

Fully Automated Flexible Docking of Ligands into Flexible Synthetic Receptors Using Forward and Inverse Docking Strategies

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The prediction of the structure of host–guest complexes is one of the most challenging problems in supramolecular chemistry. Usual procedures for docking of ligands into receptors do not take full conformational freedom of the host molecule into account. We describe and apply a new docking approach which performs a conformational sampling of the host and then sequentially docks the ligand into all receptor conformers using the incremental construction technique of the FlexX software platform. The applicability of this approach is validated on a set of host–guest complexes with known crystal structure. Moreover, we demonstrate that due to the interchangeability of the roles of host and guest, the docking process can be inverted. In this inverse docking mode, the receptor molecule is docked around its ligand. For all investigated test cases, the predicted structures are in good agreement with the experiment for both normal (forward) and inverse docking. Since the ligand is often smaller than the receptor and, thus, its conformational space is more restricted, the inverse docking approach leads in most cases to considerable speed-up. By having the choice between two alternative docking directions, the application range of the method is significantly extended. Finally, an important result of this study is the suitability of the simple energy function used here for structure prediction of complexes in organic media.

INTRODUCTION

A key problem in host–guest chemistry and molecular recognition is the development of novel synthetic receptors.^{1,2} The long-term goal is the *de novo* design of highly specific synthetic host molecules for given guests.³ The complementary problem, i.e., the design of molecular guests for natural (protein) receptors, has received considerable attention from the pharmaceutical industry, and numerous computational procedures have been devised to support ligand design.^{4–7} Both these issues can be seen as specific instances of the more general problem of optimizing molecular interactions by molecular design.

A significant prerequisite for design is the existence of methods that reliably predict the geometry of molecular receptor–ligand complexes including the conformations of the participating molecules, a problem known as docking.⁸ To this end, a number of empirical approaches have been developed. These methods, in general, utilize information on the structure of the receptor and allow for only limited flexibility or even assume the receptor to be rigid. An obvious approach for predicting the structure of the complex is a stochastic search for the global optimum, e.g. by simulated

annealing. Stochastic methods are, however, in general not very efficient since they tend to spend long times sampling irrelevant parts of the conformational space.

A particularly simple approach is the building of the ligand inside the binding site of the receptor, starting from favorable placements of rigid parts of the ligand.^{9–11} During the incremental build-up of the ligand the conformational flexibility is taken into account by sampling different possible torsion angles at each rotatable bond as it is added. Docked ligand fragments are scored, and the best conformations are kept for the following growth step. The underlying assumption is that parts of an optimal solution are nearly optimal themselves. This conformational selection leads to a significant reduction in the size of the search space with corresponding gain in efficiency.

The structure of the receptor, or more precisely the geometrical arrangement of the interacting groups of the receptor, plays an important directing role during the build-up of the ligand. This is also the case for rigid synthetic receptors. De Jong et al.¹² demonstrate this role in their study on docking of ligands into an energy-minimized structure of a large cyclodextrin dimer which was assumed to be rigid. The advantage of the directing role is highly reduced once the conformation of the receptor is not known. Often the receptor accommodates its ligand by changing its own conformation upon binding, an effect known as induced fit. For example, the naphthalene ring in the macrocyclic nucleotide receptor of Hamilton and van Engen¹³ (1 in Chart

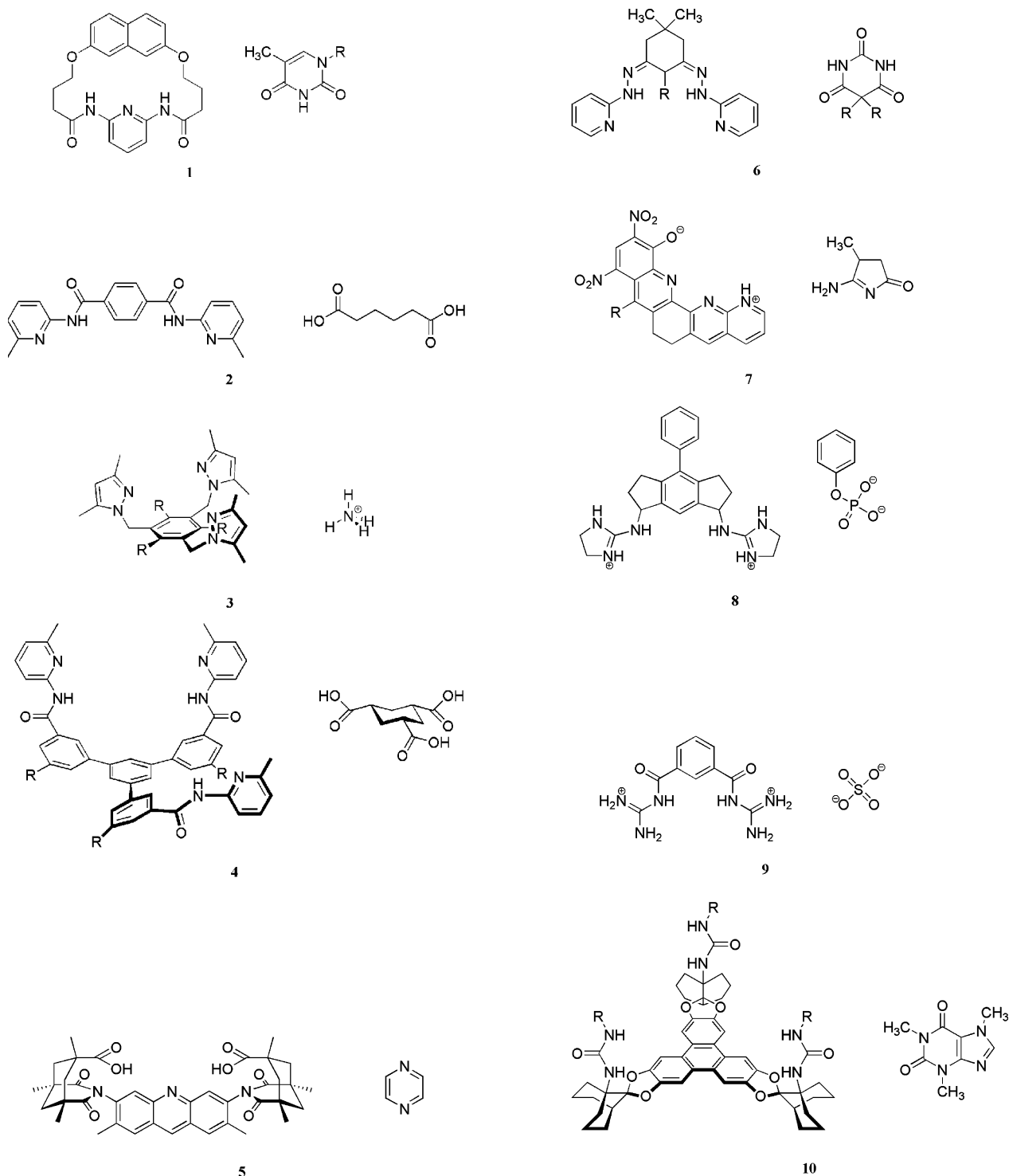
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Chart 1. Structural Formulas of the Test Data Set of Receptor–Ligand Complexes with Known Crystal Structure^a

^a Receptors are drawn on the left, ligands on the right side, respectively.

1) is oriented approximately parallel to the pyridine plane (161.6°) in the complexed state (Figure 1), whereas in the uncomplexed state the angle between the two planes amounts to 127.5°. In many cases, the conformational space of the uncomplexed host molecule is huge, and the conformation seen in the complex is only one of many possibilities the host can adopt with only a minor loss in conformational energy.

Here, we present two simple and related automated procedures for the structure prediction of complexes between flexible, low molecular weight receptors, and their ligands

based on the FlexX docking technology. Synthetic receptors are in general designed to be rigid, because rigidity confers binding specificity and higher binding affinity due to lower conformational entropy loss upon binding. Often, this concept of rigidity cannot be put into practice due to limitations of the synthesis procedure. Thus, residual flexibility is an important feature of many artificial receptors and, therefore, must be considered in reliable structure prediction of general host–guest complexes. Our procedures allow for conformational flexibility of both partners. We achieve this by docking one molecule (the guest) sequentially into the different

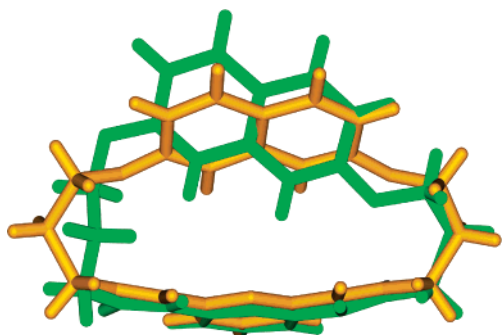


Figure 1. Superposition of the crystal structures of the synthetic receptor by Hamilton and van Engen¹³ in holo-form (complexed state, orange) and apo-form (noncomplexed state, green).

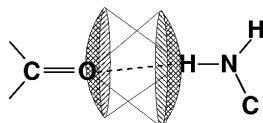


Figure 2. The FlexX interaction scheme. An interaction is recognized if the center of one interaction geometry lies approximately on the interaction surface of the counter group and vice versa.

possible conformers of the other (the host), using a discrete representation of the conformation space. The relevance of these results for structure prediction and design of receptors for molecular recognition is discussed. The methods presented are validated on a test data set of 10 host–guest complexes with known crystal structures.

COMPUTATIONAL DETAILS

Docking Algorithm. All methods are based on FlexX,^{10,11} a standard tool used for docking flexible ligands into rigid protein receptors. In FlexX the conformational flexibility of ligands is modeled by a discrete set of torsional angles at acyclic single bonds.¹⁴ Conformations of ring systems are computed with CORINA.¹⁵ FlexX describes nonbonded interactions between molecules by assigning interaction geometries (Figure 2). These are characterized by a center and a shape of an interaction surface. An interaction is recognized if the center of one interaction geometry lies approximately on the interaction surface of the counter group and vice versa. The ligand is fragmented into components by severing all acyclic single bonds. A fragment (base fragment) is selected and docked into the protein. Finally, the ligand is incrementally constructed by linking the remaining components in compliance with ligand flexibility. An empirical scoring function according to Böhm¹⁶ is used for ranking of the docking results (see below). All basic concepts of FlexX have been adapted for docking into artificial receptors. In the following, only the new developments are described. Together with a simple user interface, they have been incorporated into a new software tool FlexR based on the Flex* software library.

Host Flexibility. For synthetic receptors, the conformational adjustment of the receptor upon ligand binding typically plays a more important role than for protein receptor sites. To incorporate induced fit phenomena in the docking procedure we extended the original FlexX approach such that the host flexibility is taken into account. We address flexibility of both partners by docking the guest into the

different possible conformers of the host. In a first step, a full conformational analysis of the host is performed. Herein, the conformational freedom of acyclic single bonds is constrained to a discrete set of preferred torsion angles taken from the MIMUMBA database which contains approximately 900 molecular fragments with a central single bond.¹⁴ During conformational sampling, amide groups are constrained to trans (dihedral angle H–N–C=O of 180°) and carbonic acid groups constrained to cis (dihedral angle H–O–C=O of 0°) lowest-energy conformations. Favorable conformations for ring systems are computed with the program CORINA.¹⁵ The number of ring atoms for each elementary ring is limited to nine so far. For generation of representative conformations of macrocyclic rings, we have used CONFORT.¹⁷ Possible conformations are then generated by combining all MIMUMBA and CORINA or CONFORT results. Host conformers with intramolecular short contacts (clashes) are discarded.

The actual docking process is performed sequentially for each of the precomputed host conformations using the FlexX algorithm. The guest is split into rigid fragments which are docked into one of the host conformations. The best fragment placements are used to grow the rest of the guest in the field of the host. At every growth step, possible orientations of the newly added fragment are evaluated, and the best are kept for further build-up. The resulting structures are ranked by the scoring function.¹⁶ Finally, the results for all different host conformations are merged to a single ranked list of receptor–ligand complexes.

Forward and Inverse Docking. In complexes of two small molecules the roles of guest and host are interchangeable. Therefore, two docking directions are possible. In forward docking, the receptor takes the part of the host and the ligand corresponds to the guest. In the process of placing the base fragment of the guest (see below), we can take advantage of the structural information provided by the receptor. A frequent disadvantage of forward docking is the huge conformational space of a nonrigid receptor. Therefore, it was proposed to invert the conventional docking process.^{18,19} In this inverse docking, the receptor is treated as a guest, i.e., it is built up around the rigid ligand. This technique has been successfully used for the docking of flexible bacterial cell-wall fragments to glycopeptide antibiotics.¹⁸ Inverse docking was also proposed as a computational tool for the docking of organocatalysts to transition-state models.¹⁹ In the general case, however, it cannot be assumed that the ligand is represented well by a single conformation. In this work, we therefore present an inverse docking procedure in which both receptor and ligand are allowed full flexibility according to the MIMUMBA model. Since usually the ligand is smaller than the receptor and therefore its conformational space is much smaller, the computational requirements can be reduced drastically. Another reason for speed-up is the reduction of the number of interaction sites on the host side, i.e., the ligand. A drawback of the smaller number of interaction sites is, however, the loss of the directing role for complex build-up during construction.

Base Fragment Selection and Placement. Many artificial receptors exhibit some symmetry elements. The base fragment selection process accounts for this symmetry by removing all but one of the symmetry equivalent base

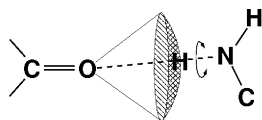


Figure 3. One-point base fragment placer. For each grid point on the interaction surface of the oxygen donor, the N–H-group is placed in a way that the interaction vector (dashed line) coincides with the N–H-bond. The angular search about this axis is performed in discrete steps.

fragments. This step is performed by analyzing the topological symmetry of the connection table of the molecule.¹⁰

In FlexX, the initial (base) fragment is placed via matching of three (triangle matching) or two (line matching) different interaction points of the guest with complementary groups in the host. In triangle matching, the position and orientation of the fragment is defined, whereas in line matching a scan of the remaining degree of freedom is performed for the placement.

In inverse docking, the host is often too small to form even two interactions with a single fragment of the guest. Therefore, it was necessary to extend the algorithms for the placement of the initial (base) fragment. In the case that even the line matching fails for a base fragment, a point matching is evoked that places the fragment by forming a single strong hydrogen-bonded interaction only. In this algorithm, a series of placements is made to find the optimal orientation of the first fragment. This is achieved by scanning through a discrete set of rotations of this new fragment about the interaction vector (see Figure 3).

Scoring Function. Throughout the docking process, ranking of (intermediate) results is performed by an empirical scoring function that models solely the interaction between the host and the guest.¹⁶ This scoring function accounts for deviations from the optimal geometry at each interaction site. Solvent screening of interactions and direct solvation effects are taken into account in a very crude approximation that has been parametrized for protein–ligand interactions in aqueous solutions.¹⁶ When the receptor structure is fixed, its self-energy is constant (disregarding effects such as solvent screening of interactions) and can therefore be neglected. When the receptor is flexible, its energy should be taken into account in deciding which conformation is most probable. Nevertheless, here we neglect the finer details of the energy landscape and only use simple clash criteria to avoid unphysical conformations. Ranking is based only on the interaction between receptor and ligand which turns out to be sufficient for the complexes studied in this work.

Test Data Set. Since full flexibility is assumed for guest and host, the first relevant question is whether the conformation of each molecule, independently, is predicted correctly.

For a validation of our methods, we have selected host–guest complexes from the Cambridge Structural Database (CSD).²⁰ As first selection criterion we have chosen the predominant interaction type. Here, we selected complexes where the intermolecular interactions are dominated by hydrogen bonds. As second selection criterion we have chosen receptor flexibility. As mentioned in the Introduction, flexibility counteracts specificity, and therefore most of the synthetic receptors in the CSD are rather rigid. Among all complexes we selected 10 relatively flexible receptors (Table 1, structural formulas of the complexes are shown in Chart 1), exhibiting up to nine rotatable bonds. The ligand set includes small heterocyclic rings (druglike or biologically relevant) and aliphatic carbonic acids as well as small cationic and anionic molecules.

The following steps were necessary to prepare receptor and ligand for docking: The structures were extracted from the CSD. The protonation state of the respective X-ray structures was used. For instance, for receptor **7** the zwitterionic structure with protonated amine and deprotonated hydroxyl group was used, while all carbonic acids (complexes **2**, **4**, and **5**) are in the protonated (i.e. neutral) state. Atom types, bond types, and formal charges were assigned automatically by a rule-based heuristic and checked manually. Long aliphatic chains that do not influence the docking (R-groups in Chart 1) were replaced by methyl groups. Only one-half of the dimeric receptor **6** was used for docking; the other half was substituted by a methyl group (also indicated by an R-group in Chart 1). Finally, the molecules were energy-minimized with the built-in force field (a recent version of the Tripos force field³⁰) to obtain low-energy conformations with suitable bond lengths and angles for the subsequent conformational analysis. The aromatic ring system of receptor **10** was kept rigid to avoid out-of-plane distortion.

EVALUATION OF DOCKING EXPERIMENTS

The predicted complex structures (Figures 4–13) contain the correct conformations of ligand and receptor even in cases of rather flexible molecules, such as complexes **2** (Figure 5) and **4** (Figure 7). More importantly, the results for the complete test set indicate that accurate solutions are found and that they are recognized by the scoring function (Table 2).

The comparison between forward and inverse docking reveals that inverse docking is as accurate, in general, as forward docking. For example, consider the root-mean-square deviation (RMSD) for the sulfate complex **9**. Here similar results are obtained with respect to RMSD in forward and

Table 1. Test Data Set of Receptor–Ligand Complexes with Known Crystal Structure

Id	short description	CSD code	lit.
1	macrocyclic nucleotide base receptor	FODTIB	Hamilton and van Engen ¹³
2	glutaric acid receptor	JEWNUU	Garcia-Tellado et al. ²¹
3	ammonium ion receptor	CUKTUX	Chin et al. ²²
4	tricarboxylic acid receptor	QIJPEE	Ballester et al. ²³
5	two-point binding receptor	POLFUR	Pascal and Ho ²⁴
6	barbiturate receptor	DAQVAS	Berl et al. ²⁵
7	creatinin receptor	ZESFEI	Bell et al. ²⁶
8	bis(guanidinium) receptor for phenyl phosphate	HASWUT	Kneeland et al. ²⁷
9	bis(guanidinium) receptor for sulfate	QAFVAV	Grossel et al. ²⁸
10	caffeine receptor	WEWTEX	Waldvogel et al. ²⁹

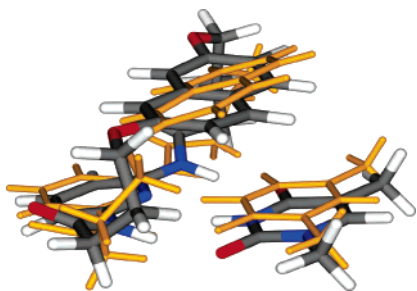


Figure 4. Docking results of complex **1** (forward docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

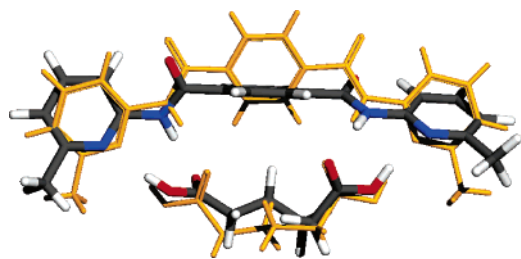


Figure 5. Docking results of complex **2** (forward docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

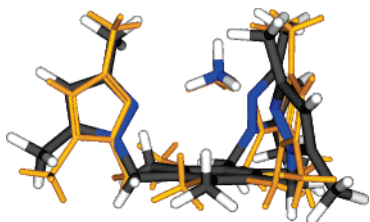


Figure 6. Docking results of complex **3** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

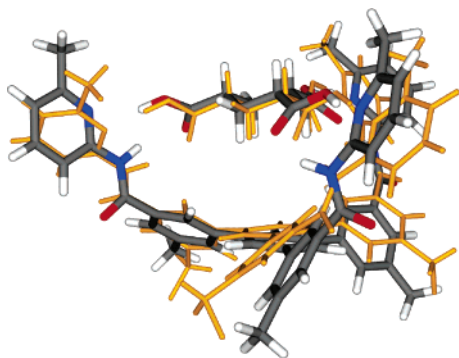


Figure 7. Docking results of complex **4** (inverse docking, rank 8, atom coloring) superimposed to X-ray structure (orange).

reverse docking. In this particular example the highest ranking solution has minimal RMSD. The RMSD of the top ranking solution is not necessarily the best criterion for the assessment of docking studies. It is much more important to find a near native pose at high ranks. In Table 2, therefore also the minimal RMSD within the 10 top-ranking solutions is displayed. For complex **4** a low RMSD can be found within the first 10 ranks but not at rank 1. In this example, the ligand around which the host is constructed is a tricarboxylic acid with a central cyclohexane unit and three freely rotatable carbonic acid groups in equatorial positions. The top-ranking solution is obtained for a different conformer of the ligand yielding the proper hydrogen-bonding pattern, however. The conformation used for docking differs from

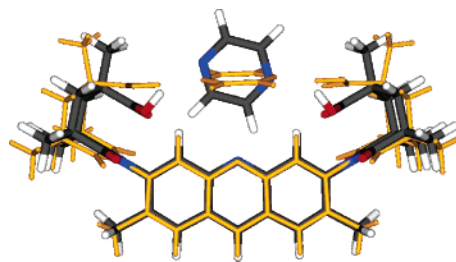


Figure 8. Docking results of complex **5** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

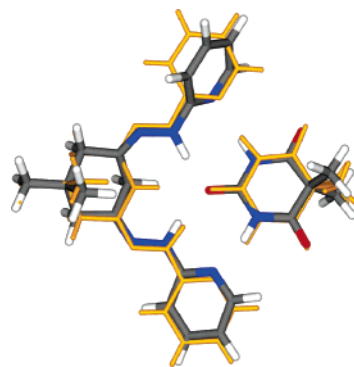


Figure 9. Docking results of complex **6** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

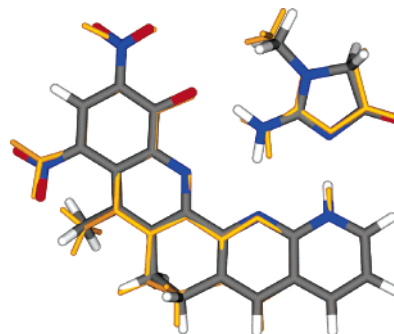


Figure 10. Docking results of complex **7** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

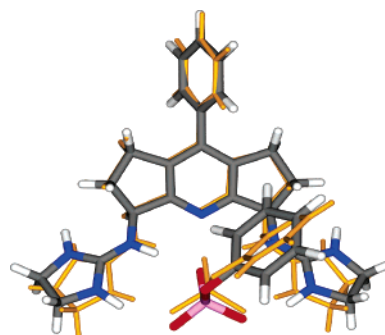


Figure 11. Docking results of complex **8** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

the crystal structure in that all three carbonic acid groups are rotated by 180° simultaneously. These two ligand conformers are isoenergetic in the free state, and the docking scores within the first 10 ranks are almost identical, as well. The near-native solution is found at rank 8. As can be seen in Table 2 all RMSD values among the first 10 solutions lie well below 2 Å, with the exception of complex **8**. In protein–ligand docking, typically an RMSD below 2 Å is considered a successful docking result. The larger deviation from the

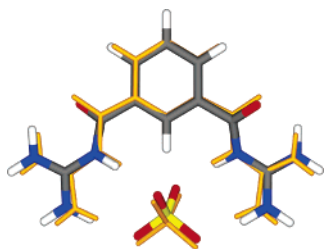


Figure 12. Docking results of complex **9** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

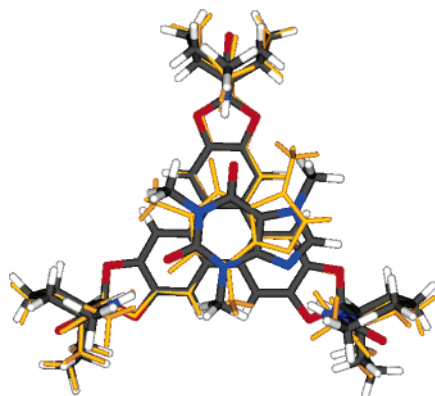


Figure 13. Docking results of complex **10** (inverse docking, rank 1, atom coloring) superimposed to X-ray structure (orange).

Table 2. Docking Results^a

Id	RMSD [Å] of first solution		min. RMSD [Å] within first 10 solutions		CPU time HH:MM:SS	no. of discrete host conformers
	complex	ligand	complex	ligand		
Forward Docking						
1	1.06	1.20	1.06	1.20	00:00:09	5
2	1.60	1.66	1.34	1.39	04:02:59	4608
3	1.44	0.58	1.24	0.44	24:36:47	45813
4						1.1·10 ⁸
5	1.32	2.02	1.06	1.28	00:20:10	81
6	2.59	3.78	0.86	1.06	00:16:54	576
7	0.78	0.93	0.74	0.93	00:00:36	24
8	1.37	2.75	1.37	2.75	02:22:18	5106
9	0.48	0.32	0.48	0.32	00:11:03	1926
10	0.71	1.19	0.54	1.07	00:02:14	27
Inverse Docking						
1	1.02	1.09	1.02	1.09	00:00:03	1
2	1.40	1.82	1.33	1.72	26:25:54	44640
3	1.63	1.26	1.20	0.47	00:00:01	1
4	2.96	4.19	1.05	0.69	03:41:42	1728
5	1.00	1.70	0.93	1.23	00:00:03	1
6	1.07	1.16	0.93	1.04	00:00:07	1
7	0.60	0.48	0.33	0.35	00:00:02	1
8	1.71	2.60	0.85	1.15	00:00:43	6
9	0.55	0.49	0.55	0.49	00:00:03	1
10	0.70	0.91	0.38	0.60	00:00:03	1

^a For each complex, we list the root-mean-square deviation (RMSD) of the first solution, the best RMSD within the first 10 solutions, the CPU time, and the corresponding number of conformers of the host. The data are listed for the forward and inverse docking procedure. We use the symmetry corrected all-atom RMSD superimposed to the whole complex. The first RMSD given in each column is that of the receptor–ligand complex, the second is the RMSD of the ligand. CPU times are obtained on an Intel Pentium 4 Xeon CPU at 3.06 GHz.

X-ray structure in the case of complex **8** originates from the freely rotatable phenyl ring. In our docking experiment, the phenyl ring has an almost planar orientation with respect to the π -system of the receptor due to π -stacking (described

by aromatic–aromatic interactions in the scoring function). Similar deviations are encountered for complex **5**. Considering the weak nature of this interaction, even a weak force caused by crystal packing effects probably is sufficient for a rotation of the phenyl ring. Investigation of the crystal structure for complex **8** reveals the phenyl ring in close proximity to an adjacent phenyl ring within the unit cell. In complex **5** the pyrazine ligand bound to the receptor is in direct vicinity to two other pyrazine molecules. From complexes **5** and **8** it can be seen that crystal packing can have a significant effect on the structure of host–guest complexes in the solid state. These forces are not accounted for by the docking score, and thus a difference between the predicted and experimental structures might be expected. Instead, our results show that the predicted structure compares well with the experimental solid-state structure. For all complexes of the test data set, the crystal structure is close to the putative structure in solution. Crystal packing appears not to change the overall receptor–ligand binding topology and has only small effect on complex geometry. This can be attributed to the fact that for all complexes of the test data set the intermolecular forces of complex formation (by hydrogen bonds) are much stronger than the crystal packing effects (van der Waals attraction and exclusion effects). For complexes dominated by nonpolar interactions, however, the crystal packing forces may have a much stronger influence.

The extension of the base placement algorithms by a point-matching technique is an essential prerequisite for the success of inverse docking. For example, for the ammonium ion binding complex **3**, the base fragment of the receptor cannot be placed by triangle or line matching since only a single directed interaction is formed at this stage. The results in Table 2 show that forward and inverse docking yield comparable RMSDs even in this case. The same is true for the two-point binding receptor **5**. Complexes **3** and **5** represent the two cases where the ligand either acts as hydrogen donor or acceptor, respectively.

During the inverse docking procedure for complexes **5** and **10**, after base fragment placement, the central, relatively weakly interacting fragments have to be placed. They do not form hydrogen bonds with the ligand. Nevertheless productive conformations are found that allow the formation of the existing hydrogen bonds between the ligand and the receptor in the later stages of the docking process.

While the accuracy of forward and inverse docking is similar, inverse docking is significantly faster in most cases. For instance, a speed-up by 5 orders of magnitude is achieved for complex **3**. For complex **4**, forward docking is not possible with the current approach because it would require on the order of 10⁴ days on a single CPU, whereas the inverse docking is completed after about 4 h. Complex **2** is the only one where inverse docking takes longer than forward docking. In this case, both ligand and receptor exhibit high flexibility, and the optimal assignment of host and guest is not obvious.

With complex **1** we demonstrate the ability of handling macrocyclic receptors. Prerequisite is the availability of an efficient tool for conformational analysis of large ring systems which returns an ensemble of ring conformations. Here, we used CORINA and CONFORT for this task. For more complex ring systems, such as cyclodextrins or cryptophanes, alternative conformational analysis tech-

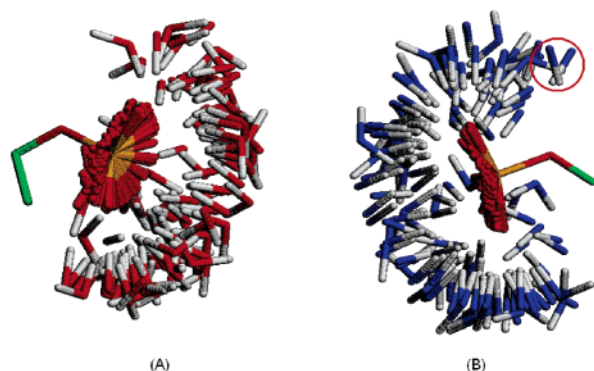


Figure 14. (A) IsoStar scatterplot, using CSD data, showing the distribution of OH contact groups around an aromatic phosphate ester group C(aromatic)–O–PO₃²⁻. (B) IsoStar scatterplot, using CSD data, showing the same distribution for NH contact groups. Only in two cases (CSD codes BORFAP and MENLEW, indicated by red circle) of the 227 fragments found, the NH-group is pointing toward the ester oxygen.

niques³¹ have to be applied, e.g. methods based on systematic search in torsion angle space. Work along this line is in progress in a collaborative project of our groups.³²

When we initially applied the methodology presented in this paper to the phenyl phosphate binding complex **8**, all top-ranking solutions contained a hydrogen bond between the phosphate ester oxygen and an NH group of the receptor. In some cases the phosphate ester oxygen even formed a bridged hydrogen bond to two NH donors. This result does not agree with the crystal structure of Kneeland et al.²⁷ This observation indicates that the interaction strength for this kind of hydrogen bond might be overestimated by the Böhm scoring function implemented in FlexX.

There is evidence from quantum chemical calculations on complexes of monomethyl phosphoric acid ester with water that the hydrogen bonding energy of the sp² oxygen acceptor outweighs the one of the sp³ ester oxygen by a factor of about 2.³³ To analyze this interaction type, we performed a systematic investigation of all occurrences of this contact with IsoStar³⁴ (Figure 14). No case was found of an OH group forming a hydrogen bond to an aromatic phosphate ester oxygen (Figure 14(A)). For NH as donor group, two cases among the 227 interactions were found (Figure 14-(B)) where the N–H vector is facing toward the ester oxygen of an aromatic phosphate ester (CSD codes BORFAP³⁵ and MENLEW³⁶). In both cases, the phenyl phosphate forms a bridge between two metal centers of binuclear metal complexes. The formation of a hydrogen bond might be due to the special nature of these metal complexes. We regard this rare occurrence (<1%) as a ‘pathological’ case that is not of general relevance. As a first remedy, we changed the rules for assigning interaction geometries in FlexR: No hydrogen acceptor geometry is assigned to the latter type of oxygen. After this modification, the hydrogen-bonding pattern in both forward and inverse docking of complex **8** was predicted correctly.

DISCUSSION

Our results demonstrate that docking of ligands of various types into synthetic receptors can be performed with great success employing the Flex* suite of programs. In contrast to protein–ligand docking, full conformational flexibility has

to be addressed due to the inherent flexibility of most synthetic receptors. The straightforward adaptation of protein–ligand docking techniques was successful in 9 out of 10 cases. Due to the necessary conformational sampling of the receptor molecule, the forward docking approach often is too time-consuming to be generally applicable. Furthermore, for receptors with a huge conformational space such as in complex **4** this algorithm is not viable. The interchange of host and guest makes this complex accessible to docking. The feasibility of inverse docking in this case originates from the fact that the conformational space of the receptor is searched with the FlexX *k*-greedy strategy.¹⁰ That means that at each growing step only the *k* best solutions are kept for the next stage. This leads to a well-defined search which disregards the largest part of the search space. For the same reason, inverse docking is often significantly faster than the standard forward docking. In our test set there is only one example for which the latter docking direction is superior. In this particular case, a simple count of the respective number of rotatable bonds in the two molecules could be used as a simple guidance for the docking direction.

The range of applications for both docking directions is limited by the conformational analysis step. Sequential docking can be performed, if the number of enumerated host conformations does not exceed a few 10 000 structures. This is best characterized by the number of rotatable bonds of the host molecule. For instance, the forward docking of complex **4** is infeasible (9 rotatable bonds), while forward docking of complex **3** (6 rotatable bonds) and inverse docking of complex **2** (5 rotatable bonds) are both feasible. As a rule of thumb, docking in one direction can be performed, if the number of rotatable bonds in the respective host is below 7. However, to perform docking on the time scale of minutes, the number of rotatable bonds in the host should not exceed 3.

The incremental build-up of a large receptor molecule around a small ligand in inverse docking works astoundingly well. In forward docking, typically the receptor provides much more interaction anchors which guide the incremental construction of the ligand and thus severely limit its conformational space and simplify the search. As shown by our results, the lack of directional interactions when docking around a small ligand does not seem to pose a serious problem. The explanation for this finding is that receptor conformations not forming further interactions with the ligand are discarded by the *k*-greedy search at an early stage, if better options exist. This is significantly different for protein–ligand complexes where docking works considerably better for deeply buried pockets than for ligands binding to the surface. In the pocket, the *k*-greedy algorithm mainly excludes solutions with severe overlap (excluded volume effects).

The simple interaction term used for ranking performs surprisingly well, considering that the neglect of the intramolecular interactions and direct solvation effects results in a crude approximation. It might be expected that the receptor self-energy dominates the energetics and determines the structure of the complex and therefore needs to be taken into account for the final ranking. There are a number of reasons why this turns out not to be necessary. First, part of the self-energy is taken into account indirectly during the generation of conformations. Clashes are avoided, and at the

same time the values used for the torsion angles have been derived from a database of stable structures. Second, the energy landscape of molecules of this size in nonpolar solvents may well be rather flat, with many minima of similar energy. Upon complexation the conformation is adopted that can accommodate the ligand best.

Furthermore, the scoring function contains a term for lipophilic interactions, which in the case of complexes that form in nonpolar environments may seem unnecessary, or even wrong. We attribute the good results obtained for the structure prediction to the following fact: The lipophilic interaction of the FlexX energy function is relatively short-range and thus rewards structures which maximize the interface between host and guest. Together with the other terms in the scoring function it leads to complex conformations with high steric and chemical complementarity.

The scores of the top-ranked conformations are dominated by the hydrogen-bond term, as is expected by our selection process for the complexes. In some complexes only hydrogen bonds (e.g. complex **3**) and no lipophilic interactions are formed, while in other complexes a combination of hydrogen bonds and lipophilic contacts is observed (e.g. the largest lipophilic contribution to the score is seen in complex **10**). Even in this case, the major contribution to the interaction energy is due to hydrogen bonds (−16.3) and not due to lipophilic interactions (−13.3). The comparison of the contributions to the score of the best-ranked solution in all 10 complexes shows that in each case polar interactions outweigh nonpolar interactions.

The overall prediction accuracy of the binding geometries is excellent as might be expected for FlexX. However, it is well-known that the FlexX scoring function does not provide a reliable estimate of the binding affinity. For this reason we have not tabulated score values here. Moreover, the FlexX scoring function is implicitly tailored to describing protein–ligand interactions in aqueous solution. Most of the synthetic receptors chosen in our test set have been designed for organic solvents. Therefore, it cannot be expected that the binding energies are reproduced accurately. A scoring function for different solvents and with a more specific description of the intermolecular interaction terms is currently under development.³³

It is interesting to note that the largest and most flexible of the artificial receptors in this study have the size of typical protein binding pockets. Thus, the larger artificial receptor–ligand complexes are idealized model systems for protein–ligand binding. Therefore, the results from studies on artificial receptor docking can be used to improve algorithms and scoring functions for protein–ligand docking. As an example case for this improvement we presented the new base placement algorithm and the modified interaction assignment relevant for complex **8**.

SUMMARY AND CONCLUSIONS

In this work, we have suggested two related approaches (forward and inverse docking) for predicting the structure of binary complexes between artificial receptors and their ligands, taking into account the conformational flexibility in both molecules. For the selected test cases, excellent agreement between predicted and experimental structures is observed for both methods. By having the choice between

the two docking directions, the application range of the method is significantly extended.

The high efficiency of incremental build-up approaches originates from the fact that the conformational space is searched with a *k*-greedy strategy. As a result CPU time is often drastically reduced in inverse docking. Comparing the results of the forward and inverse docking procedure for the same system provides a consistency check: The finding that two significantly different paths for the search lead to practically the same structures lends credibility to the approach. Further, it demonstrates that inverse docking is generally applicable for docking into synthetic receptors and not limited to the special case of rigid receptors. Finally, it should be noted that the incremental build-up method for docking has evolved from earlier methods for de novo ligand design.³⁷ The success of the present study suggests that similar procedures can be utilized for combinatorial design of artificial receptors.

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