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CRDOCK: An Ultrafast Multipurpose Protein–Ligand Docking Tool

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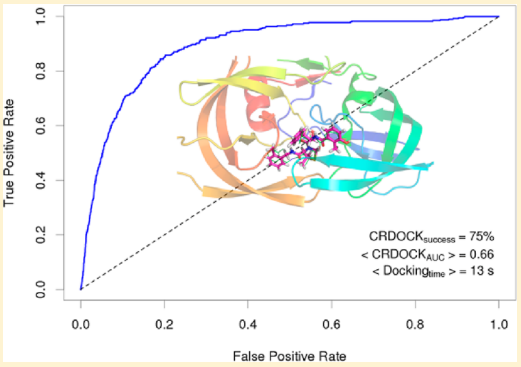
S *Supporting Information*

ABSTRACT: An ultrafast docking and virtual screening program, CRDOCK, is presented that contains (1) a search engine that can use a variety of sampling methods and an initial energy evaluation function, (2) several energy minimization algorithms for fine tuning the binding poses, and (3) different scoring functions. This modularity ensures the easy configuration of custom-made protocols that can be optimized depending on the problem in hand. CRDOCK employs a precomputed library of ligand conformations that are initially generated from one-dimensional SMILES strings. Testing CRDOCK on two widely used benchmarks, the ASTEX diverse set and the Directory of Useful Decoys, yielded a success rate of ∼75% in pose prediction and an average AUC of 0.66. A typical ligand can be docked, on average, in just ∼13 s. Extension to a representative group of pharmacologically relevant G protein-coupled receptors that have been recently cocrystallized with some selective ligands allowed us to demonstrate the utility of this tool and also highlight some current limitations. CRDOCK is now included within VSDMIP, our integrated platform for drug discovery.



1. INTRODUCTION

Docking and virtual screening (VS) strategies have acquired a relevant role in modern drug discovery since the pioneering work of Kuntz et al.[1] back in the early 1980s. However, and despite many advances carried out in the field during the past decade, this methodology is still far from perfect.[2] To increase its usefulness, more accurate methods are needed that can not only predict the native pose of a ligand within a protein in a crystallographic structure at the top of the list of possible solutions, as done in docking studies, but also discriminate true binders from a pool of decoys, as done in VS. Moreover, a modern docking tool needs to be fast because the number of molecules in currently used chemical libraries is well above 10^6. It is clear then that different objectives are pursued in docking and VS. In the former, native pose prediction is the main goal; in the latter, as long as true binders are separated from decoys, less accurate binding poses can be tolerated. In an ideal case, however, both criteria should be met because success in VS for the wrong reasons is unlikely to be reproducible and does not contribute to advancing the field.[3] More commonly, the goodness of fit between the ligand and the receptor is evaluated by means of an energy function composed of different terms that attempt to account for the forces driving the binding event. Although the underlying physical laws describing the binding process are well understood, accuracy and computational resources (mainly time) evolve in opposite directions, and fine tuning the appropriate balance between them is by no means an easy task. Therefore, accuracy is normally sacrificed for speed, especially in VS, and very often too simplistic scoring functions are employed.

Prior to scoring it is also necessary to sample the binding site of the receptor as exhaustively as possible. To this end and to save computer time, the space is usually discretized on a three-dimensional (3D) lattice and probe interaction energies at the grid points are calculated and stored.[4] Then, the molecule under study is translated and rotated at each lattice node along the three dimensions of the box, and interaction energies with the protein are estimated for each pose using the data stored on the grid points. Depending on the docking tool, the conformers can be generated within the binding site itself "on the fly" or created beforehand and stored to be reused again as many times as needed. The former method is more computer intensive, but as an advantage, it can generate strained

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conformations that adapt better to the active site environment. If the latter method is employed, a collection of all allowed conformers is quickly generated only once following some predefined rules, but the drawback is that a relevant conformation can be missed. For example, AutoDock[5] and GOLD[6] produce conformers in situ, whereas Glide[7,8] and FRED[9] use a pregenerated database of conformers. Our original in-house docking tool, CDOCK,[10] belongs to this second category because it was developed bearing in mind its potential use in VS, where millions of small molecules, some of them with hundreds of possible conformers, are available for docking in different projects. CDOCK was implemented in our open-access Virtual Screening Data Management on an Integrated Platform (VSDMIP) which has been recently extended to cover not only receptor-based VS[11] but also ligand-based[12] and fragment-based VS.[13]

Among the challenges still to be faced, some are more technical and related to computational times and correct implementation of tools to configure distinct VS protocols while others have to do with explicit inclusion of entropy,[14] solvent effects,[15,16] and receptor flexibility,[17,18] which appear to be necessary to get more accurate estimates of binding free energies. Indeed, theoretical approaches that tackle these problems continue to be developed and improved but are usually impractical for large VS campaigns because of the huge number of compounds that need to be evaluated. These computational efforts can be highly reduced if a prioritized list of compounds is available to be passed on to the most demanding calculations. This should be, in fact, the final objective of a docking program: saving time without increasing the false positive and negative rates compared to random selection. Unfortunately, not many currently available tools meet this requirement.

Bearing these facts in mind and in an attempt to improve the sampling and scoring capabilities of CDOCK, we present here CRDOCK, an ultrafast ligand docking program that was tested on two widely used benchmarks: the ASTEX diverse set (ADS, for docking)[19] and the Directory of Useful Decoys (DUD, for VS).[20] The latter was subsequently expanded with a representative group of recently available and pharmacologically very relevant G protein-coupled receptors (GPCR) in complex with some selective ligands. Being aware of the importance of water-mediated interactions in receptor−ligand binding, a water selection algorithm was implemented that is based on interaction energy calculations on a 3D grid, as pioneered by Peter Goodford in his renowned GRID program.[4] We also detected, discussed, and in most cases solved some widely reported problems[19,21] involving several well-known ligand−receptor complexes.

2. METHODS

Our CRDOCK tool contains (1) a search engine that can use a variety of sampling algorithms and an initial energy evaluation function for placing the ligand in the binding site, (2) several energy minimization algorithms for fine tuning the binding poses, and (3) different scoring functions for ligand ranking. Different methods are available for each of these components, and they can be independently chosen and combined.

2.1. Ligand Preparation. The ligands present in the complexes studied were prepared in two different ways. To test the docking engine alone, their X-ray coordinates from the PDB files were extracted and the ligand-free protein was used as the target (rigid ligand docking). Protonation and tautomeric states for these ligands were assigned with Open Babel 2.3.0[22] assuming a pH of 7.0, and no further manipulation of the coordinates was performed so as to preserve the native bound conformations. As a more realistic alternative and to check the efficiency of our in-house conformer generator, we also started from scratch, in the absence of any information about the ligand's 3D structure (flexible ligand docking). To this end, each ligand from the previous step was converted into a 1D simplified molecular-input line-entry system (SMILES) string and then inserted into the VSDMIP database following our standard protocol: (a) automated conversion from SMILES to 3D MOL2 format using CORINA,[23] (b) atomic charge calculations with MOPAC[24] (AM1 ESP method) on every single structure provided by CORINA, (c) atom-type assignment according to the AMBER force field,[25] and (d) conformer generation using ALFA.[26]

2.2. CRDOCK Constituent Parts. The three main components of the CRDOCK tool are depicted in Figure 1.

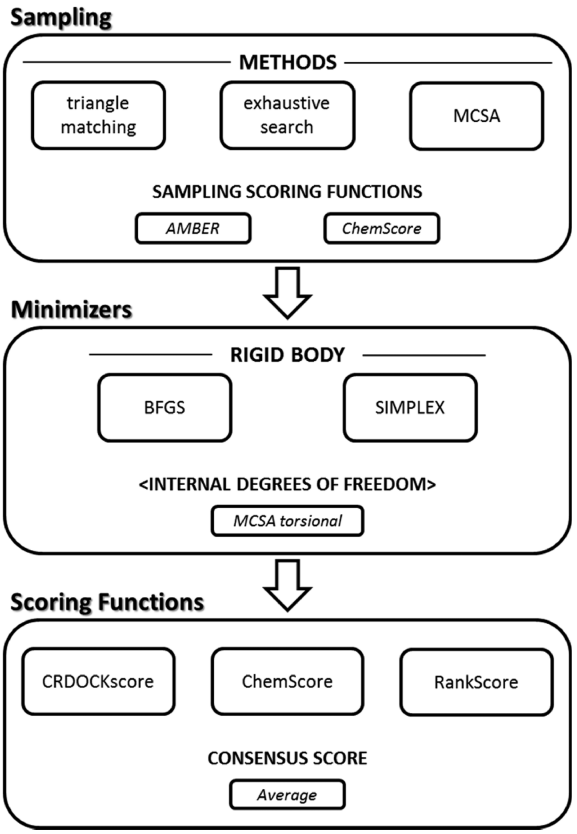

Figure 1. Graphical overview of CRDOCK components and workflow. Method enclosed in angle brackets is optional.

The different methods integrated in each step can be independently selected and later combined to configure different custom-made workflows. This is of special interest in those cases where a researcher faces computational restrictions.

2.2.1. Sampling. CRDOCK implements three sampling strategies: (a) triangle matching, (b) exhaustive search, and (c) Monte Carlo simulated annealing (MCSA).

a. Triangle Matching. CRDOCK determines the interaction points for a given ligand conformation using the functional groups present in its structure. All possible combinations of 3 different interaction points (for each conformer) are generated

applying a cutoff to avoid very short edges (4.5 Å by default). These are the ligand interaction triangles. The same combination is performed for the receptor interaction points (see receptor active site analysis below) to obtain the receptor interaction triangles. The program then looks for the best superimpositions between the receptor's and the ligand's interaction triangles, evaluating each one with an AMBER-like (12−6 Lennard−Jones potential with an electrostatic term modeled with a sigmoidal dielectric screening function) or the ChemScore[27] empirical scoring function. If none of the ligand conformers can be used to build triangles, a lower cutoff for the edges is employed (2.5 Å). Finally, if no triangle can be built with this reduced distance, the exhaustive search or MCSA algorithms (see below) will be used instead.

b. Exhaustive Systematic Search. In this case each ligand conformer is translated over each single grid point and rotated in all directions with steps of 30° on each axis. The interaction energy for each generated pose is evaluated with the energy functions described in point a above. Because this search is quite time consuming, it is performed only if the number of conformers is ≤5. For the remaining cases the MCSA method described below is employed.

c. Monte Carlo Simulated Annealing. Random translations and rotations of the ligand are generated to determine a new pose from the last accepted pose (the first pose is generated randomly). The new pose is accepted or rejected depending on a probability managed by the temperature (Metropolis criterion). By default and on the basis of results from internal tests, 23 temperature rounds are performed with a maximum number of generated poses per round of 725 000 to avoid redundancy. The algorithm starts at 773 K, and this temperature is scaled down at each round by 20%. The probability of change for each parameter defining a pose was set to 0.8.

Regardless of the sampling method, the best 512 poses per conformer are saved in a stack up to a maximum of 5000 per molecule. The final result is a collection of the best ligand poses (5000 by default) which can be passed on to the next step to be refined by energy minimization. In order to promote diversity, it is possible to apply a root-mean-square deviation (rmsd)-based filter to check whether or not a new pose is added to the list.

2.2.2. Minimization. Prior to scoring, the above selected poses can be refined either by rigid-body energy minimization (translations and rotations) or by changing the internal degrees of freedom (torsional angles) to fine tune their fit within the receptor binding site. This process is carried out expediently using precalculated AMBER-like potential interaction energies stored in a 3D grid.[28,29] Three algorithms have been implemented: (a) Broyden−Fletcher−Goldfarb−Shanno (BFGS, for rigid-body minimizations), a deterministic method that belongs to the family of quasi-Newton methods; (b) Amoeba or downhill SIMPLEX (for rigid-body minimizations), the stochastic algorithm from Nelder and Mead;[30] and (c) MCSA torsional (MCSAtor, for internal degrees of freedom [torsions] minimizations), a stochastic method analogous to the described MCSA algorithm but that only optimizes molecular torsions. By default, 200 steps of BFGS rigid-body minimization are performed.

2.2.3. Scoring Functions. The final ranking of the resulting poses from the previous step can be evaluated with either a single scoring function or a combination of several of them ("consensus scoring"). The available options are (a) CRDOCKscore, (b) ChemScore, and (c) RankScore.

a. CRDOCKscore. This is a modified version of GlideScore,[31] the scoring function implemented in program Glide (eq 1), that combines van der Waals (E_{vdW}) and electrostatic (E_{qq}) energy terms from the AMBER force field with lipophilic (E_{lipo}) and hydrogen-bonding (E_{hb}) terms from ChemScore.[27] The AMBER terms are scaled by weighting factors α and β for E_{vdW} and E_{qq}, respectively, as defined in GlideScore.

$$\text{CRDOCKscore} = \alpha E_{vdw} + \beta E_{qq} + E_{lipo} + E_{hb} \quad (1)$$

where α = 0.065 and β = 0.130. No additional modifications were made to the AMBER or ChemScore lipophilic and hydrogen-bonding terms.

b. ChemScore. ChemScore is our implementation of the well-known ChemScore[27] scoring function.

c. RankScore. RankScore is a statistical potential scoring function specifically derived for VS classification from known sets of compounds (DUD).[32]

2.2.4. Water Selection Algorithm. Very often water-mediated interactions between the ligand and the receptor binding site are key for accurate fitting. We employed a modified version of cGRILL (see receptor active site analysis) to generate water affinity maps using a probe representing a water molecule that can act as a hydrogen-bond acceptor and donor. For this purpose we employed the concept of *extended atom* using an AMBER-like energy function for an oxygen atom endowed with a van der Waals term, a partial charge of −0.12 au and a hydrogen-bond block function based on an ideal H-acceptor distance of 1.8 Å and a donor−H-acceptor angle of 180°. Then the program clusters similar interaction areas using an energy cutoff which is one-half of the maximum value of the scoring function.

2.3. Benchmarks. Three different test sets were used: (a) ADS for pose prediction, (b) DUD for VS, and (c) GPCR for pose prediction and VS. The standard criterion to validate the ability to predict the native pose was the heavy atoms rmsd between the docking solution and the native conformation for each ligand in the crystal structure. In common with other similar studies, we chose an rmsd value of 2 Å as the upper limit for a solution to be considered correct. VS performance was assessed by means of the area under the curve (AUC) of the generated receiver operating characteristics (ROC) plots.[33]

All receptors (except otherwise stated) were prepared for both docking and VS following the same protocol, namely, for each one, all species other than the protein itself, cofactors, and metals were removed. Hydrogen atoms were added using the pdb2pqr[34] tool and adapted to the AMBER 03 force field using GROMACS v.4.5.3. One thousand steps of steepest descent were followed by 2000 steps of Polak−Ribiere conjugate gradient energy minimization where only hydrogen atoms were allowed to move. No energy minimization was performed for DUD targets so that our results could be compared with those published in other DUD-related publications. The cubic grid for cGRILL calculations was defined as the space delimited by the axis-parallel box containing the cocrystallized ligand, augmented by 5 Å in each axis direction.

a. ASTEX Diverse Set (ADS). The ADS is composed of 85 protein−ligand complexes that can be downloaded from the Protein Data Bank (PDB).

b. Directory of Useful Decoys (DUD). The DUD is composed of 40 different targets with known 3D structures and a set of true/fake binders for each target.

c. G Protein-Coupled Receptors (GPCR) Set. We enlarged the original DUD by including such pharmacologically relevant targets from the GPCR family as the adrenergic β_2, the dopaminergic D_3, the muscarinic M_2, the histamine H_1, and the opioid μ receptors, all of which have been cocrystallized in the presence of antagonists or inverse agonists at a resolution ≤ 3.1 Å. For both self-docking and VS tests we used the ligand-bound protein structures found in PDB entries 2RH1 (β_2), 3PBL (D_3), 3UON (M_2), 3RZE (H_1), and 4DKL (μ). In the latter case, the covalent bond between the morphinan ligand and Lys233 was broken and an alternate nonclashing rotamer was selected for Lys233 using PyMOL.[35] SMILES strings for known true ligands for these targets (Supporting Information, Table S1) were taken from the DrugBank database.[36] To select a suitable set of decoys, we followed a similar procedure to that reported in the original DUD description: (a) the clean drug-like set of small molecules was downloaded from ZINC[37] (9 542 593 SMILES strings); (b) Molecular ACCess System (MACCS) fingerprints were calculated for each SMILES string using OpenBabel;[22] (c) a Tanimoto cutoff of 0.4 was used as a filter to select the most topologically dissimilar compounds as compared to the known ligands (415 636); (d) Qikprop[38] was used to calculate physicochemical properties for each compound and select those most similar to the true ligands; and (e) 30 decoys per ligand were saved for the VS experiments.

2.4. Receptor Ligand-Binding Pocket Analysis. Given a suitably prepared receptor structure, the next steps are to energetically characterize the active site and to determine its main interaction points.

a. Energetic Characterization. This is performed with the cGRILL program, an improved version of our CGRID code[10] that relies on interaction energy calculations on 3D grids as pioneered by Goodford in his well-known GRID program.[4] cGRILL uses the AMBER 12−6 Lennard−Jones term for C, N O, H, S, and P probe atoms and an electrostatic term modeled with a sigmoidal dielectric screening function, together with other terms from ChemScore (lipophilic, H-bond acceptor, H-bond donor, "mixture", metal, and clash). Finally, a grid containing clash-free points was added for accelerating the sampling process during docking. All grid maps can be inspected using PyMOL molecular visualization software.[35] Residues, cofactors, and other species are parametrized automatically using an AMBER force field-like atom-typing scheme.

b. Interaction Points. The best interaction areas ("hot spots") are mapped by sampling the active site with different molecular probes, an idea already introduced in other docking programs.[39] The calculations are similar to those in cGRILL for AMBER grids but using polyatomic probes instead, which are allowed to rotate at each grid point with a step size of 180° in each axis. The molecular probes are CH_4 to detect lipophilic regions and NH and CO to detect H-bond-accepting and -donating partners, respectively. The best result for any of the probes at each grid point is selected. The generated set is postprocessed to avoid redundancy. For a given area, only the best probe in a radius of 2 Å for lipophilic or 1.5 Å for hydrogen-bond probes is kept. Then, these nonredundant probes are rescored by summing up their neighbors' energetic scores.[39] The best probe with the highest new score is selected as the best point in the active site, and all the surrounding points with a distance less than 4 Å are also selected. The process continues adding points within 4 Å from the selected ones until no more points fulfilling the distance restraints are available. The selected docked probes are exported as a PDB file.

2.5. Alternative Docking Protocols. Two different alternatives were explored.

(a) Alternative 1. Our original CDOCK code with default parameters: MCSA/exhaustive sampling, SIMPLEX refinement, and the scoring function as the sum of van der Waals and electrostatic terms from the AMBER force field, the electrostatic contributions to ligand and receptor desolvation, the nonelectrostatic part of the desolvation modeled as a linear relationship with the solvent accessible surface area lost once the complex has been formed, and an explicit term to account for hydrogen-bonding interactions. In the preparation of the receptor, water molecules involved in the binding event are always kept and full complex relaxation is accomplished using energy minimization.

(b) Alternative 2. A different CRDOCK configuration consisting of (1) default sampling (triangle matching or MCSA), (2) BFGS and MCSAtor for fine tuning, and (3) nonbonding energy terms (E_{vdW} and E_{qq}) from the AMBER force field for pose scoring. For the VS experiments, ChemScore, AMBER, and RankScore were used as the scoring functions as well as a simple average of the three ("consensus scoring").

3. RESULTS AND DISCUSSION

Different CRDOCK configurations were tested for both data sets. After analyzing the results (Table 1), the combination that yielded the best performance was triangle matching and AMBER energy evaluation, BFGS as the rigid body minimizer without torsional optimization, and the CRDOCK scoring function. The results shown in Table 2 were obtained with this CRDOCK configuration.

Table 1. Summary of the Results from Alternative Protocol Using the ADS and the DUD

protocol	no. complexes with rmsd ≤ 2.0 Å (ADS)	average AUC (DUD)
CRDOCK	62	0.66
alternative 1 (CDOCK)	64[e]	0.60
alternative 2 FF[a]	59[f]	0.56
alternative 2 CS[b]	59[f]	0.58
alternative 2 RS[c]	59[f]	0.57
alternative 2[d]	59[f]	0.58

[a]AMBER force field scoring function. [b]ChemScore. [c]RankScore. [d]Consensus scoring = average between AMBER, Chemscore, and Rankscore scoring functions. [e]See methods. [f]Selected poses are always the same, and the difference is due to the final scoring function applied.

3.1. Docking and Scoring: Pose Prediction and Errors Found. Although with some controversy,[40] the rmsd between a docking solution and the crystallographic coordinates is still the most widely accepted criterion as a metric of success. A docking solution with an rmsd value ≤ 2.0 Å is regarded as a correct pose. Considering the receptor as a rigid entity, two different docking experiments were conducted: (a) rigid ligand docking, where the ligand conformation is taken directly from the structure of the complex, and (b) flexible ligand docking, where

Table 2. Summary of the VS Statistics Related to the AUC Values Grouped by Protein Families

target[a] family	average	SD[b]	median	max	min
global	0.66	0.15	0.69	0.97	0.32
kinases[c]	0.63	0.11	0.63	0.77	0.47
serineproteases[d]	0.78	0.06	0.76	0.84	0.74
NHR[e]	0.71	0.19	0.74	0.97	0.42
metalloenzymes[f]	0.61	0.15	0.63	0.76	0.41
folateenzymes[g]	0.83	0.15	0.83	0.94	0.72
rest	0.61	0.26	0.59	0.80	0.32

[a]ACE, angiotensin-converting enzyme; AChE, acetylcholinesterase; ADA, adenosine deaminase; ALR2, aldose reductase; AmpC, AmpC β-lactamase; AR, androgen receptor; CDK2, cyclin-dependent kinase 2; COMT, catechol *O*-methyltransferase; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; DHFR, dihydrofolate reductase; EGFR, epidermal growth factor receptor; ER_{ago}, estrogen receptor (agonist-bound conformation); ER_{antago}, estrogen receptor (antagonist-bound conformation); FGFR1, fibroblast growth factor receptor kinase; FXa, factor Xa; GART, glycinamide ribonucleotide transformylase; GPβ, glycogen phosphorylase β; GR, glucocorticoid receptor; HIVPR, HIV protease; HIVRT, HIV reverse transcriptase; HMGR, hydroxymethyl-glutaryl-CoA reductase; HSP90, human heat shock protein 90; INHA, enoyl ACP reductase; MR, mineralocorticoid receptor; NA, neuraminidase; P38 MAP, P38 mitogen-activated protein; PARP, poly(ADP-ribose) polymerase; PDE5, phosphodiesterase 5; PDGFRB, platelet-derived growth factor receptor kinase; PNP, purine nucleoside phosphorylase; PPARγ, peroxisome proliferator activated receptor γ; PR, progesterone receptor; RXRα, retinoic X receptor α; SAHH, *S*-adenosyl-homocysteine hydrolase; SRC, tyrosine kinase SRC; Thr, thrombin; TK, thymidine kinase; VEGFR2, vascular endotelial growth factor. receptor; [b]Standard deviation. [c]CDK2, EGFR, FGFR1, HSP90, P38 MAP, PDGFRB, SRC, TK, and VEGFR2. [d]FXa, Thr, and trypsin. [e]AR, ER_{ago}, ER_{antago}, GR, MR, PPARγ, PR, and RXRα. [f]ACE, ADA, COMT, and PDE5. [g]DHFR and GART.

a precalculated set of conformers is generated from scratch. In the former we challenge, on one hand, whether the sampling algorithms are able to generate the correct pose and, on the other hand, whether the scoring function is able to recognize it as its best solution. In the latter we also test the conformer generator. Our results (Figure 2) indicate that CRDOCK correctly identifies 93% (79 out of 85) of the native poses in the rigid docking experiment. However, this percentage falls to 73% (62 out of 85) when the ligand coordinates are generated from the SMILES strings using CORINA and ALFA.[26] Both results are comparable to those obtained with the most widely used docking programs. For example, in the original ADS study the authors described a very similar performance for GOLD (around 70−80% of complexes with an rmsd $\leq$ 2.0 A) and almost the same decrease in performance when the ligand 3D structure was generated from scratch instead of using the crystallographic pose.[19] Li et al. used a set of 195 diverse high-resolution ligand−protein complexes to compare Glide, GOLD, LigandFit, and Surflex docking programs and obtained a variable range from 60% to 80% in most cases.[41] Cross et al., using a subset of high-resolution structures from the CCCD/Astex set, compared GLIDE, ICM, PhDock, FlexX, and Surflex docking tools. The 70−90% rate of successfully docked poses when the native ligand was reduced to 50−77% when the ligand structure was generated from scratch using CORINA.[42] More recently and using ADS, performances of 60−80% for Surflex-Dock[43] and ~74% for LeadFinder[44] were reported. All benchmarks confirm than the results currently obtained with CRDOCK are comparable to those obtained with other modern docking programs. Next, we looked for the reasons for failure.

Common Errors. Some errors that we encountered in flexible ligand docking appear to be commonly reported by others[19] using different docking engines and scoring functions.

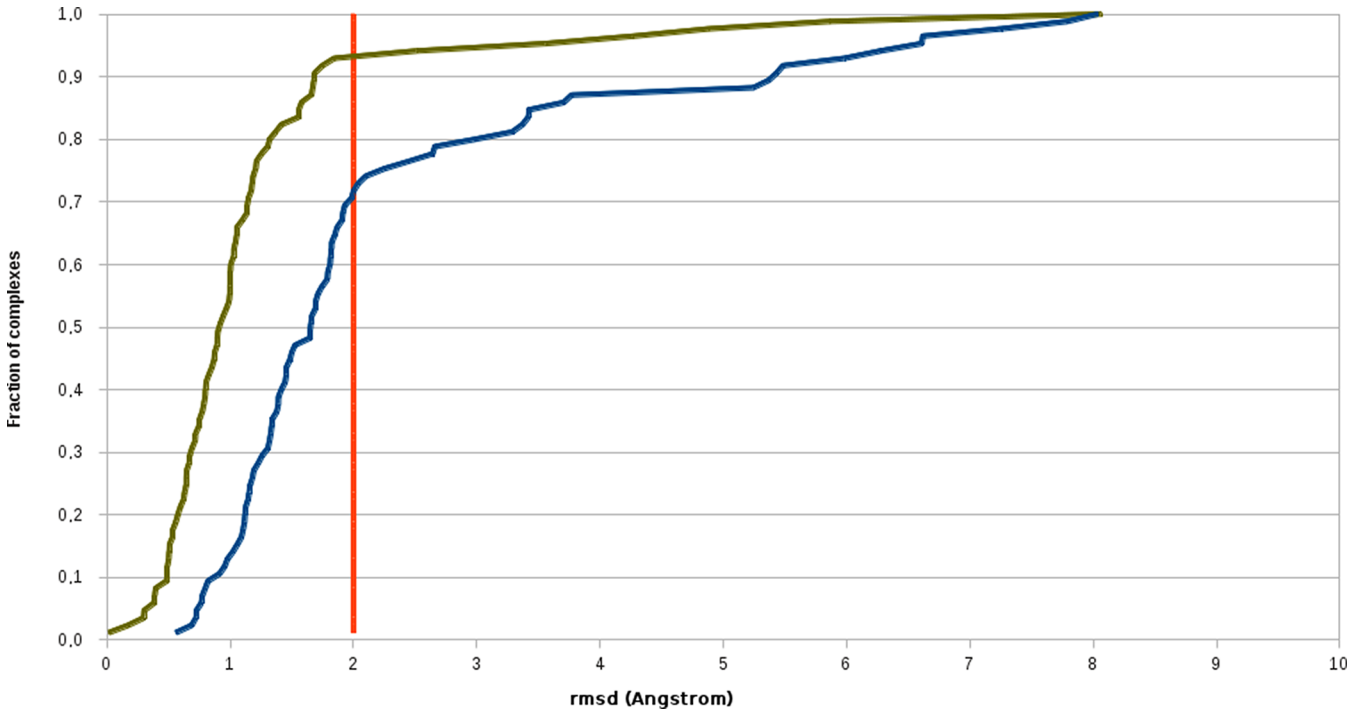

Figure 2. Pose prediction performance obtained using CRDOCK in rigid (green) and flexible (blue) ligand docking. Red line indicates a success threshold of rmsd = 2 Å.

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The ligands in PDB entries 1JJE, 1SJ0, 1YVF, and 1TZ8 belong to this category (Supporting Information, Figure S1). The ligand in complex 1JJE displays an almost planar and quasi-symmetrical conformation with a central diacetal moiety chelating a zinc ion. Due to these properties it is not unreasonable that the docking program, while being able to capture the native interactions, selects a pose that is rotated by 180° compared to the X-ray structure. The same is observed for the symmetric ligand in the 1TZ8 complex. The best docking solution reproduces all of the contacts present in the X-ray structure, but its rmsd (2.64 Å) is (only apparently) beyond the success cutoff. The native pose for the ligand in the 1YVF complex has a carboxylic group directly exposed to the solvent whose location is strongly penalized by the scoring function. Therefore, the native pose is not promoted to the top of the ranking list. Finally, the ligand in the 1SJ0 complex presents, in its central ring, a large substituent in the axial position and a small substituent in the equatorial position. This peculiar arrangement is not considered stable enough to be selected by CORINA as a representative conformation, and therefore, the docking algorithm fails in reproducing the native pose.

Another common error is the sulfonamide ligand in 1JD0, which was not docked correctly into human carbonic anhydrase XII using the rigid docking protocol (rmsd = 5.7 Å) but, rather surprisingly, was successfully docked (rmsd = 1.85 Å) when starting from scratch.

Ligand Conformational Sampling Errors. These errors are related to our ligand preparation step. In some cases the generated set of conformers does not properly cover the conformational space of a ligand. In the present study, the vast majority of the problematic cases are those in which the bound ligand in the experimental structure exhibits torsional angles whose values are away from those considered as ideal. These torsions are, to some extent, forced by the binding site environment. Since ideal angles are taken as rules in our conformer generator code ALFA, these particular conformations will not be present in the conformer population. Within the ADS, the ligands found in PDB entries 1Q1G, 1R58, 1UML, 1UNL, and 1UOU fall into this category.

To test whether or not this was, in fact, the source of error, these ligands were parametrized (GAFF, General Amber force field), immersed in cubic boxes of TIP3P water molecules, energy minimized (2000 steps of steepest descent followed by 2000 additional steps of Polak−Ribiere conjugate gradient), and simulated (constant temperature [300 K] and pressure [1 atm]) for 15 ns. Torsional angles were monitored over the whole trajectory and compared to those found in the crystal. In all five cases the relative population of conformers whose torsional angles were within 5° of the crystallographic structure was below 7%. This means that the native conformation is rarely sampled, and therefore, algorithms based on rules are very unlikely to generate near-native conformations.

Errors Due to Missing Water-Mediated Interactions. There are five cases of failure in which water molecules crucially mediate ligand−protein interactions (water-mediated bridges). As the usual protocol consists of eliminating, among other species, all water molecules present in a binding site, docking algorithms are unable to reproduce the native pose. This problem occurs in PDB entries 1GM8, 1G9V, 1GPK, 1HVY, and 1XM6 (Supporting Information, Figure S1) and was also found in the rigid ligand docking experiments. The ligand in complex 1GM8 has a β-lactam ring that interacts with residue Ser386 through a water-mediated bridge (WAT2460) and a benzylic amide buried at the bottom of the binding pocket. CRDOCK correctly reproduces the X-ray position of the latter moiety, while it places the β-lactam ring in an alternate location (rmsd = 3.37 Å). In the 1G9V complex, WAT916 mediates the interaction between Asn308 and the nitrogen atom of the ligand's amide group whereas WAT927 and WAT983 interact with the ligand's carboxylate. Both functional groups are wrongly positioned by CRDOCK in the absence of the water molecules (rmsd = 3.77 Å). The ligand in the 1GPK complex has a charged amino group interacting with a water molecule (WAT2529). When the water molecule is not considered as part of the binding site, the amino group is attracted by Glu199 and an alternative docking pose is favored (rmsd = 3.71 Å). The ligand in complex 1HVY has two different sources of error. First, the conformational sampling of the ligand fails because the closest conformer found is 1.47 Å away from the X-ray structure; second, the absence of two water molecules (WAT477 and WAT1017) precludes the correct orientation of the two carboxylic moieties at one end of the molecule (rmsd = 3.36). Finally, the ligand in the 1XM6 complex is unable to correctly orientate the oxazolidinone ring if WAT1009, which chelates the Zn^{2+} ion, is not present in the binding site. In its absence, the ligand's carbonyl group is strongly attracted by the Zn^{2+} ion (rmsd = 2.45).

The "missing water" problem could be easily solved in some cases using cGRILL to calculate the water affinity map within the binding site and place the relevant water molecule(s). These were correctly identified for both 1G9V and 1XM6, and upon incorporation into the protein structure, the rmsd of the ligands was notably reduced relative to the "dry" docking solution (from 3.77 to 1.51 Å and from 2.45 to 1.90 Å for 1G9V and 1XM6, respectively). For the remaining cases adding the water molecules helped only in part. In the 1HVY complex the ligand's carboxylate groups were correctly positioned upon addition of the water molecules, but the conformational problem still persisted (rmsd = 2.75 Å). Proper docking of the ligand in the 1GM8 complex improved significantly following incorporation of the water molecules; most of the contacts with the target were reproduced, but the rmsd was still 2.67 Å. On the other hand, for the same ligand in the "rigid" approach the rmsd decreased from 4.27 to 0.61 Å. Finally, for the 1GPK complex, addition of the relevant water molecules resulted in a pose with rmsd = 1.39 Å, although this solution was the second best on the ranking list.

Other Errors. Some compounds were classified as misdocked because of an rmsd slightly above 2.0 Å despite the fact that the top scoring pose faithfully reproduced the main interaction points that are observed within the binding site in the X-ray crystal structure. Five complexes belong to this category of "soft errors": 1HP0 (rmsd = 2.57 Å), 1N2V (rmsd = 2.86 Å), 1MMV (rmsd = 2.84 Å), 1XOQ (rmsd = 2.10 Å), and 2BM2 (rmsd = 2.25 Å). Finally, the ligands in complexes 1IG3, 1OQ5, 1P62, and 2BSM were not correctly positioned and no clear reason could be found to account for the deviations, which are presumably due to several combined factors such as insufficient or deficient ligand conformational sampling and/or inaccuracies in the scoring function.

3.2. Virtual Screening: Discriminating True Binders from Decoys. VS calculations were performed with the 40 targets and their associated sets of true binders and decoys from DUD. Global as well as family-wise statistics (AUCs from the ROC plots) were compiled and are summarized in Table 2. The average AUC values are comparable to those already

reported,[45] and there is a clear variability depending on the target. The 5 top scores are obtained for RXRα (AUC = 0.97), DHFR (AUC = 0.94), ER_{ago} (AUC = 0.86), trypsin (AUC = 0.84), and COX2 (AUC = 0.80). These targets are related neither functionally nor structurally, except for ER_{ago} and RXRα which belong to the family of nuclear hormone receptors (NHR). The same lack of obvious connection applies to the worst scoring targets, with the exception of the two NHR members PR and PPARγ: TK (AUC = 0.47), PR (AUC = 0.45), PPARγ (AUC = 0.42), ACE (AUC = 0.41), and AmpC (AUC = 0.32). The trend that CRDOCK performs better than average on folate enzymes and serine proteases has been observed with other docking programs.[45] In the case of kinases our method outperforms other docking tools reporting on the same data set[45] even though the average AUC value for this family is still below the global AUC average.

We took a closer view at the ROC curves (Supporting Information, Figure S2) to shed more light into those targets for which VS performance was worse than random: AChE (AUC = 0.49), TK (AUC = 0.47), PR (AUC = 0.47), ACE (AUC = 0.41), InhA (AUC = 0.48), and AmpC (AUC = 0.32). AmpC appears to be one of most problematic targets for all docking programs.[42] AChE[46] and TK[47] are two well-known enzymes in which the flexibility of some residues within the active site plays a crucial role in ligand recognition. As our docking tool does not include receptor flexibility, it is not entirely surprising that the VS protocol that we used fails in these cases, as do many other programs.[42] Although the global AUC values were below random for PR, ACE, and InhA, early enrichments (as detected in other studies)[42] were clearly apparent because 3/5, 4/9, and 13/17 true binders, respectively, were present on the top 0.5% of the rank-ordered list.

Different docking programs using DUD as a test set afforded values similar to those reported here for CRDOCK. Cross et al.[42] found average AUC values of 0.55 (DOCK), 0.59 (PhDock), 0.61 (FlexX), 0.63 (ICM), 0.66 (Surflex), and 0.72 (Glide). Finally, Marco et al.[48] reported a median AUC of 0.69 (very similar to CRDOCK) and Novikov et al.[44] an average AUC around 0.70.

3.3. GPCR: Pose Prediction and Virtual Screening. The original ADS was supplemented with five GPCR complexes, and self-docking experiments were carried out as described in the pose prediction section. The ligands in PDB entries 3PBL, 3UON, 2RH1, and 3RZE were correctly positioned within the binding site (rmsd $\leq$ 2.0 Å) in both the rigid and the flexible ligand docking tests. In the case of the μ opioid receptor, however, the first solution had rmsd = 2.44 Å, but it has to be borne in mind that in the crystal structure (PDB entry 4DKL) the funaltrexamine ligand is covalently bonded to Lys233. In the CRDOCK solution (Supporting Information, Figure S3) the main deviation lies precisely on the flexible chain that is covalently bonded to the amino group of Lys233 in the experimental structure, whereas the morphinane core reproduced the native pose accurately (rmsd = 1.27 Å). Therefore, although the overall rmsd is above the canonical cutoff value of 2.0 Å, the docking error can be considered "soft" in light of the conserved important interactions.

To explore the ability of CRDOCK to predict selectivity, we performed a simple cross-docking VS experiment using these five GPCR complexes, that is, every ligand was docked into every receptor to check whether the best score was assigned to the true ligand–receptor couple. In addition, by superimposing the seven transmembrane helices of all the GPCR studied we were able to calculate and compare the rmsd for all docking poses (Table 3). Without exception, the lowest rmsd was found

Table 3. RMSD Values (in Angstroms, top) and Scores (kcal mol^{-1}, bottom) for Cross-Docking Studies on GPCRs

	ligands				
	β_2	D_3	H_1	M_2	μ
GPCR[a]					
β_2	**1.10**	3.81	5.67	4.58	5.42
D_3	3.80	**2.00**	5.10	6.80	7.18
H_1	4.29	3.98	**1.61**	4.48	8.11
M_2	4.67	4.60	**2.16**	**1.15**	5.93
μ	6.03	5.21	8.51	4.72	**1.27**
GPCR[b]					
β_2	**−101.3**	−86.1	−85.6	−82.8	−46.2
D_3	−83.9	**−94.0**	*−98.2*	−91.0	−73.7
H_1	−111.2	−97.3	**−117.5**	*−123.0*	−107.7
M_2	−87.8	−83.5	−104.7	**−115.4**	−57.2
μ	−61.6	−77.5	−72.7	−88.0	**−92.1**

[a]Numbers in bold along the diagonal highlight the lowest rmsd values for the ligand bound to its cognate receptor and, in addition, for the H_1 ligand (doxepin) bound to the M_2 receptor. [b]Numbers in bold along the diagonal highlight the binding energies for the cognate ligands, which are sometimes less favorable (in the same row) than for a noncognate ligand (values in italics).

for the native ligand in its own receptor. In addition, a very low rmsd at the M_2 receptor was found for doxepin, an H_1 receptor antagonist which is also known to bind with high affinity to muscarinic,[49] α_1-adrenergic,[50] and some mosquito dopamine[51] receptors. Regarding the ranking, the native ligands received the top scores at the β_2, M_2, and μ opioid receptors but those of the D_3 and H_1 receptors got the second best. At the D_3 receptor it was doxepin that appeared in the first position, and at H_1 it was not doxepin, as expected, but 3-quinuclidinyl benzilate, the prototypical anticholinergic agent with high selectivity for M_2 receptors. These findings raised a note of caution and prompted us to perform further studies.

When all true (as annotated in the DrugBank[36]) GPCR ligands studied were merged into a single set and used in an expanded VS experiment against the five GPCR, the AUC values from the obtained ROC curves were 0.50 (D_3), 0.53 (β_2), 0.56 (M_2), 0.71 (μ), and 0.61 (H_1). More informative, however, is the number of true binders that are recovered for each target at the top 10 of the ordered list: 6 are correctly identified for the β_2, D_3, and H_1 receptors, 5 for the M_2 receptor, and 2 for the μ opioid receptor.

Finally, when the set of ligands for each GPCR consisted of 30 decoys per true binder, the AUC from the resulting ROC curves were 0.50 (D_3), 0.56 (β_2), 0.64 (M_2), 0.80 (μ), and 0.82 (H_1). The average value of 0.67 compares very well with the findings reported above for the DUD. Nonetheless, it has to be noted that the number of true binders for each GPCR varied greatly, from 7 for the opioid receptor to 74 for the H_1 receptor (Supporting Information, Tables S1–S5), and this wide difference can be largely responsible for the diversity of the outcome. After completion of this work, we became aware of a recent compilation of 147 GPCR targets and a ligand library (agonists + antagonists) that included 39 decoy molecules for each true binder.[52] Application of the CRDOCK VS protocol to our selected GPCRs and this alternative compound

collection resulted in a small improvement for the β_2 receptor (0.1 AUC units), roughly the same performance for the D_3 receptor, and a slight decrease for μ (0.2 AUC units), H_1 (0.2 AUC units), and M_2 (0.1 AUC units) receptors. These differences, which amount to an average decrease in AUC from 0.67 to 0.56, can be expected due to the distinct way decoys and true ligands were selected and also to the fact that all receptors used are in the antagonist-bound conformation.

Altogether, these results are encouraging but indicate that to study selectivity among related GPCR with an acceptable degree of accuracy further improvements in CRDOCK and receptor preparation (e.g., incorporation of bound water molecules)[52] will be necessary.

3.3. Alternative Docking Protocols with Flexible Ligands. Two other alternatives were tested: our old in-house docking engine CDOCK and a different CRDOCK configuration.

From the results obtained for VS using DUD, CRDOCK represents a significant improvement over the rest (Table 1), which confirms the importance of the hybrid scoring function. However, performance on an individual target is in some cases strongly dependent on the selected scoring function. For example, in the case of the PDB code 1XGJ (β-lactamase AmpC) the AUC for the hybrid scoring function is as low as 0.32, but this figure is increased to 0.71when a force-field-based scoring function is used. Therefore and in agreement with other studies, we believe that trying to develop a universal scoring function[53,54] can be a daunting task indeed. Instead, more satisfactory results for the problem in hand can be achieved if a tailor-made target-dependent scoring function is used.

3.4. Benchmarking. The average time to perform the docking of a single flexible ligand using the reported combination of pieces that yielded the best performance is ~10 s on a 64-bit 3.3 GHz Intel Core i5 processor. We observed that the triangle matching approach could be used for around 91% of the compounds in the database. For the remaining ligands either exhaustive search or MCSA was used, and this took ~26 s on average to complete, leaving the docking average time in ~13 s. Therefore, with a modest cluster of 100 processors it should be possible to screen more than one-half million compounds per day in a typical VS campaign.

4. CONCLUSIONS

We introduce CRDOCK, a new computational tool that performs reasonably well in both pose prediction (docking) and true binder discrimination (VS). CRDOCK has demonstrated its abilities on two widely used benchmarking tests: the ADS for pose prediction and the DUD for VS. The docking failures found, some in common with other published reports, were analyzed in detail, and several solutions were found. In addition, five representative ligand−GPCR complexes were studied, and the results were in line with those obtained from DUD. Self-docking was always satisfactory with rmsd values below 2.0 Å, cross-docking was correct in 3 out of 5 cases, and the VS results provided encouraging results that support previous evidence[55] suggesting the feasibility of carrying out successful VS campaigns on pharmacologically important GPCR.[52,56] It is expected that future work on the remaining caveats will enhance CRDOCK performance.

Besides its accuracy, an additional advantage of CRDOCK is its reduced computational cost, once the conformational library for the ligands has been generated. It should be noted that this process is done only once, and the resulting conformers can be employed in different VS campaigns, so that millions of compounds can be screened thereafter using a relatively modest computational infrastructure. CRDOCK is open source and can be downloaded free of charge to noncommercial parties following registration at the CBM Bioinformatics Unit's web page (http://ub.cbm.uam.es/).

■ ASSOCIATED CONTENT

Supporting Information

Figures containing a structural depiction of docking errors, ROC curves corresponding to those targets for which VS performance was worse than random, and X-ray and docking solution for the ligand present in PDB entry 4DKL; tables listing the β_2, D_3, H_1, M_2, and μ binders used in the VS of GPCRs as well as the AUC values per DUD target. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AUC, area under the curve; BFGS, Broyden−Fletcher−Goldfarb−Shanno; DUD, Directory of Useful Decoys; MCSA, Monte Carlo simulated annealing; rmsd, root-mean-square deviation; ROC, receiver operating characteristic; VSDMIP, Virtual Screening Data Management on an Integrated Platform

■ REFERENCES

(1) Kuntz, I. D.; Blaney, J. M.; Oatley, S. J.; Langridge, R.; Ferrin, T. E. A geometric approach to macromolecule-ligand interactions. *J. Mol. Biol.* **1982**, *161*, 269−288.

(2) Woltosz, W. S. If we designed airplanes like we design drugs. *J. Comput.-Aided Mol. Des.* **2012**, *26*, 159−163.

(3) Kolb, P.; Irwin, J. J. Docking screens: right for the right reasons? *Curr. Top. Med. Chem.* **2009**, *9*, 755−770.

(4) Goodford, P. J. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. *J. Med. Chem.* **1985**, *28*, 849−857.

(5) Goodsell, D. S.; Olson, A. J. Automated docking of substrates to proteins by simulated annealing. *Proteins* **1990**, *8*, 195−202.

(6) Jones, G.; Willett, P.; Glen, R. C. Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *J. Mol. Biol.* **1995**, *245*, 43−53.
(7) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J. Med. Chem.* **2004**, *47*, 1750−1759.
(8) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.
(9) McGann, M. R.; Almond, H. R.; Nicholls, A.; Grant, J. A.; Brown, F. K. Gaussian docking functions. *Biopolymers* **2003**, *68*, 76−90.
(10) Pérez, C.; Ortiz, A. R. Evaluation of docking functions for protein-ligand docking. *J. Med. Chem.* **2001**, *44*, 3768−3785.
(11) Gil-Redondo, R.; Estrada, J.; Morreale, A.; Herranz, F.; Sancho, J.; Ortiz, A. R. VSDMIP: virtual screening data management on an integrated platform. *J. Comput.-Aided Mol. Des.* **2009**, *23*, 171−184.
(12) Cabrera, Á. C.; Gil-Redondo, R.; Perona, A.; Gago, F.; Morreale, A. VSDMIP 1.5: an automated structure-and ligand-based virtual screening platform with a PyMOL graphical user interface. *J. Comput.-Aided Mol. Des.* **2011**, *25*, 813−824.
(13) Cortes-Cabrera, A.; Gago, F.; Morreale, A. A reverse combination of structure-based and ligand-based strategies for virtual screening. *J. Comput.-Aided Mol. Des.* **2012**, *26*, 319−327.
(14) Marshall, G. R. Limiting assumptions in structure-based design: binding entropy. *J. Comput.-Aided Mol. Des.* **2012**, *26*, 3−8.
(15) Yuriev, E.; Agostino, M.; Ramsland, P. A. Challenges and advances in computational docking: 2009 in review. *J. Mol. Recognit.* **2011**, *24*, 149−164.
(16) Mysinger, M. M.; Shoichet, B. K. Rapid context-dependent ligand desolvation in molecular docking. *J. Chem. Inf. Model.* **2010**, *50*, 1561−1573.
(17) Cozzini, P.; Kellogg, G. E.; Spyrakis, F.; Abraham, D. J.; Costantino, G.; Emerson, A.; Fanelli, F.; Gohlke, H.; Kuhn, L. A.; Morris, G. M.; Orozco, M.; Pertinhez, T. A.; Rizzi, M.; Sotriffer, C. A. Target flexibility: an emerging consideration in drug discovery and design. *J. Med. Chem.* **2008**, *51*, 6237−6255.
(18) Kokh, D. B.; Wade, R. C.; Wenzel, W. Receptor flexibility in small-molecule docking calculations. *Wiley Interdisciplinary Reviews: Computational Molecular Science*; Wiley: New York, 2011; Vol. *1*, pp 298−314.
(19) Hartshorn, M. J.; Verdonk, M. L.; Chessari, G.; Brewerton, S. C.; Mooij, W. T. M.; Mortenson, P. N.; Murray, C. W. Diverse, high-quality test set for the validation of protein-ligand docking performance. *J. Med. Chem.* **2007**, *50*, 726−741.
(20) Huang, N.; Shoichet, B. K.; Irwin, J. J. Benchmarking sets for molecular docking. *J. Med. Chem.* **2006**, *49*, 6789−6801.
(21) Liebeschuetz, J.; Cole, J.; Korb, O. Pose prediction and virtual screening performance of GOLD scoring functions in a standardized test. *J. Comput.-Aided Mol. Des.* **2012**, Epub ahead of print.
(22) O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel: An open chemical toolbox. *J. Cheminf.* **2011**, *3*, 1−14.
(23) *Corina Molecular Networks*; GmbH Computerchemie Langemarckplatz 1, E., Germany, 2000.
(24) Stewart, J. J. MOPAC: a semiempirical molecular orbital program. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1−105.
(25) Case, D. A.; Darden, T. A.; Cheatham, I., T.E.; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Merz, K. M.; Wang, B.; Pearlam, D. A.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, J. W.; Ross, W. S.; Kollman, P. A. *AMBER 8*; University of San Francisco: San Francisco, 2004.
(26) Gil-Redondo, R. Master Thesis. UNED, Madrid, 2006.
(27) Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 425−445.
(28) Gschwend, D. A.; Kuntz, I. D. Orientational sampling and rigid-body minimization in molecular docking revisited: on-the-fly optimization and degeneracy removal. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 123−132.
(29) Wang, J.; Kollman, P. A.; Kuntz, I. D. Flexible ligand docking: a multistep strategy approach. *Proteins* **1999**, *36*, 1−19.
(30) Nelder, J. A.; Mead, R. A simplex method for function minimization. *Comput. J.* **1965**, *7*, 308.
(31) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Daniel, T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.
(32) Fan, H.; Schneidman-Duhovny, D.; Irwin, J. J.; Dong, G.; Shoichet, B. K.; Sali, A. Statistical Potential for Modeling and Ranking of Protein-Ligand Interactions. *J. Chem. Inf. Model.* **2011**, *51*, 3078−3092.
(33) Triballeau, N.; Acher, F.; Brabet, I.; Pin, J. P.; Bertrand, H. O. Virtual screening workflow development guided by the "receiver operating characteristic" curve approach. Application to high-throughput docking on metabotropic glutamate receptor subtype 4. *J. Med. Chem.* **2005**, *48*, 2534−2547.
(34) Dolinsky, T. J.; Czodrowski, P.; Li, H.; Nielsen, J. E.; Jensen, J. H.; Klebe, G.; Baker, N. A. PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. *Nucleic Acids Res.* **2007**, *35*, W522−W525.
(35) DeLano, W. L. *The PyMOL molecular graphics system*; Schrodinger Inc.: Cambridge, MA, 2002.
(36) Knox, C.; Law, V.; Jewison, T.; Liu, P.; Ly, S.; Frolkis, A.; Pon, A.; Banco, K.; Mak, C.; Neveu, V. DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. *Nucleic Acids Res.* **2011**, *39*, D1035.
(37) Irwin, J. J.; Shoichet, B. K. ZINC-a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **2005**, *45*, 177−182.
(38) Jorgensen, W. L. *QikProp*; Schrödinger LLC: New York, 2006.
(39) Ruppert, J.; Welch, W.; Jain, A. N. Automatic identification and representation of protein binding sites for molecular docking. *Protein Sci.* **1997**, *6*, 524−533.
(40) Baber, J. C.; Thompson, D. C.; Cross, J. B.; Humblet, C. GARD: a generally applicable replacement for RMSD. *J. Chem. Inf. Model.* **2009**, *49*, 1889−1900.
(41) Li, X.; Li, Y.; Cheng, T.; Liu, Z.; Wang, R. Evaluation of the performance of four molecular docking programs on a diverse set of protein-ligand complexes. *J. Comput. Chem.* **2010**, *31*, 2109−2125.
(42) Cross, J. B.; Thompson, D. C.; Rai, B. K.; Baber, J. C.; Fan, K. Y.; Hu, Y.; Humblet, C. Comparison of several molecular docking programs: pose prediction and virtual screening accuracy. *J. Chem. Inf. Model.* **2009**, *49*, 1455−1474.
(43) Spitzer, R.; Jain, A. N. Surflex-Dock: Docking benchmarks and real-world application. *J. Comput.-Aided Mol. Des.* **2012**, Epub ahead of print.
(44) Novikov, F. N.; Stroylov, V. S.; Zeifman, A. A.; Stroganov, O. V.; Kulkov, V.; Chilov, G. G. Lead Finder docking and virtual screening evaluation with Astex and DUD test sets. *J. Comput.-Aided Mol. Des.* **2012**, Epub ahead of print.
(45) von Korff, M.; Freyss, J.; Sander, T. Comparison of ligand- and structure-based virtual screening on the DUD data set. *J. Chem. Inf. Model.* **2009**, *49*, 209−231.
(46) Alberts, I. L.; Todorov, N. P.; Dean, P. M. Receptor flexibility in de novo ligand design and docking. *J. Med. Chem.* **2005**, *48*, 6585−6596.
(47) Fischer, B.; Merlitz, H.; Wenzel, W. Increasing diversity in in-silico screening with target flexibility. *Comput. Life Sci.* **2005**, 186−197.
(48) Neves, M. A.; Totrov, M.; Abagyan, R. Docking and scoring with ICM: the benchmarking results and strategies for improvement. *J. Comput.-Aided Mol. Des.* **2012**, Epub ahead of print.

(49) Ehlert, F. J.; Delen, F. M.; Yun, S. H.; Liem, H. A. The interaction of amitriptyline, doxepin, imipramine and their N-methyl quaternary ammonium derivatives with subtypes of muscarinic receptors in brain and heart. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 13−19.
(50) Richelson, E.; Nelson, A. Antagonism by antidepressants of neurotransmitter receptors of normal human brain in vitro. *J. Pharmacol. Exp. Ther.* **1984**, *230*, 94−102.
(51) Meyer, J. M.; Ejendal, K. F.; Avramova, L. V.; Garland-Kuntz, E. E.; Giraldo-Calderon, G. I.; Brust, T. F.; Watts, V. J.; Hill, C. A. A "Genome-to-lead" approach for insecticide discovery: pharmacological characterization and screening of *Aedes aegypti* D(1)-like dopamine receptors. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1478.
(52) Gatica, E. A.; Cavasotto, C. N. Ligand and decoy sets for docking to G protein-coupled receptors. *J. Chem. Inf. Model.* **2012**, *52*, 1−6.
(53) Moitessier, N.; Englebienne, P.; Lee, D.; Lawandi, J.; Corbeil, C. R. Towards the development of universal, fast and highly accurate docking/scoring methods: a long way to go. *Br. J. Pharmacol.* **2008**, *153* (Suppl 1), S7−26.
(54) Tarasov, D.; Tovbin, D. How sophisticated should a scoring function be to ensure successful docking, scoring and virtual screening? *J. Mol. Model.* **2009**, *15*, 329−341.
(55) Carlsson, J.; Coleman, R. G.; Setola, V.; Irwin, J. J.; Fan, H.; Schlessinger, A.; Sali, A.; Roth, B. L.; Shoichet, B. K. Ligand discovery from a dopamine D3 receptor homology model and crystal structure. *Nat. Chem. Biol.* **2011**, *7*, 769−778.
(56) Mysinger, M. M.; Weiss, D. R.; Ziarek, J. J.; Gravel, S.; Doak, A. K.; Karpiak, J.; Heveker, N.; Shoichet, B. K.; Volkman, B. F. Structure-based ligand discovery for the protein-protein interface of chemokine receptor CXCR4. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 5517−5522.