The placebas routine was then used to select the base fragment and place it onto the reference coordinates defined by mapref. An option within placebas allowed perturbation such that a set of slightly different placements was generated; this also gave the receptor description some influence on these placements.

(c) The remainder of the ligand was built up using the standard incremental construction algorithm. If a solution could not be found, step b was repeated using the automatic base placement in FlexX.

(d) The best solution was scored according to its interaction with the protein, and the rms deviation from the reference ligand was calculated.

3. Docking with the Original FlexX Algorithm. (a) The test ligand and receptor description file were read into FlexX.

Table 1. Statistics of the Docking Test; Results for the 194 Successfully Docked Complexes are Shown

	FlexX	FlexS/FlexX	FlexS	ref
average score	−27.51	−21.82	−12.16	−17.82
STDEV score	15.07	15.45	16.78	16.71
average RMSD	3.98	1.65	0.87	0.00
STDEV RMSD	3.81	2.79	1.05	0.00
no. solns <2.5 Å (%)	101 (**52.1**)	136 (**70.1**)	188 (**96.9**)	N/A
no. nolns <2.0 Å (%)	91 (**46.9**)	122 (**62.9**)	182 (**93.8**)	N/A
no. nolns <1.5 Å (%)	69 (**35.6**)	100 (**51.6**)	171 (**88.1**)	N/A
no. nolns <1.0 Å (%)	44 (**22.7**)	69 (**35.6**)	152 (**78.4**)	N/A

The placebas algorithm was used with the triangle matching technique to generate base placements. All base placements were taken forward to the following step.

(b) The remainder of the ligand was then built up using the standard incremental construction algorithm in FlexX.

(c) The best solution was scored and the rms deviation from the reference ligand calculated.

4. Superposition Using FlexS Followed by Scoring the Ligands in the Receptor. (a) The test and reference ligands were read into FlexS, and the placebas algorithm was used to select and superimpose the base fragment. The standard triangle placement algorithm was used; if this failed, then the optimization algorithm was attempted. All base placements were taken forward to the following step.

(b) The remainder of the ligand was then built up using the standard incremental construction algorithm in FlexS.

(c) The best solution was then read into FlexX, scored in the presence of the receptor, and the rms deviation from the reference ligand calculated.

5. Scoring the Reference Ligands in the Receptor. (a) The reference ligand was read into FlexX, scored in the presence of the receptor, and the rms deviation from the reference ligand calculated.

IV. RESULTS ON THE 200-COMPLEX DATASET

Table 1 summarizes the statistics of the different methods' performance against the 200-complex dataset. A total of 194 of the complexes successfully gave results with all three methods, and six complexes gave no results at all with the hybrid approach. Only the top-ranked pose from FlexX has been considered for these results. The reference ligands were also scored in the receptor and included as a fourth column.

The performance of standard FlexX is similar to that reported in the original study where 46.5% of the top-scoring solutions had an acceptable rms deviation of <2.0 Å. The authors also reported that if one took the top 10 solutions provided by FlexX, 70% of these solutions contained a placement of <2.0 Å. A conclusion of the work was that further work could be done to improve the scoring function with the aim of getting these "best" solutions to rank 1. Although it is possible to examine a number of poses when analyzing the results of a single ligand docking, it is often more difficult if many ligands are being investigated. In this case, we have focused on the top-ranked pose as output by FlexX to see if our approach can improve the number of "correct" solutions (<2.0 Å RMSD).

The performance of FlexS demonstrates its utility as a superposition tool; 93.8% of the superpositions were successful in overlaying the ligand over its reference with an rms deviation of <2.0 Å. When scored within the protein complex, however, these solutions do not score as well; the average score of the results (−12.16) is significantly higher than that obtained with standard FlexX (−27.51). It is also interesting that the average scores of the reference ligands (−17.82) when scored within the receptor are also fairly high compared with the docked solutions. However, this is to be expected because of the steepness of the potentials used in the scoring function. Thus, any slight perturbation away from the local energy minimum will significantly affect the score.

For our combined FlexS/FlexX approach, we are docking into the protein active site and using some knowledge about the original cocrystallized ligand. This yields significant performance improvement over the standard FlexX algorithm; 62.9% of the solutions have an rms deviation of <2.0 Å, a 34% increase over standard FlexX. Also, more solutions have an rms deviation of <1.5 Å (51.6%) with our approach than have an rms deviation of <2.0 Å with the FlexX approach. Table 2 presents the docking performance data for each protein complex considered. Examples from the table will be described below.

Comparison of Methods. The overall statistics show that the combined FlexS/FlexX approach performed better than

Table 2. Results of the Docking Experiments for All 200 Complexes

RMSD (Å)	examples at rank 1
<1.0	121p 1abe 1abf 1acj 1acm1 1aha 1avd 1coy 1cps 1dbb 1dbj 1dbk 1dwb 1dwc 1ele 1etr1 1fki 1ghb 1hdc 1hgh 1hgi 1hgj 1hsl 1hti 1hvr 1imb 1ivb 1ivc 1ivd 1lah1 1lcp 1lic 1lst 1mbi 1mdr 1mrg1 1pbd 1phd 1phf 1ppc 1srj 1stp 1tnh 1tnj 1tnl 1tph 1trk 1ulb 1wap 1xid1 1xie 2ada 2cht 2cpp 2gbp 2mth3 2phh 2ypi 3aah 3hvt 3ptb 4fab 4ts1 5abp 5cpp 5tim 6abp 6rsa 8atc
>1.0, ≤ 1.5	1aaq 1aco 1atl 1baf 1cbx 1cil 1did 1dr1 1dwd 1ela 1frp 1hyt 1ive 1mmq 1nis 1nsc 1phg 1tnk 1tyl 1ukz 2ctc 2lgs 2r04 3cpa 4cts 4fxn 4hmg 4tim 5p2p 6rnt 7tim
>1.5, ≤ 2.0	1ack 1apt 1azm 1cdg 1dbm 1die 1fkg 1glp 1ida 1igj 1ivf 1mcr 1ppm 1tdb 1tka 2pk4 2r07 2sim 3cla 4phv 5tmn 6tim
>2.0, ≤ 2.5	1ake 1blh1 1hri 1ldm 1mld 1mrk 1rne 1snc 1tng 2cmd 2dbl 3gch 4tln 4tmn
>2.5	1aec 1ase 1bbp 1bma 1byb 1cbs 1cde 1com 1ctr 1eap 1eed 1elb 1elc 1eld 1epb 1eta 1glq 1hef 1hfc 1hgg 1icn 1lmo 1lna 1lpm 1mup 1nco 1poc 1pph 1ppi 1ppk 1ppl 1pso 1rbp 1sts 1rnt 1rob 1slt 1sty 1tlp 1tmn 1tni 1tpp 2ak3 2cgr 2er6 2mcp 2plv 2tmn 2yhx 4dfr 4est 4fbp 4hvp 5cts 6cpa 6tmn 8gch 9hvp
failed	1fen 1hdy 1pha 2xis 3tpi 7cpa

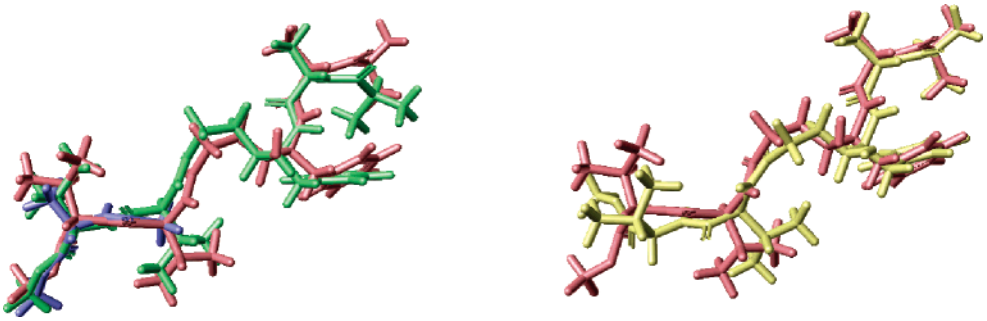

Figure 1. Ligand from 1aaq. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

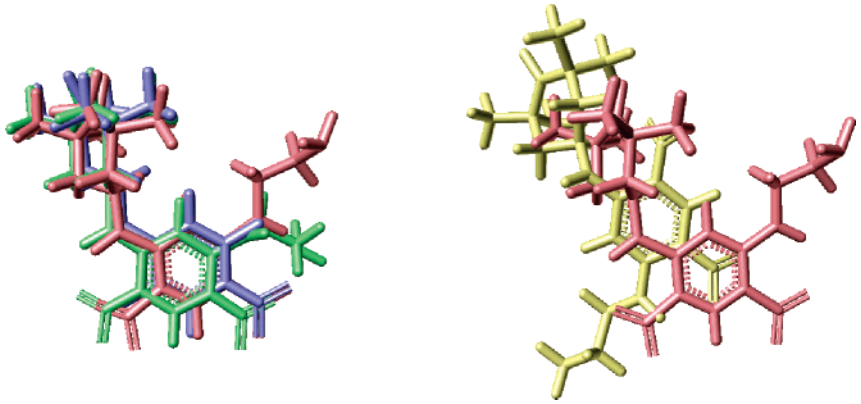

Figure 2. Ligand from 1baf. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

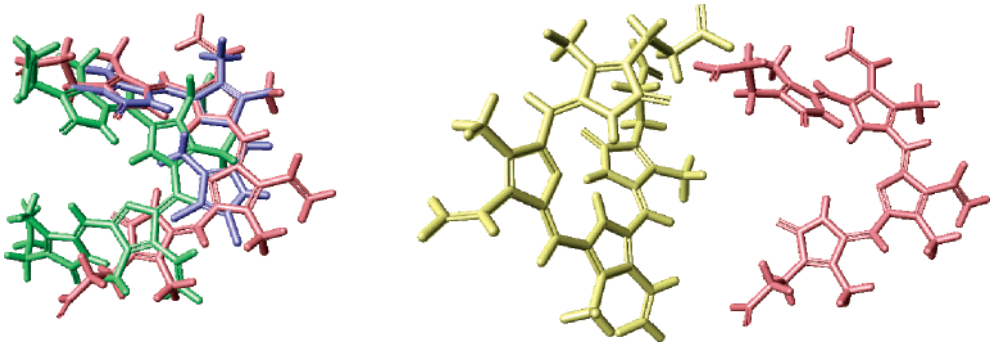

Figure 3. Ligand from 1bbp. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

standard FlexX. This is intuitive given that FlexX is docking "blind", whereas the combined FlexS/FlexX approach is guided by the original ligand placement.

Figure 1 illustrates an example where both approaches perform almost equivalently well (1.12 and 1.34 Å RMSD for the FlexS/FlexX and FlexX methods, respectively). This is an HIV protease example where the ligand is a synthetically modified polypeptide; there are a number of donor/acceptor atoms spaced out through the ligand, and these interact well with corresponding groups on the protein and the key water molecule in the binding pocket. Consequently, there is limited flexibility in where the ligand can bind to make these interactions, and both approaches perform well.

Figure 2 shows an example from a F_{AB} fragment complexed with its hapten, where FlexX does poorly (5.11 Å RMSD). The combined approach gives a much better solution (1.46 Å RMSD), although the terminal ethyldiaminyl group is positioned incorrectly. This better solution is due to the core fragment selection (which includes all of the molecule apart from the ethyldiaminyl group) and superposition by FlexS. The FlexX solution, however, is scored as −25.41, whereas the FlexS/FlexX docked solution is scored as −16.28, which demonstrates that the scoring function needs improving to select the better solution during standard FlexX.

Figure 3 shows the ligand from bilin binding protein (biliverdin IX); in this example, FlexX completely fails to dock the ligand into the binding pocket, perhaps because of steric effects from unusually close packing between the protein and the ligand in the crystal structure. The combined approach selects and overlays a significant portion of the ligand onto itself as a template; however, the subsequent perturbation and complex building appears to force the ligand out of the binding site to some extent, although the best solution is still in the right part of the cavity (4.92 Å RMSD).

Figure 4 illustrates an example where the combined approach does not perform as well as standard FlexX; here, the protein is a retinoic acid binding protein and the ligand

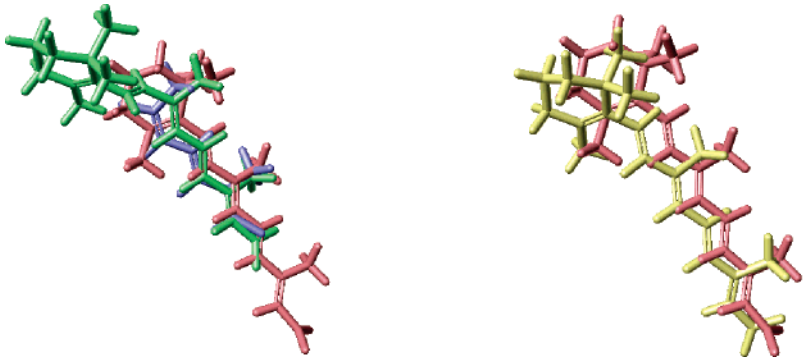

Figure 4. Ligand from 1cbs. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

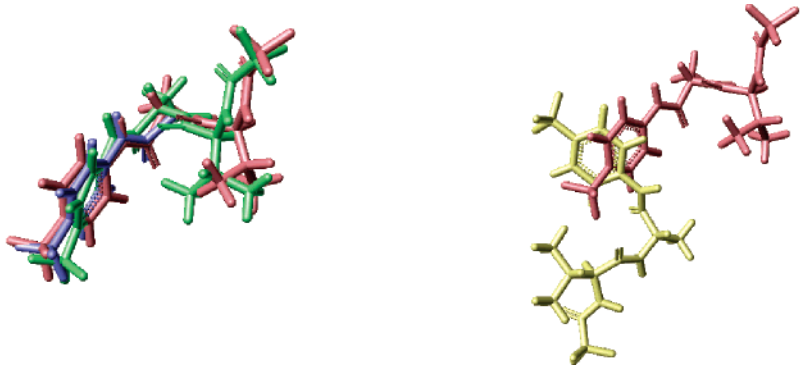

Figure 5. Ligand from 1ele. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

is all-trans retinoic acid. FlexX does a good job of reproducing the complex (1.69 Å RMSD); however, for the combined approach, the chosen base placement is positioned incorrectly (although positioned over a structurally similar part of the template). Essentially, the template has a repeating isoprene unit; FlexS overlays the base fragment over the wrong isoprene unit, which leads to a reasonable base fragment overlay but gives a complex that is incorrectly docked (4.95 Å RMSD). This highlights the importance of placing the base fragment correctly, and for the combined FlexS/FlexX approach, it may be improved by sampling the FlexS placements rather than using only the top-ranked solution.

Figure 5 shows the ligand from 1ele, elastase, where the ligand cavity is very shallow. Here, the FlexS core placement is good, and the corresponding FlexS/FlexX docked solution is also good (0.96 Å RMSD). For this complex, FlexX does not find a good solution (10.71 Å RMSD) and docks the ligand on the surface of the protein rather than in the cavity where the original ligand docks. These results reinforce the importance of providing FlexX with a good base placement; for the FlexS/FlexX docking, the base placements are generally better and, consequently, the final solution is also; where the results are not good, it is generally because the base placement from FlexS has been positioned poorly. It is possible that for proteins with shallow cavities, there are many more potential base/ligand placements, and for FlexX, it is more difficult to rank the correct base placement above the other possibilities. This, in turn, would lead to an "incorrect" solution where FlexX does not reproduce the binding mode found in the crystal structure. It is these cases where it is valuable to examine more than one solution from FlexX, as there may well be alternative binding modes, and the "correct" placement is more likely to be found in the top 10 solutions.[24]

Figure 6 is an example where the ligand, hexadecanesulfonic acid, is very flexible and hydrophobic. The binding pocket from 1lic, adipocyte lipid-binding protein, is also fairly open. FlexX manages to get the sulfonic acid group in the right region of the pocket; however, the hexadecane portion of the ligand wraps around the pocket in the opposite direction, giving a poor solution (5.77 Å RMSD). Using the FlexS/FlexX approach, the sulfonic acid group is superimposed very closely onto that in the reference ligand such that it predetermines the direction that the incremental construction algorithm builds the hexadecane tail. Consequently, this leads to a very good solution (0.94 Å RMSD). In this example, the solution space is very large (ligand has 15 rotatable bonds) and there is not much information to guide the FlexX algorithm to the correct solution (ligand is very hydrophobic). Using the key information in the sulfonic acid headgroup to determine the base placement and orientation via FlexS helps to focus the incremental construction in FlexX toward the correct solution.

V. CROSS-DOCKING EXPERIMENTS

As in the original FlexX200 paper,[24] we also examined various targets by cross-docking experiments. The results of this experiment are even more important with respect to our approach, given that using information from the cocrystallized ligand during docking and then docking the same ligand is a fairly biased test. The most important factor is whether you can use the information from the cocrystallized ligand to dock new ligands more accurately.

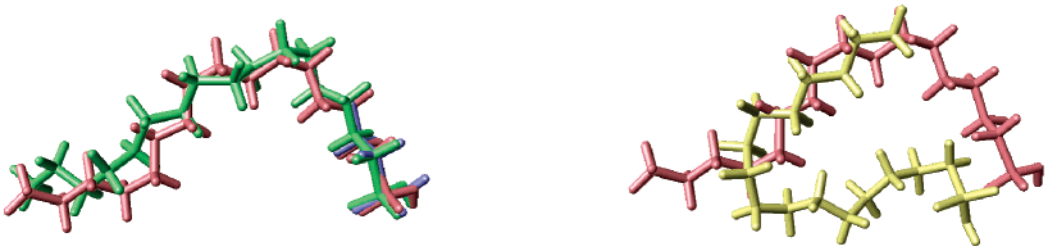

Figure 6. Ligand from 1lic. Crystal structure position (pink), FlexX/FlexS docked structure (green), FlexS core placement (blue), and FlexX docked structure (yellow).

In this case, docking a ligand from one crystal structure into the same target protein that has been cocrystallized with a different ligand is the best way to test the approach. The crystal structures for a protein complexed with different ligands are superimposed using the protein backbone, each ligand is then docked into each non-native structure, and the rms deviation is calculated compared with its position in its native structure.

In this case, seven proteins were considered: trypsin, α-thrombin, carboxypeptidase A, cytochrome P-450, a FAB fragment, L-arabinose-binding protein, and triosephosphate isomerase. All ligands of one group were docked into all protein structures of the same group. Tables 3 and 4 present the results of the cross-docking experiments using the combined FlexS/FlexX approach and the FlexX method alone, respectively. Each table shows the rms deviations and scores of the highest-ranked docked solutions of a ligand in its own complex structure (first three columns) and the best rms deviation and corresponding score in a non-native structure (second three columns).

For the combined FlexS/FlexX approach (Table 3), 32 of the 35 ligands show an rms deviation of <2.0 Å when docked into their own structure; the 3 ligands that do not perform as well are 1pph, 3ptb, and 7cpa. Complex 1pph also shows a poor rms deviation with standard FlexX (4.52 Å RMSD), and complex 7cpa fails to dock altogether.

When docked into a foreign structure using the FlexS/FlexX approach, only for 4 of the 35 structures (1dwc, 1dwd, 1phg, 2cpp) are the results significantly worse and is an rms deviation of >2.0 Å shown. Of these, for 1dwc, the FlexX results for the native ligand are almost as poor (Table 4), and for 1phg, FlexX fails to find a solution.

Figure 7 shows the solutions from the cross-docking experiment for 6cpa using the ligand from 7cpa. FlexX fails to find a solution for the ligand in any of the carboxypeptidase A structures listed; using our approach, the ligand in 6cpa provides enough information such that the 7cpa ligand can at least be docked, although the placement is not so accurate (3.23 Å RMSD). The 6cpa ligand, however, is fairly similar to that of 7cpa, so perhaps this is not so surprising. The poor rms deviation is mainly due to the algorithm placing the terminal phenyl ring (top of figure) at 180° to the crystal structure position; aside from this, the docked solution is positioned fairly well compared with the crystal structure.

Figure 8 shows the solutions from cross docking the ligand from 1phf (cytochrome P-450) into 1pha. Again, the FlexS/FlexX solution is fairly close to the crystal structure (1.24 Å RMSD), the main difference being that the phenyl ring is almost orthogonal to that in the reference crystal structure. The base fragment here is the entire ligand, and this is overlaid onto the template from 1pha well. FlexX gives a solution that is not very close to the reference (4.38 Å RMSD) and is positioned with an incorrect orientation.

Figure 9 illustrates another cytochrome P-450 example where the ligand from 1phg has been docked into 1pha. Here, the solutions from both methods are identical and also poor compared with the reference crystal structure (5.21 Å RMSD). The docked solutions are almost flipped by 180° in the plane of the carbonyl group; both the pyridyl rings and the geminal dimethyl groups are close to the reference

Table 3. Cross-Docking Results with FlexS/FlexX

original complex			best non-native receptor structure		
PDB code	RMSD [Å]	score	PDB code	RMSD [Å]	score
		trypsin			
1ppc	0.68	−28.21	1tnj	2.24	−45.22
1pph	4.04	−8.34	1tnj	1.81	−34.25
1tng	1.94	−18.67	1tnh	0.73	−19.31
1tnh	0.20	−24.20	1tng	0.58	−22.29
1tni	1.87	−15.23	1tnl	1.16	−15.09
1tnj	0.93	−17.03	1tnk	0.96	−18.59
1tnk	1.25	−18.86	1tnl	1.15	−17.09
1tnl	0.66	−15.80	1tng	0.75	−5.48
3ptb	3.61	−9.33	1ppc	0.47	−31.84
		α-thrombin			
1dwb	0.35	−17.63	1dwd	0.45	−17.16
1dwc	0.88	−20.00	1dwb	4.52	−21.26
1dwd	0.88	−22.15	1dwb	8.25	−30.34
		carboxypeptidase A			
1cbx	1.06	−47.89	7cpa	1.10	−39.92
1cps	0.45	−31.33	6cpa	0.73	−32.91
2ctc	1.47	−27.22	3cpa	0.85	−22.69
3cpa	1.35	−30.65	1cbx	1.67	−41.66
6cpa	1.36	−32.87	1cps	2.97	−31.17
7cpa	4.47	−43.45	6cpa	3.23	−25.84
		cytochrome P-450			
1pha			2cpp	7.05	5.62
1phd	0.47	−10.17	1pha	1.61	−4.39
1phf	0.46	−8.68	1pha	1.24	−5.85
1phg	1.25	7.48	1pha	5.22	−9.25
2cpp	0.38	−3.56	1pha	2.88	−1.01
5cpp	0.41	−7.03	2cpp	0.65	−6.04
		FAB fragment			
1dbb	0.53	−7.10	2dbl	0.48	−7.92
1dbj	0.21	−6.45	1dbk	0.61	−3.47
1dbk	0.78	−4.91	1dbj	0.53	−7.64
1dbm	1.97	−10.26	2dbl	2.14	−7.32
2dbl	1.56	−4.99	1dbm	1.19	−4.87
		L-arabinose-binding protein			
1abe	0.66	−19.02	5abp	0.38	−13.56
1abf	0.81	−10.73	5abp	0.30	−14.48
5abp	0.48	−18.68	1abf	0.49	−16.38
		triosephosphate isomerase			
4tim	1.06	−20.55	6tim	0.87	−28.14
5tim	0.34	−11.98	4tim	1.54	−18.50
6tim	0.72	−23.75	4tim	1.47	−21.52

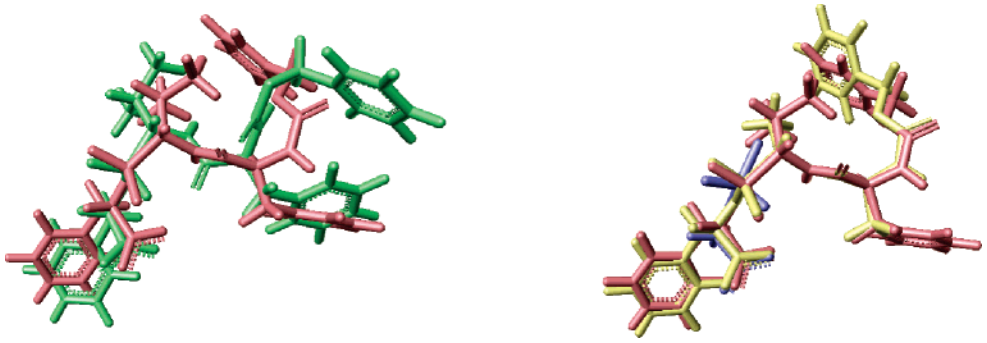

Figure 7. Ligand from 7cpa docked into 6cpa. Crystal structure position (pink), FlexX/FlexS docked structure (green), and FlexS core placement (blue). The 6cpa template ligand is also shown (yellow).

Table 4. Cross-Docking Results with FlexX

original complex			best non-native receptor structure		
PDB code	RMSD [Å]	score	PDB code	RMSD [Å]	score
		trypsin			
1ppc	3.12	−46.73	1tnj	2.24	−45.22
1pph	4.52	−37.71	3ptb	1.43	−36.79
1tng	1.89	−18.67	1tni	0.49	−18.44
1tnh	0.55	−20.34	1tni	0.47	−21.61
1tni	2.67	−15.23	1tnk	2.53	−14.53
1tnj	1.30	−16.80	1tnl	1.09	−16.52
1tnk	1.85	−18.86	1tni	1.14	−15.73
1tnl	0.62	−17.72	1tnj	0.63	−17.44
3ptb	0.70	−30.31	1ppc	0.33	−28.20
		α-thrombin			
1dwb	0.55	−14.90	1dwd	0.50	−17.58
1dwc	6.68	−18.31	1dwd	1.31	−27.34
1dwd	4.81	−29.12	1dwc	8.19	−27.63
		carboxypeptidase A			
1cbx	6.31	−45.29	2ctc	1.30	−47.85
1cps	0.96	−37.18	1cbx	1.06	−40.19
2ctc	1.97	−39.85	6cpa	1.87	−26.96
3cpa	2.52	−35.56	2ctc	1.72	−44.72
6cpa	5.92	−35.22	1cps	2.77	−40.70
7cpa					
		cytochrome P-450			
1pha			2cpp	7.39	−7.10
1phd			1pha	1.73	−7.84
1phf	4.54	−12.41	1phd	4.38	−6.33
1phg			2cpp	5.21	−12.56
2cpp	2.92	−4.50	1phf	0.47	−5.89
5cpp	1.34	−7.82	1phd	0.75	−7.24
		FAB fragment			
1dbb	6.64	−4.96	1dbj	1.18	−9.91
1dbj	8.32	−9.25	1dbk	4.57	−8.41
1dbk	6.31	−7.34	1dbb	6.36	−8.44
1dbm	6.45	−3.72	1dbj	2.45	−10.89
2dbl	5.61	−4.31	1dbm	1.12	−4.05
		L-arabinose-binding protein			
1abe	1.20	−27.39	1abf	1.18	−24.55
1abf	0.92	−24.72	5abp	0.58	−23.70
5abp	0.55	−26.33	1abf	1.19	−25.89
		triosephosphate isomerase			
4tim	4.09	−30.91	6tim	0.96	−30.70
5tim	1.99	−15.65	4tim	1.20	−16.88
6tim	1.62	−29.86	5tim	0.75	−19.09

structure. It is also interesting that the base placement (the whole molecule, in this case) from the FlexS superposition has been perturbed significantly during the subsequent docking step, resulting in the same solution as that from standard FlexX. The carbonyl group in the docked solution is forming a hydrogen-bonding interaction with a threonine residue (THR101), and this appears to be preferred over the interaction of the pyridyl group with the zinc atom in the heme group, which is missing. So, although the rms deviation, when compared with the reference structure, is not good in this case, the solution is still a reasonable one.

Importantly, these results show that the combined FlexS/FlexX approach can be applied to cases when the template ligand is different from the test ligand, can give solutions where standard FlexX can fail, and can give solutions that are correctly placed in the binding pocket with a higher probability than when using standard FlexX.

The use of FlexS to guide the base fragment selection and placement is, therefore, validated in these cases; it is also important to note that this base placement is only guided and not determined explicitly by ligand superposition; FlexX still perturbs the placement to favor interactions with the receptor, as in the case of the cytochrome P-450 example described above (Figure 9).

In most of these examples, the core fragment from the test ligand is the same or very similar to part of the best template ligand. Because FlexS superimposes the core fragment onto the template on the basis of fields representing physicochemical properties and volume overlap, there is no requirement for the core fragment of the test ligand to be present in the template ligand, although in cases where the template ligand does not contain a similar fragment, the resulting docked solutions are less accurate. However, in these cases, you can still get a reasonable docked solution, whereas FlexX alone may have failed (7cpa, 1pha, 1phd, 1phg).

VI. CONCLUSIONS AND FUTURE WORK

When the hybrid FlexX/FlexS approach is used, and only the top-ranked solutions are judged, 62.9% of the complexes of this dataset can be reproduced with acceptable accuracy (<2.0 Å RMSD). On average, the scores for these placements were higher (less favorable) than the scores obtained by standard FlexX docking (46.9% of the complexes with <2.0 Å RMSD), although the placements were more accurate. This highlights the importance of the base fragment selection and placement for FlexX and also the importance of the scoring function.

The cross-docking experiments illustrate that the hybrid approach is applicable to cases where the template ligand is different from the ligand being docked, that it can give results where FlexX fails to find a solution, and that it tends to give solutions that are better placed.

This is an important result, given that reproducing known crystal structures is not the aim of docking algorithms, but

Figure 8. Ligand from 1phf docked into 1pha. Crystal structure position (pink), FlexX/FlexS docked structure (green), and FlexS core placement (blue). The 1pha template ligand is also shown (yellow), and so is the FlexX docked structure (cyan).

Figure 9. Ligand from 1phg docked into 1pha. Crystal structure position (pink), FlexX/FlexS docked structure (green), and FlexS core placement (blue). The 1pha template ligand is also shown (yellow).

instead, the aim is the prediction of binding for new ligands. With this approach, we are assuming that the binding mode will be similar to that of a known cocrystallized ligand template, or at least that some portion of the ligand will bind in the same location as that of the template. FlexX is provided with a base fragment placement that is in the same location as the template ligand; the docking method is, therefore, focused in this region of the binding pocket and can sample it more thoroughly, giving a greater likelihood of finding the best solution.

There are several aspects of this work that can be extended and will be subsequently investigated. The first of these is a refinement of the base placement and selection method. Currently, we are using the rank 1 solution from FlexS ligand superposition as the base placement for FlexX; as a standard, both FlexS and FlexX create and use many different base placements before incremental construction. We have highlighted the importance of base placement; if the top-ranked solution from FlexS is not optimal, it is likely to have a dramatic impact on the docking results, and this is more likely to happen if the template ligand is dissimilar to the test ligand. Given this, we will investigate whether it would be more advantageous to take a selection of FlexS base placements through into the FlexX docking process to try and avoid this potential pitfall. It may also be interesting to examine cases where there are multiple known ligands that can be used as reference structures; in this case, we would look to obtain a selection of base placements for each template, rank the subsequent list, and feed these into the incremental construction step.

The second aspect to be extended is the use of this approach for virtual screening. As the speed of computation increases, pharmaceutical companies are increasingly performing high-throughput docking experiments with the aim of enriching actives from a collection of compounds with unknown activity against a specific target. FlexX-PHARM[11] has already been shown to improve the results of FlexX in virtual screening by using pharmacophore constraints during the docking process; our technique would be complementary to this approach.

The third aspect that would be interesting to investigate would be to incorporate protein flexibility using FlexE,[7] as here we have only considered the protein as a rigid entity.

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Supporting Information Available: Figures S1−S7 provide chemical structures for the ligands used in the cross-docking experiments from Tables 3 and 4. This material is available free of charge via the Internet at http://pubs.ac.org.

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