Journal of Molecular Recognition

Received: 7 June 2010, Revised: 20 July 2010, Accepted: 21 July 2010, Published online in Wiley Online Library: 23 October 2010

(wileyonlinelibrary.com) DOI:10.1002/jmr.1077

Challenges and advances in computational docking: 2009 in review

Elizabeth Yuriev[a]*, Mark Agostino[a] and Paul A. Ramsland[b,c,d]*

Docking is a computational technique that places a small molecule (ligand) in the binding site of its macromolecular target (receptor) and estimates its binding affinity. This review addresses methodological developments that have occurred in the docking field in 2009, with a particular focus on the more difficult, and sometimes controversial, aspects of this promising computational discipline. These developments aim to address the main challenges of docking: receptor representation (such aspects as structural waters, side chain protonation, and, most of all, flexibility (from side chain rotation to domain movement)), ligand representation (protonation, tautomerism and stereoisomerism, and the effect of input conformation), as well as accounting for solvation and entropy of binding. This review is strongly focused on docking advances in the context of drug design, specifically in virtual screening and fragment-based drug design. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: computational docking; protein-ligand complexes; binding mode prediction; scoring; receptor flexibility

INTRODUCTION

Generally speaking, docking is a computational technique that places a small molecule (ligand) in the binding site of its macromolecular target (receptor) and estimates its binding affinity. The purpose of the current review is not to cover the principles of the docking methodology and its applications, but to address the state-of-the-art advances in this field. We particularly aimed to focus on the more challenging, and sometimes controversial, aspects of this promising computational discipline. This review is not limited to, but is strongly focused on docking advances in the context of drug design, specifically in the areas of virtual screening (VS) (1) and fragment-based drug design (FBDD). The area of protein–protein docking will not be covered in this review (but references are provided here for readers' convenience) (2–11). Miscellaneous case studies, except where important methodological developments are described, are also beyond the scope of this review.

Generating a receptor-ligand structure *in silico* involves two main components (sometimes inaccurately referred to as "steps"): docking and scoring. Docking per se entails conformational and orientational sampling of the ligand within the constraints of the receptor binding site. Scoring function selects the best pose (i.e., ligand conformation, orientation, and translation) for a given molecule and rank orders ligands, if a ligand database is docked/screened. To be successful, docking must accurately predict two things relative to experimentally available information: ligand structure (pose prediction) and its binding propensity (affinity prediction).

In this review we will cover methodological developments that have occurred in the docking field in 2009. These developments aim to address the main challenges of docking: receptor representation (such as structural waters, side chain protonation, and, most of all, flexibility (from side chain rotation to domain movement)), ligand representation (protonation, tautomerism and stereoisomerism, and the effect of input conformation), as well as accounting for solvation and entropy of binding. These challenges of docking are very well reviewed by Corbeil *et al*. (12)

RECEPTOR REPRESENTATION IN DOCKING

Receptor source

The common source of receptor structures for docking is X-ray crystallography and NMR. However, the growing gap between the sequence and structure availability points the practitioners of docking towards receptor structures that are modeled (by homology modeling, threading, and *de novo* methods). In that respect, the quality of such models for the purposes of docking generally and virtual screening specifically has to be evaluated. Fan *et al*. (13) have carried out such an evaluation using 38 targets and the Database of Useful Decoys (DUD) (14). In general, they found that comparative models significantly outperformed random selection, but on average were no more enriching than the corresponding templates. Typically, the holo crystal structure templates gave the best enrichments, but the modeled structures

* *Correspondence to: E. Yuriev , Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia.*
E-mail: Elizabeth.Yuriev@pharm.monash.edu.au

P. A. Ramsland, Centre for Immunology, Burnet Institute, GPO Box 2284, Melbourne, VIC 3001, Australia.
E-mail: pramsland@burnet.edu.au

a *E. Yuriev, M. Agostino*
Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

b *P. A. Ramsland*
Centre for Immunology, Burnet Institute, Melbourne, VIC 3004, Australia

c *P. A. Ramsland*
Department of Surgery Austin Health, University of Melbourne, Heidelberg, VIC 3084, Australia

d *P. A. Ramsland*
Department of Immunology, Monash University, Alfred Medical Research and Education Precinct, Melbourne, VIC 3004, Australia

were often competitive. Importantly, they found that none of the tested sequence or structural attributes (e.g., the overall target-template sequence identity) could reliably predict the accuracy of ligand docking. However, they have shown that the docking screens can be improved by employing multiple models instead of a single model. Their results have suggested that to best exploit comparative models in molecular docking screens one should use consensus enrichment calculations that include multiple models as well as templates. Others have also addressed issues of docking into homology models (13,15–17); for docking into homology models of G-Protein Coupled Receptors (GPCRs), see below.

Receptor flexibility

In many popular docking methods the ligand is treated as flexible but the protein conformation is kept rigid. This relies on the Lock-and-Key hypothesis for protein ligand binding. However, it is now widely accepted that ligand binding is not a static event but a dynamic process, in which both the ligand and protein may undergo conformational changes. In docking, incorporating protein flexibility exponentially expands the potential search space and quickly becomes impractical. Therefore, properly accounting for receptor flexibility is much more computationally expensive than doing that for ligands (18). Docking programs have only recently started to offer receptor flexibility during docking. Several software advances published in 2009 have specifically addressed the issue of receptor flexibility.

Receptor flexibility by Monte Carlo (MC) simulations and rotamer libraries

RosettaLigand offers one of the most extensive receptor flexibility treatments developed to date (19,20). In this stochastic MC approach, binding site side chain rotamers are optimized using a simulated annealing procedure and the backbone is minimized subject to restraints.

AutoDock 4 fully models the flexibility of selected portions of the protein (21). User-selected side chains are separated from the protein and treated explicitly during the simulation, allowing rotation around torsional degrees of freedom. In the MADAMM procedure, the protein is "flexibilized" by the side chain rotamer libraries of the InsightII (22). Schrodinger's Induced Fit Docking (IFD) Workflow (23,24) involves rigid receptor docking with Glide (25,26), combined with protein-ligand complex minimization with the homology modeling module Prime (24). IFD has been successfully used for studies of kinases (27,28), HIV-1 Integrase (29), heat shock protein 90 (30), monoacylglycerol lipase (31).

Receptor ensembles by molecular dynamics (MD)

Most methods that employ ensembles of protein structures generate them by MD. Huang and Wong (32) have developed a new MD-based "two-reference" modeling approach where a protein can adopt conformations between two experimentally observed extremes, such as a fully opened apo form and the most closed ligand-bound structures of protein kinase A. They have docked four diverse ligands, which previously evaded successful pose prediction, and found that the ligands docked successfully with proper conformations of the protein induced.

Armen *et al.* (33) have carried out extensive evaluation of different degrees of introducing explicit all-atom receptor flexibility into docking using both MD and torsion angle MD. They have measured the effects of different degrees of receptor flexibility on docking accuracy by cross-docking. The compared models involved flexible side chains, flexible loops, multiple flexible backbone segments, and entirely flexible targets. Interestingly, they found that, at least for the studied target p38a mitogen-activated kinase, the introduction of flexible side chains and backbone fragments leads to superior results in docking accuracy, while the incorporation of fully flexible protein reduces accuracy due to the increased "noise" affecting the scoring function.

Other methods to account for receptor flexibility

Abagyan and Totrov (34) have developed the 4D-docking protocol for Internal Coordinate Mechanics (ICM), where the receptor conformation is the fourth dimension (35,36). In this protocol, multiple grids represent multiple receptor conformations and each is represented as a variable in the global optimization. This approach demonstrated increased accuracy with no loss in effectiveness compared to single grid methods.

Developers of many programs aim to achieve "on-the-fly" receptor generation. Within Surflex (37,38), each pose is re-scored using all of the provided receptor conformations (39), while the evolution implementation of Flexibility Induced Through Targeted Evolutionary Description (FITTED) allows for cross-over and mutations of multiple side chain and backbone conformations (40).

Fuhrmann *et al.* (41) have suggested applying gradient-based optimization algorithms by the use of exponential mapping to define the molecular orientation, which then helps to calculate the orientational gradient. In their work, the local minimization algorithm is adopted to efficiently change the orientational parameters while preserving the molecular orientation.

Issues of efficiency in receptor flexibility

Whichever method is used to generate receptor ensembles, docking ligand databases or even individual ligands against large ensembles is computationally expensive. Anderson and co-workers have looked at this problem from two points of view. In the absence of general agreement about how to weight scores generated by individual ensemble members, they have carried out an extensive evaluation of different weighting schemes using structures of *Candida albicans* dihydrofolate reductase (CaDHFR) and influenza A neuranimidase from MD simulations (42). The schemes they have tested and their respective ligand ranking accuracies included simple averaging (36%), Boltzmann weighting without (60%) and with (72%) structural minimization, and taking initial structures from independent MD runs (61%). Their results suggest that ligands are more accurately assessed when docked to the minimized ensemble from a single MD simulation, an improvement due to more than just error minimization. They have also developed an efficient method to evaluate and select the most "contributive" ensemble members prior to docking for targets with conserved binding site cores (43).

An alternative to MD is to generate receptor ensembles by normal mode analysis. Abagyan and co-workers have demonstrated that the elastic network model (ENM) is a method that may initiate not only local conformational changes, such as those of side chains, but the movement of the protein backbone. They have also demonstrated that the ENM can be significantly more efficient than MD (44).

Gohlke and co-workers (45) have developed an accurate grid-based representation of intermolecular interactions, which evaluates interaction energies via lookup tables even for a

moving protein. The efficiency is achieved by adapting a 3D grid with pre-calculated potential field values, derived from the initial receptor conformation, to another conformation by moving the points in space, but keeping the values.

LIGAND REPRESENTATION IN DOCKING

Effect of input ligand conformation

Cross and co-workers have investigated the effect of input conformation (crystal vs. CORINA-generated structures, advocated by Kirchmair *et al*., (46)) on the accuracy of pose prediction (47). Their unsurprising, at first glance, finding was that crystal structures used as an input typically produced better overall results, however not always. Corbeil and Moitessier (40) have compared the docking accuracy of several programs using 100 crystal structures of 18 diverse proteins. Similar to the above study, they found that docking accuracy of FlexX (48), GOLD (49,50), Glide, and Surflex was 10–15% worse for OMEGA-generated ligand input structures, compared to crystal ligand input structures. For FITTED, the docking accuracy was comparable for both types of input structures, possibly due to the introduction of conformational treatment of rings (40). Therefore, leaving aside the issue of program-dependence and the effect of other factors such as solvation, protein flexibility, and the quality of the benchmark (see Crystal Structure As a Validation Benchmark Section), these studies have suggested that sampling algorithms are, at least potentially, biased towards the input ligand conformation.

Conversely, Feher and Williams' (51) results present a rather opposing picture. They have also used a representative set of commercially available docking programs (GOLD, MOE (52), Glide, FlexX, and Surflex), 14 protein-ligand test systems, and assessed several methods for generating input ligand conformation: the X-ray structures, the minimized CORINA structures, as well as structures from conformational searches and from MD. They found that even small changes in the ligand input conformation can lead to drastic differences in the geometries and scores of docked poses. No one method and no ligand starting geometry were found to produce the most accurate docking pose. The authors' prudent (although, computationally costly) recommendation was to always use multiple input.

Methods for conformational treatment of ligands

Precomputed conformations

In the TrixX Conformer Generator (TCG), introduced by Griewel *et al*., (53) conformational ensembles are built incrementally in a best-first-search process, which employs an internal root mean square deviation (RMSD) clustering and conformational energy as a scoring function. To address the accuracy versus ensemble size issue, TCG allows the user to set a trade-off. Using TCG, Griewel *et al*. have demonstrated that an average of 20 conformers per ensemble is sufficient to achieve an average accuracy of 1.13 Å.

Systematic sampling

Generally speaking, the only method to comprehensively sample conformational space is to carry out a systematic search in torsional space. However, this method quickly becomes computationally prohibitive. In the MOLSDOCK algorithm (54), the exhaustive search is carried out using mutually orthogonal Latin squares. However it is computationally efficient due to subsequent sampling by a procedure similar to the mean-field technique, which allows identifying the optimal structure. MOLSDOCK has been tested against 45 protein-ligand complexes and was shown to perform as well as AutoDock 4 (21).

Incremental construction

Incremental construction involves building the ligand "on-the-fly" within the constraints of the binding site, while simultaneously addressing ligand flexibility.

In the *E-Novo* protocol (Enumerated *de Novo* Design) (55), a scaffold core is generated within the binding site, using a ligand-bound protein structure. Ligands of interest are then generated from a scaffold using an R-group fragmentation/enumeration. Inhibitors of six targets have been used to test the protocol. The applicability of this method is limited to the cases where experimental ligand-bound structures are available, or the ligands to be docked are not too diverse.

In the TrixX BMI (56), the ligands are split into fragments, which are enumerated in a relational database using triangular descriptors. During screening, the target-based query descriptors are used to extract the initial matches and then to reconstruct them incrementally. TrixX BMI has been tested against 85 protein-ligand complexes of the Astex Diverse Set (57) and predicted the bound mode (to less than 2 Å) in 80% of the cases.

Kuntz and co-workers (58) have developed a new ranking-based sampling algorithm within the anchor-and-grow ligand sampling method of DOCK 6. By softening the vdW interaction energy, improving the performance of the bump filter (to remove clashes with the receptor) and by only ranking (without clustering) the layers of ligand growth, they managed to guide the sampling algorithm towards the correct pose.

Genetic algorithms (GA)

GAs are based on Darwin's theory of evolution and natural selection. In a docking program, a population (of poses) evolves with favorable genes passed on to the next generation and unfavorable eliminated.

Kang *et al*. (59) have developed an improved adaptive GA, which incorporates such advances as a multi-population genetic strategy, entropy-based searching with self-adaptation, and the quasi-exact penalty. They have tested their algorithm against the data set of 134 complexes and obtained good pose prediction ($<$2 Å) in approximately 70% of cases.

FITTED incorporates a Lamarckian genetic algorithm, which uses conjugate-gradient energy minimization as the local search method. The evolution process, implemented in FITTED, limits the fraction of the offspring progressing through the optimization. To reduce computational cost, FITTED uses a funnel approach to create a "high" quality initial population. The recent development in FITTED has addressed the somewhat underserved issue of ring flexibility through the application of a corner flap algorithm (40). However, no restrictions have been imposed on the adjacent atoms in the ring, a usual drawback of the corner flap approach.

Monte Carlo

MC methods use random modifications to generate alternative ligand poses. RosettaLigand uses stochastic Monte Carlo to generate ligand conformations, which are pre-enumerated but

also subjected to torsion space minimization during the docking simulation (19,20). A highly effective search algorithm has been implemented in AutoDock-Vina, which combines MC-like perturbations with local search methods (60).

Effect of ligand protonation, tautomerism, and stereoisomerism

Baker and co-workers (19) have commented on the problem of sampling tautomeric and protonation states, given the possible difference of free and bound ligand states in these respects. They suggested enumeration of tautomeric and protonation states as a possible solution but have warned about the potentially prohibitive computational cost. Another suggested alternative included segmentation and incremental construction of the docked ligand, whereby the protonation and tautomerism "decisions" are independent and hence decrease the problem size.

ten Brink and Exner (61) have investigated the influence of ligand protonation, tautomerism, and stereoisomerism on docking results. They have carried out cognate docking of crystal structures (redocking) and virtual screening experiments for three targets and the Astex dataset. They demonstrated that two docking programs, GOLD and Protein-Ligand ANT System (PLANTS), had problems in identifying the correct protomer/tautomer and, to a lesser extent, the correct stereoisomer. ten Brink and Exner have recommended that, until scoring functions can overcome the problem of incorrect identification of protomer/tautomer, a preselection of plausible protomers/tautomers should be routinely performed. They have developed Structure PrOtonation and REcognition System (SPORES) – a tool for preprocessing of protein and protein-ligand complexes and for the setup of 3D ligand databases. SPORES performs rule-based assignment of atom types and generates protonation and tautomer as well as stereoisomer states, based on these assignments.

Kalliokoski *et al*. (62) have addressed the same problem by using AutoDock 4 (21) and studying the effect of ligand protonation and tautomerization on 19 targets and the publicly available DUD decoy set. Specifically, they have compared two approaches: enumeration of all protonation and tautomeration ligand forms versus using a single reasonable ligand form. Their results have indicated that the two approaches can result in comparative enrichment in structure-based virtual screening. However, as expected, the latter approach significantly increased computational efficiency.

SCORING FUNCTIONS

Entropy

Entropic contributions form an important component of binding energy and are notoriously difficult to account for in docking applications.

Kongsted and Ryde (63) have attempted to improve the calculation of entropies within the MM/PBSA (molecular mechanics with Poisson-Boltzmann surface area) framework by introducing a buffer region of approximately 4 Å outside the protein, included into the calculation. This buffer region, which contains water molecules, is kept fixed during the calculation, reducing extensive changes in the molecular geometry and ensuring that the entropy term does not limit the precision of the MM/PBSA predictions. This method was tested on 17 complexes and produced improved predictions of binding affinities.

Conversely, Coutinho and co-workers (64) have endeavored to directly calculate the entropy loss, corresponding to the loss of torsional, vibrational, rotational and translational free energies of the ligand upon binding with the receptor. They have estimated this entropy loss, resulting from reduced conformational flexibility upon receptor binding, using the Searle–Williams method by assigning an amount of 0.7 kcal/mol to every freely rotatable (i.e., single) bond, excluding the terminal methyl groups.

Wang and co-workers (65) have improved the prediction of binding affinities of XIAP-Smac mimetics complexes (X-linked inhibitor of apoptosis; second mitochondria-derived activator of caspase) by using the modified MM/GBSA function (molecular mechanics with generalized Born surface area). The modification involved the inclusion of the free energy change between the free and bound states of the ligand, or "ligand reorganization energy." This study has demonstrated that ligand reorganization, and not only the induced fit of receptors, is important for correct prediction of binding affinities and should be evaluated for other protein-ligand systems and included into newly developed scoring functions.

Solvation

Receptors bind to their ligands in solution and the solvation aspects are commonly treated implicitly, that is, by the use of implicit solvents, knowledge-based scoring functions or by modification or calibration of other scoring functions.

Fong *et al*. (66) have investigated the inclusion of a desolvation penalty into their QM/MM scoring, using a Generalized Born solvent model, and found that it resulted in improved pose prediction. Cincilla *et al*. (67) have modified the solvation treatment in the scoring function of AutoDock 3 (68) to improve the predictions of weak complexes containing ligands with polar atoms lacking a matching partner in the binding site. Specifically, they have removed the constant hydrogen bonding energy term for the polar ligand atoms and introduced the Stouten free energy desolvation term. The modified function has also differentiated between the "polar" and "non-polar" heteroatoms on the basis of hybridization and connectivity.

Kuntz and co-workers (58) have used two implicit solvent scoring functions AMBER/GBSA and AMBER/PBSA, implemented in DOCK 6, for docking small molecules to RNA. Sodium ions were used to neutralize the backbone charge and a double shell of explicit water was used to shield the charges. They have found that the quality of pose prediction increased from 70% to 80% for moderately flexible ligands ($<$7 rotatable bonds) and from 26% to 50–60% for highly flexible ligands (7–13 rotatable bonds).

Huang and Wong have tested the performance of a simple implicit solvent method (a distance dependent dielectric model) in comparison to a version of the Generalized Born method (GBMV) and found it to produce better pose prediction results for a fraction of the computational cost (69).

Most methods of treating solvation do not take into account the effects that could be exercised by "structural" water molecules, that is, those that provide stabilization and/or recognition through specific hydrogen bonding and even van der Waals interactions. Villacanas *et al*. (70) have reviewed the effect of structural water molecules on docking and concluded that the general feeling in the literature is that explicit water molecules improve docking outcomes, both in pose prediction

Journal of Molecular Recognition

and virtual screening. Englebienne and Moitessier (71) have shown that the consideration of displaceable water molecules, implemented in FITTED, improves pose prediction, but does not significantly affect scoring accuracy. They have suggested that the latter is most likely the outcome of most scoring functions having been developed for "dry" proteins.

Empirical functions

Korb *et al*. (72) have developed the $PLANTS_{CHEMPLP}$ and $PLANTS_{PLP}$ scoring functions, which are based on parts of other, already published functions. They have used the function parameterization procedure, which allowed improving pose prediction in large test sets: 87% in Astex diverse set and 77% in Cambridge Crystallographic Data Centre (CCDC)/Astex clean list.

Englebienne and Moitessier (73) have developed empirical scoring functions RankScore 2.0, 3.0, and 4.0 within FITTED, derived from crystal and docked structures as well as trained from VS data. They have fine tuned their functions using an iterative approach by optimizing the correlations between observed actives and calculated scores and by optimizing the Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) for the discrimination of actives and inactives. They have validated the functions against Wang's set of 100 complexes (74).

Interestingly, Tarasov and Tovbin (75) have developed an extremely simple empirical scoring function *NScore* with the aim of estimating how sophisticated a scoring function should be in order to be successful in docking, scoring and ligand ranking. *NScore*, based on basic physical principles without any adjustment, training or experimental bias, has performed comparably to programs with sophisticated scoring functions (ICM, GOLD, DOCK, and Glide).

Quantum mechanical/molecular mechanical (QM/MM) scoring functions

There have been several papers in 2009, exploring the potential of QM/MM scoring (66,76–81). Fong *et al*. (66) have tested three functions (HF/6-31G*, AM1d, and PM3) for ligand treatment in combination with AMBER, GoldScore, and ChemScore for successful pose prediction of six HIV protease inhibitors. Gleeson and Gleeson (76) have used the combination of B3LYP/6-31G** and Universal Force Field (UFF) for successful cross-docking and re-scoring of nine kinase ligands. Chung *et al*. (77) have combined QPLD, a QM/MM docking program, with SiteMap (82), a binding site classification module. They have used 455 protein-ligand complexes and demonstrated a scoring improvement, over Glide, for three possible binding site types (hydrophilic, hydrophobic, and metalloproteins). Cho and co-workers have tested QM/MM scoring for different types of binding sites, namely for those with polar groups, hydrophobic groups, and metalloproteins (78,81). In metalloproteins (78), they have extended the QM region to include the protein atoms surrounding the binding site along with metal ions and ligand atoms. This extension helped the charge scaling on metal ions and improved binding mode prediction.

Consensus scoring

Many scoring functions perform very well for the purpose of pose prediction, but a goal of predicting binding affinities using scoring functions is still unfulfilled. While the scoring function advances described above improve their performance in that respect, it is also clear that the currently available functions could be used more efficiently in combination. Commonly, a consensus scoring involves multiple rescoring of a docked pose with different scoring functions or a combination thereof (83).

Caflisch and co-worker (84) have applied consensus scoring in their fragment-based virtual screening against Plasmepsins (targets for malaria). They have used the median rank of four force-field-based energy functions: the binding free energy approximated by the linear interaction energy with continuum electrostatics (LIECE), CHARMm electrostatic interaction energy, CHARMm van der Waals (vdW) efficiency, and the TAFF interaction energy. Their consensus scoring yielded the highest enrichment for the first 1000 compounds, while the TAFF function worked the best when the whole library was considered. LIECE and electrostatic interaction energy performed slightly worse than consensus scoring, and vdW efficiency did not do much better than random selection.

Cheng *et al*. (85) have combined ASP, PLP, DrugScore, GlideScore, LigScore, and ChemScore in their assessment of scoring functions, which used a set of 195 diverse high resolution crystal structures with reliable binding constants from PDBbind database. They found that consensus scoring schemes have improved the success rate of pose prediction from 70% to 80% or even higher (based on the cutoff of 2 Å).

It has been recognized that consensus scoring is strongly dependent on the initial parameters. To address this issue, a new algorithm, SeleX-Consensus Scoring (SeleX-CS), has been devised (86). In SeleX-CS, a subset of scoring functions is initially allowed to form a consensus score, and that subset is consequently optimized using the Monte Carlo/Simulated Annealing procedure. This method was successfully tested in a virtual screen against two GPCRs. Another consensus scoring optimization protocol has been developed by Li *et al*. (87). Their multi-objective optimization method (MOSFOM) simultaneously considers the energy (AMBER) and the contact score of DOCK, and was demonstrated to improve enrichment in virtual screening. Contrary to the above examples, in some studies, it has been noted that consensus scoring lead to none or only moderate improvement in docking accuracy and/or overall enrichment (71,88).

Thus, whereas there are several examples of consensus scoring delivering better outcomes compared to using a single scoring function (84–87), the poorly combined scoring functions can impair the results. Ultimately, the *ab initio* principles of combining functions for consensus scoring are unclear and in most cases the combination is achieved empirically and tested retrospectively. To address the lack of understanding of the basic principles of rescoring and consensus scoring O'Boyle *et al*. (89) have used three scoring functions associated with GOLD (49,50) (ChemScore, GoldScore, ASP) and the Astex Diverse Set to test two alternative proposals. Their "consensus hypothesis" postulates that a combination of two scoring functions works by correcting for false positives (the averaging effect). They found that, while it may hold true for an experiment where ranks from scoring and rescoring functions are combined, for an experiment where the rescoring function is used to rank molecules, which have been docked by another function, it does not hold. Their "complementary hypothesis" suggests that a combination works because various scoring functions have different strong points. They have established that an improvement in ranking of actives in virtual screening can indeed be achieved if one scoring function works better for pose prediction (i.e., better

scoring of different poses of the same ligand), while another works better for affinity prediction or relative scoring of different ligands.

Other scoring developments

Fingerprinting

Interaction fingerprints (IFs) and profile-based methods have been applied to scoring and virtual screening to identify interaction patterns that can guide and improve predictions. The Glide XP (extra precision) scoring function descriptors have been used to locate key pharmacophoric features of docked fragments, which have been shown to be consistent with features from known tight binding compounds (90). Perez-Nueno *et al.* (91) have improved GoldScore results in VS by supplementing this scoring function with binding site "fingerprinting." Nandigam *et al.* (92) have introduced weighted Structural Interaction Fingerprint (w-SIFt) to gain insight into which interactions are critical in determining inhibitor potency. w-SIFt incorporates an empirically determined weight fit from inhibitor potency data.

Combination with experimental data

Gonzalez-Ruiz and Gohlke (93) have combined DrugScore function with experimental data, specifically NMR amide proton chemical shift perturbations (CSP). They have implemented this hybrid scoring scheme in AutoDock 3, applied it to 70 protein-ligand complexes, and have observed an improvement in pose prediction from 71% (without CSP) to 99% (with CSP).

Targeted scoring functions

Seifert reviewed targeted scoring functions for virtual screening (94). Unlike an accurate universal scoring function, still a major goal of the field, targeted functions, which capitalize on prior knowledge, are actually quite effective. The review covers such approaches as extending existing functions, recalibrating those using binary data and optimizing by statistical methods, fingerprint scoring functions, and target-specific filtering. Using an ensemble of protein kinases, he also demonstrated that a targeted scoring function can be tuned to multiple targets of a target class, leading to improved robustness of the resulting scoring function parameters (95).

OTHER METHODOLOGICAL ADVANCES

Improving pose prediction

In most cases, docking algorithms produce a list of docked poses ranked by a scoring function(s). Since scoring functions are only approximate representations of the underlying biochemical and biophysical phenomena, the ranking is often less than perfect, with "correct" binding modes occasionally failing to be ranked at the top of the list. While some advances in the scoring functions (see above) give promise to better ranking, other methodological developments have occurred in 2009 to address this issue.

Kolb and Irwin (96) have looked at pose prediction in the context of virtual screening and asked an important, although often overlooked question: when we dock, do we predict the binding modes correctly or we are just lucky in finding binders? A number of "complete" studies they have reviewed indicated that indeed docking can often, but not always, predict the binding mode correctly, increasing confidence in newly identified ligands ("right for the right reasons"). They concluded that while there is no universal docking method, it is clear that docking works best for (i) small binding sites and/or small ligands ("small is beautiful for docking"), (ii) binding sites with small orienting constraints, (iii) cases with specific knowledge about the receptor, and (iv) high levels of a practitioner's experience. They finish with a passionate plea to investigators to, whenever possible, experimentally solve structures for their predicted ligands and compare them to the predictions (prospective validation).

Baker and co-workers (19) have carried out a blind docking study of pharmaceutically relevant ligands and have identified several challenges associated with pose prediction: multiple deep, tight fitting binding pockets and directional polar interactions (could be overcome by fragment docking followed by linking); spurious receptor flexibility leading to the binding site adopting non-native shapes with false binding pockets (could be dealt with by introducing an energy bonus to native side chain conformations). While these findings were specifically associated with RosettaLigand (20), they are clearly relevant to other docking methods.

Interaction fingerprints (see above) and profile-based approaches to analyzing docked poses are based on the hypothesis that an active site contains a set of interaction points exploited by binding ligands. Wallach and Lilien (97) have proposed an algorithm where docking is combined with pharmacophore modeling. In their approach, the list of docked poses is examined to identify those that are maximally self-consistent with the pharmacophore map generated from the same poses. They have extensively applied their method to several protein systems and demonstrated improved pose prediction. A new atom-pairs-based IF (APIF) considers the relative positions of pairs of interacting atoms. It has been developed by Perez-Nueno *et al.* (91) for post-processing of docking results and tested in a virtual screen against a range of targets and using a range of scoring approaches. The IF-based scoring has demonstrated superior enrichment and pose prediction. Novikov *et al.* (98) have improved enrichment in VS against poly-(ADP-ribose)-polymerase (PARP) by choosing poses conforming to a predetermined interaction criterion (structural filtering).

Agostino *et al.* (99) have developed a docking-based site mapping of antibody binding sites with respect to their binding to carbohydrate antigens. They have validated their method against high resolution crystal structures and found site mapping to be very accurate and robust. For a panel of xenoreactive carbohydrate-binding monoclonal antibodies they have identified most likely antigen binding modes (100), by applying interaction-based filters derived from the antibody site maps and selecting the carbohydrate poses exhibiting the most preferred binding characteristics. Thus identified binding modes were found to be in agreement with experimentally determined binding profiles and were able to explain the relevant binding phenomena.

Yasuo *et al.* (101) have used modified MM/PBSA analysis to select the most energetically stable pose among docking solutions. The modification involved ignoring the entropic term ($-T\Delta S$), based on the assumption that different conformations of the same ligand, unlike different ligands, would only have variations in the entropy term. They found that a CoMFA model based on the alignment of thus generated poses of 20 known CYP inhibitors had good statistics, which they accredited to the quality of pose prediction.

QM modifications of/additions to otherwise MM-based scoring have the potential to improve correct binding mode identification. The improvement of binding mode predictions in metalloproteins has been achieved with the use of modified QM/MM scoring (see above) (78). Calculation of charges with the semi-empirical PM6 method (as compared to the Gasteiger charges) on both ligands and proteins has significantly increased docking accuracy in AutoDock 4 (102). However, this modification has not affected the binding affinity estimation, suggesting that a new scoring function for AutoDock is needed.

Fragment-based docking

The use of molecular docking to assist in Fragment-Based Drug Design (FBDD) (103,104) is logically obvious. However, due to the low affinity and non-specific nature of fragment binding, it is also questionable. Thus, Shoichet and co-workers (105,106) set out to address this apparent paradox and investigate the relevant questions: can docking prioritize fragments and predict their binding modes? Can it lead to the emergence of specific inhibitors? Can they be optimized for specificity and affinity? How does *in silico* fragment-based screening compare to full molecule screening? Docking their fragment libraries into β-lactamases failed to identify any lead-like inhibitors. However, it did lead to the discovery of low affinity fragment inhibitors and novel chemotypes. Most significantly, docking predictions for fragment binding modes have been compellingly confirmed by the subsequent crystallographic investigations – an encouraging finding for other docking-assisted FBDD projects.

One of the main concerns with respect to fragment based docking is the applicability of docking programs and scoring functions, optimized for "larger small molecules," to small fragments. To address this issue, Kawatkar *et al*. (107) have tested Glide, one of the most widely used docking packages, for fragment docking from self-docking, cross-docking, and enrichment perspectives. They tested nine different scoring schemes, associated with Glide. For prostaglandin D2 synthase and DNA ligase, they found that Glide standard precision (SP) with either GlideScore or Emodel ranking performed the best, with docking accuracy similar to that generally reported for lead-like molecules. They also found that using MM/GBSA did not improve the results, suggesting that the success of MM/GBSA re-scoring may be system dependent. Sherman and co-workers (90) have demonstrated that Glide XP is also successful in predicting fragment poses, with an RMSD of <1 Å. Impressively, they have also developed fragment-specific docking settings to generate poses that explore miscellaneous pockets of a binding site, while maintaining the docking accuracy of the top ranked pose.

Rarey and co-workers (108) have approached the issue of predictability in fragment-based docking by devising a retrospective validation scheme, not a straightforward matter in the *de novo* design field. They have used 188 crystal structures of complexes, belonging to eight different protein families with diverse functions and attempted to reconstruct ligands from fragments using FlexNovo. They have demonstrated that, in five out of eight cases, native ligands could be successfully reconstructed. In these cases, the ligands were ranked within the first five candidates.

The Fragment Screening by Replica Generation (FSRG) method of Fukunishi *et al*. (109) also attempts to address the potentially poor surface complementarity between proteins and small compounds. In this method, several side chains are attached to the fragment being docked to produce a set of molecules of increased size. In a way, this method computationally combines usually separate steps of fragment-based design: identification of fragments and their evolution. Fukunishi *et al*. (109) have tested their procedure on six targets and shown its potential for retrieving known actives.

Moriaud *et al*. (110) have developed a computational fragment-based approach by protein local similarity. Working at the complete PDB scale, they have generated a database of MED-Portions (new structural objects encoding protein-fragment binding sites), derived from mining all available experimental protein-ligand structures. They have combined this database with the MED-SuMo (software to superimpose similar protein interaction surfaces) and MED-Hybridize (a toolkit for recombining chemical moieties into putative ligand molecules) so that pools of matching MED-Portions could be retrieved for any binding site query. This approach has then been applied to two important drug design target superfamilies (protein kinases and GPCRs) and its potential for retrieving active known molecules has been demonstrated (110).

Several docking-based methods have now been developed in order to gain binding information for proteins in the absence of experimentally determined crystal structures of their complexes. The computational fragment mapping approach, FTMap, was developed to identify protein regions suitable for drug targeting (111,112). It has been tested on DJ-1 and GCase, implicated in Parkinson's and Gaucher's diseases, respectively (111), and renin, a long-standing pharmaceutical target for the treatment of hypertension (112). Comparison to the data derived from experimental multiple solvent crystal structures has shown that FTMap, a computational method for the identification of fragment binding hot spots, is a robust and accurate complement and alternative to the expensive experimental approaches.

Several studies have come out of the Caflisch group, which described their novel fragment-based docking strategy, using a very large library (millions of molecules), such as ZINC (113) or iResearch (ChemNavigator, Inc., 2006), as a starting point. Each molecule of the library is decomposed into mainly rigid fragments, which are then docked. The library molecules are then "re-assembled" by docking them flexibly, based on the positions of their fragments as anchors. They have also used NMR and MD to complement the *in silico* work and to guide the selection of candidates for further testing. In the screen against Plasmepsins (targets for malaria), several inhibitors showed very low micromolar activity; these have been identified in the NCI database, but not in malaria-related collections (84). Testing against the West Nile virus (WNV) NS3 protease (a target for WNV and dengue viral infections) has identified novel micromolar inhibitors (114,115). The significance of these studies is not only in developing a novel drug design strategy, but also in validating it prospectively, that is, by predicting novel inhibitors and testing their predictions.

Covalent docking

Docking of covalently bound ligands is important for gaining insight of enzymatic processes and designing superior covalent ligands.

Along with improving receptor flexibility (see above), developers of AutoDock 4 have implemented two methods for docking covalently bound ligands: a grid-based method and a modification of the flexible side chain approach (21). While the former

method showed distinct sensitivity to the choice of Gaussian map(s), the latter performed excellently on the test cases used.

Juhl *et al*. (116) have developed a predictive and robust protocol, termed "substrate-imprinted docking," where covalent docking of reaction intermediates and geometry optimization of the resulting enzyme-substrate complex produces binding site structures suitable for putative ligands. This method has been applied to model substrate specificity and enantioselectivity of lipase and esterases and reproduced experimentally determined differences in selectivity and specificity with an accuracy of 81%.

Covalent docking has also been applied to oligopeptidase (117) and proteasome (118) inhibitors, and glycoside hydrolyses ligands (119).

Combining docking with molecular dynamics

In many studies, docking has been combined with MD in a sequential fashion to investigate the dynamic behavior of the complexes (120); this should be distinguished from using MD for generating receptor ensembles (see above). The following is a small selection of docking/MD combination studies, addressing the issues of improved VS performance as well as a better understanding of binding phenomena.

In the study of binding of an RNA polymerase II to an isomerase enzyme, an all atom unconstrained MD in an explicit solvent was applied to a docked complex to simulate an experimentally known significant loop-bending event, occurring upon binding (121). Kranjc *et al*. (122) have used MD in explicit solvent to predict the free energy of dissociation in a ligand-prion protein docked system and found values in good agreement with binding assays and NMR. MD trajectories of docked DNA-ligand systems have been used to discriminate between optimal and non-optimal intercalation modes (123). Combination of MD with covalent docking provided insights into the dynamic behavior of docked complexes: enzyme-substrate (119) and 20S proteasome-peptide aldehyde (118). Caflisch and co-workers (124) have used MD to validate the kinase-ligand binding mode determined by fragment-based docking, discussed in detail above. In the study of human cytochrome P450 2A6, the docking/MD arrangement was used to investigate the question of how mutations affect the enzymatic mechanism (125).

Docking/MD partnership has even been used as a pre-screening tool in the development and optimization of new drug delivery systems. Specifically, it has been used to model and predict polymer–drug interactions in self-assembled nanoparticles, producing binding energies well correlated with experimental maximum drug loading values (126).

Virtual screening

The focus of virtual screening studies continues to be on improving performance of docking programs/scoring functions, as well as fine-tuning the ways to estimate said performance.

How to measure success?

This question is one of the key issues in virtual screening and has been widely discussed and debated in the field; see, for example, *Journal of Computer-Aided Molecular Design* Special Issue on "Evaluation of Computational Methods," 2008 (3-4).

Existing measures of VS success include enrichment factors (EF), ROC/AUC, Robust Initial Enhancement (RIE). Which one performs the best is a contentious issue (127). Hevener *et al*. (88) have developed sum of the sum of log rank (SSLR) – a simple statistical method to more accurately assess scoring performance by including the inhibition constants of known actives into the virtual screening evaluation.

Mackey and Melville (127) have focused on another important, and until recently poorly addressed, issue – the ability to quantify chemotype retrieval in virtual screening. They have developed and evaluated two metrics: "cluster averaging," where the contribution of each active to the scoring metric is proportional to the number of other actives of the same chemotype, and "first found," where only the first active of a given chemotype contributes to the score. Using DOCK, DUD and eight targets, they have shown "cluster averaging," while less intuitively appealing than "first found," to be a more reliable alternative when performing VS for scaffold hopping purposes.

Early rejection approaches

In a paper aptly entitled "Knowing when to give up: early rejection stratagems in ligand docking," Skone *et al*. (128) have addressed the issue of reducing the computational cost of virtual screening. They have applied the "lazy evaluation" principle used elsewhere in computer science to the problem of scoring thresholds in molecular docking. This paradigm is that a calculation that makes no contribution to the final outcome should be avoided. They found that applying either of the two investigated methods – threshold-based function approximation and quota-based ligand rejection – has proved beneficial by reducing the run times of database docking without a substantial deterioration in ligand placement. They concluded that this principle should be easily implemented in a wide range of docking programs.

Automation

Even with the recent advances, docking remains a manually intensive method and still requires expert handling and decision making (129). For virtual screening to become truly useful for medicinal chemists, it should become fully automatable. Irwin *et al*. (129) have investigated the feasibility of such automation using their DOCK Blaster server (blaster.docking.org) and tested it for pose fidelity and enrichment. Useful results, that is, good pose fidelity and good enrichment were obtained in 25–40% of the cases. While these results are relatively poor, especially compared to expert studies, DOCK Blaster offers a way to automatically leverage the exponentially increasing structural protein data for drug discovery.

Other advancements in automation of virtual screening include the development of integrated computational platforms, such as Virtual Screening Data Management on an Integrated Platform (VSDMIP) (130).

Distributed docking/virtual screening

To address the issue of data management in parallel applications in structure-based design, several advances have been made in the use of high performance computing for docking and virtual screening.

The Docking@Home project (131) has been developed to distribute scientific calculations, such as virtual screening against drug targets, among volunteer/general public computers, connected to the Internet (120). Zhou and Caflisch (132) have developed a distributed VS data management system (DVSDMS),

which performs the data handling and job distribution using MySQL, the structured query language database. The core idea of DVSDMS is the separation of the data management from the docking and ranking applications. Garcia-Sosa *et al.* (133) have combined paradigms of grid computing and collaborative research in their Chemomentum computing environment.

Other developments in VS

Several approaches have been tested to improve enrichment in VS, including: application of interaction fingerprints for post-processing of docking results (91,98,134), consensus (84,86) and QM/MM (79) scoring, combining docking with pharmacophore perception (90,134–138) or other ligand-based methods (64,139–141), accounting for receptor flexibility (142–144). and use of machine-learning strategies (67,145). These advances are discussed in some detail elsewhere in this review.

GPCRs as a case study for docking and virtual screening

In 2009, GPCRs have arguably been the hottest target for structure-based drug design. Their importance is due to them being targeted by a very large number of drugs (146). The increased interest in the virtual screening against GPCRs is thanks to the 2007–2008 determination of the crystal structures of the β_2- and β_1-adrenergic (β_2-AR and β_1-AR, respectively) and adenosine A_{2A} receptors (147–151). In one of the first virtual screens against β_2-AR, both potent and novel chemotypes have been found (152). These new structures have also delivered an opportunity for virtual screening against the homology models of other GPCRs, generated using these crystal structures as templates. This prospect has been tested in "GPCR Dock 2008" – a community-wide blind assessment of the prediction algorithms (153).

One of the very important challenges for structure-based drug design (SBDD) is the ability to predict different pharmacological profiles, that is, antagonists versus agonists versus inverse agonists for different GPCRs (154). Another main challenge of designing GPCR ligands is the robustness of SBDD methods with respect to their potential to generate subtype-specificity. Katritch *et al.* (155) have demonstrated such potential by β_2-AR receptor optimization in the presence of docked ligands and the resultant improved prediction of agonist binding affinities. Generally, optimization of GPCR binding sites by flexible receptor docking, particularly as a measure to improve and test the quality of GPCR homology model, has evolved as a mainstream theme in the GPCR field as exemplified by a number of recent studies (156–159).

Many docking studies have been published where improved GPCR ligands were identified. The following examples represent a snapshot of this exciting and rapidly developing field. Virtual screening against two GPCRs (CB1, cannabinoid receptor 1; CCR2, chemokine receptor 2) has been a testing ground for the new consensus scoring algorithm SeleX-CS (86), which delivered promising outcomes. Computational fragment-based approach by protein local similarity has then been applied to GPCRs, and its potential for retrieving active known molecules was demonstrated (110). Dailey *et al.* (160) have carried out a VS experiment against CXCR4 using Surflex-Dock (38) and identified low micromolar inhibitors. Dong *et al.* (161) have used docking with ICM (34) to elucidate the structural basis of natural peptide binding to a family A GPCR, the type 1 cholecystokinin receptor. The resulting agonist-occupied GPCR models were in agreement with experimental data.

VALIDATION STUDIES

Structural validation issues

A program's ability to predict a ligand pose within a reasonable RMSD to the experimental structure (usually, 2 Å (96,162)) as its top or highly ranked pose is considered a docking success. While this approach is the field's *de facto* method of structural validation and usually works very well for the problem at hand, it raises several issues. Firstly, is a crystallographically determined pose always a good benchmark? More specifically, is a given crystal structure a "good" structure to be used for benchmarking? And, generally speaking, do static crystal structures give us a correct picture of a dynamic binding process? The last question brings us to the second issue to be raised with respect to binding modes predicted by docking. Namely, should we only consider the top ranked docked pose or attempt to ascertain the dynamic nature of binding afforded by considering multiple alternative binding modes generated by docking? And finally, is RMSD an appropriate and sufficient measure to be used for the judgment of pose prediction quality or should we use alternative and/or complementary assessment metrics? Below we will briefly discuss the latest developments with respect to these issues.

Crystal structure as a validation benchmark

What must be always remembered when using crystal structures of receptor-ligand complexes as benchmarks of docking performance is that the positioning of small ligands within protein binding sites is still a challenge in X-ray crystallography, especially for structures solved at moderate resolution. As has been shown by Malde and Mark (163), the higher disorder associated with ligands, compared to the surrounding protein, makes it difficult (and, sometimes, impossible) to orient the ligand correctly. What makes ligand positioning particularly complicated is the lack of correlation between atom type and electron density. As a result, the ligand positioning is often subjective and frequently incorrect. Bound ligands in crystal structures, which are "assumed to be experimental," (163) can be positioned incorrectly.

Furthermore, one issue, which is often overlooked when validating a docked outcome, is the presence or absence of crystal-induced artifacts and water-mediated contacts in the crystal structure, to which it is compared. Sondergaard *et al.* (164) have analyzed ligands in the PDBBind 2007 refined data set and found that 36% of ligands were influenced by crystal contacts and that the performance of X-Score scoring function was affected by these contacts. The authors have suggested two solutions that may help to overcome the influence of crystallographic contacts: either through the representation of the protein-ligand complex as a "solution-like" ensemble (e.g., via MD; see above) or by exclusion of the affected crystal structures from validation and development data sets.

Pose prediction measures

As a measure of pose prediction, RMSD suffers from a range of drawbacks. Any pose above the cutoff (usually, 2 Å) is considered incorrect, which leads to a loss of potentially useful information,

and a very small number of bad poses may skew the overall outcome. Most importantly, poses with low RMSD may incorrectly describe intermolecular interactions and *vice versa*. A distinct example of how the incorrect placement of a flexible solvent-exposed portion of a ligand may affect an RMSD of an otherwise nearly perfectly docked ligand has been described by Agostino *et al*. (165)

Alternative/complementary prediction metrics have been proposed in the past, such as the relative displacement error RDE (166) and interaction-based accuracy classification IBAC (167). The advantage of the latter measure is that it captures information about intermolecular interactions encapsulated in the experimental binding mode and reproduced in the docked structure, however it has been mentioned (46) that it is not amenable to automation. Site-mapping approach of Agostino *et al*. (99) is based on the intermolecular interactions in an ensemble of docked poses and addresses the lack of automation in the IBAC scheme. This site-mapping method has been validated against a range of crystal structures and applied to the investigation of antibody–carbohydrate interactions important for xenoantigen recognition.

Several measures, which are based on RMSD but designed to overcome the associated drawbacks, have been proposed. Baber *et al*. (162) have developed Generally Applicable Replacement for RMSD (GARD), which is based on normalizing RMSD to the unit interval. Docking failures are given a low value (close to 0) of GARD and good poses are valued close to unity. Another method has been proposed by Brooks and co-workers (120), where docking accuracy is calculated as the frequency of finding high-quality docked poses among a collection of low energy ligand conformations.

Huang and Wong (32) have addressed the issue of measuring pose prediction in flexible receptor docking, where the structure of the protein as well as that of the ligand can change upon docking. They have generated distance matrices to characterize the relative positioning of the receptor and the ligand and have used the correlation coefficient between elements of distance matrices from docked and experimental structures as the measure of success. This approach is independent of the choice of protein atoms, that otherwise would be needed to be made for superimposition, and allows monitoring whether the proper structural change is induced in the receptor upon ligand binding.

Zavodszky *et al*. approached the assessment of pose prediction quality from a very different perspective. They have postulated that, as long as a scoring function is performing adequately, the scores should correlate with distances (between docked and experimental binding modes) (168). They have applied thus derived correlation coefficient, called correlation-based score (CBS), to redocking of 50 protein-ligand systems with SLIDE and found it to be a good measure of pose prediction.

Validations for different target and ligand types

Most scoring functions in use today have been developed for protein–ligand interactions, where ligands are mostly small organic molecules. It has been long recognized in the docking field that various docking programs/scoring functions perform differently for different targets (169). Cross *et al*. (47) have identified trends in program performance when testing them for pose prediction and VS against specific protein families. Furthermore, macromolecules other than proteins, for example, DNA, are increasingly becoming interesting drug design targets. Similarly, docking studies for ligands other than small organic molecules, offer novel ways to investigate mechanisms of interactions in systems, such as enzyme-carbohydrate complexes. Novel docking programs, and more importantly – scoring functions, need to be developed for these systems. Alternatively, existing programs/functions need to be tested for these systems before they can be used reliably.

Nucleic acids

DNA significantly differs from proteins as a ligand receptor due to its high charge density and solvent exposure of its binding site as well as the sequence-dependent nature of its location.

Ricci and Netz (170) have tested AutoDock 4 for different scenarios of DNA-ligand docking: cognate and cross docking as well as intercalation, major groove binding and covalent binding. They have evaluated AutoDock in cognate and cross-docking and have demonstrated that the current limitations of docking methods, with respect to nucleic acids as targets, can be overcome by a proper choice of target conformation. They have shown that, by using a modified canonical B-DNA as a target and artificially introducing an intercalation gap, they could use AutoDock to efficiently, if only qualitatively, evaluate ligand–DNA interactions.

Kuntz and co-workers (58) have tested DOCK 6 for modeling RNA-small molecule complexes using a test set of 70 complexes. With the optimized parameters and a minimal scoring function, they have successfully predicted experimentally determined binding modes: in 70% of the cases for moderately flexible ligands ($<$7 rotatable bonds) and in 26% of the cases for highly flexible ligands (7–13 rotatable bonds).

Several other groups have investigated docking to nucleic acids to search for quadruplex binders (171–173) and to study intercalation (123,174–176).

Metalloproteins

Ligand binding to metalloproteins can be dramatically different compared to other proteins, particularly because it could be covalent in nature. While there have been recent advances in covalent docking (discussed elsewhere in this review), the validation of docking protocols for these systems is particularly important. Michielin and co-workers (177) have tested their EADock 2 program for docking ligands to heme proteins and introduced the Morse-like metal binding potentials, fitted to reproduce DFT calculations (178). Compared to a standard docking protocol, where the iron-ligand interactions are underestimated, the pose prediction improved approximately two-fold as a result (i.e., from 28% to 62% of cases).

Carboranes

Boron-containing ligands are becoming increasing important as therapeutic and diagnostic agents, particularly in cancer. However, their structure-based design is hampered by the lack of boron atom type parameters in most molecular modeling packages. Tiwari *et al*. (179) have developed simple and efficient strategies to overcome this hurdle by the replacement of boron atom types with carbon atom types. They have validated this approach by cognate ligand docking into the human dihydrofolate reductase (hDHFR) and comparing the poses and binding energies to the experimental structures.

Carbohydrates

There are some peculiarities to protein–carbohydrate interactions, compared to general protein–ligand interactions, which make carbohydrate docking particularly challenging: extreme flexibility, a large number of hydroxyl groups, extensive hydrogen bonding networks, and the formation of crucial CH-π contacts. Therefore, widely used docking programs and scoring functions, which account differently for these types of interactions, may not perform as well for protein–carbohydrate complexes. Agostino *et al*. (165) have evaluated several docking programs (Glide, AutoDock, GOLD, and FlexX) by cognate and cross-docking antibody–carbohydrate simulations and demonstrated that generally docking has been performed well by Glide. GOLD and AutoDock had several problems and FlexX performed poorly.

Glide appears to be a popular choice for docking carbohydrates, and this choice is supported by a range of studies where carbohydrate docking into diverse proteins has been structurally validated. Kolomiets *et al*. (180) have cognately docked *C*-fucosyl dipeptide into *P. aeruginosa* lectin LecB and confirmed the accuracy of their result against the crystal structure. Benltifa *et al*. (181) have used Glide XP to dock a series of glucose-based spiro-isoxazolines into glycogen phosphorylase b. The docked structures were found to be in excellent agreement with experiment, both in terms of docked pose/crystal structure RMSDs and docked score/experimental free binding energies correlations.

COMPARISON STUDIES

A universal docking tool (algorithm plus scoring function) that outperforms all others *on every system* does not exist at the moment (19,61,85,169). Therefore, objective assessment and

Table 1. Docking program/scoring functions comparison studies

Programs/functions compared[a]	Test set	Pose prediction	Affinity prediction/VS performance	Performance measure	Refs.
DOCK, FlexX, **Glide**, **ICM**, PhDOCK, **Surflex**, **Surflex Ringflex**	Astex with additional kinase and nuclear receptor target	Y	N	RMSD, GARD	(162)
DOCK, FlexX, **Glide**, GOLD, **Surflex**; ChemScore, D-Score, F-Score, G-Score, **GlideScore**, GoldScore, Grid-Score, PMF-Score, X-Score, **Surflex-Score**	Crystal structure of dihydropteroate synthase (DHPS). Ligand decoy sets: Schrodinger, ZINC, and ACD, seeded with 65 known actives	Y	Y	RMSD, enrichment factors, and ROC curves	(88)
Kang's GA, GOLD, **Glide**, **Surflex**, DOCK6	GOLD data set of 134 crystal structures	Y	N	RMSD	(59)
DOCK, FlexX, **Glide**, ICM, PhDOCK, **Surflex**	A set of 68 diverse high resolution crystal structures; DUD	Y	Y	RMSD, ROC factors, and ROC AUCs	(47)
AutoDock, **Glide**, FlexX, Surflex	Crystal structure of hDHFR with carboranyl ligands	Y	Y	RMSD	(179)
ASP, **ChemScore**, D-Score, **DrugScore**, F-Score, G-Score, **GlideScore**, GoldScore, Jain, **LigScore**, LUDI, **PLP**, PMF, PMF-Score, **X-Score**	A set of 195 diverse high resolution crystal structures; binding constants from PDBbind database	Y	Y	RMSD, binding affinity	(85)
APIF, GoldScore, **CHIF**	Trypsin, rhinovirus, HIV protease, carboxypeptidase, estrogen receptor-α; Maybridge ligand database	Y	Y	RMSD, enrichment factors	(91)
ChemScore, DockScore, **DrugScore**, **eHiTS**, FlexX, **Glide**, **GOLD**, Hammerhead, LigScore, **PLP**, PMF, **RankScore (FITTED)**, Surflex, **X-Score**	58 complexes (HIV protease, thrombin, trypsin, and matrix metalloproteases)	N	Y	Binding affinity, rank ordering	(71)
QM/MM combinations of: **AMBER**, GoldScore, ChemScore; **HF/6-31G***, AM1d, PM3	Six HIV protease-inhibitor complexes	Y	N	RMSD	(66)

[a] Top performing programs/scoring functions indicated by bold font.

comparison of docking programs and scoring functions (together or separately for the latter) is a critical ongoing theme in the field. Such comparisons are usually focused on the ability of programs to (i) successfully recapitulate the experimentally determined binding mode (as discussed in detail above) and/or (ii) predict ligands binding affinities or, at least, correctly rank ligands with respect to their binding affinities. It is widely accepted that meaningful comparison of docking approaches (algorithms and/or scoring functions) is difficult, non-trivial (182), and sometimes "intriguing" (85). It has also been shown that expert use of the software, compared to the novice, default, "out-of-the box" implementation, may have a significant effect on the docking results (47).

Cheng *et al*. (85) have carried out an extensive assessment and comparison of scoring functions and their performance from the points of view of "Docking Power" (ability to identify the correct pose among computer-generated decoys), "Ranking Power" (ability to correctly rank different ligands), and "Scoring Power" (ability to correlate scores to experimental binding affinities, ideally – linearly). They combined poses from several docking programs and clustered them to produce a non-redundant, low energy set of poses for each protein. Thus, the authors of this study have cleverly separated the issue of docking performance from scoring performance, therefore evaluating just the functions and not the programs, by which they are driven. They have concluded, based on their findings, that today's scoring functions generally perform better in pose prediction than in affinity prediction. However, some functions did better in terms of docking power, while others performed better in terms of ranking/scoring. Therefore, the authors have emphasized that it is important to select a function in accordance with a specific purpose. Cross *et al*. (47) came to a similar conclusion in their comparison study, where some programs performed better in pose prediction, while others in virtual screening.

A summary of docking and/or scoring comparative studies, published in 2009, and their findings is given in Table 1. According to these studies, two packages that consistently outperform other ones are Glide (23–26) and Surflex (37,38). However, as has already been pointed out in this review, docking program/scoring function performance is both target- and ligand-dependent and should always be evaluated and validated for the problem at hand.

CONCLUDING REMARKS

It is evident from docking literature, that accounting for flexibility and successful scoring remain significant challenges. However, important advances are being made.

In respect to receptor flexibility, Armen *et al*. (33) have observed various effects of receptor flexibility on docking accuracy. While this result is not surprising, it is reasonable to expect that the effect of the degree of incorporated/allowed flexibility on the docking accuracy is different for different proteins and will depend on the extent of conformational change/induced fit occurring during binding. It will be interesting to watch the field in anticipation of more studies of that type to have a more comprehensive view on the "required" or "optimal" degree of receptor flexibility in docking and virtual screening.

The issues of ligand flexibility have recently come back to the forefront. For as long as ligand flexibility has been incorporated into docking algorithms, it appeared that the issue of input ligand conformation is resolved and cannot create major problems. It has been generally assumed in the field that the input ligand conformation does not affect the docking results, provided that it is a reasonable one. Does it? In the study by Feher and Williams (51), no ligand starting geometry (experimental structures, as well as CORINA, conformational search, or MD structures) was found to consistently produce the most accurate docking pose. The authors seemed to be surprised that the crystal structure, when used as an input, could produce worse result than a random conformation. Given the work by Cross *et al*. (47) and Corbeil and Moitessier (40), this indeed should seem unexpected. However, if anything, this outcome should have been treated as an advantage of the conformational ligand treatment considered: even if not successful in redocking, it clearly demonstrated no bias based on the input structure. Thus, in our opinion, the jury is still out and the potential bias of docking results towards input conformation requires further evaluation. Notwithstanding, the implications of Feher and Williams' (51) findings, and the questions they raise, could be significant: while using a single input conformation and performing a single docking run is likely to decrease docking accuracy, having multiple ligand input does not guarantee said accuracy.

Similar to input ligand conformation, the aspects of input speciation (i.e., ligand protonation, tautomerism, and stereo-isomerism) have also been tested in the recent docking efforts. The studies by ten Brink and Exner (61) and Kalliokoski *et al*. (62) represent somewhat contradictory outcomes and, therefore, recommendations. Since these studies have used different programs and different data sets, it is difficult to compare their findings and validity of their recommendations. Thus, more work is needed. In this future work, the influence of the structures being validated/evaluated against should be taken into account (see Crystal Structure As a Validation Benchmark Section). Ultimately, the dilemma of multiple versus single input form is, to some extent, similar to considering stochastic versus systematic methods in conformational analysis. Similar to that problem, the solution will most likely be in superior algorithms and scoring functions (as has been suggested by ten Brink and Exner (61)), rather than in deciding which input approach to use.

With respect to scoring, the matters of consensus scoring continue to be actively evaluated and debated. Friedman and Caflisch (84) have found that their consensus scoring improved early enrichment in virtual screening. To give more weight to the value of consensus scoring (at least in that study), it must be remembered that only a limited number of top-ranking compounds is usually given visual inspection and even a smaller number is tested experimentally. Thus, when thinking of early, rather than full, enrichment, it seems that consensus scoring is indeed making a difference. Another, still unresolved, matter with respect to consensus scoring is the lack of understanding of its basic principles. O'Boyle *et al*. (89) have assessed the factors underlying the success of rescoring and consensus scoring using three "related" functions. It would be interesting to see more work in that direction to ascertain how unrelated functions, at least those from different developers, would behave in a similar scenario.

Acknowledgements

This work was funded in part by grants ID566722 and ID543317 (to P.A. Ramsland) from the National Health and Medical Research

Council of Australia (NHMRC). M. Agostino is a recipient of an Australian Postgraduate Award (APA). P.A. Ramsland is a recipient of an R. Douglas Wright Career Development Award (ID365209) from the NHMRC. The authors gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

REFERENCES

1. Villoutreix BO, Eudes R, Miteva MA. 2009. Structure-based virtual ligand screening: recent success stories. *Comb. Chem. High Throughput Screen.* **12**: 1000–1016.
2. Yoshikawa T, Tsukamoto K, Hourai Y, Fukui K. 2009. Improving the accuracy of an affinity prediction method by using statistics on shape complementarity between proteins. *J. Chem. Inf. Model.* **49**: 693–703.
3. Mosca R, Pons C, Fernandez-Recio J, Aloy P. 2009. Pushing structural information into the yeast interactome by high-throughput protein docking experiments. *PLoS Comput. Biol.* **5**: e1000490.
4. Das R, Andre I, Shen Y, Wu Y, Lemak A, Bansal S, Arrowsmith CH, Szyperski T, Baker D. 2009. Simultaneous prediction of protein folding and docking at high resolution. *Proc. Natl. Acad. Sci. USA* **106**: 18978–18983.
5. Beglov D, Lee CJ, De Biasio A, Kozakov D, Brenke R, Vajda S, Beglova N. 2009. Structural insights into recognition of beta2-glycoprotein I by the lipoprotein receptors. *Proteins* **77**: 940–949.
6. Schug A, Weigt M, Onuchic JN, Hwa T, Szurmant H. 2009. High-resolution protein complexes from integrating genomic information with molecular simulation. *Proc. Natl. Acad. Sci. USA* **106**: 22124–22129.
7. Garzon JI, Lopez-Blanco JR, Pons C, Kovacs J, Abagyan R, Fernandez-Recio J, Chacon P. 2009. FRODOCK: a new approach for fast rotational protein-protein docking. *Bioinformatics* **25**: 2544–2551.
8. Nussinov R, Schreiber G (eds). 2009. Computational Protein–Protein Interactions. CRC Press: Boca Raton.
9. Hwang H, Pierce B, Weng Z. 2009. Protein–protein docking. In Nussinov R, Schreiber G (eds). Computational Protein–Protein Interactions. CRC Press: Boca Raton; 147–165.
10. Liu X, Kai M, Jin L, Wang R. 2009. Computational study of the heterodimerization between mu and delta receptors. *J. Comput. Aided Mol. Des.* **23**: 321–332.
11. Vajda S, Kozakov D. 2009. Convergence and combination of methods in protein–protein docking. *Curr. Opin. Struct. Biol.* **19**: 164–170.
12. Corbeil CR, Therrien E, Moitessier N. 2009. Modeling reality for optimal docking of small molecules to biological targets. *Curr. Comp. Aided Drug Des.* **5**: 241–263.
13. Fan H, Irwin JJ, Webb BM, Klebe G, Shoichet BK, Sali A. 2009. Molecular docking screens using comparative models of proteins. *J. Chem. Inf. Model.* **49**: 2512–2527.
14. Huang N, Shoichet BK, Irwin JJ. 2006. Benchmarking sets for molecular docking. *J. Med. Chem.* **49**: 6789–6801.
15. Sherbiny FF, Schiedel AC, Maass A, Muller CE. 2009. Homology modelling of the human adenosine A_{2B} receptor based on X-ray structures of bovine rhodopsin, the *beta*$_2$-adrenergic receptor and the human adenosine A_{2A} receptor. *J. Comput. Aided Mol. Des.* **23**: 807–828.
16. Sivasubramanian A, Sircar A, Chaudhury S, Gray JJ. 2009. Toward high-resolution homology modeling of antibody Fv regions and application to antibody-antigen docking. *Proteins* **74**: 497–514.
17. Hartmann C, Antes I, Lengauer T. 2009. Docking and scoring with alternative side-chain conformations. *Proteins* **74**: 712–726.
18. B-Rao C, Subramanian J, Sharma SD. 2009. Managing protein flexibility in docking and its applications. *Drug Discov. Today* **14**: 394–400.
19. Davis IW, Raha K, Head MS, Baker D. 2009. Blind docking of pharmaceutically relevant compounds using RosettaLigand. *Protein Sci.* **18**: 1998–2002.
20. Davis IW, Baker D. 2009. RosettaLigand docking with full ligand and receptor flexibility. *J. Mol. Biol.* **385**: 381–392.
21. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. 2009. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **30**: 2785–2791.
22. Cerqueira NM, Bras NF, Fernandes PA, Ramos MJ. 2009. MADAMM: a multistaged docking with an automated molecular modeling protocol. *Proteins* **74**: 192–206.
23. Sherman W, Beard HS, Farid R. 2006. Use of an induced fit receptor structure in virtual screening. *Chem. Biol. Drug Des.* **67**: 83–84.
24. Sherman W, Day T, Jacobson MP, Friesner RA, Farid R. 2006. Novel procedure for modeling ligand/receptor induced fit effects. *J. Med. Chem.* **49**: 534–553.
25. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. 2004. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **47**: 1739–1749.
26. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. 2004. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J. Med. Chem.* **47**: 1750–1759.
27. Zhong H, Tran LM, Stang JL. 2009. Induced-fit docking studies of the active and inactive states of protein tyrosine kinases. *J. Mol. Graph. Model.* **28**: 336–346.
28. Cho YY, Yao K, Pugliese A, Malakhova ML, Bode AM, Dong Z. 2009. A regulatory mechanism for RSK2 NH(2)-terminal kinase activity. *Cancer Res.* **69**: 4398–4406.
29. Barreca ML, Iraci N, De Luca L, Chimirri A. 2009. Induced-fit docking approach provides insight into the binding mode and mechanism of action of HIV-1 integrase inhibitors. *ChemMedChem* **4**: 1446–1456.
30. Lauria A, Ippolito M, Almerico AM. 2009. Inside the Hsp90 inhibitors binding mode through induced fit docking. *J. Mol. Graph. Model.* **27**: 712–722.
31. King AR, Dotsey EY, Lodola A, Jung KM, Ghomian A, Qiu Y, Fu J, Mor M, Piomelli D. 2009. Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chem. Biol.* **16**: 1045–1052.
32. Huang Z, Wong CF. 2009. Conformational selection of protein kinase A revealed by flexible-ligand flexible-protein docking. *J. Comput. Chem.* **30**: 631–644.
33. Armen RS, Chen JH, Brooks CL. 2009. An evaluation of explicit receptor flexibility in molecular docking using molecular dynamics and torsion angle molecular dynamics. *J. Chem. Theory Comput.* **5**: 2909–2923.
34. Abagyan R, Totrov M. 1994. Biased probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins. *J. Mol. Biol.* **235**: 983–1002.
35. Bottegoni G, Kufareva I, Totrov M, Abagyan R. 2009. Four-dimensional docking: a fast and accurate account of discrete receptor flexibility in ligand docking. *J. Med. Chem.* **52**: 397–406.
36. Abagyan R, Kufareva I. 2009. The flexible pocketome engine for structural chemogenomics. *Methods Mol. Biol.* **575**: 249–279.
37. Jain AN. 2003. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **46**: 499–511.
38. Jain AN. 2007. Surflex-Dock 2.1: robust performance from ligand energetic modeling, ring flexibility, and knowledge-based search. *J. Comput. Aided Mol. Des.* **21**: 281–306.
39. Jain AN. 2009. Effects of protein conformation in docking: improved pose prediction through protein pocket adaptation. *J. Comput. Aided Mol. Des.* **23**: 355–374.
40. Corbeil CR, Moitessier N. 2009. Docking ligands into flexible and solvated macromolecules. 3. Impact of input ligand conformation, protein flexibility, and water molecules on the accuracy of docking Programs. *J. Chem. Inf. Model.* **49**: 997–1009.
41. Fuhrmann J, Rurainski A, Lenhof HP, Neumann D. 2009. A new method for the gradient-based optimization of molecular complexes. *J. Comput. Chem.* **30**: 1371–1378.
42. Paulsen JL, Anderson AC. 2009. Scoring ensembles of docked protein:ligand interactions for virtual lead optimization. *J. Chem. Inf. Model.* **49**: 2813–2819.
43. Bolstad ES, Anderson AC. 2009. In pursuit of virtual lead optimization: pruning ensembles of receptor structures for increased efficiency and accuracy during docking. *Proteins* **75**: 62–74.

44. Rueda M, Bottegoni G, Abagyan R. 2009. Consistent improvement of cross-docking results using binding site ensembles generated with elastic network normal modes. *J. Chem. Inf. Model.* **49**: 716–725.
45. Kazemi S, Kruger DM, Sirockin F, Gohlke H. 2009. Elastic potential grids: accurate and efficient representation of intermolecular interactions for fully flexible docking. *ChemMedChem* **4**: 1264–1268.
46. Kirchmair J, Markt P, Distinto S, Wolber G, Langer T. 2008. Evaluation of the performance of 3D virtual screening protocols: RMSD comparisons, enrichment assessments, and decoy selection—what can we learn from earlier mistakes? *J. Comput. Aided Mol. Des.* **22**: 213–228.
47. Cross JB, Thompson DC, Rai BK, Baber JC, Fan KY, Hu Y, Humblet C. 2009. Comparison of several molecular docking programs: pose prediction and virtual screening accuracy. *J. Chem. Inf. Model.* **49**: 1455–1474.
48. Rarey M, Kramer B, Lengauer T, Klebe G. 1996. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **261**: 470–489.
49. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. 2003. Improved protein-ligand docking using GOLD. *Proteins* **52**: 609–623.
50. Jones G, Willett P, Glen RC, Leach AR, Taylor R. 1997. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **267**: 727–748.
51. Feher M, Williams CI. 2009. Effect of input differences on the results of docking calculations. *J. Chem. Inf. Model.* **49**: 1704–1714.
52. Anon A. 2010. *MOE (Molecular Operating Environment)*. http://www.chemcomp.com/index.htm [4 June 2010].
53. Griewel A, Kayser O, Schlosser J, Rarey M. 2009. Conformational sampling for large-scale virtual screening: accuracy versus ensemble size. *J. Chem. Inf. Model.* **49**: 2303–2311.
54. Viji SN, Prasad PA, Gautham N. 2009. Protein-ligand docking using mutually orthogonal Latin squares (MOLSDOCK). *J. Chem. Inf. Model.* **49**: 2687–2694.
55. Pearce BC, Langley DR, Kang J, Huang H, Kulkarni A. 2009. E-novo: an automated workflow for efficient structure-based lead optimization. *J. Chem. Inf. Model.* **49**: 1797–1809.
56. Schlosser J, Rarey M. 2009. Beyond the virtual screening paradigm: structure-based searching for new lead compounds. *J. Chem. Inf. Model.* **49**: 800–809.
57. Hartshorn MJ, Verdonk ML, Chessari G, Brewerton SC, Mooij WT, Mortenson PN, Murray CW. 2007. Diverse, high-quality test set for the validation of protein-ligand docking performance. *J. Med. Chem.* **50**: 726–741.
58. Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC, Thomas V, Rizzo RC, Case DA, James TL, Kuntz ID. 2009. DOCK 6: combining techniques to model RNA-small molecule complexes. *RNA* **15**: 1219–1230.
59. Kang L, Li H, Jiang H, Wang X. 2009. An improved adaptive genetic algorithm for protein-ligand docking. *J. Comput. Aided Mol. Des.* **23**: 1–12.
60. Trott O, Olson AJ. 2009. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**: 455–461.
61. ten Brink T, Exner TE. 2009. Influence of protonation, tautomeric, and stereoisomeric states on protein-ligand docking results. *J. Chem. Inf. Model.* **49**: 1535–1546.
62. Kalliokoski T, Salo HS, Lahtela-Kakkonen M, Poso A. 2009. The effect of ligand-based tautomer and protomer prediction on structure-based virtual screening. *J. Chem. Inf. Model.* **49**: 2742–2748.
63. Kongsted J, Ryde U. 2009. An improved method to predict the entropy term with the MM/PBSA approach. *J. Comput. Aided Mol. Des.* **23**: 63–71.
64. Dhaked DK, Verma J, Saran A, Coutinho EC. 2009. Exploring the binding of HIV-1 integrase inhibitors by comparative residue interaction analysis (CoRIA). *J. Mol. Model.* **15**: 233–245.
65. Yang CY, Sun H, Chen J, Nikolovska-Coleska Z, Wang S. 2009. Importance of ligand reorganization free energy in protein-ligand binding-affinity prediction. *J. Am. Chem. Soc.* **131**: 13709–13721.
66. Fong P, McNamara JP, Hillier IH, Bryce RA. 2009. Assessment of QM/MM scoring functions for molecular docking to HIV-1 protease. *J. Chem. Inf. Model.* **49**: 913–924.
67. Cincilla G, Vidal D, Pons M. 2009. An improved scoring function for suboptimal polar ligand complexes. *J. Comput. Aided Mol. Des.* **23**: 143–152.
68. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **19**: 1639–1662.
69. Huang Z, Wong CF. 2009. Docking flexible peptide to flexible protein by molecular dynamics using two implicit-solvent models: an evaluation in protein kinase and phosphatase systems. *J. Phys. Chem. B* **113**: 14343–14354.
70. Villacanas O, Madurga S, Giralt E, Belda I. 2009. Explicit treatment of water molecules in protein-ligand docking. *Curr. Comp. Aided Drug Des.* **5**: 145–154.
71. Englebienne P, Moitessier N. 2009. Docking ligands into flexible and solvated macromolecules. 4. Are popular scoring functions accurate for this class of proteins? *J. Chem. Inf. Model.* **49**: 1568–1580.
72. Korb O, Stutzle T, Exner TE. 2009. Empirical scoring functions for advanced protein-ligand docking with PLANTS. *J. Chem. Inf. Model.* **49**: 84–96.
73. Englebienne P, Moitessier N. 2009. Docking ligands into flexible and solvated macromolecules. 5. Force-field-based prediction of binding affinities of ligands to proteins. *J. Chem. Inf. Model.* **49**: 2564–2571.
74. Wang R, Lu Y, Wang S. 2003. Comparative evaluation of 11 scoring functions for molecular docking. *J. Med. Chem.* **46**: 2287–2303.
75. Tarasov D, Tovbin D. 2009. How sophisticated should a scoring function be to ensure successful docking, scoring and virtual screening? *J. Mol. Model.* **15**: 329–341.
76. Gleeson MP, Gleeson D. 2009. QM/MM as a tool in fragment based drug discovery. A cross-docking, rescoring study of kinase inhibitors. *J. Chem. Inf. Model.* **49**: 1437–1448.
77. Chung JY, Hah JM, Cho AE. 2009. Correlation between performance of QM/MM docking and simple classification of binding sites. *J. Chem. Inf. Model.* **49**: 2382–2387.
78. Cho AE, Rinaldo D. 2009. Extension of QM/MM docking and its applications to metalloproteins. *J. Comput. Chem.* **30**: 2609–2616.
79. Anisimov VM, Bugaenko VL. 2009. QM/QM docking method based on the variational finite localized molecular orbital approximation. *J. Comput. Chem.* **30**: 784–798.
80. Sgrignani J, Bonaccini C, Grazioso G, Chioccioli M, Cavalli A, Gratteri P. 2009. Insights into docking and scoring neuronal alpha4beta2 nicotinic receptor agonists using molecular dynamics simulations and QM/MM calculations. *J. Comput. Chem.* **30**: 2443–2454.
81. Cho AE, Chung JY, Kim M, Park K. 2009. Quantum mechanical scoring for protein docking. *J. Chem. Phys.* **131**: 134108.
82. Halgren T. 2007. New method for fast and accurate binding-site identification and analysis. *Chem. Biol. Drug Des.* **69**: 146–148.
83. Charifson PS, Corkery JJ, Murcko MA, Walters WP. 1999. Consensus scoring: a method for obtaining improved hit rates from docking databases of three-dimensional structures into proteins. *J. Med. Chem.* **42**: 5100–5109.
84. Friedman R, Caflisch A. 2009. Discovery of plasmepsin inhibitors by fragment-based docking and consensus scoring. *ChemMedChem* **4**: 1317–1326.
85. Cheng T, Li X, Li Y, Liu ZC, Wang R. 2009. Comparative assessment of scoring functions on a diverse test set. *J. Chem. Inf. Model.* **49**: 1079–1093.
86. Bar-Haim S, Aharon A, Ben-Moshe T, Marantz Y, Senderowitz H. 2009. SeleX-CS: a new consensus scoring algorithm for hit discovery and lead optimization. *J. Chem. Inf. Model.* **49**: 623–633.
87. Li H, Zhang H, Zheng M, Luo J, Kang L, Liu X, Wang X, Jiang H. 2009. An effective docking strategy for virtual screening based on multi-objective optimization algorithm. *BMC Bioinform.* **10**: 58.
88. Hevener KE, Zhao W, Ball DM, Babaoglu K, Qi J, White SW, Lee RE. 2009. Validation of molecular docking programs for virtual screening against dihydropteroate synthase. *J. Chem. Inf. Model.* **49**: 444–460.
89. O'Boyle NM, Liebeschuetz JW, Cole JC. 2009. Testing assumptions and hypotheses for rescoring success in protein-ligand docking. *J. Chem. Inf. Model.* **49**: 1871–1878.
90. Loving K, Salam NK, Sherman W. 2009. Energetic analysis of fragment docking and application to structure-based pharmacophore hypothesis generation. *J. Comput. Aided Mol. Des.* **23**: 541–554.
91. Perez-Nueno VI, Rabal O, Borrell JI, Teixido J. 2009. APIF: a new interaction fingerprint based on atom pairs and its application to virtual screening. *J. Chem. Inf. Model.* **49**: 1245–1260.
92. Nandigam RK, Kim S, Singh J, Chuaqui C. 2009. Position specific interaction dependent scoring technique for virtual screening based on weighted protein–ligand interaction fingerprint profiles. *J. Chem. Inf. Model.* **49**: 1185–1192.

93. Gonzalez-Ruiz D, Gohlke H. 2009. Steering protein–ligand docking with quantitative NMR chemical shift perturbations. *J. Chem. Inf. Model.* **49**: 2260–2271.
94. Seifert MH. 2009. Targeted scoring functions for virtual screening. *Drug Discov. Today* **14**: 562–569.
95. Seifert MH. 2009. Robust optimization of scoring functions for a target class. *J. Comput. Aided Mol. Des.* **23**: 633–644.
96. Kolb P, Irwin JJ. 2009. Docking screens: right for the right reasons? *Curr. Top. Med. Chem.* **9**: 755–770.
97. Wallach I, Lilien R. 2009. Predicting multiple ligand binding modes using self-consistent pharmacophore hypotheses. *J. Chem. Inf. Model.* **49**: 2116–2128.
98. Novikov FN, Stroylov VS, Stroganov OV, Kulkov V, Chilov GG. 2009. Developing novel approaches to improve binding energy estimation and virtual screening: a PARP case study. *J. Mol. Model.* **15**: 1337–1347.
99. Agostino M, Sandrin MS, Thompson PE, Yuriev E, Ramsland P. 2009. *In silico* analysis of antibody-carbohydrate interactions and its application to xenoreactive antibodies. *Mol. Immunol.* **47**: 233–246.
100. Agostino M, Sandrin MS, Thompson PE, Yuriev E, Ramsland PA. 2010. Identification of preferred carbohydrate binding modes in xenoreactive antibodies by combining conformational filters and binding site maps. *Glycobiology* **20**: 724–735.
101. Yasuo K, Yamaotsu N, Gouda H, Tsujishita H, Hirono S. 2009. Structure-based CoMFA as a predictive model—CYP2C9 inhibitors as a test case. *J. Chem. Inf. Model.* **49**: 853–864.
102. Bikadi Z, Hazai E. 2009. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J. Cheminform.* **1**: 15.
103. Law R, Barker O, Barker JJ, Hesterkamp T, Godemann R, Andersen O, Fryatt T, Courtney S, Hallett D, Whittaker M. 2009. The multiple roles of computational chemistry in fragment-based drug design. *J. Comput. Aided Mol. Des.* **23**: 459–473.
104. Warr WA. 2009. Fragment-based drug discovery. *J. Comput. Aided Mol. Des.* **23**: 453–458.
105. Chen Y, Shoichet BK. 2009. Molecular docking and ligand specificity in fragment-based inhibitor discovery. *Nat. Chem. Biol.* **5**: 358–364.
106. Teotico DG, Babaoglu K, Rocklin GJ, Ferreira RS, Giannetti AM, Shoichet BK. 2009. Docking for fragment inhibitors of AmpC beta-lactamase. *Proc. Natl. Acad. Sci. USA* **106**: 7455–7460.
107. Kawatkar S, Wang HM, Czerminski R, Joseph-McCarthy D. 2009. Virtual fragment screening: an exploration of various docking and scoring protocols for fragments using Glide. *J. Comput. Aided Mol. Des.* **23**: 527–539.
108. Zaliani A, Boda K, Seidel T, Herwig A, Schwab CH, Gasteiger J, Claussen H, Lemmen C, Degen J, Parn J, Rarey M. 2009. Second-generation de novo design: a view from a medicinal chemist perspective. *J. Comput. Aided Mol. Des.* **23**: 593–602.
109. Fukunishi Y, Mashimo T, Orita M, Ohno K, Nakamura H. 2009. In silico fragment screening by replica generation (FSRG) method for fragment-based drug design. *J. Chem. Inf. Model.* **49**: 925–933.
110. Moriaud F, Doppelt-Azeroual O, Martin L, Oguievetskaia K, Koch K, Vorotyntsev A, Adcock SA, Delfaud F. 2009. Computational fragment-based approach at PDB scale by protein local similarity. *J. Chem. Inf. Model.* **49**: 280–294.
111. Landon MR, Lieberman RL, Hoang QQ, Ju SL, Caaveiro JMM, Orwig SD, Kozakov D, Brenke R, Chuang GY, Beglov D, Vajda S, Petsko GA, Ringe D. 2009. Detection of ligand binding hot spots on protein surfaces via fragment-based methods: application to DJ-1 and glucocerebrosidase. *J. Comput. Aided Mol. Des.* **23**: 491–500.
112. Brenke R, Kozakov D, Chuang GY, Beglov D, Hall D, Landon MR, Mattos C, Vajda S. 2009. Fragment-based identification of druggable 'hot spots' of proteins using Fourier domain correlation techniques. *Bioinformatics* **25**: 621–627.
113. ZINC. 2010. *ZINC*. http://zinc.docking.org/ [4 June 2010].
114. Ekonomiuk D, Su XC, Ozawa K, Bodenreider C, Lim SP, Otting G, Huang D, Caflisch A. 2009. Flaviviral protease inhibitors identified by fragment-based library docking into a structure generated by molecular dynamics. *J. Med. Chem.* **52**: 4860–4868.
115. Ekonomiuk D, Su XC, Ozawa K, Bodenreider C, Lim SP, Yin Z, Keller TH, Beer D, Patel V, Otting G, Caflisch A, Huang D. 2009. Discovery of a non-peptidic inhibitor of west nile virus NS3 protease by high-throughput docking. *PLoS Negl. Trop. Dis.* **3**: e356.
116. Juhl PB, Trodler P, Tyagi S, Pleiss J. 2009. Modelling substrate specificity and enantioselectivity for lipases and esterases by substrate-imprinted docking. *BMC Struct. Biol.* **9**: 39.
117. Lawandi J, Toumieux S, Seyer V, Campbell P, Thielges S, Juillerat-Jeanneret L, Moitessier N. 2009. Constrained peptidomimetics reveal detailed geometric requirements of covalent prolyl oligopeptidase inhibitors. *J. Med. Chem.* **52**: 6672–6684.
118. Zhang S, Shi Y, Jin H, Liu Z, Zhang L. 2009. Covalent complexes of proteasome model with peptide aldehyde inhibitors MG132 and MG101: docking and molecular dynamics study. *J. Mol. Model.* **15**: 1481–1490.
119. Moura-Tamames SA, Ramos MJ, Fernandes PA. 2009. Modelling beta-1,3-exoglucanase-saccharide interactions: structure of the enzyme-substrate complex and enzyme binding to the cell wall. *J. Mol. Graph. Model.* **27**: 908–920.
120. Taufer M, Armen R, Chen J, Teller P, Brooks C. 2009. Computational multiscale modeling in protein-ligand docking. *IEEE Eng. Med. Biol. Mag.* **28**: 58–69.
121. Ng CA, Oehme DP, Kato Y, Tanokura M, Brownlee RTC. 2009. Binding of an RNA pol II Ligand to the WW domain of Pin1 using molecular dynamics docking simulations. *J. Chem. Theory Comput.* **5**: 2886–2897.
122. Kranjc A, Bongarzone S, Rossetti G, Biarnes X, Cavalli A, Bolognesi ML, Roberti M, Legname G, Carloni P. 2009. Docking ligands on protein surfaces: the case study of prion protein. *J. Chem. Theory Comput.* **5**: 2565–2573.
123. Perez-Montoto LG, Santana L, Gonzalez-Diaz H. 2009. Scoring function for DNA-drug docking of anticancer and antiparasitic compounds based on spectral moments of 2D lattice graphs for molecular dynamics trajectories. *Eur. J. Med. Chem.* **44**: 4461–4469.
124. Lafleur K, Huang D, Zhou T, Caflisch A, Nevado C. 2009. Structure-based optimization of potent and selective inhibitors of the tyrosine kinase erythropoietin producing human hepatocellular carcinoma receptor B4 (EphB4). *J. Med. Chem.* **52**: 6433–6446.
125. Li WH, Ode H, Hoshino T, Liu H, Tang Y, Jiang HL. 2009. Reduced catalytic activity of P450 2A6 mutants with coumarin: a computational investigation. *J. Chem. Theory Comput.* **5**: 1411–1420.
126. Costache AD, Sheihet L, Zaveri K, Knight DD, Kohn J. 2009. Polymer–drug interactions in tyrosine-derived triblock copolymer nanospheres: a computational modeling approach. *Mol. Pharm.* **6**: 1620–1627.
127. Mackey MD, Melville JL. 2009. Better than random? The chemotype enrichment problem. *J. Chem. Inf. Model.* **49**: 1154–1162.
128. Skone G, Voiculescu I, Cameron S. 2009. Knowing when to give up: early-rejection stratagems in ligand docking. *J. Comput. Aided Mol. Des.* **23**: 715–724.
129. Irwin JJ, Shoichet BK, Mysinger MM, Huang N, Colizzi F, Wassam P, Cao Y. 2009. Automated docking screens: a feasibility study. *J. Med. Chem.* **52**: 5712–5720.
130. Gil-Redondo R, Estrada J, Morreale A, Herranz F, Sancho J, Ortiz AR. 2009. VSDMIP: virtual screening data management on an integrated platform. *J. Comput. Aided Mol. Des.* **23**: 171–184.
131. 2010. *Docking@Home*. http://docking.cis.udel.edu [4 June 2010].
132. Zhou T, Caflisch A. 2009. Data management system for distributed virtual screening. *J. Chem. Inf. Model.* **49**: 145–152.
133. Garcia-Sosa AT, Sild S, Maran U. 2009. Docking and virtual screening using distributed grid technology. *QSAR Comb. Sci.* **28**: 815–821.
134. Kumar A, Chaturvedi V, Bhatnagar S, Sinha S, Siddiqi MI. 2009. Knowledge based identification of potent antitubercular compounds using structure based virtual screening and structure interaction fingerprints. *J. Chem. Inf. Model.* **49**: 35–42.
135. Salam NK, Nuti R, Sherman W. 2009. Novel method for generating structure-based pharmacophores using energetic analysis. *J. Chem. Inf. Model.* **49**: 2356–2368.
136. Gimenez-Oya V, Villacanas O, Fernandez-Busquets X, Rubio-Martinez J, Imperial S. 2009. Mimicking direct protein–protein and solvent-mediated interactions in the CDP-methylerythritol kinase homodimer: a pharmacophore-directed virtual screening approach. *J. Mol. Model.* **15**: 997–1007.
137. Peach ML, Nicklaus MC. 2009. Combining docking with pharmacophore filtering for improved virtual screening. *J. Cheminform.* **1**: 6.
138. Ahmed A, Choo H, Cho YS, Park WK, Pae AN. 2009. Identification of novel serotonin 2C receptor ligands by sequential virtual screening. *Bioorg. Med. Chem.* **17**: 4559–4568.
139. Perez-Pineiro R, Burgos A, Jones DC, Andrew LC, Rodriguez H, Suarez M, Fairlamb AH, Wishart DS. 2009. Development of a novel virtual screening cascade protocol to identify potential trypanothione reductase inhibitors. *J. Med. Chem.* **52**: 1670–1680.
140. Zavodszky MI, Rohatgi A, Van Voorst JR, Yan H, Kuhn LA. 2009. Scoring ligand similarity in structure-based virtual screening. *J. Mol. Recognit.* **22**: 280–292.

141. Shanmugasundaram K, Rigby AC. 2009. Exploring novel target space: a need to partner high throughput docking and ligand-based similarity searches? *Comb. Chem. High Throughput Screen.* **12**: 984–999.
142. Barakat KH, Torin Huzil J, Luchko T, Jordheim L, Dumontet C, Tuszynski J. 2009. Characterization of an inhibitory dynamic pharmacophore for the ERCC1-XPA interaction using a combined molecular dynamics and virtual screening approach. *J. Mol. Graph. Model.* **28**: 113–130.
143. Rastelli G, Degliesposti G, Del Rio A, Sgobba M. 2009. Binding estimation after refinement, a new automated procedure for the refinement and rescoring of docked ligands in virtual screening. *Chem. Biol. Drug Des.* **73**: 283–286.
144. Okimoto N, Futatsugi N, Fuji H, Suenaga A, Morimoto G, Yanai R, Ohno Y, Narumi T, Taiji M. 2009. High-performance drug discovery: computational screening by combining docking and molecular dynamics simulations. *PLoS Comput. Biol.* **5**: e1000528.
145. Plewczynski D, von Grotthuss M, Rychlewski L, Ginalski K. 2009. Virtual high throughput screening using combined random forest and flexible docking. *Comb. Chem. High Throughput Screen.* **12**: 484–489.
146. Kolb P, Ferreira RS, Irwin JJ, Shoichet BK. 2009. Docking and chemoinformatic screens for new ligands and targets. *Curr. Opin. Biotechnol.* **20**: 429–436.
147. Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK. 2007. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* **318**: 1266–1273.
148. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. 2007. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **318**: 1258–1265.
149. Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK. 2007. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **450**: 383–387.
150. Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF. 2008. Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature* **454**: 486–491.
151. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC. 2008. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* **322**: 1211–1217.
152. Kolb P, Rosenbaum DM, Irwin JJ, Fung JJ, Kobilka BK, Shoichet BK. 2009. Structure-based discovery of beta2-adrenergic receptor ligands. *Proc. Natl. Acad. Sci. USA* **106**: 6843–6848.
153. Michino M, Abola E, Brooks CL III, Dixon JS, Moult J, Stevens RC. 2009. Community-wide assessment of GPCR structure modelling and ligand docking: GPCR Dock 2008. *Nat. Rev. Drug Discov.* **8**: 455–463.
154. Reynolds KA, Katritch V, Abagyan R. 2009. Identifying conformational changes of the beta(2) adrenoceptor that enable accurate prediction of ligand/receptor interactions and screening for GPCR modulators. *J. Comput. Aided Mol. Des.* **23**: 273–288.
155. Katritch V, Reynolds KA, Cherezov V, Hanson MA, Roth CB, Yeager M, Abagyan R. 2009. Analysis of full and partial agonists binding to beta2-adrenergic receptor suggests a role of transmembrane helix V in agonist-specific conformational changes. *J. Mol. Recognit.* **22**: 307–318.
156. Yuzlenko O, Kiec-Kononowicz K. 2009. Molecular modeling of A1 and A2A adenosine receptors: comparison of rhodopsin- and beta2-adrenergic-based homology models through the docking studies. *J. Comput. Chem.* **30**: 14–32.
157. Miller KJ, Wu GY, Varnes JG, Levesque P, Li J, Li D, Robl JA, Rossi KA, Wacker DA. 2009. Position 5.46 of the serotonin 5-HT_{2A} receptor contributes to a species-dependent variation for the 5-HT_{2C} agonist (*R*)-9-ethyl-1,3,4,10*b*-tetrahydro-7-trifluoromethylpyrazino[2,1-*a*]isoindol- 6(*2H*)-one: impact on selectivity and toxicological evaluation. *Mol. Pharmacol.* **76**: 1211–1219.
158. McRobb FM, Capuano B, Crosby IT, Chalmers D, Yuriev E. 2010. Homology modeling and docking evaluation of aminergic G protein-coupled receptors. *J. Chem. Inf. Model.* **50**: 626–637.
159. Gregory KJ, Hall NE, Tobin AB, Sexton PM, Christopoulos A. 2010. Identification of orthosteric and allosteric site mutations in M2 muscarinic acetylcholine receptors that contribute to ligand-selective signaling bias. *J. Biol. Chem.* **285**: 7459–7474.
160. Dailey MM, Hait C, Holt PA, Maguire JM, Meier JB, Miller MC, Petraccone L, Trent JO. 2009. Structure-based drug design: from nucleic acid to membrane protein targets. *Exp. Mol. Pathol.* **86**: 141–150.
161. Dong M, Lam PC, Pinon DI, Abagyan R, Miller LJ. 2009. Elucidation of the molecular basis of cholecystokinin Peptide docking to its receptor using site-specific intrinsic photoaffinity labeling and molecular modeling. *Biochemistry* **48**: 5303–5312.
162. Baber JC, Thompson DC, Cross JB, Humblet C. 2009. GARD: a generally applicable replacement for RMSD. *J. Chem. Inf. Model.* **49**: 1889–1900.
163. Malde AK, Mark AE. 2009. Binding and enantiomeric selectivity of threonyl-tRNA synthetase. *J. Am. Chem. Soc.* **131**: 3848–3849.
164. Sondergaard CR, Garrett AE, Carstensen T, Pollastri G, Nielsen JE. 2009. Structural artifacts in protein-ligand X-ray structures: implications for the development of docking scoring functions. *J. Med. Chem.* **52**: 5673–5684.
165. Agostino M, Jene C, Boyle T, Ramsland PA, Yuriev E. 2009. Molecular docking of carbohydrate ligands to antibodies: structural validation against crystal structures. *J. Chem. Inf. Model.* **49**: 2749–2760.
166. Abagyan RA, Totrov MM. 1997. Contact area difference (CAD): a robust measure to evaluate accuracy of protein models. *J. Mol. Biol.* **268**: 678–685.
167. Kroemer RT, Vulpetti A, McDonald JJ, Rohrer DC, Trosset JY, Giordanetto F, Cotesta S, McMartin C, Kihlen M, Stouten PF. 2004. Assessment of docking poses: interactions-based accuracy classification (IBAC) versus crystal structure deviations. *J. Chem. Inf. Comput. Sci.* **44**: 871–881.
168. Zavodszky MI, Stumpff-Kane AW, Lee DJ, Feig M. 2009. Scoring confidence index: statistical evaluation of ligand binding mode predictions. *J. Comput. Aided Mol. Des.* **23**: 289–299.
169. Warren GL, Andrews CW, Capelli AM, Clarke B, LaLonde J, Lambert MH, Lindvall M, Nevins N, Semus SF, Senger S, Tedesco G, Wall ID, Woolven JM, Peishoff CE, Head MS. 2006. A critical assessment of docking programs and scoring functions. *J. Med. Chem.* **49**: 5912–5931.
170. Ricci CG, Netz PA. 2009. Docking studies on DNA-ligand interactions: building and application of a protocol to identify the binding mode. *J. Chem. Inf. Model.* **49**: 1925–1935.
171. Cosconati S, Marinelli L, Trotta R, Virno A, Mayol L, Novellino E, Olson AJ, Randazzo A. 2009. Tandem application of virtual screening and NMR experiments in the discovery of brand new DNA quadruplex groove binders. *J. Am. Chem. Soc.* **131**: 16336–16337.
172. Redman JE, Granadino-Roldan JM, Schouten JA, Ladame S, Reszka AP, Neidle S, Balasubramanian S. 2009. Recognition and discrimination of DNA quadruplexes by acridine-peptide conjugates. *Org. Biomol. Chem.* **7**: 76–84.
173. Wu P, Ma D-L, Leung C-H, Yan S-C, Zhu N, Abagyan R, Che C-M. 2009. Stabilization of G-Quadruplex DNA with Platinum(II) Schiff base complexes: luminescent probe and down-regulation of c-*myc* oncogene expression. *Eur. J. Chem.* **15**: 13008–13021.
174. Loza-Mejia MA, Castillo R, Lira-Rocha A. 2009. Molecular modeling of tricyclic compounds with anilino substituents and their intercalation complexes with DNA sequences. *J. Mol. Graph. Model.* **27**: 900–907.
175. Holt PA, Ragazzon P, Strekowski L, Chaires JB, Trent JO. 2009. Discovery of novel triple helical DNA intercalators by an integrated virtual and actual screening platform. *Nucleic Acids Res.* **37**: 1280–1287.
176. Byler KG, Wang C, Setzer WN. 2009. Quinoline alkaloids as intercalative topoisomerase inhibitors. *J. Mol. Model.* **15**: 1417–1426.
177. Grosdidier A, Zoete V, Michielin O. 2009. Blind docking of 260 protein-ligand complexes with EADock 2.0. *J. Comput. Chem.* **30**: 2021–2030.
178. Rohrig UF, Grosdidier A, Zoete V, Michielin O. 2009. Docking to heme proteins. *J. Comput. Chem.* **30**: 2305–2315.
179. Tiwari R, Mahasenan K, Pavlovicz R, Li C, Tjarks W. 2009. Carborane clusters in computational drug design: a comparative docking evaluation using AutoDock, FlexX, Glide, and Surflex. *J. Chem. Inf. Model.* **49**: 1581–1589.
180. Kolomiets E, Swiderska MA, Kadam RU, Johansson EM, Jaeger KE, Darbre T, Reymond JL. 2009. Glycopeptide dendrimers with high affinity for the fucose-binding lectin LecB from Pseudomonas aeruginosa. *ChemMedChem* **4**: 562–569.
181. Benltifa M, Hayes JM, Vidal S, Gueyrard D, Goekjian PG, Praly JP, Kizilis G, Tiraidis C, Alexacou KM, Chrysina ED, Zographos SE, Leonidas DD, Archontis G, Oikonomakos NG. 2009. Glucose-based spiro-isoxazolines: a new family of potent glycogen phosphorylase inhibitors. *Bioorg. Med. Chem.* **17**: 7368–7380.
182. Cole JC, Murray CW, Nissink JW, Taylor RD, Taylor R. 2005. Comparing protein-ligand docking programs is difficult. *Proteins* **60**: 325–332.