Predicting the structure of protein complexes: a step in the right direction

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In a blind test of protein-docking algorithms, six groups used different methods to predict the structure of a protein complex. All six predicted structures were close enough to the experimental complex to be useful; nevertheless, several important details of the experimental complex were missed or only partially predicted.

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Interactions between macromolecules are at the heart of biological activity and specificity, and are vital for signal transduction, cell-cycle control, the regulation of transcription and translation, DNA repair, and many other processes. The question of how molecules such as proteins recognize each other has consequently received much attention.

The physical bases for the interactions between proteins have been understood for some time. In 1940, Linus Pauling [1] attributed the association of biological macromolecules to forces including:

> van der Waals attraction and repulsion, electrostatic interactions, hydrogen-bond formation etc., which are now rather well understood. These interactions are such as to give stability to a system of two molecules with complementary structures in juxtaposition... A general argument regarding complementariness may be given. Attractive forces between molecules vary inversely with a power of distance, and maximum stability of a complex is achieved by bringing molecules as close together as possible, in such a way that positively charged groups are brought near to negatively charged groups, electric dipoles are brought into suitable mutual orientations, etc... (In) order to achieve maximum stability, the two molecules must have complementary surfaces, like die and coin, and also a complementary distribution of active groups.

Since Pauling's time, structural biologists have tried to apply the physical chemistry of molecular interactions to predict exactly how these complementary surfaces might come together, with mixed results.

What has come to be called the 'protein docking problem' [2] has its origins in the large size and complex shape of protein molecules. Everyone came to agree with Pauling that stable complexes were formed when molecules could match complementary functionalities. What remained difficult was identifying exactly which of the many 'knobs and holes' [3] present on the surface of a protein should be fit together. Often, more than one fit seemed possible. Distinguishing among the different possible fits required the proper evaluation of their interaction energies. Quantum mechanical evaluation, although well developed for small molecules, was not feasible for proteins — simpler methods for calculating interaction energies were required. These methods had to consider the possibility of cooperative effects on binding. Such effects included changes in the conformation of the interacting molecules and changes in their potential surfaces. These cooperative effects made the problem of distinguishing among the different possible fits especially difficult.

How to tell right from wrong

The effect of these uncertainties has been evident since the first protein docking simulations were conducted in the late 1970s. In a seminal paper published in 1978, Wodak and Janin showed that with modern computers, simplified force-fields and simplified protein representations, they could fit two proteins together, regenerating the experimental configuration of the complex [4]. But their simulation also fit the proteins together in ways that differed from the X-ray crystal structure. Originally, it was believed that these non-native configurations would be excluded by using functional criteria, better representations of the protein and more sophisticated force-fields [5]. The problem has turned out to be more difficult to solve than was anticipated; despite the use of increasingly complex energy schemes and protein representations [6–15], the problem of multiple solutions continued to arise. The problem was especially acute when the conformations used in docking calculations were from proteins crystallized without their partner (for an example, see Fig. 1) [6,7]. Determining which of the several calculated complexes is likely to correspond to the experimentally determined complex, and accounting for conformational change on binding, are still considered major challenges.

Testing our assumptions

A recent paper [16] gives grounds for renewed, but cautious, optimism. The crystallography group of Strynadka and James challenged investigators who had developed docking software to predict the structure of a complex

Figure 1
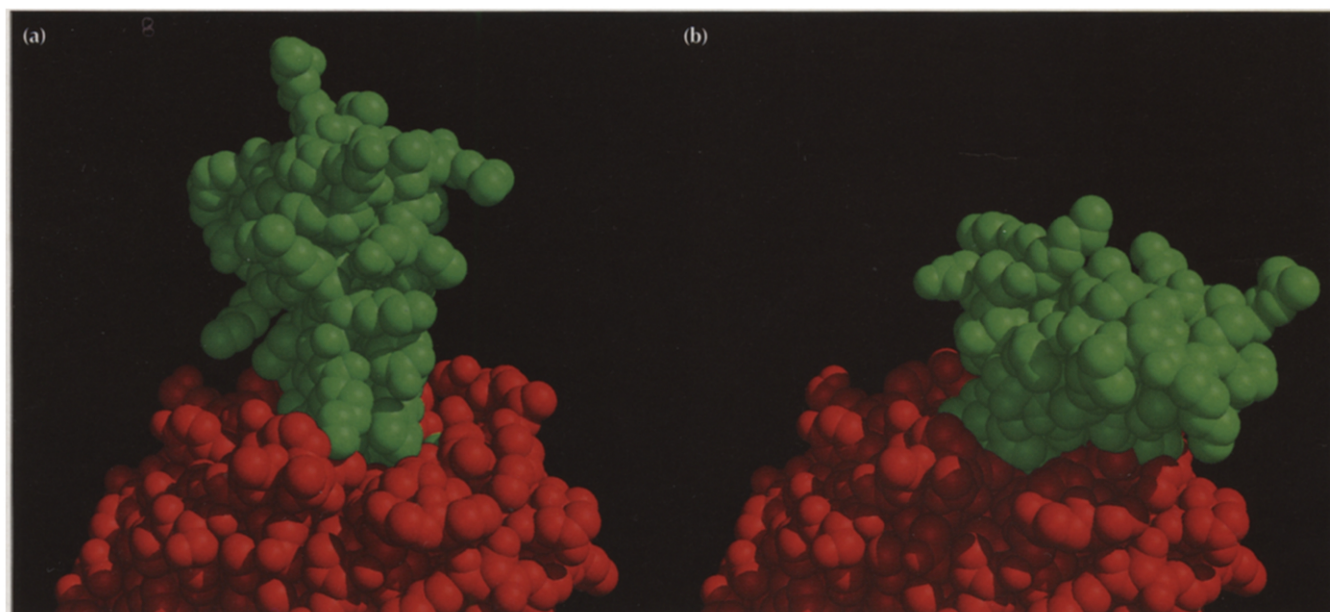
Two dockings of BPTI (green) with trypsin (red) [6]. **(a)** A configuration 0.52 Å from the experimental structure. **(b)** A configuration 21.41 Å from the experimental structure. Both complexes have large, complementary interfaces that are difficult to distinguish between by several different measurements of complementarity.

before it was solved. The dockers were given the crystal structures of the two proteins, the β-lactamase TEM-1 (263 amino acids) and the β-lactamase inhibitor protein (BLIP; 165 amino acids), in their uncomplexed forms. TEM-1 had been well studied, but BLIP was a new protein about which little was known except for its three-dimensional structure and the fact that it binds tightly to TEM-1. The residues involved in the interface between TEM-1 and BLIP were unknown, nor was it known whether the conformations of the molecules changed on binding.

Each of the six groups who accepted the crystallographers' challenge was able to predict the structure of the complex [16] (Table 1). Five of these groups used fundamentally different algorithms to generate the predicted complexes [6–9,17], and each group used different energy evaluation schemes to judge among the different possibilities calculated [6–9,17–21]. (For an excellent discussion of some of the technical aspects of the docking calculations performed by the six groups, see [22].) Five of the six groups submitted more than one prediction. Four of these five picked as their most likely prediction the one that turned out to resemble the experimental structure most closely. A fifth group did not rank their structures. The sixth group submitted one structure only; it closely resembled the experimental structure (Table 1). Visual comparison of the predicted and experimental structures showed a high degree of similarity (Fig. 2). The predictions were judged to have been good enough to have

helped solve the experimental structure using molecular replacement techniques [16]. The similarities between the predicted and experimental structures, and the wide range of docking methods used to generate them, suggest

Table 1

Comparison of the computer-docked complexes to the X-ray crystal structure [16].

Group	RMS of best prediction (Å)[a]	Rank of best prediction[b]	Number of predictions
Eisenstein & Katchalski-Katzir	1.10	1	3
Shoichet & Kuntz	1.80	1	15
Jackson & Sternberg[c]	1.89	1	1
Abagayan & Totrov	1.91	1	3
Duncan, Rao & Olson	1.92	1	14
Janin, Cherfils & Zimmerman	2.47	unranked	4

[a]When compared to the X-ray structure, comparing all main-chain atoms.
[b]The predictions were ranked by the individual groups in order of the likelihood to correspond to the crystallographic result.
[c]Using the complexes generated by Shoichet & Kuntz, evaluated with a different energy scheme [18].

Figure 2
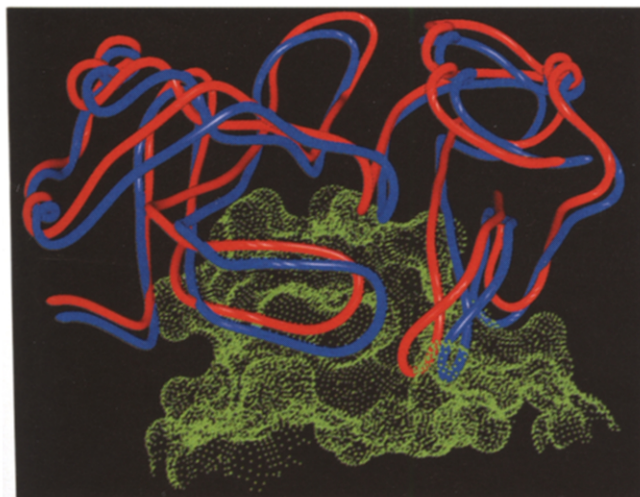
The overlap of a predicted BLIP–TEM-1 complex with the one solved by X-ray crystallography. A main-chain representation of the predicted orientation for BLIP is shown in blue, that for the crystallographically determined orientation of BLIP is shown in red. The molecular surface of TEM-1, from the X-ray structure of the complex, is shown in green. The rms between the main-chain atoms of the two complexes is 1.80 Å.

that, for at least some proteins, there is enough information in the positions of the side-chain and main-chain atoms that do not undergo conformational change to specify the structures of their complexes.

Was it a fair test?

This paper represents the first blind test of docking algorithms, and the skeptical reader might wonder whether these results reflect peculiarities of the test system. In some ways, the test was an easy one. First, neither of the two proteins showed dramatic conformational changes on complex formation. Second, BLIP does occlude the active site of TEM-1 in the complex, as most of the groups expected. This is a reasonable expectation for a 100 pM inhibitor [23], but it is not always true.

In other ways this test case was quite difficult. In contrast to most proteins of determined three-dimensional structure, little was known about BLIP. As it turned out, the structure of the complex was unusual. In most complexes studied to date, the enzyme presents a concave surface to the convex surface of the inhibitor. In the complex between BLIP and TEM-1, the interface was mixed; BLIP positions loops in the concave active site of TEM-1, but the majority of the interface is composed of a convex TEM-1 surface matched to a concave inhibitor surface (Fig. 2). The two proteins buried 2636 Å^2 of surface area in complex [16], an unusually large amount (1200–1700 Å^2 is more common [24]). The complex retained many of the traditional sources of uncertainty in protein docking calculations. Both proteins showed a small degree of overall

conformational change. Between the native and complexed forms of TEM-1, the main-chain root-mean-square (rms) deviation was 0.35 Å, while for BLIP it was 0.70 Å. Individual residues in the binding interfaces experienced more significant conformational changes. The largest of these changes were in the reading-head loops of BLIP that make the most intimate contacts with TEM-1. Like the specificity loops of many proteinacious inhibitors, these loops in the unbound conformation of BLIP had high temperature factors, suggesting that they were either poorly determined or highly flexible — in either case somewhat untrustworthy for docking purposes.

Room for improvement

Conformational changes such as those seen in the BLIP–TEM-1 complex are examples of degrees of freedom present in two interacting macromolecules that the current docking algorithms either do not treat, or do not treat well. The failure to account for them explains many of the errors present in the predicted structures. None of the predictions suggested the global aspects of conformational change present in the complex, and none of the methods predicted even local conformational changes successfully [16]. For this reason, many of the specific polar contacts seen in the crystal structure are missing in the docked structures. Our own top-ranking structure (Fig. 2), for instance, includes only three of ten hydrogen bonds observed crystallographically between BLIP and TEM-1, and includes only one of four ion pairs. None of the waters seen crystallographically was predicted in the docked complexes. Waters are often involved in molecular complexes, but, because they can in principle occupy many sites, they are hard to treat computationally and their possible specific roles are usually ignored.
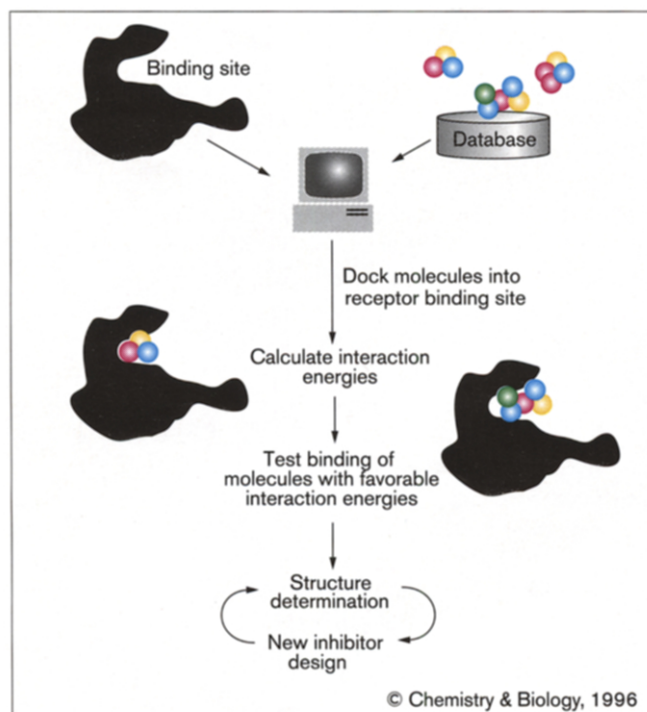
Docking calculations can thus, in favorable circumstances, predict protein complexes to 'low resolution'. The overall features of a protein complex can be anticipated, but many of the details, the 'high resolution' features, will only emerge with the experimental structure. What are the prospects for improving the 'resolution' of the calculations? A longstanding difficulty is that of calculating interaction energies [6,7]. Predicting the correct polar interactions, for instance, is often a matter of distinguishing which of the several possible bonding partners will contribute most strongly to the interaction energy. Calculating the energy of even known polar interactions remains an area of active research [25]; our ability to choose reliably among different possible interactions must await advances in energy-evaluation techniques. A second major challenge is that of accounting for conformational freedom. Proteins are flexible and have many possible conformations, even while retaining a particular global fold. Several algorithms now exist to explore local flexibility: these range from varying the conformations of individual residues [26] to loop-building techniques [27] to molecular dynamics or Monte Carlo

simulations to genetic algorithms [28]. Some docking algorithms already include such techniques [9,17,28,29]. While potentially helpful, these methods cannot account for major hinge motions sometimes seen in molecular association. To do so efficiently will require fundamental advances in our algorithms.

Structure-based inhibitor discovery

A second area where molecular docking is used is in structure-based inhibitor discovery. We distinguish between two applications: discovering new molecules based on the structure of a known inhibitor with a receptor, and discovering novel inhibitors based on the structure of the receptor alone. In the first area considerable progress has been made. Starting with the structures of a ligand–receptor complex, several groups have developed potent inhibitors that are candidates for clinical trials [30–33]. In several cases these molecules inhibit their target receptors in the low picomolar concentration range, a considerable improvement over the starting inhibitors. Computational approaches that allow investigators to model the effects of derivatization [34] or that identify potential binding sites

Table 2

Properties of inhibitors discovered using a database search and docking approach.

Receptor	Affinity of lead (μM)[a]	Affinity of follow-up (μM)[a]	Ref.
HIV protease	100	5	[44,47]
Thymidylate synthase	5	0.5	[46]; P. Costi, unpublished
Hemagglutinin	500	5	[48]
Cercarial elastase	3		[49]
Malarial protease	8	0.1	[49]
CD4–gp120	5	1	M. McGregor, unpublished
DHFR	7		D. Gschwend, unpublished

[a]The initial leads were from DOCK searches of molecular databases. Follow-up inhibitors were synthesized on the basis of the initial lead.

Figure 3

Searching molecular databases for novel inhibitors using a docking algorithm. A docking program fits molecules from a database of small molecule structures into the the receptor binding site. Thousands of fits are often calculated for each molecule. Fits are evaluated for complementarity, typically by calculating van der Waals and electrostatic energies. Promising compounds are bought and tested for their ability to bind to the receptor. The structure of the inhibitor–receptor complex can then be determined by X-ray crystallography, and this information can be used to design new inhibitors.

in the receptor [35] have played an important role in these efforts [30,36]. Perhaps more important has been the use of iterative cycles of synthesis and the determination of X-ray crystal structures [33]. Notwithstanding these successes, certain problems persist. Errors in calculated interaction energies limit our ability to predict the effects of particular substitutions to inhibitors. Even computationally intensive thermodynamic cycle methods [37] have errors of no less than 1 kcal mol^{-1}, which translates to a five-fold difference in K_i.

Several groups have developed methods to aid in the discovery of novel inhibitors based on the structure of the receptor alone. One approach has been to use molecular fragments to build up new molecules in receptor sites [34,38–40]. This approach has lead to the discovery of several new inhibitors [38,41]. Another possibility is to screen molecular databases for novel inhibitors that can complement a receptor of known structure [42–44] (Fig. 3). This task differs from that of predicting a complex between two molecules, although the algorithms used may be the same. Rather than asking 'what is the structure of the complex between two molecules known to associate?', the problem becomes 'which of the > 100 000 molecules in the database can be expected to bind tightly to the receptor, given the three-dimensional structure of each?' All the problems of the first question are included in the second, but the second must also consider whether the balance between solvation energies and receptor binding favors complex formation, and further must rank this balance for each molecule in the database. This would seem to be a difficult problem. Nevertheless, Bartlett and co-workers have used this approach to discover novel

inhibitors for several receptors [42], and we and our colleagues have been able identify novel ligands for seven proteins of known structure (Table 2). These ligands bind in the μM concentration range and do not resemble the previously known inhibitors or substrates for the proteins. These successes are explained in part by the smaller size of the database molecules when compared to BLIP, which considerably simplifies the conformational aspect of the docking problem, and also by how the task has been framed. The goal is to discover a novel inhibitor, not every novel inhibitor in the database; false negatives are acceptable. In our experience, these false negatives will usually be flexible molecules that exist in the database in a different conformation from the one adopted when they bind to the receptor. Control experiments comparing the results from a docking calculation and a random screen of database molecules remain to be performed; these would be very desirable, and would give an indication of how many possible inhibitors are missed. False positives are tolerable, and easily screened out, if the screen is restricted to commercially available compounds by using a database such as the Available Chemicals Directory (supplied by MDL Ltd, San Leandro, CA).

Improving docking algorithms for structure-based inhibitor discovery requires advances in several areas. Our treatment of ligand and receptor solvation, so important for evaluating whether a ligand will bind to a receptor or not, are presently crude. As in macromolecular docking, better treatments of conformational flexibility and the role of specific water molecules and ions will also be important. Predicting where on a receptor surface novel ligands might bind needs further consideration. Several recent X-ray studies suggest that it may be possible for ligands to bind at unexpected sites [45–47].

The progress in structure-based inhibitor discovery, and the recent success in predicting the structure of a protein–protein complex, are reasons for cautious optimism in molecular docking. Still, it is humbling to recognize how difficult it has been to fit complementary groups together, "like dye and coin", or like "balls into sockets". Our conceptualization of the problem has not changed much since Pauling and Crick first suggested these analogies, though our ability to numerically simulate biological complementarity certainly has. One of the most surprising lessons has been that the complementary groups present on molecular surfaces may be fitted together in more than one way. Because of uncertainties in energy methods, and because the existing programs do not yet consider the full range of conformational flexibility, docking calculations retain many ambiguities. We are not yet at the stage where investigators can naively present two structures to a docking program and expect to have returned a definitive prediction of how they will bind, or if they will bind. What docking calculations can do is to present investigators with

sensible hypotheses that can be experimentally tested. Used in this spirit, docking programs will be useful tools for investigators interested in understanding the structural basis of biological recognition.

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