Review Article

Predicting Protein-Protein Binding Affinity by *In silico* Docking

Valery G Veresov*

Department of Biophysics and Engineering of Cell, Institute of Biophysics and Cell Engineering of NAS of Belarus, Belarus

Abstract

Protein-protein interactions, which result in either transient or long-lived complexes, play a central role in the processes happening in the cells. Perturbations in those interaction networks can lead to disease. Understanding the mechanisms of proteinprotein binding is inherently difficult due to the range of potential interactions, the molecular rearrangement associated with binding, and the time and length scales involved. In silico protein docking is a valuable tool in determination of the structures of protein-protein complexes that complements experimental methods with a level of details that are unattainable in experiment. Although the precise prediction of the structure of protein-protein complex is sufficient to describe the interaction in atomic details, it is the knowledge of affinity of the components for each other is necessary for the prediction of the existence of this assembly and whether it is transient or permanent. Recently, a structure-based binding affinity benchmark Version 1.0, which includes a non redundant set of 144 protein-protein complexes that have highresolution structures available for both the complexes and their unbound components, and for which dissociation constants have been measured by biophysical methods, and the updated version, which contains 179 entries, have been presented. Because for all protein complexes in this benchmark the unbound structures of component proteins are available, these benchmarks allow for the assessment of conformational changes upon protein-protein binding. We use this assessment together with experimental data to explain failures and successes in prediction structures and affinity by in silico docking using the combination of rigid docking and RosettaDock refinements.

ABBREVIATIONS

FFT: Fast Fourier Transform; PRE: Paramagnetic Relaxation Enhancement; NMR: Nuclear Magnetic Resonance; PLS: Partial Least Squares; SVM: Support-Vector Machine; GP: Gaussian Process; RMSD: Root-Mean- Square-Deviation

INTRODUCTION

Relating structure to function is a major objective of structural biology. Although current structural biology tools have broaden essentially our knowledge in structures, dynamics and functions of individual proteins, the situation differs significantly in the case of protein-protein complexes while protein-protein interactions that result in either transient or long-lived complexes play a fundamental role in many biological functions and sometimes also result in diseases. Understanding the mechanisms of protein-protein binding is inherently difficult due to the range of potential interactions, the molecular rearrangement associated with binding, and the time and length scales involved. Physicalchemical and structural studies of molecular recognition between

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*Corresponding author

Valery Veresov, Department of Biophysics and Engineering of Cell, Institute of Biophysics and Cell Engineering of NAS of Belarus, Ackademicheskaya Str. 27, Minsk, Belarus, Tel: 375-172842252; Fax: 375172842359: Email: veresov@ibp.org.by

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proteins are essential to understand protein function and hence life processes. Given the experimental difficulties for having information on protein-protein interactions at atomic level, computational docking is increasingly used as a complementary tool to predict the structure of a specific complex formed by two given interacting proteins. Although in recent years the field has experienced a rapid improvement, major challenges remain, such as the treatment of flexibility and reliable scoring (see recent reviews on protein-protein docking [1-8]). Most of the available docking methods treat the interacting proteins as rigid bodies during the whole process, or at least in a first stage. The majority of rigid-body docking methods are based on an exhaustive sampling of the rotational and translational space in search for geometric surface correlation mainly through FFT (Fast Fourier Transform), spherical polar Fourier, or geometric hashing algorithms. Although the precise prediction of the structure of protein-protein complex is sufficient to describe the interaction in atomic details, it is the knowledge of affinity of the components for each other is necessary to predict whether the assembly actually exists under a given condition of temperature, pH, and

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protein concentration, and whether it is transient or permanent. Even if docking methods are highly promising tools for modeling protein-protein complexes, they do not yet allow a reliable estimation of the binding affinity of the complex and successful docking procedures often give equally good scores for proteins that do not interact experimentally [9]. Therefore, the design of ideal computational tools for protein-protein complex modeling that would also predict the binding affinity of a complex is one of the challenges in structural bioinformatics. Such computational tools would open the route to *in silico*, large-scale annotation and prediction of complete interactomes.

The process of protein-protein complex formation comprises at least two steps [10]. During an initial diffusive approach possibly guided by electrostatic interactions the proteins encounter each other many times before an intermediate loosely bound state near the native binding geometry is reached [10] (also termed 'encounter complex'). Upon formation of additional interactions and conformational changes at the interface regions the partners form the native complex with often (but not always) a high steric interface complementarity. The nature of the encounter state has been elusive for a long time, due to a lack of experimental methods to probe it [11]. Recently, new tools to study this preliminary step in complex formation have been reported that changed the view of this state and of proceeding from the encounter complex towards the final structure. The major progress is due to the application of NMR paramagnetic relaxation enhancement (PRE), a technique that is exquisitely sensitive to the presence of lowly populated states in the fast exchange regime [12-14].

Recently, a structure-based affinity benchmark of Kastritis *et al* (version 1.0), which includes a non redundant set of 144 protein–protein complexes that have high-resolution structures available for both the complexes and their unbound components, and for which dissociation constants have been measured by biophysical methods [15], has been presented and very recently its updates by Vreven *et al.* (structure-based affinity benchmark version 2.0) has been reported [16]. Because for all these complexes the unbound structures of component proteins are available, these two benchmarks allow for the assessment of conformational changes upon protein-protein binding. We use the data from these benchmarks together with other experimental and theoretical data to gain structural insights into failures and successes in a structure and affinity prediction by *in silico* docking.

Protein-protein association. What rigid-body docking models describe?

The structure of a protein complex together with information about its affinity and other thermodynamic characteristics provide a "frozen" view of the complex and ignores the kinetic nature of protein-protein association. Since all interactions are of relatively short range, proteins, before their association, diffuse randomly in solution performing Brownian motion until they reach an area, known as "the steering region" or "longrange electrostatic steering region", where mutual electrostatic attraction leads them to a 'macrocollision', resulting sometimes in the formation of a specific low-free energy encounter complexes

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[10,17-19]. The nature of these encounter complexes has been a matter of considerable research and debates [11, 20]. However, the encounter complex structures and configurations were elusive over many years to conventional structural and biophysical methods because their populations are low, their lifetimes are short, and they are difficult to trap. Considerable progress has been achieved with application of novel experimental and improved computational methods, developed during the last decade. One of the most promising approaches for studying the formation of encounter complexes is 'paramagnetic relaxation enhancement' (PRE) [12-14]. In this technique certain areas in one of the proteins are labeled with magnetic particles, which produce signals when the two proteins are close to each other. Repeating the measurement several times with the magnetic particles in different positions provides information about the overall structure of the complex. Computational modeling can then be used to work out the fine details of the structure, including the shapes of the intermediate structures made by the proteins as they interact.

Encounter complexes are best described as low free energy ensembles of states that are close to the final complex and in which the two molecules can rotationally diffuse along each other, or participate in a series of 'microcollisions' that properly accommodate the reactive surface groups (Figure 1) [11,20]. The encounter complexes are stabilized by a combination of electrostatic forces and desolvation, and are destabilized by unfavorable entropy. Specific short-range interactions do not seem to play an important role at this stage [10]. The formation of the final complex from the encounter complex requires desolvation and structural rearrangement of the side chains, thus lowering further the free energy of interaction (Figure 1). The macrocollisions that result in the formation of encounter complex seem at first glance as not requiring interaction forces between the macromolecules. However, the lifetime of such a macrocollision will often not be sufficient for a macromolecule to find a small binding site on the partner and the fraction of macrocollisions that results in a productive complex will be small. Electrostatic interactions have long been recognized as the dominant factor used by Nature in order to enhance protein association beyond



Figure 1 Successive steps in a possible mechanism for protein–protein association, compatible with PRE data. A:B_{ES} stands for ensembles of encounter complexes between proteins A and B. Formation of transient encounter complexes is executed by nonspecific collisions, guided mostly by electrostatic interactions. Many encounter complexes separate rapidly. Some productive encounter complexes (A:B_{ES,prod}) reorient and come closer to the final, specific orientation with final fitting of interacting surfaces (AB), guided mostly by desolvation, as water molecules move away from the protein surfaces.

the basal rates dictated by diffusion [10]. In the diffusional search, it is well known from kinetics studies that components that have charge complementarity and a strong dipole associate much faster than would be expected from random diffusional encounters. This is most readily visualized simply as a 'funnel' in the spatial and orientational degrees of freedom, directing the incoming component towards the interface that will bind it and orientating it correctly. The association of a pair of proteins can be described as a two-step reaction:

$$A + B \underset{k_{-1}}{\overset{\leftarrow}{\rightarrow}} A : B \underset{k_{-2}}{\overset{\rightarrow}{\rightarrow}} AB$$

Where A and B are the free proteins, A:B is the encounter complex, and AB is the final complex. According to this scheme, the first step includes Brownian diffusion, macrocolision and the formation of the encounter complex (complexes). The second step of association consists of conformational rearrangements to the final complex. The macroscopic rate constant for formation of the productive complex is $k_{on}=k_1k_2/(k_1+k_2)$, and for dissociation, $k_{off}=k_1k_2/(k_1+k_2)$ [10]. In (Figure 2) energy diagrams are shown to illustrate various possibilities. The encounter complex is often thought of as an ensemble of conformationally diffuse along each other, or participate in a series of 'microcollisions' that properly accommodate the reactive surface groups.

It is well known that, for most protein a global search via rigid docking gives low energy structures in several regions of the conformational space, some of which are far from the structure of the native complex. Physics-based energy functions are expected yet to be globally valid for modeling interactions between proteins, including the non-native states. Thus, one can assume that the rigid-body solutions with the energy values that are low relative to the average energy but still exceed the energy at the global minimum may represent relatively short-lived encounter complexes along the association pathways. Recently, exhaustive sampling of the conformational space in proteinprotein association using a physics-based energy function has



Figure 2 Energy diagrams. A: Final complex which is reached without favorable electrostatic forces and without formation of transient (encounter) complexes between the proteins. B: Final complex formed due electrostatic attraction without formation of encounter complexes; C: Proceeding to a stable final complex (AB) through formation of encounter complex (A:B); D: Like C, with a high-energy barrier between encounter complex and the final complex. The proteins can get trapped in a non-productive local energy minimum (non-productive encounter complex).

been carried out [21]. The study has shown the agreement between experimental PRE data and theoretical PRE profiles calculated from the ensemble of structures generated by docking thus confirming the hypothesis that the structures of encounter complexes can be obtained simply as byproducts of docking. While this result seems not unexpected, in view of the limited structural information available on encounter complexes it is of great practical significance.

Predicting protein-protein affinity

Protein docking is generally applied to individual pairs of proteins that are known to interact, to model the threedimensional structure of the complexes they form. Docking programs usually comprise two standard steps: generation of thousands of alternative poses to sample all possible interaction modes, followed by scoring these poses using a 'pseudo-energy' function. A set of solutions is generated by the first step, containing often near-native solutions, but scoring functions often fail to rank them properly and pick out truly best poses [9]. Yet, the potential to use protein docking algorithms to infer protein-protein binding affinities has long been used and a number of algorithms have been proposed to relate the binding affinity of two proteins to the structure of their complex. These algorithms vary dramatically from one another in terms of their physical relevance, accuracy and computational cost. Exact methods such as free energy perturbation and thermodynamic integration [22], and free energy pathway methods such as the linear interaction energy (LIE) and molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) methods [23] are in principle highly accurate. However, because they use extensive molecular dynamics or Monte Carlo simulations their application is extremely limited and is usually only applicable where the bound and unbound states have significant overlap. Empirical energy functions are much faster, and either take into account the physical chemical properties of the interface between subunits, and assess the free energy of their interaction, or they use statistical potentials, or they rely on empirical scoring functions developed for protein-protein docking and use linear regression to obtain a weighted function of physical or knowledge-based terms, although machine-learning algorithms have been applied as well [24-30]. Such models can also use biological information on the conservation of the protein sequence or the effect of point mutations. Horton and Lewis obtained a high correlation coefficient of 0.96 with experimental measurements, using only three terms in a linear combination [24]. Later works using different methods and datasets also reported high correlation coefficients, ranging up to 0.95 values [25-30]. However, as outlined in several recent papers, these high accuracy predictions are due to limitations in the data sets used for training and testing the algorithms [9]. When a large number of approaches were tested on a larger and more reliable dataset no correlations higher than 0.53 was observed. A community-wide effort on the computational discrimination between binding and nonbinding protein pairs highlighted the critical role that the dataset plays for a balanced assessment of the methods [9,31]. Docking algorithms compute the structure of protein complexes, so in principle, they should be able to distinguish pairs of proteins by their binding affinities. However, given their poor performance in ranking correct conformations, it has widely been thought

that docking programs are not yet accurate enough to predict binding affinities [9]. The main molecular origin why current models are limited in predicting binding affinities is thought to be a conformational flexibility of binding proteins [32].

In recent years several new affinity prediction algorithms have been proposed. The pyDock [33] uses a simple approach to scoring of rigid-body docking poses, which is based on Coulombic electrostatics with distance dependent dielectric constant, and implicit desolvation energy with atomic solvation parameters previously adjusted for rigid-body protein-protein docking. This scoring function is not highly dependent on specific geometry of the docking poses and therefore can be used in rigid-body docking sets generated by a variety of methods. The protocol has been tested in a large benchmark set of 80 unbound docking cases. The method was able to detect a near-native solution from 12,000 docking poses and place it within the 100 lowest-energy docking solutions in 56% of the cases, in a completely unrestricted manner and without any other additional information. More specifically, a near-native solution will lie within the top 20 solutions in 37% of the cases.

The original program ZDOCK performed a grid-based docking search using Fast Fourier Transform (FFT), and its scoring includes desolvation, electrostatics, and a shape complementarity function. The current ZDOCK approach [34] executes efficient global search with rescoring and refinement, by combining the tools ZDOCK, ZRANK and RosettaDock. The combination of these techniques has led to increased success on a docking benchmark and suggests that this is a promising avenue for further improving protein complex prediction success.

The SIPPER [35] (scoring by intermolecular pairwise propensities of exposed residues) scoring uses statistical potentials extracted from intermolecular pairs of exposed residues in known complex structures, which were then used to score protein-protein docking poses. SIPPER combines the value of residue desolvation based on solvent-exposed area with the propensity-based contribution of intermolecular residue pairs. This scoring function has found a near-native orientation within the top 10 predictions in nearly one-third of the cases of a standard docking benchmark and proved to be also useful as a filtering step, drastically reducing the number of docking candidates needed by energy-based methods like pyDock.

Moal *et al* have proposed a QSAR-like approach MARS (Multivariate Adaptive Regression Splines) [36] based on a large set of molecular descriptors using commonly available tools, introducing the use of energetic factors associated with conformational changes and disorder to order transitions, as well as features calculated on structural ensembles. The descriptors were used to train and test a binding free energy model using a consensus of four machine learning algorithms, whose performance constitutes a significant improvement over the other state of the art empirical free energy functions tested.

ZAPP (Zlab Affinity for Protein–Protein interaction) predicts protein–protein binding free energies using a linear combination of nine energy terms and a constant [37]. Only one term uses the unbound structures in addition to the complex structures, while the other eight terms only require the complex structure. SPA-PP (specificity and affinity of the protein–protein interactions) has been developed by incorporating both the specificity and affinity into the optimization strategy [38]. The authors of the method report testing results and comparisons with other scoring functions showing fine performance of the method on both predictions of binding poses and affinity.

Zhou et al have used a biomacromolecular QSAR (Bio QSAR) scheme with 101 structural descriptors categorized into five groups to extract abundant information from a proteinprotein interface: (1) constitutional descriptors (such as the numbers of different amino acids, groups and atoms present at the interface, the ratio of the residues at the interface to all residues in the complex, the quantity of hotspot residues, etc.) (2) Contacting descriptors (such as atomic contact vectors, residue pair numbers, residue interaction indices, empirical contact potentials, etc.) (3) Geometrical descriptors (such as the accessible surface area, interfacial connectivity index, interface volume, etc.) (4) physicochemical descriptors (such as electrostatic potential, interfacial polarity, molar refractivity, hydrophilicity/hydrophilicity, etc.) and (5) non bonded descriptors(such as the number of hydrogen bonds, hydrophobic forces, salt bridges, water-mediated hydrogen bonds, etc.) [39]. Three sophisticated regression methods, i.e. partial least squares (PLS), support-vector machine (SVM) and Gaussian process (GP), were employed to establish the linear and nonlinear correlations between the interface descriptors and binding affinity of the 144 studied protein complexes from the structure affinity benchmark [15]. The modeling performance and predictive power of Bio QSAR were reported to be comparable to or even better than that of traditional knowledge-based strategies, mechanismtype methods and empirical scoring algorithms, while Bio QSAR possesses certain additional features compared to the traditional methods, such as adaptability, interpretability, deep-validation and high-efficiency

The minimal affinity model of Janin takes into account only two structural features of the complex, the size of its interface, and the amplitude of the conformation change between the free and bound subunits [40].

Comparison of the Pearson coefficient R among several methods, where the same data set [15] was used is given in (Table 1).

Luo *et al* have carried out a systematic physico-chemical and conformational studies with interfaces involved in different PPIs, including crystal packing, weak transient heterodimers, weak transient homodimers, strong transient heterodimers and homodimers. The comparative analysis has shown that the interfaces tend to be larger, less planar, and more tightly packed with the increase of the interaction strength. Meanwhile the strong interactions undergo greater conformational changes than the weak ones involving main chains as well as side chains. Finally, using 18 features derived from such an analysis, a support vector regression model was developed to predict the binding affinity [41].

SolveBind is a binding affinity prediction method based on the global surface model of *Kastritis et al.* [42], using information on the number of atoms in the interface (NAtomsINT) and the

Table	1:	Comparison	of	the	Pearson	coefficient	R	among	various
methods, where the same data set [15] is used.									

Method	R	Feature	Ref.	
ZAPP	0.63	Regression with nine terms	[37]	
MARS	0.52	Machine learning	[36]	
BIOQSAR	0.82	Machine learning	[39]	
SPA-PP	0.39	Statistical potential	[38]	
ROSETTADOCK	0.42	Regression with nine terms	[53]	
DFIRE	0.35	Statistical potential	[52]	
Minimalistic Solvation-based Predictor	0.48	Solvation	[43]	
Minimal Model of Janin	0.63 for small I_rmsd	BSA and I_rmsd	[40]	

I_rmsd stands for Interface Root- Mean- Square - Deviation

percentages of charged and polar residues in the non-interacting surface (%AAcharNIS and %AApolNIS). Properties of the non-interacting surface were found to correlate with affinity [42] and may regulate solvation and electrostatic contributions to binding affinity [42].

Shakhnovich *et al* have proposed a minimalistic solvationbased model for predicting protein binding energy using the solvation factor described by a simple linear combination of buried surface areas according to amino-acid types [43]. The authors report that their minimalistic model demonstrates a predictive power comparable to more complex methods, making the proposed approach the basis for high throughput applications.

Moal and Fernandez-Recio [44] have used atomic and residue contact potentials derived directly from experimental binding free energy changes following mutation. The first set of potentials is obtained by unweighted least-squares fitting and bootstrap aggregating. The second set is calculated using a weighting scheme optimized against absolute binding affinity data, so as to account for the overrepresentation of certain complexes, residues, and families of interactions. More recently Fernandez-Recio and coworkers proposed a simplified model ('surface energy model') also parameterized using empirical changes in free energy upon mutations [45]. The model correlates well with empirical binding free energies of a functionally diverse set of rigid-body interactions (r = 0.66). When used to rerank docking poses, it could place near-native solutions in the top 10 for 37% of the complexes evaluated, and 82% in the top 100. The method shows that hydrophobic burial is the driving force for protein association, accounting for 50-95% of the cohesive energy

FireDock [46] is an efficient method for the refinement and rescoring of rigid-body docking solutions. The refinement process consists of two main steps: (1) rearrangement of the interface side-chains and (2) adjustment of the relative orientation of the molecules. The method accounts for the observation that most interface residues that are important in recognition and binding do not change their conformation significantly upon complex formation. While allowing full side-chain flexibility is a common procedure in many refinement methods, often causing excessive conformational changes and distorting preformed structural patterns, which have been shown to be important for binding recognition, FireDock restricts side-chain movements, and thus manage to reduce the false-positive rate noticeably. In the later stages of FireDock procedure (orientation adjustments and scoring) it smooths the atomic radii. This allows for the minor backbone and side-chain movements and increases the sensitivity of the algorithm. FireDock succeeded in ranking a near-native structure within the top 15 predictions for 83% of the 30 enzyme-inhibitor test cases, and for 78% of the 18 semi unbound antibody-antigen complexes.

Very recently Vangone and Bonvin have demonstrated that the network of inter-residue contacts (IRCs) between two interacting proteins is a good descriptor for the binding affinity [47]. Using the structure-based binding affinity benchmark of Kastritis et al. [15] and removing the cases with ambiguity in the exact Kd values and all complexes with missing or unresolved residues (>2) at the interface it has been observed a good correlation between IRCs and experimentally determined binding affinity data (Kd or ΔG) for protein complexes (bound forms). The most relevant contributions to BA are the number of IRCs made by charged and polar residues (IRCs_charged/charged and IRCs_ polar/polar), while the apolar residues are only counted when interacting with charged and polar ones (IRCs_charged/apolar and IRCs_polar/apolar). A model combining IRCs contribution and that of non-interacting surfaces (IRCs/NIS-based model) has been reported to possess the best performance of any model developed so far, with R=-0.73 and RMSE=1.89 kcal mol⁻¹ [47]. The correlation between the number of interfacial contacts and binding affinity is consistent both with the previously reported evidence that interfaces with a significant interaction strength are large and tightly packed [48] and with the simple BSA models introduced by Chothia and Janin [49] and Horton and Lewis [24]. Although BSA and the number of contacts at the interface are of course somewhat related, Vangone and Bonvin have shown that the number of interface contacts shows much better correlations with binding strength than the BSA. The comparison of the performance of 16 different predictors given in this work is presented in (Figure 3).

Recently Erijman et, basing on the same benchmark of Kastritis *et al* [15], have carried out the exploration how different biophysical features derived from a protein-protein structure correlate with protein-protein binding affinity [55]. The features that showed the highest correlation with binding affinity were the total number of H bonds, geometric complementarity measured by the van der Waals energy, and side chain conformational changes for high-resolution X-ray structures [55].

Benchmarking RosettaDock with the last structureaffinity Benchmark 1.0 of Kastritis et al [15]

Many protein docking algorithms are divided into several steps: the initial global search and subsequent steps to improve these initial predictions. The global search is a full search of the orientations of the two proteins, typically keeping the larger protein (referred to as the receptor) fixed, while moving the smaller protein (the ligand). This is often a rigid-body search



Figure 3 Comparison of the performance of different predictor models reported by Vangone and Bonvin [47], Moal *et al* [36]., and the CCHarPPI web server [50]. The performance is expressed as Pearson's Correlation coefficient between experimental and predicted binding affinities. Predictions were made on the common set of 79 complexes. The considered models include: (1) the IRCs/NIS model of Vangone and Bonvin [47]; (2) the 'global surface model' of Kastritis *et al* [42]. (3) The BSA-based model of Horton and Lewis [24]; (4-6) the top three best performing models reported by Moal *et al* [36]. (their consensus model, DFIRE [51] and PMF [29]); the models based on composite scoring functions reported by the CCHarPPI web server [50], such as (7) ZRANK [34]; (8) ZRANK2 [34]; (9) RosettaDock [52]; (10) PyDock [33]; (11) SIPPER [35]; (12) PISA [53]; (13-15) FireDock, FireDock _AB and FireDock_EI, respectively [46]; (16) PIE [54].

in six dimensions, utilizing a fast Fourier transform (FFT) for efficiency and softness for small overlaps [32]. Subsequently, one or more refinement and scoring steps of a set of preselected rigid docking solutions are added to achieve closer agreement with the native geometry and to recognize near-native docking solutions preferentially either as the best or among the best scoring complexes. The accuracy and speed of flexible refinement and rescoring of preselected docked protein structures are important for the success of the multistage docking protocol. A rescoring of docking decoys using more elaborate energy functions and filters has been shown to improve the selection of models [32]. Several strategies for scoring and structural refinement of docked complexes have been developed [56-67]. In earlier works structural flexibility was treated by soft potentials [56], multicopy representations of side chains [57], or flexible loops [58,59], by using conjugate gradient minimization [60] followed by clustering to identify near-native structures. In an approach to refine a large number of complexes obtained by the FFT-based ZDOCK program [68] a combination of several descriptors showed significant improvement [65]. Often a single descriptor (e.g. surface complementarity) or a single binding energy component (e.g. vdWaals or electrostatic energy) is insufficient to distinguish near-native and non-native complexes. A combination of different surface and interface descriptors was found to be able to enrich the number of near-native solutions in the pool of best scoring docking solutions [36,47,55,65]. The increase in the number of experimentally solved protein-protein complex structures in recent years allows extraction of more data on the statistical residue and atom contact preferences at protein-protein interfaces. New effective knowledge-based scoring functions have been developed that are based on contact preferences of amino acids at known interfaces compared to interfaces of non-native decoy complexes [47, 64,65]. An iterative approach has been designed for the derivation of knowledge-based scoring functions that optimally distinguish between native binding modes and possible decoy poses [66]. The resulting distance dependent pair-potentials significantly improved scoring of near-native complexes for bound and unbound docking.

One more possibility to directly use computationally rapid rigid docking algorithms is to indirectly account for receptor flexibility by representing the receptor target as an ensemble of structures. The structural ensemble can, for example, be a set of structures obtained experimentally (e.g. from nuclear magnetic resonance (NMR) spectroscopy) or can be formed by several structural models of a protein. Docking to an ensemble increases the computational demand and due to the large number of protein conformations may also increase the number of false positive docking solutions. A variety of ensemble-based approaches have been developed in recent years in the field of small-molecule docking (reviewed in [70]). Cross docking to ensembles from MD simulations have also been used to implicitly account for conformational flexibility in protein docking [71]. Mustard and Ritchie generated protein structures deformed along directions compatible with a set of distance constraints reflecting largescale sterically allowed deformations [72]. Subsequently, the structures were used in rigid body docking searches to identify putative complex structures. Conformer selection and induced-fit mechanism of protein-protein association have been compared by ensemble docking methods using the RosettaDock approach [73] that allows side chain and limited backbone relaxation during docking refinement [74]. The method was able to successfully select binding-competent conformers out of the ensemble based on favorable interaction energy with the binding partner [73].

In a recent paper of *Kozakov et al* it has been shown using Principal Component Analysis that the energy landscape of 42 interacting proteins, at least within the 10 Å IRMSD

neighborhood of the native state, always includes a permissive subspace ('tunnel') along which the conformation of the complex can substantially change without crossing significant energy barriers and that the energy landscape is smooth funnel in a two dimensional permissive subspace [21]. In all cases this subspace captured at least 75% of the total motion as the two molecules approach the native state. For each of the 42 complexes a high energy subspace has been detected, which reduces the dimensionality of the space available to encounter complexes along the association pathways. This suggests that methods such as molecular dynamics (MD) or Monte Carlo (MC) simulations that start from productive encounter complexes should fairly quickly converge to native structures (or near-native ones because of some inaccuracy of scoring functions) making these strategies as promising tools of the efficient refinement. The Monte Carlo approach is especially attractive as being much less computationally expensive as compared with MD. Instead of screening all possible conformations with a Fouriertransformable energy function, random starting decoys are refined by applying random translational and rotational moves and deciding on their acceptance using the Metropolis criterion [75,76]. While FFT methods have the advantage of great speed and complete sampling of the conformational space, Monte Carlo methods are able to generate more physical decoy distributions, can involve arbitrary energy functions, and might allow for structural flexibility.

Several docking protocols including rigid-body moves and Monte Carlo refinement have been proposed [75-80]. In ICM-DISCO [77], the simulations start with a rigid-body docking searched by a pseudo-Brownian Monte Carlo simulation. The second step is the Monte Carlo refinement of ligand side-chain torsion angles. In RosettaDock [75], the simulations start from random ligandreceptor orientations, followed by a low-resolution rigid-body docking. In a second step, RosettaDock optimizes side chains and rigid-body orientations simultaneously, based on the simulated annealing Monte Carlo simulation. In both approaches, the rigidbody docking is performed by Monte Carlo searches. In the first stage of RosettaDock the proteins are represented as backbones plus side-chain centroids, and the search is guided by a residuescale interaction potential. Benefiting from the simplified protein representation, the method was later extended to account for loop flexibility [80]. Lorenzen and Zhang [78] refined initial docking estimates of protein complex structures, generated by an FFT-based method, using a Monte Carlo approach including rigid body moves and side-chain optimization. During the simulation they gradually shifted from a smoothed van der Waals potential, which prevented trapping in local energy minima, to the standard Lennard-Jones potential. Following the simulation, the conformations were clustered to obtain the final predictions. The refinement procedure was able to generate near-native structures (interface rmsd<2.5 Å) as first model in 14 of 59 cases in the benchmark set. Pierce and Weng [79] refined global docking predictions from ZDOCK using RosettaDock, and selected the best models based on their ZRANK score. Refining docking benchmark predictions from ZDOCK led to improved structures of top ranked hits in 20 of 27 cases, and an increase from 23 to 27 cases with hits in the top 20 predictions. In addition, the ZRANK energy function was optimized using the refined models. With the new energy function, the numbers of cases with hits ranked at number one increased from 12 to 19 and from 7 to 15 for two different ZDOCK versions. These results show that combinations of independently developed docking protocols (ZDOCK/ZRANK and RosettaDock) can substantially improve protein-docking results.

Several studies show convincingly that the accuracy and reliability of docking results can be often significantly improved by combining different classes of methods. For example, *Kozakov et al* have studied the 30 clusters generated by FFT-based docking by starting RosettaDock runs from random points around the cluster centers, and observing whether a certain fraction of trajectories converge to a small region within the cluster [81]. A cluster was considered stable if such a strong attractor existed and contained a low-energy structure. It was shown that all clusters close to the native structure are stable, and that restricting considerations to stable clusters eliminate around half of the false positives. More generally, improving model accuracy using Monte Carlo methods enables the use of potentials that are more accurate, but also more sensitive to structural errors.

The analysis of docking predictions by the community-wide experiment on the Critical Assessment of Predicted Interactions (CAPRI) shows that Monte Carlo based methods can yield highly accurate models, but the search is restricted to a neighborhood of the starting structures [31]. In principle, a fairly accurate structure of the complex contains information about binding energy/affinity and a number of structural features have been shown to correlate with affinity [47]. We asked whether it is possible to predict affinity by rigid-body approaches followed by one or several RosettaDock refinements if productive encounter complex structure is known. The Version 1 structure-affinity Protein Docking Benchmark of four laboratories [15], which is a nonredundant set of 144 protein-protein complexes that have high-resolution structures available for both the complexes and their unbound components, and for which dissociation constants have been measured by biophysical methods, and updates by Vreven et al [16] was used to assess the performance of RosettaDock refinements. The set is diverse in terms of the biological functions it represents, with complexes that involve G-proteins and receptor extracellular domains, as well as antigen/ antibody, enzyme/inhibitor, and enzyme/substrate complexes. It is also diverse in terms of the partners' affinity for each other, with Kd ranging between 10⁻⁵ and 10⁻¹⁴ M. Nine pairs of entries represent closely related complexes that have a similar structure, but a very different affinity, each pair comprising a cognate and a noncognate assembly. For sake of simplicity we considered only the cases either of small conformational changes (I_rmsd <0.6Å) or large ones (I_rmsd >2.5 Å).

With the aim of assessment the performance of RosettaDock refinements the unbound structures were superimposed over the bound complex and the resulting superposed structure was used as the starting structure for local docking. We first prepared each docking partner in isolation, optimizing their side-chain conformations prior to docking using docking_local_refine option. The same procedure was applied to the structures of complexes. -11.782 For the assessment of RosettaDock performance we used the convergence of the starting structures to the structure

of the bound complex. To do that we calculated the number of refinement runs resulting in convergence (NRDcnv). We judged the RosettaDock refinements as convergent if I_rms deviation from the bound state was <0.5Å and the distinct funnel took place. If such convergence was not reached during 10 refinement runs and the deviation from the bound state remained stable during last five steps the refinement process was judged as nonconvergent. Our results also show a good correlation between binding affinity and interface energy score values (I_sc), which are the total score differences between the components together and the components pulled far apart from each other without their relaxation (repacking). While in our recent paper [82] we used the RosettaDock binding score (RDBS), which is the total score difference between the components together and the components pulled far apart after their relaxation (repacking), to rank different docking solutions by affinities, affinity prediction based on I_sc values seems to be more convenient because I_sc calculations are free from the problems associated with global minimization of pulled apart individual components; these problems are especially grave when membrane proteins or protein complexes are docked. The results of analysis of RosettaDock refinements performance are given in (Table 2). It is seen from the Table that the convergence was not achieved for five complexes that display large conformation changes, and also for one complex with small conformational changes.

In the initial global search some unspecific sterical overlap between docking partners is typically tolerated to implicitly account for conformational adjustment of binding partners. For the success of a multistep docking strategy it is necessary that the set of selected rigidly docked structures would contain solutions sufficiently close to the native structure in order to allow for further improvement during the refinement process. Overall, good discrimination of near-native docking poses from the very early stages of rigid-body protein docking is essential step before applying more costly interface refinement to the correct docking solutions. Hence, the initial scoring has to be powerful enough to recognize and preselect a binding mode sufficiently close to the native position and it has to simultaneously tolerate possible inaccuracies, such as atomic overlaps at the interface. An ideal scoring function should recognize favorable native contacts as found in the bound complex and discriminate those from nonnative contacts with lower scores. However, even a best possible arrangement of rigid unbound partner structures (superimposed on the corresponding bound structures in the native complex) can result in small number of native contacts in case of significant backbone and side changes at the interface [5]. This indicates that in case of significant backbone and side changes at the interface the contacting residue or atom pairs of rigidly docked proteins may show little correspondence to the native interface structure. Thus, near-native poses might be overlooked during the rigid docking search even with a powerful scoring function. Recently,

Table 2: Performance of Rosetta Dock refinements on 25 Version 1 Structure-affinity Benchmark [15] complexes.									
ref ^(a)	Complex PDB	Unbound component_1 PDB	Unbound component_1 PDB	Kd (M)	I_rmsd [7]	I_sc bound and funnel (f) presence	nRDcnv ^(b)	I_sc unbound superimposed on bound after Ncnvb refinement runs and funnel (f) presence	
7	1AVX_A:B	1QQU_A	1BA7_B	4.8E-10	0.47	-10.3 (f)	3	-8.2 (f)	
8	1AVZ_B:C	1AVV_A	1FYN_A	1.6E-05	0.73	-7.0 (f)	3	-6.2(f)	
9	1AY7_A:B	1RGH_B	1A19_B	2.0E-10	0.54	-7.4 (mf)	8	-6.5(mf)	
12	1BRS_A:D	1A2P_A	1A19_B	2.0E-13	0.42	-12.4(f)	4	-8.9(f)	
13	1BUH_A:B	1HCL_A	1DKS_A	7.7E-08	0.75	-6.9(mf)	6	-7.5(f)	
15	1BVN_P:T	1PIG_A	1HOE_A	9.2E-12	0.87	-12.5(f)	2	-8.0(f)	
23	1E96_A:B	1MH1_A	1HH8_A	2.7E-06	0.71	-7.5(mf)	7	-6.6(mf)	
24	1EAW_A:B	1EAX_A	9PTI_A	5.0E-11	0.54	-6.4(mf)	2	-6.6(mf)	
26	1EFN_B:A	1AVV_A	1FYN_A	3.8E-08	0.90	-7.8(mf)	5	-5.8(f)	
28	1EWY_A:C	1GJR_A	1CZP_A	3.6E-06	0.80	-7.5(mf)	5	-5.2(mf)	
30	1F34_A:B	4PEP_A	1F32_A	1.0E-10	0.93	-8.6(f)	3	-6.3(f)	
31 ^(f)	1F6M_A:C	1CL0_A	2TIR_A	2.7E-06	4.90	-6.1(bf)	Ncnv10		
35	1FQJ_A:B	1TND_A	1FQI_A	6.7E-08	0.91	-10.2(f)	4	-6.7(f)	
37	1GCQ_B:C	1GRI_B	1GCP_B	1.7E-05	0.92	-8.3(f)	7	-5.8(f)	
40	1GPW_A:B	1THF_D	1K9V_F	5.0E-9	0.65	-10.0(f)	4	-9.0(f)	
46	1HE8_B:A	821P_A	1E8Z_A	3.2E-06	0.92	-5.8(bf)	3	-6.0(f)	
48	1I2M_A:B	1QG4_A	1A12_A	2.5E-12	2.12	-13.4(f)	Ncnv10		

51	1IBR_A:B	1QG4_A	1F59_A	1.0E-09	2.54	-16.6(f)	Ncnv10	
54	1J2J_A:B	103Y_A	10XZ_A	1.1.E-6	0.63	-6.2(f)	7	-6.0(bf)
58	1JTG_B:A	3GMU_B	1ZG4_A	4.0E-10	0.49	-12.8(f)	4	-9.8(f)
64	1KTZ_A:B	1TGK_A	1M9Z_A	2.0E-07	0.39	-7.3(f)	3	-6.2(bf)
66	1KXQ_H:A	1KXQ_H	1PPI_A	3.5E-09	0.72	-9.3(f)	4	-7.1(f)
68	1M10_A:B	1AUQ_A	1M0Z_B	5.8E-09	2.10	-8.0(f)	3	-6.8(f)
69	1MAH_A:F	1J06_B	1FSC_A	2.5E-11	0.61	-8.3(f)	3	-6.7(f)
75	1NVU_R:S	1LF0_A	2II0_B	1.9E-06	3.09	-16.4 (f)	Ncnv10	
80	1PPE_E:I	2PTN_A	1LU0_A	3.0E-12	0.34	-10.8(f)	2	-6.5(f)
81	1PVH_A:B	1BQU_A	1EMR_A	8.0E-06	0.34	-7.2(f)	3	-6.9(f)
82	1PXV_A:C	1X9Y_A	1NYC_A	3.1E-10	2.63	-14.8 (mf ^(d))	7	-8.4 (f)
83	1QA9_A:B	1NHF_A	1CCZ_A	9.0E-06	0.73	-4.6(bf)	3	-4.9(mf)
84	1R0R_E:I	1SCN_E	2GKR_I	2.9E-11	0.45	-11.1(mf)	7	-6.0(f)
89	1T6B_X:Y	1ACC_A	1SHU_X	1.7E-10	0.62	-8.9(f)	7	-7.3(f)
91	1UUG_A:B	3EUG_A	2UGI_B	<1E-13	0.77	-10.9(f)	7	-6.9(f)
99	1YVB_A:I	2GHU_A	1CEW_I	6.5E-09	0.51	-9.8(f)	7	-8.1(f)
99	20UL_A:B	3BPF_A	2NNR_A	1.7E-09	0.53	-8.7(f)	4	-7.7(f)
100	1Z0K_A:B	2BME_A	1YZM_A	7.7E-06	0.53	-9.6(mf)	7	-7.3(f)
101	1ZHI_A:B	1M4Z_A	1Z1A_A	2.0E-07	0.68	-7.8(f)	5	-7.2(f)
102	1ZLI_A:B	1KWM_A	2JTO_A	1.3E-09	2.53	-13.8	Ncnv10	
103	1ZM4_A:B	1N0V_C	1XK9_A	1.3E-06	2.94	-6.7(bf)	4	-7.2(f)
104	2A9K_A:B	1U8Z_A	2C8B_X	6.0E-08	0.85	-11.6(f)	7	-7.2
106	2AJF_A:E	1R42_A	2GHV_E	1.6E-08	0.65	-5.8(bf)	9	-6.7(f)
108	2B42_A:B	2DCY_A	1T6E_X	1.1E-09	0.72	-14.5(f)	7	-9.1(f)
110	2BTF_A:P	1IJJ_B	1PNE_A	2.3E-06	0.75	-6.2(f)	5	-5.3(f)
111	2COL_A:B	1FCH_A	1C44_A	1.1E-07	2.62	-6.7	5	-7.0
113	2GOX_A:B	1C3D_A	2GOM_A	1.4E-09	0.60	-8.2(f)	4	-7.9(f)
118	219B_E:A	1YWH_A	219A_A	3.3E-10	3.79	-12.9(bf)	Ncnv10	-5.7(bf)
123	203B_A:B	1ZM8_A	1J57_A	3.2E-12	3.13	-7.5(f)	Ncnv10	-5.6(bf)
124	200B_A:B	200A_A	1VJ1_A	6.0E-05	0.85	-6.7(f)	8	-5.2(f)
127	2PCB_A:B	1CCP_A	1HRC_A	1.0E-05	0.45	-4.9(bf)	5	-5.1(f)
128	2PCC_A:B	1CCP_A	1YCC_A	1.6E-06	0.39	-4.9(bf)	3	-4.9(f)
129	2PTC_E:I	2PTN_A	9PTI_A	6.0E-14	0.28	-9.7 (f)	Ncnv10	-5.5 (f)
130	2SIC_E:I	1SUP_A	3SSI_A	1.7E-11	0.36	-12.9 (f)	2	-9.9(f)
131	2SNI_E:I	1UBN_A	2CI2_I	2.0E-12	0.35	-9.4 (f)	4	-8.3 (f)
132	2TGP_Z:I	1TGB_A	9PTI_A	2.4E-06	0.57	-8.9 (f)	5	-5.5 (f)
133	2UUY_A:B	2PTN_A	2UUX_A	5.6E-09	0.44	-8.9 (f)	5	-7.0 (f)
134	2VDB_A:B	3CX9_A	2J5Y_A	1.5E-10	0.47	-10.4(f)	3	-7.4 (f)
139	3SGB_E:I	2QA9_E	20V0_A	1.8E-11	0.36	-10.7(f)	5	-7.7

^(a) Stands for number in structure-affinity benchmark Version 1.0 [15]

^(b) Norvb stands for number of Rosetta Dock refinements runs resulting in I_rms- deviation from bound conformation < 0.5 Å ^(c) (f) denotes the presence of funnel

(d) (mf) stands for moderate funnel (e) (bf) stands for bad funnel

^(f) Complexes with I_rmsd >2.0 are presented in bold

the limitations of rigid docking strategies combined with a rescoring step have been systematically investigated by Pons et al., [83]. The authors applied a combination of rigid FFT-correlation based docking and rescoring using the pyDock approach. The protocol showed very good performance for most proteins that undergo minor conformational changes upon complex formation (<1A° Rmsd between unbound and bound structures) but unsatisfactory results for cases in which binding induced significant conformational changes or applications that involved homology modeled proteins. These data and considerations of precedent section suggest that if conformational changes upon binding are moderate and if the productive encounter complex is reliably determined by rigid-body global docking it is possible to predict affinity with a fairly good accuracy. One may expect that the use of several successive refinements taking into account flexibility of side chains may be a promising strategy to predict structure of protein-protein complex and assess affinity. This raises at least two questions: (i) how to determine that some preselected pose determined by rigid docking represents a nearnative productive encounter complex; and (ii) how to assess the conformational flexibility in the absence of experimental data on the structures of both unbound proteins and their complexes. As to the latter question it is appropriate either to use direct experimental data on protein flexibility of proteins, or use implicit reasons based on protein selectivity [84], or alternatively make theoretical estimates [5]. The protein selectivity is a promising feature because it has been determined for a broad number of proteins and strongly correlates with the protein rigidity upon protein-protein association.

The most difficult task is the establishment of the structure and affinity of a protein-protein complex when the transfer from encounter complex to final one is coupled with significant changes of backbone structure. Several current programs possess the capacity to take into account backbone flexibility. For example, in Fiber Dock, backbone mobility is modeled by an unlimited number of low and frequency normal modes [85], while in ATTRACT, it is modeled by the first few lowest frequency modes [86].

Classification of transient protein-protein interactions by RosettaDock I_sc scores

Nearly a decade ago, Nooren and Thornton have proposed a two-class classification of transient protein-protein interactions based on the lifetime (or stability) of the protein-protein complex [48]. In this classification weak transient interactions that feature a dynamic oligomeric equilibrium in solution, where the interaction is broken and formed continuously, and strong transient interactions that require a molecular trigger to shift the oligomeric equilibrium have been distinguished. More recently, another classification, based on the binding affinities, has been reported [9]. In accordance with this classification the binding affinities have been categorized into five classes: Very High $(K_d < 10^{-10}M)$, High $(10^{-10}M < K_d < 10^{-8}M)$, Medium $(10^{-8}M < K_d < 10^{-10}M)$ ⁶M), Low ($10^{-6}M < K_d < 10^{-5}M$), and Very Low ($K_d > 10^{-5}M$). Because in accordance with the classification of Nooren and Thornton and experimental data available (reviewed in [9]) Very High and Very Low affinities correspond to permanent binding and weak transient interactions, respectively, whereas the interactions of the remaining three classes can be assigned to Strong Transient ones as requiring a trigger for protein-protein complex dissociation, we have recently categorized all interactions into five classes (Permanent ($K_d < 10^{-10}$ M), High - Strong transient (10^{-10} M) 10 M< K_d<10⁻⁸M), Medium - Strong transient (10⁻⁸M< K_d<10⁻⁶M), Low - Strong transient ($10^{-6}M < K_d < 10^{-5}M$), and weak transient $(K_{a}>10^{-5}M)$ [82]. Although a good correlation between binding affinity and scores is absent for most computational docking algorithms, we used an approximate correlation between RosettaDock binding score (RDBS), which was calculated by subtracting the corresponding score of complexes from those of the individual chains, and binding affinity [9] to assign transient interactions to the above four transient interaction classes. RDBS values were used for the qualitative ranking between different complexes by affinities also because we have revealed that for a number of protein-protein complexes from the Protein-Protein Docking Benchmark 3.0 [88], affinity data of which have been measured with isothermal titration calorimetry , the interactions with (RDBS > -5), (-5> RDBS >-30), (-30> RDBS >-80), (-80> RDBS >-180) might be assigned, to a fairly good approximation, to Weak transient (K_d>10⁻⁵M), Low - Strong transient (10⁻⁶M< K_a<10⁻⁵M), Medium - Strong transient (10⁻ $^{8}M < K_{4} < 10^{-6}M$), and High - Strong transient ($10^{-10}M < K_{4} < 10^{-8}M$) interactions, respectively ('Unpublished results'). In this paper we propose a new five-class classification of transient proteinprotein interactions by RosettaDock I_sc values. We categorize all transient interactions into five classes (Very High-Strong transient (10⁻¹⁴M<K_d<10⁻¹⁰M), High -Strong transient (10⁻¹⁰M< $K_{a} < 10^{-8}$ M), Medium - Strong transient (10^{-8} M < $K_{a} < 10^{-6}$ M), Low -Strong transient ($10^{-6}M < K_d < 10^{-5}M$), and weak transient ($K_d > 10^{-5}M$) ⁵M)). The data given in (Table 2) support a quite satisfactory and fairly universal interrelation between RosettaDock interface energy score (I_sc) and the above five classes of transient interaction, whereby for a significant number of protein-protein complexes the interactions with (I_sc > -4), (-4> I_sc >-5.5), (-6.5> I_sc >-8.5), (-8.5> I_sc >-12), (-16<I_sc<-12) might be assigned, to a fairly good approximation, to Weak transient ($K_d > 10^{-5}M$), Low - Strong transient (10⁻⁶M< K_d<10⁻⁵M), Medium - Strong transient $(10^{-8}M < K_d < 10^{-6}M)$, High - Strong transient $(10^{-10}M < K_d < 10^{-8}M)$ and Very High-Strong transient interactions (10⁻¹⁴M<K_d<10⁻¹⁴M ¹⁰M), respectively ('unpublished results'). Such a correlation allows the approximate qualitative ranking between different complexes by affinities through their ranking by the RosettaDock interface energy score (I_sc). The chief merit of using I_sc over the RosettaDock binding score (RDBS) is that I_sc ranking is free from the problems associated with global minimization of pulled apart individual components; these problems are especially grave when membrane proteins or protein complexes are docked.

DISCUSSION & CONCLUSION

Protein– protein docking aims to predict the threedimensional structure of a complex from the knowledge of the structure of the individual proteins in aqueous solution. Many docking methods are now able to predict the three-dimensional structure of binary assemblies if the protein partners do not display important conformational changes between their bound and unbound forms. However, large-scale motion upon binding is still a major and unresolved problem in the absence of experimental constraints. This motion generally involves the

displacement or the internal rearrangement of loops or domains and can also be characterized by the simultaneous movement of several flexible parts. However, even though the motion upon binding is moderate protein docking often requires the effective usage of several steps to produce accurate predictions. The refinements using approaches sampling conformation space more thoroughly as compared with rigid docking methods, even though in 'semi flexible manner', such as this does Monte Carlo minimization method being applied to side chains in RosettaDock protocols [75], present efficient approach to produce accurate predictions of 3D-structure of the protein-protein complexes and affinity. However when the conformational changes upon protein binding are large there are no universally reliable tools to assure accurate predictions. In order to avoid futile attempts to dock flexible proteins using rigid methods, even though with semi flexible refinements, it is necessary to develop and use methods that can predict protein flexibility [89-91] and such approaches allowing the assessment of the flexibility should be used prior to docking.

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