Protein–protein association kinetics and protein docking

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Rigid body protein docking methods frequently yield false positive structures that have good surface complementarity, but are far from the native complex. The main reason for this is the uncertainty of the protein structures to be docked, including the positions of solvent-exposed sidechains. Substantial efforts have been devoted to finding near-native structures by rescoring the docked conformations and employing various filters. An alternative approach emulates the process of protein–protein association, that is, first finding the region in which binding is likely to occur and then refining the complex while allowing for flexibility.

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Abbreviations

CAPRI	Critical Assessment of Predicted Interactions
FFT	fast Fourier transform
rmsd	root mean square deviation

Introduction

Protein–protein interactions play a central role in various aspects of the structural and functional organization of the cell. Genome-wide proteomics studies, primarily yeast two-hybrid assays, provide an increasing list of interacting proteins, but only a small fraction of the potential complexes will be amenable to direct experimental analysis. Thus, it is important to develop both docking methods that can elucidate the details of specific interactions at the atomic level and computational tools that provide information on the kinetics of protein–protein association in various environments. Although this section of *Current Opinion in Structural Biology* includes reviews both on docking (Smith and Sternberg, this issue, pp 28–35) and on protein association kinetics (Schreiber, this issue, pp 41–47), we believe that understanding the process of protein binding also helps to develop more powerful docking algorithms and focus on the kinetics of protein–protein association and on docking approaches that emulate some elements of the binding process. Such algorithms first solve the recognition problem and find broad surface regions in which the interactions are most likely to occur, and then refine these broadly defined sites into high-affinity complex structures. Both the recognition and refinement steps may entail the challenge of going beyond the rigid view of protein structures, thereby avoiding some of the discrimination problems inherent to rigid body methods. In addition to recent publications, references will be made to papers that were presented at the conference *Modeling of Protein Interactions in Genomes* (16–19 June 2001, Charleston, South Carolina, USA; http://reco3.ams.sunysb.edu/conference/) and are now in press.

Rigid body docking with reranking of docked conformations

Current protein docking methods generally consist of a rigid body search that generates a large number of docked conformations with favorable surface complementarity, followed by the reranking of the conformations using a potential approximating the free energy. The most widely used rigid body search is based on the fast Fourier transform (FFT) correlation approach, introduced by Katchalski-Katzir and associates [1] in 1992. The approach provides an efficient way of systematically exploring the space of docked conformations, enabling one to perform large-scale docking studies [2], but restricts the potential to a correlation function form. In spite of this constraint, the original shape complementarity target function has been extended to include electrostatic interactions [3,4•] or both electrostatic and solvation terms [5]. A recent improvement of the method is the use of polar Fourier correlations that accelerate the search for candidate low-energy conformations [6•]. Other approaches to rigid body protein docking include the use of computer vision concepts [7], Boolean operations [8] and the genetic algorithm [9,10]. All rigid body procedures generally result in good (i.e. near-native) docked conformations when starting from co-crystallized protein structures; however, applications involving unbound (separately crystallized) proteins yield both near-native structures and many false positives that have good surface complementarity (and electrostatics if included in the potential), but are far from the native complex.

Substantial efforts have been devoted to developing methods that can rank the docked conformations and select ones close to the native complex, usually using a potential that accounts for the chemical activity between the molecules, and possibly refining the interacting surfaces [4•,11•,12]. The discrimination between near-native and other structures can be further improved by employing filters based on additional information, including interfacial statistics extracted from the analysis of known protein complexes [13–19,20•], predictions of functional sites either from sequence profiles [21] or using the evolutionary trace approach [22], empirical relationships approximating the binding free energy [23•] and additional structural data [24]. These procedures improve the discrimination such that conformations with less than 5 Å rmsd are generally found within the top 10–100 structures; however, no current method can eliminate all false positives and the highest-ranking docked conformations may be as far as 20 Å from the native complex. Thus, these methods have limited value as research tools when applied to unbound protein

structures, unless additional information is available to choose among the best candidates. Improving robustness is particularly important because docking methods will certainly be applied to both experimental structures and protein models (A Tovchigrechko, CA Wells, IA Vakser, personal communication).

Improving discrimination is far from trivial; for example, Norel *et al.* [19] found no correlations between the rmsd of docked conformations and properties such as buried nonpolar and polar surface areas, the number of unsatisfied buried charges and the number of hydrogen bonds in the interface. More recently, it was found that both the electrostatic interaction energy and residue preferences in the interface provide useful filters [20•]. These filters generally improve discrimination for complexes of enzymes with their inhibitors, but are less successful for antigen–antibody complexes [3,10,20•]. This is not surprising because the properties of the interface in protease–inhibitor and antibody–antigen complexes differ substantially in terms of amino acid composition, charge–charge interaction and flexibility [13,25,26]. More generally, it may be an oversimplification to expect that the same recognition rules will apply to all protein complexes, irrespective of their functions.

Binding mechanisms and sidechain effects

It has been established, both experimentally [27] and theoretically [28•,29], that the mechanism of protein binding is highly dependent on the particular system. For instance, it has been shown that, depending on the ion concentration, the association rate of fast binders ($k_{on} > 10^8\ M^{-1}s^{-1}$) can change by as much as five orders of magnitude [26,29,30], whereas the association rates of slow binders ($k_{on} < 10^7\ M^{-1}s^{-1}$) change by less than one order of magnitude [28•]. A rationalization of this behavior was first presented in an analysis of the free energy landscapes of association for four receptor–ligand systems [29]. It was shown that a fast binding process was consistent with a binding mechanism controlled by long-range electrostatic steering to the binding site, thus the strong dependence on ion concentration [30–35], whereas slow binders show little or no specific long-range steering [28•]. Instead, the latter correlate well with the shorter-range affinity for the correct binding region due to desolvation forces that are much less dependent on ion concentration than long-range electrostatics.

The differentiation between fast and slow binders is also important from the point of view of protein–protein docking. Indeed, experience tells us that it is easier to predict protein complexes in systems in which desolvation, mostly nonpolar, is important [11•]. Surface complementarity is often a good indicator in these complexes, because the native structure usually represents the state that maximizes the contact area between the molecules. Therefore, rigid body methods that optimize surface complementarity work relatively well for these systems. Complexes in which long-range electrostatic interactions play a dominant role are harder to predict. The main reason is that lysine and arginine sidechains, many of them poorly resolved in the X-ray structure of the unbound protein, may substantially differ between unbound and bound conformations. Thus, key sidechains may be in the wrong position when docking unbound proteins and hence electrostatic and hydrogen bonding interactions are frequently less favorable in near-native docked conformations than in structures that are far from the native complex, resulting in false positives.

The role of critical sidechains in protein binding cannot be underestimated. Indeed, one or two wrong sidechains are enough to completely blur the binding affinity between receptor and ligand. Although this at first might seem like a contradiction for successful *in vivo* binding, Kimura *et al.* [36•] have hinted that, in solution, some key sidechains might well be in their right conformers, such that when presented with their corresponding substrate, the chemical affinity would be able to stabilize the encounter complex at the binding region. The intrinsic deficiencies of crystal structures emphasize the need for methods that, before or parallel to docking, can refine the surfaces of interacting proteins, including sidechains, in order to improve receptor–ligand affinities. Although currently overshadowed by the focus on rigid body methods, the importance of adjusting the conformation of key sidechains during protein docking has been clearly recognized [37]. Docking with sidechain prediction was recently generalized by DM Lorber *et al.* (DM Lorber, MK Udo, BK Shoichet, personal communication), who searched through a precalculated sidechain rotamer library in every step of a rigid body docking algorithm. According to results presented at the *Modeling of Protein Interactions in Genomes* conference, the method avoids false positives better than the rigid body approaches and remains computationally feasible.

Relationships between protein docking and association kinetics

The binding mechanisms also highlight the importance of long-range electrostatic and desolvation forces in bringing the receptor and ligand onto a productive pathway. As there exist relatively robust estimates of these free energy terms, docking methods can use them to identify potential binding regions; however, the calculated electrostatic and desolvation forces are typically not specific enough, and usually lead to targets of the order of 10 Å rmsd from the final complex structure [3,11•]. Interestingly, Schreiber and colleagues [38••] have used this indirect route to the binding site by appropriately mutating complementary electrostatic interactions outside the binding pocket of the complex between TEM-1 β-lactamase and its inhibitor protein BLIP, reporting increases in the association rate of as much as 200-fold.

The transition from the 'recognition' stage, whereby some components of the free energy broadly define the interface

between the receptor and ligand, to the final complex structure is much more difficult to determine computationally [39•]. The main reason is that the intertwining of the interacting surfaces involves an increasing role for van der Waals forces and other short-range interactions. These are much harder to evaluate as states separated by a few angstroms in the configurational space may be separated by large steric barriers in energy space. Thus, straightforward (local) energy minimization certainly does not help and the problem requires the use of algorithms that employ some smoothing of the target function [40,41].

The two-stage mechanism of protein binding suggests an approach to docking that substantially differs from the generation/scoring paradigm of rigid body docking. With some similarity to the way proteins bind, the first step of the algorithm is to identify the broad binding region on the protein surface and the second step deals with the actual formation of the complex. This approach has been implemented by Totrov and Abagyan [42,43] using pseudo-Brownian rigid body docking first, followed by biased probability Monte Carlo minimization where the search is restricted to sidechains in the interface. Recently, this algorithm has been used for docking a much larger set of proteins with remarkable success [44••].

Several other initiatives are currently underway in order to improve the automatic identification of the broad binding region between molecules, to a certain degree emulating the diffusional search of the ligand for its target on the receptor surface. Before establishing substantial surface contacts, receptor–ligand association is governed by electrostatic and desolvation interactions, and hence the approximate binding region can be found by mapping these smooth components of the free energy in the conformational space of encounter complexes [29]. Another possibility is the use of low-resolution docking methods [2]. Preliminary results suggest that these methods can identify surface regions in which binding is most likely to occur. In many applications, the search for such regions can be restricted to parts of the protein surface by biochemical information; for example, in antibody–antigen interactions, we clearly restrict consideration to the hypervariable loops of the antibody.

The second step consists of the refinement of the binding region to atomic scale. Due to the inherently rugged energy surface in this step, the key to success is the use of appropriate smoothing procedures [40,41]. We have recently proposed a tunable docking method that samples the six-dimensional space of receptor–ligand structures around a 10 Å rmsd cluster of encounter complexes [45••]. The sampling is initially biased only by the smooth desolvation and electrostatic components of the free energy, optimizing the chemical affinity between the molecules. Improved discrimination is achieved by slowly adding the van der Waals interaction to the force field in the course of the conformational search.

Conclusions

For almost a decade, most efforts in protein–protein docking focused on rigid body methods, primarily FFT correlation techniques. As rigid body docking can systematically explore the shape complementarity between proteins, this interest is well justified; however, from the very beginning, it was clear that surface complementarity is very sensitive to small structural perturbations and hence rigid methods may not work well for the realistic problem of docking separately crystallized proteins [1]. This sensitivity is even more critical if the goal is to dock protein models. The hope was to use the rigid body search to generate a number of conformations with good surface complementarity and then to rescore them using empirical potentials and various filters; however, eliminating all false positives is very difficult and improving discrimination is still an open problem.

Problems in rigid body docking may well lead to the comeback of flexible approaches that are inherently slower and generally examine far fewer docked conformations than the rigid body methods. However, they can emulate the real binding process, focusing first on the identification of the broad surface regions in which binding is most likely to occur (i.e. solve the recognition problem) and then on the refinement of these broadly defined sites into high-affinity complex structures. Both of these binding steps entail the challenge of going beyond the rigid view of protein structures. A number of flexible protein docking algorithms have already been published [41,44••,45••] and others have been presented at the recent *Modeling of Protein Interactions in Genomes* conference. An important result produced by this conference was the launching of the docking challenge Critical Assessment of Predicted Interactions (CAPRI), which had a successful start soon after the conference with three target complexes and 19 participating groups [46]. As we described, the development of protein docking methods may be at an important junction and hence evaluating the results of CAPRI will be very useful; however, results for only three complexes are likely to provide a biased view and thus the value of the CAPRI experiment depends on experimentalists making further targets available. In parallel to the blind tests in CAPRI, docking algorithms are being tested on a benchmark set of published protein–protein complexes (see [46] for further information).

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