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MINIREVIEW

Integrative computational modeling of protein interactions

João P. G. L. M. Rodrigues and Alexandre M. J. J. Bonvin

Computational Structural Biology Group, Bijvoet Center for Biomolecular Research, Utrecht University, the Netherlands

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Correspondence
A. M. J. J. Bonvin, Computational Structural Biology Group, Bijvoet Center for Biomolecular Research, Faculty of Science – Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands
Fax: +31 30 253 7623
Tel: +31 30 253 3859
E-mail: a.m.j.j.bonvin@uu.nl
Website: http://nmr.chem.uu.nl/~abonvin

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Protein interactions define the homeostatic state of the cell. Our ability to understand these interactions and their role in both health and disease is tied to our knowledge of the 3D atomic structure of the interacting partners and their complexes. Despite advances in experimental method of structure determination, the majority of known protein interactions are still missing an atomic structure. High-resolution methods such as X-ray crystallography and NMR spectroscopy struggle with the high-throughput demand, while low-resolution techniques such as cryo-electron microscopy or small-angle X-ray scattering provide data that are too coarse. Computational structure prediction of protein complexes, or docking, was first developed to complement experimental research and has since blossomed into an independent and lively field of research. Its most successful products are hybrid approaches that combine powerful algorithms with experimental data from various sources to generate high-resolution models of protein complexes. This minireview introduces the concept of docking and docking with the help of experimental data, compares and contrasts the available integrative docking methods, and provides a guide for the experimental researcher for what types of data and which particular software can be used to model a protein complex.

Introduction

The cell is a busy environment: several thousand molecules constantly meet and exchange information to define its metabolic state. Of all contributors to cellular homeostasis, proteins are the most abundant and active. Therefore, eavesdropping on their interactions and learning how information is being shared are pivotal for a complete understanding of the cell. Further, this also provides the first step towards rational development of therapeutics for many deadly or incapacitating diseases [1].

Cellular and molecular biology have evolved and delivered powerful methods to identify and pinpoint the cellular location of proteins and their interactions. Yet, only structural biology can provide definite answers on the mechanisms of these interactions by revealing the high-resolution atomistic structures of the underlying biomolecular complexes [2]. Experimental determination of the structure of biomolecular interactions can, however, be a laborious, time-consuming and costly endeavor [3]. The growing gap between the universe of known sequences and that of determined structures is evidence that high-throughput structural biology remains a dream [4,5]. This gap widens when considering the number of available structures of biomolecular complexes [6]. Computational structural biology, by contrast, has the potential to deliver (high-resolution) models of protein–protein interactions. It has, however, struggled with inaccuracies since its inception in the late 1960s. As a result, models have often been met with skepticism. Fortu-

Abbreviations
CAPRI, critical assessment of predicted interactions; FFT, fast Fourier transform; SANS, small-angle neutron scattering; SAXS, small-angle X-ray scattering.

nately, recent decades have seen fascinating developments in both software and hardware [7], and computational structure prediction is now routinely considered an integral part of research. The docking field, in particular, has thrived in the last decade since the inception of the critical assessment of predicted interactions (CAPRI) experiment [8]. Several rounds of blind predictions have stimulated discussions and resulted in the development of a wide variety of docking algorithms, some of which have consistently met with considerable success [9–12]. Interestingly, the most successful participants of the latest CAPRI rounds fall within the category of 'data-driven', 'information-driven' or 'integrative modeling' algorithms [13]. These approaches were developed to counter the inaccuracies in computational sampling and scoring methods by feeding them whatever experimental information is available for a given interaction. As recent CAPRI assessments have shown, this synergy is often enough to drive the docking calculations toward the right answer. As such, computational modeling of complexes has grown into a well-accepted complementary method to classical experimental techniques such as NMR spectroscopy and X-ray crystallography.

This minireview is dedicated to docking approaches for the modeling of biomolecular complexes, with a focus on integrative and information-driven algorithms. It first explores what computational docking is and its limitations, then explains which types of information docking methods best benefit from and why including information is a valuable strategy. Alternative methods to predict the structure of protein–protein complexes are then discussed. The minireview ends with an overview of the current and future challenges for the docking community.

Computational molecular docking

Molecular docking is usually defined as a prediction of the structure of a molecular complex starting from the individual structures of its participants. Despite their differences, docking algorithms share three common elements: (a) 3D structural models of the individual components must be available, whether experimentally determined or computationally predicted; (b) they must explore the conformational landscape of the interaction and generate structural models of the complex, what is called sampling; and (c) they must assess the generated models and select those that are more likely to be representatives of the native complex, what is called scoring.

3D structures of the components are usually obtained experimentally via X-ray crystallography or NMR spectroscopy. Nevertheless, considering that the number of known protein sequences dwarfs the number of available 3D structures, it is rather common to obtain a model of the protein in question through homology modeling methods [6,14].As for sampling and scoring, these are the 'Achilles' heel' of docking and remain two very difficult problems given the natural properties of biomolecules, in particular their flexibility and our limited understanding of their thermodynamics [15–17].

Sampling: exploring the conformational and interaction landscape

Simply put, sampling is the enumeration of all possible orientations and conformations that the monomers of a complex can assume in 3D space. If we assume both molecules to be rigid, i.e. that their conformations pre- and post binding do not differ substantially (approximately < 1 Å r.m.s.d. of the coordinates of the backbone atoms), then there are methods that can efficiently cover the entire search space [18]. These are usually based on grid searches using fast Fourier transforms (FFT), as pioneered by Katchalski-Katzir *et al.* [19] and widely used nowadays [20–22], geometric hashing, first developed and still used by Wolfson and colleagues [23,24], or spherical harmonics, as introduced and used by Ritchie and co-workers [25,26]. FFT techniques represent the protein surfaces in a Cartesian grid model that favors close contacts, i.e. overlap of the surfaces, and penalizes overlap of the core, performing exhaustive rigid-body conformational searches very efficiently (typically in a few hours). Geometric hashing techniques divide the molecular surface into interaction patches and match them across the interacting molecules. Spherical harmonics-based methods also calculate fast Fourier correlations but are computationally more efficient because of the use of a combination of spherical harmonic functions to describe the protein shapes, and the calculation of docking orientations via scalar products of rotated and translated coefficient vectors [25]. All these methods have been designed to evaluate molecular shape complementarity, but often incorporate simple energy functions to bias the scoring, for example, based on electrostatics (e.g. ZDOCK [27]), desolvation (e.g. pyDock [28]) or statistical potentials (PIPER [29], IRAD [30]). Other methods are less computationally efficient, but nevertheless powerful. HADDOCK [31] uses a derivative-based search method in Cartesian space – rigid-body energy minimization – that acts directly on an energy function represented by a sum of

electrostatics, van der Waal's and restraint energies, therefore, targeting specific patches on the molecular surface deemed favorable by the energy function. A relatively recent method, SwarmDock [32], incorporates normal-mode analysis – an approach pioneered by ATTRACT [33] – into a Particle Swarm Optimization meta-heuristic, effectively docking while optimizing conformation, position and orientation simultaneously.

Unfortunately, proteins tend not to be rigid [34] and their flexibility causes challenges for docking [15,35,36]. Sampling the full conformational landscape of a biomolecule is a well-known problem in protein folding: it is time-consuming, suffers from inaccuracies related to the energy functions used to describe the landscape, and usually requires an army of computing CPU cores or specialized hardware [7]. Therefore, most docking methods refrain from using traditional molecular dynamics simulations in Cartesian space to sample the flexibility of the interacting partners upon binding. Instead, they often implement other (more efficient) search methods such as Monte Carlo (e.g. RosettaDock [37]), normal-mode analysis (e.g. ATTRACT [33], SwarmDock [32] and FiberDock/FlexDock [24], simulated annealing in torsion angle space (e.g. HADDOCK [31]) and/or use simplified representations of the system such as coarse-grained models (e.g. ATTRACT [38,39] and RosettaDock [37]). Finally, some methods have been developed to tackle the particular problem of dealing with large domain motions, such as the flexible multidomain approach of Karaca & Bonvin [15] that can describe extremely large conformational changes (up to 19.5 Å r.m.s.d. between the free and the bound state).

Molecular dynamics in Cartesian space is often still used to refine the docking models, in some cases, in explicit solvent to better represent the cellular environment, either as last step in the docking protocol (e.g. HADDOCK [31]) or as an additional step (e.g. ATTRACT [39]).

Scoring: discriminating right from wrong

The so-called 'holy grail' of docking is to develop a method – scoring function – that is able to not only discriminate near-native conformations from others, but also accurately estimate the binding affinity of the interacting molecules [16,40].

Quantum mechanics descriptions of the molecules and the interaction medium (e.g. aqueous solution) could provide such discrimination but are too computationally demanding to be applied to protein–protein complexes, let alone the thousands of models produced by docking methods. Consequently, docking programs implement scoring schemes based on simpler molecular mechanics, empirical observations, evolution/homology or a combination of these [40]. Furthermore, scoring functions tend to be adapted to particular sampling schemes; i.e. sampling methods that generate a very large number of models (e.g. FFT-based, geometric hashing, rigid-body docking) tend to use simple but fast-to-compute scoring functions, whereas methods that generate a smaller number of models (e.g. simulated annealing, molecular dynamics, Monte Carlo) can afford to employ more elaborate, and therefore slower, scoring functions. In line with this, different stages of the same docking approach often use different scoring functions, typically increasing the complexity (and CPU costs) of the scoring function used while reducing the number of models considered.

Electrostatic interactions are commonly included in scoring functions through a Coulomb potential, even in fast, exhaustive methods such as FFT-based Hex [26], ZDOCK [27] and pyDock [28]. Most methods also include an atomic repulsion term to avoid steric clashes, via implementation of molecular mechanics Lennard–Jones potentials (e.g. HADDOCK [31], RosettaDock [41] and ATTRACT [38]) or identify clashes by measuring the molecular overlap (e.g. FFT-based methods and PatchDock [42]). The tendency of molecules to bury part of their solvent-accessible area upon interaction is also valuable in discriminating near-native models and is implemented in several docking methods, either by valuing large complementary patches (FFT-based and geometric hashing methods) or explicitly via calculation of the buried surface area (e.g. HADDOCK [31]). Related to the buried surface area, several algorithms also incorporate a desolvation energy term that attempts to model the thermodynamic cost or gain associated with changes in the solvation layer of the individual molecules upon binding (e.g. FastContact [43], pyDock [28], HADDOCK [44], ZDOCK [45] and PatchDock [42]/FireDock [46]). Another common strategy in scoring is to use knowledge-based functions derived from statistical analysis of experimentally determined structures. A recent study [47] identified several knowledge-based scoring functions that seem to discriminate near-native models particularly well: SIPPER [48], DECK [49] and PISA/PIE [50]. In addition to these recently developed functions, others such as ZRANK [51] and DFIRE [52] have been in use for years in the docking community. Interestingly, coarse-grained scoring functions perform remarkably well, particularly for complexes that undergo some conformational changes upon binding (e.g. PIE [50]). Another interesting and successful

knowledge-based scoring function is SPIDER [53], developed from an analysis of geometrical networks of residue contacts at protein–protein interfaces. Contrasting with the previous approaches, some research groups have recently developed methods to score protein complexes based on homology and evolutionary information, which have performed remarkably well [54], although the idea is not novel [55]. Finally, some methods have also used the entire pool of models generated by the sampling stage to perform a statistical analysis of the pairwise residue contacts and rank the models based on these observations (e.g. CONSRANK [56]). In general, the application and implementation of these functions is not trivial because they are often tied to a particular docking software or approach. Several scoring functions are available as user-friendly web-servers (e.g. ZRANK [51] and DFIRE [57]) or through a standalone application that users can download and execute locally (e.g. DECK [49], DCOMPLEX [52], PIE/PISA [50] and SPIDER [53]). Also, the combination of scores from different functions is not necessarily additive, given the redundancy of their individual terms, and requires special attention and expert knowledge [47].

Despite all the developments in scoring functions, it is still unwise to blindly choose the best scoring model as the representative of the native state. The assumption that near-native models populate wide low-energy wells in the energy landscape has led to the development and application of clustering algorithms to molecular simulations [58] and, naturally, to docking predictions [59]. ClusPro [60] was the first automated docking method using clustering for near-native model selection. The results of the rigid-body sampling stage are clustered using a structural similarity measure that assesses the differences at the interface of the smaller (ligand) molecule (ligand r.m.s.d., used in CAPRI as an evaluation measure [8]) and then the centroids of the clusters are ranked and provided as final models. Other docking approaches have since implemented clustering algorithms [24,31,61], most using analogous similarity measures, whereas others have developed similarity measures specific for docking (e.g. fraction of common contacts [62]).

Using experimental information in docking

In an effort to address the limitations of both sampling and scoring, the docking community started developing algorithms that use, somehow, experimental or predicted information about the interaction (Fig. 1) [13,63,64]. The information is used to bias the sampling and/or scoring. This consequently increases the accuracy of the final models, but depends on the quality of the information used. Fortunately, incomplete or partially incorrect information can still be used to great advantage for the prediction [14]. Depending on the use of one or several types of information in the calculations, these methods are designated 'data- or information driven' or 'integrative', respectively.

How the experimental data are included in the docking calculations differs from method to method, and sometimes between the different stages within a method as well. The simplest implementation is by means of a filter: after generating a number of models, each is evaluated on its agreement with the provided experimental data, and those models that do not agree or agree only poorly are removed or ignored in the later stages of the protocol. Another way of incorporating experimental data is through a restraint energy

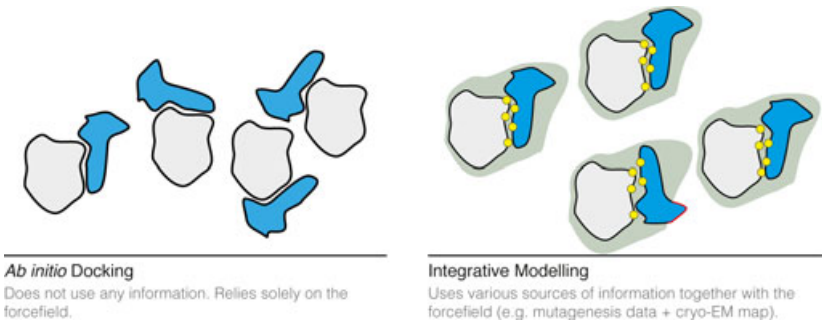

Fig. 1. *Ab initio* vs. integrative docking. Docking algorithms can use information extracted from experiments or (bioinformatics) predictions to narrow down the possible orientations of the complex. By adding external information, the sampling can be limited to the regions defined in the data and the number of wrong solutions can be reduced. Multiple sources of information can be used to solve ambiguities and improve the convergence of the calculations to a few conformations. Nevertheless, the inclusion of experimental/prediction data does not guarantee that all solutions will be correct.

term explicitly included in the sampling. This has the benefit of narrowing the conformational space search and avoids the production of models that are in obvious disagreement with the data [63]. Nevertheless, if the data are wrong or irrelevant for the interaction mode under study, the system will explore a wrong region of the interaction space and native-like solutions are unlikely to be sampled. When combined with flexible protocols, direct inclusion of data as restraints might also facilitate the modeling of conformational changes occurring upon binding, albeit with limited efficiency (see Fig. 2 in de Vries *et al*. [65]). The feasibility of implementing a certain type of data as a restraint depends, however, on the sampling method. At each step, Monte Carlo methods evaluate an energy function and use the result to calculate the probability of that particular step being accepted. However, derivative-based methods such as conjugate gradient energy minimization or molecular-dynamics-based simulated annealing require an energy term that can be described by a continuous function, fully derivable. For example, the buried surface area, calculated empirically using grids, cannot be represented by a differentiable function. Consequently, such functions cannot be implemented as a restraint in an energy minimization protocol, but are perfectly suitable for filter-based approaches or restraints-based approach using nonderivative sampling methods such as Monte Carlo searches or genetic algorithms.

State-of-the-art of integrative modeling software

The benefits of integrating experimental or prediction data into docking are clearly exposed by the latest CAPRI assessment report [12]. Table 1 summarizes the docking approaches of the five most successful research groups in the 'prediction' category of the most recent CAPRI round (HADDOCK, SwarmDock, ClusPro, GRAMM-X and pyDock), as well as other consistently good performers (ZDOCK, ATTRACT, RosettaDock, HEX and PatchDock). This list is by no means complete and the methods described therein are not necessarily the best for every possible case. For a better compilation of the existing docking approaches, readers should refer to the latest CAPRI assessment report [12] and to the review by Moreira *et al*. [64]. All the listed methods offer the possibility to integrate data into their algorithms, although they differ in the stage at which the data can be incorporated and in their implementation. FFT-based approaches (ClusPro [66], pyDock [21], GRAMM-X [67], ZDOCK [61] and HEX [26]) evaluate very large numbers of interaction modes and implement the data as a scoring bias

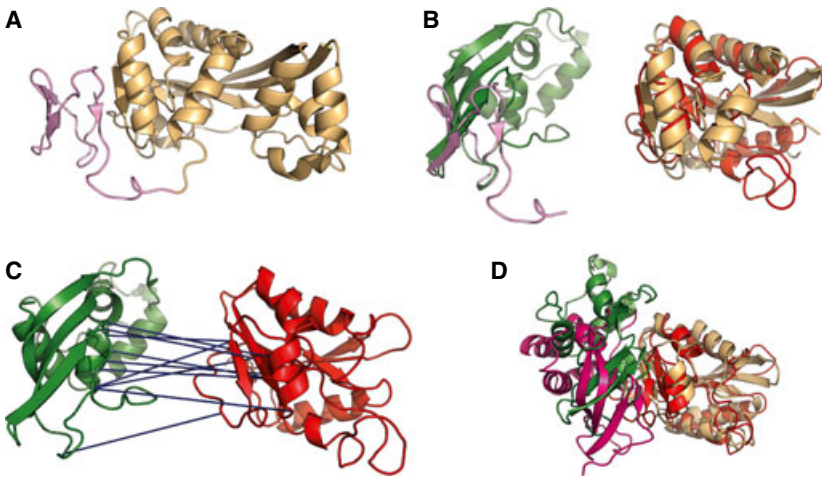

Fig. 2. Using information from structural homologues as restraints: HADDOCKing the Mtq2/Trm112 complex. (A) The zinc-binding domain (pink) and methyltransferase (orange) domain of RlmA(I) (PDB 1P91 [112]). (B) Superimposition of the starting models of Mtq2 (red) and Trm112 (green) on the respective distant structural homologous domains of RlmA(I). A simple superposition of the proteins onto the template would not have resulted in models that would qualify as acceptable under CAPRI criteria. (C) Distance restraints derived from Ca–Ca contacts between the domains of RlmA(I) and mapped onto the Mtq2 and Trm112 models. Only 10 restraints are shown for simplicity, the CAPRI prediction used 475. For a detailed description of the docking parameters refer to Rodrigues *et al*. [14]. (D) Submitted HADDOCK model (red/green) superimposed on the native structure (PDB 3Q87 [108]) (gold/magenta). The interface root mean square deviation from the native structure is 3.43 Å, as calculated by the CAPRI assessors. The restraints, together with the flexible refinement, were instrumental in bringing the model under the 4 Å acceptable RMSD cut-off for CAPRI.

Table 1. List of top-performing docking approaches participating in CAPRI.

Name	Protocol	Strengths and weaknesses	Integration of data	Public Web Server
ATTRACT [39]	Energy minimization in translational and rotational degrees of freedom using a reduced protein model and normal-mode analysis to allow conformational changes upon binding.	+ Fast derivative-based search method. + Conformational changes upon binding (local and global motions). + Support for Cryo-EM density maps. – Not available via a web server	Implements interface data by adding atom/residue-specific weights, which can be negative (repulsive). Also offers the option to dock using Cryo-EM density maps.	None
ClusPro [61]	Rigid-body search via a FFT correlation approach (PIPER), followed by structural similarity (RMSD) based clustering to find the most popular interaction modes, and final refinement of selected structures using CHARMM.	+ Best automated served in the latest CAPRI evaluation [12]. + Fast and exhaustive protocol. + Several docking 'modes' depending on the biological function (antibody/antigen, multimer, others). – Cannot handle flexible complexes.	Regions/residues in the binding partners can be introduced to bias the scoring of the models. Noteworthy option of 'negative' (repulsion) contacts.	http://cluspro.bu.edu/
GRAMM-X[a][68]	Grid-based FFT rigid body docking approach using a softened Lennard–Jones potential function. The top predictions are minimized and re-scored using a soft Lennard–Jones potential, an evolutionary conservation term, a statistical residue–residue potential, and the volume of the local energy minima in the grid.	+ Fast. + Uses CONSURF to determine evolutionary conserved residues. – Cannot handle flexible complexes.	Regions/residues in the binding partners can be introduced to bias the scoring of the models.	http://vakser.bioinformatics.ku.edu/resources/gramm/grammx/
HADDOCK [32]	Rigid-body energy minimization followed by semi-flexible (interface) refinement and final optimization in explicit solvent. Returns clusters of models ranked by HADDOCK score.	+ Best performing team in the latest CAPRI evaluation [12]. + Restraint-based integration of ambiguous experimental/prediction data. + Explicit flexibility of the interface. + Powerful user-friendly web interface. – Slower than FFT-based methods.	Several types of restraints allow integration of different sources of data: distances, orientations, radius of gyration, symmetry type, etc. Directly integrates several NMR-derived data.	http://haddocking.org
Hex [27]	Spherical polar Fourier approach using rotational	+ Extremely fast approach (~ 15 s using	Can restrict the rotational search around an	http://hexserver.loria.fr/

Table 1. (Continued).

Name	Protocol	Strengths and weaknesses	Integration of data	Public Web Server
	correlations generates a very large number of putative models, which are then re-scored using a shape correlations or shape plus electrostatic correlations.	2 graphical processing units). – Cannot handle flexible complexes.	intermolecular axis defined by a pair of residues, one on each interacting monomer. The angular range of the search can also be defined.	
PatchDock [25]	The surface of the molecules is divided into patches (concave, convex and flat) and only those containing 'hot-spot' residues are kept. The patches are then matched using geometric hashing and pose-clustering techniques and the candidate models are examined (to remove extreme clashes) and scored.	+ Extremely efficient and fast protocol. + Integrated suite of docking tools [24]. – Fragmentation of protocol in very specialized tools requires *a priori* knowledge of their limitations.	Regions/residues in the binding partners can be introduced to bias the scoring of the models.	http://bioinfo3d.cs.tau.ac.il/PatchDock/
pyDock [22]	Rigid-body search via a FFT search method (custom optimized FTDOCK) followed by scoring with a combined electrostatics and desolvation energy function.	+ Fast protocol. + Integration of different modules (pyDockSIPPER, pyDockRST, pyDockSAXS) to improve predictions. – Cannot handle flexible complexes.	PyDockRST module scores models based on agreement with user-defined distances. Implements SAXS scoring by generating synthetic SAXS curves of the models to user-provided data.	http://life.bsc.es/servlet/pydock/home/
RosettaDock [38]	Low-resolution, rigid-body, Monte Carlo search followed by simultaneous optimization of backbone displacement and side-chain conformations using Monte Carlo minimization.	+ Scoring based on the Rosetta energy function. + Very powerful refinement protocol. - Poor (direct) support of interface information. – Computationally demanding and slow protocol.	Molecules can be positioned manually in space (e.g. using PyMOL). Interfaces with other Rosetta tools (RosettaInterface) to validate mutagenesis data.	http://antibody.graylab.jhu.edu/docking
SwarmDock [33]	Local docking and particle swarm optimization of partner position and orientation, using normal modes to model induced fit, and final energy minimization. Uses the DComplex scoring function but the final models are re-ranked with a centroid potential prior to clustering.	+ Search method that explicitly models global flexibility upon binding. – Slow. Predictions can take days unless a local version and sufficient computational resources are available.	Residues belonging to the binding site can be selected to bias the starting positions of the binding partners.	http://bmm.cancerresearchuk.org/~SwarmDock
ZDOCK [28]	FFT-based rigid-body search using a scoring function composed of desolvation energy, electrostatics, and	+ Fast protocol + Robust performance over several CAPRI rounds.	Allows user-defined selection of 'blocked' and 'binding' residues that influence the	http://zdock.umassmed.edu/

Table 1. (Continued).

Name	Protocol	Strengths and weaknesses	Integration of data	Public Web Server
	grid-based shape complementarity. Recent versions implement knowledge-based scoring functions and clustering to analyze the energy landscape of binding.	– Cannot handle flexible complexes.	scoring of the FFT-based search.	

[a]The research group responsible for GRAMM-X has recently developed an alternative technique to predict the structure of protein–protein complexes based on structural similarity with (distant) homologues [104]. This method is reviewed in greater detail in the 'Beyond Docking' section of this article.

during the FFT search or as filter at the end. PyDock uses the data in a final analysis step, adding an 'agreement' score to the models that depends on the type of data. In the case of small-angle X-ray scattering (SAXS) data, pyDock uses CRYSOL to generate synthetic scattering curves and then calculate the fit to the experimentally determined curve [68]; in the case of distance restraints, pyDock calculates the percentage of user-provided distances respected by the model [69]. ClusPro translates interface data into regions that its search algorithm can either favor or avoid. For the 'attractive' restraints, the scoring of the FFT method is changed and 'favored' atoms contribute more to the score if in the interface. Note that this does not exclusively restrict the search to the predefined area; if another surface patch is very energetically favorable (i.e. electrostatic complementarity or hydrophobic packing), then some models might overlook the provided information and prefer this binding mode. A particular feature of ClusPro is the possibility of defining 'repulsive' regions. These allow one to specify regions of the interacting proteins that should not be included in the interface, effectively creating a 'negative' restraint that, unlike the 'attractive' restraints, must be respected. Both options are available in the public web server. GRAMM-X also allows the definition of regions of the partners that should be in the interface of the predicted models. The user can also define how many pairs of restraints must be satisfied, thus providing control over the strictness of the filtering algorithm [67].

Approaches like SwarmDock [32] or HADDOCK [31] use different sampling algorithms and implement the data differently. SwarmDock places the starting molecules with the interfacial residues 'in line of sight' of each other, effectively preorienting them according to the data. The standard optimization and refinement then take place, without any contribution of the data.

HADDOCK is the pioneer of 'data-driven' docking. It draws its philosophy from NMR-based structure calculation software and incorporates the data directly into the calculations as an additional restraint energy term. More importantly, the data are used throughout the entire protocol, even during the flexible refinement stages. In HADDOCK, the molecules are first separated in space, their orientation randomized and then the energy of the system is minimized using a function that includes electrostatics, van der Waal's forces and the data as distance [31] (e.g. nuclear Overhauser effect, chemical cross-links, electron paramagnetic resonance distances, mutagenesis) and orientation (e.g. residual dipolar couplings [70], pseudo-contact shifts [71]) restraints. Other types of data that cannot be directly added to the energy function to be minimized, such as SAXS profiles, are implemented as an additional term in the scoring function [72]. To deal with the high level of ambiguity in some types of experimental information, such as NMR chemical shift perturbations, which define patches of interacting residues but not the pairwise relationships between them, HADDOCK implements and extends the ambiguous distance restraint concept used in NMR structure calculations [73] as ambiguous interaction restraints. These ambiguous interaction restraints are essentially one-to-many relationships between residues of different interacting molecules (HADDOCK supports simultaneous docking of up to six molecules [74]) and have the benefit of allowing the sampling method to choose energetically favored binding modes (electrostatics and van der Waal's) that agree with (only a portion of) the information. What truly sets HADDOCK apart from all other docking approaches is the use of the same (or different) data as restraints during the more accurate refinement stages (Fig. 2). In the default HADDOCK protocol, the rigid-body energy minimization stage produces and scores 1000 models (effectively sampling

10 000 models – an internal scoring being already performed). The best 200 are then optimized through semi-flexible simulated annealing in torsion angle space that allows for small conformational changes of up to 2 Å, typically [65] (although improvements of up to ~ 5 Å have been observed for flexible peptides [75]), mimicking the induced fit mechanism of binding. User-provided interface data are also used in this stage, 'pulling' the interacting residues towards each other during the flexible optimization of the interface. The final refinement stage – a short restrained molecular dynamic simulation in explicit solvent – also uses the same information. All in all, the use of experimental or predicted interface data in HADDOCK pervades the entire protocol, not only during the orientation of the binding partners as in most other data-friendly approaches, but also in the flexible refinement of the modeled conformations.

In addition to the top performing approaches in the latest round of CAPRI, other traditionally well-performing approaches are worth mentioning. The coarse-grained ATTRACT docking developed by Zacharias [38] was recently extended to allow docking using cryo-electron microscopy (cryo-EM) density maps [76]. The very efficient docking suite by Wolfson and colleagues [24] is split into different applications that perform specific tasks: PatchDock [42] generates rigid-body models based on geometric hashing techniques, FlexDock [77] introduces flexibility around hinges and FireDock [24] refines the docked models. Other specific tools deal with symmetrical assemblies [42,78]. PatchDock can use interface information in a similar way to ClusPro, allowing 'attractive' and 'repulsive' regions to be defined, as well as offering the possibility of setting distance constraints. The same group has also developed methods to position proteins in cryo-EM density maps (MULTIFIT [79]). The RosettaDock approach of Gray *et al.* [80] makes use of the well-known Rosetta framework and can include experimental data in distance-based filters to bias the Monte Carlo sampling. Users of RosettaDock can also manually position the binding partners in space and then restrict the protocol to sample and refine only around these. The spherical polar Fourier approach HEX by Ritchie [26] has been programmed to make use of graphical processing units and therefore boasts a spectacular speed (~ 15 s for a typical docking run). It can also make use of experimental data, by specifying residues to define an intermolecular axis around which the rotational search will take place. Finally, ZDOCK, developed by Chen & Weng [27] and one of the oldest participants in CAPRI, has been continuously developed and improved over the years, and includes docking-specific knowledge-based scoring functions (e.g. ZRANK [51], IRAD and IFACE [30]) to complement a exhaustive FFT-based search powered by a new 3D convolution library [22].

Sources of experimental data used in docking

As elaborated previously, the most successful docking approaches in CAPRI, as well as many others, recognized the power of adding experimental data into the algorithm to bias the sampling and/or scoring. For experimentalists, this is a tremendous advantage, but one that requires careful planning because poor quality data will produce poor quality models ('garbage in, garbage out'). Having data from several sources is useful, but only when the information is not redundant.

Unambiguous high-resolution data on the interface are obviously the best information to use with docking. It maps one atom/residue on a monomer to another atom/residue on another monomer with, or without, a specific distance. Short (up to ~ 6 Å) interproton distances from NMR, based on the nuclear Overhauser effect, are a classic example. Other experiments are able to provide unambiguous distances over larger distances, such as Förster resonance energy transfer [81], chemical cross-linking [82] and electron paramagnetic resonance spectroscopy [83], but in many cases, these are of limited accuracy. It is important to note that, although distances are usually used to bring two atoms/residues into contact, they can also be used to keep them apart: if two residues are observed to have a very large minimum distance between them, this can also be valuable information for the docking. Information about the involved interface residues is somewhat easier to obtain and is especially powerful when multiple sources are combined. For example, mutagenesis, NMR chemical shift perturbation and cross-saturation, hydrogen/deuterium exchange and limited proteolysis all allow narrowing of the interacting surface of the monomers to (small) regions. Most data-driven docking approaches offer support for this sort of ambiguous information, implementing it such that an energetic 'bonus' is given to models that place the selected regions at the interface. This leniency allows a portion of the data to be not respected, which is useful in the case of false positives in the experimental data. In particular, HADDOCK randomly removes half (by default – can be defined) of the ambiguous interaction restraints for each docking trial. This means that each model is generated with a slightly different set of restraints, decreasing the odds that erroneous information forces the docking into sampling

and scoring wrong conformations. Shape information from SAXS, small-angle neutron scattering (SANS) and cryo-EM also provides valuable data, particularly for asymmetric complexes and assemblies of many partners. Although not trivial to implement, explicitly using density maps obtained from cryo-EM and fitting the interacting molecules into them is extremely helpful in reducing the conformational search. This of course depends on the resolution of the density maps. The same is valid for SAXS and SANS. The resulting scattering curves, or the radius of gyration derived from them, can be used for scoring. The latter can also be used directly as a restraint in docking [84]. SANS, in particular, is specifically useful for protein–nucleic acid complexes, as shown in a recent study on a large molecular mass protein–protein–RNA complex [85]. Collision cross-section data obtained from ion mobility-MS [86] can also be valuable for describing protein complex shape, although the information content for smaller systems might be rather limited [68,72].

Predicting from predictions: bioinformatics interface predictions

Biological systems such as protein–protein complexes are not always researcher-friendly and more often than not refuse to behave under the experimental conditions required to obtain structural data. In such cases, bioinformatics prediction methods may provide hints that can be used instead of experimental information to drive the docking calculations (Table 2). Predictions are also extremely useful when only a tiny amount of experimental information is available. Much like experimental techniques, bioinformatics interface predictors provide different types of data depending on their algorithms and theoretical principles. Most methods use sequence/structure conservation to define residues that might be important for interactions (e.g. WHISCY [87], PredUs [88], Consurf [89]). Other methods analyze the structural properties of the monomers, such as surface-exposed residues, and/or employ propensity scales for pairs of residues in protein complexes (e.g. InterProSurf [90], SPPIDER [91], ProMate [92]). Others yet, combine both types of information or several different methods to offer a consensus or meta-prediction, which has been shown to be more accurate than the individual methods (e.g. CPORT [93], PresCont [94], meta-PPISP [95]). Surprisingly, most prediction servers take into account only the monomer sequence and/or structure for their analysis, i.e. the predictions are not partner specific. The predictions tend then to be hardly specific and a give rise to large and diffuse potential interaction patches on the surface of the monomers. To increase specificity, some research groups developed partner-aware or partner-specific methods [96,97]. Motivated by the colossal amount of sequence information from mass sequencing efforts, methods have also been developed to dig deep into the evolutionary history of protein sequences hidden in multiple sequence alignments. By correlating residues across potentially interacting sequences, unambiguous interaction information at the residue level can be obtained. To date, this has been used to score the models [54,55], but recent developments hint at a potential application in other stages of docking [98]. Last, but not least, docking programs have been used to predict binding regions for other docking programs. The idea that given enough sampling and an energy function, the true interface will be sampled more often than at random has garnered quite a following, despite the obvious deficiencies of the sampling/scoring mentioned at the beginning of this review. Nevertheless, some of these approaches (e.g. RCF/ZDOCK [99]) have been applied in the latest CAPRI round and have shown interesting results which hint that statistical analysis of contacts in large pools of models gives some degree of predictive information. However, this applies only for very large pools of models in which the sampling is exhaustive, and relies exclusively on energy functions that have to be simple to assess such number of models within a reasonable period. As a rule of thumb, and as some of these prediction servers openly advise, the predictions should be analyzed carefully and critically, preferably in light of some experimental or functional data.

Beyond docking: other approaches for protein interaction modeling

Docking is not the only method to model protein interactions because, in principle, any structure prediction can be applied to the docking problem. Among these, a few particular methods deserve praise: the integrative modeling platform [100] and the modeling of complexes based on experimentally determined homologues [101–104].

The integrative modeling platform is a multiscale umbrella approach that combines several different scripts to model structures from various sources of information, most notably cryo-EM and SAXS. It is likely to be the method of choice for modeling very large assemblies, because most docking methods are limited to a few interacting partners and very few support the low-resolution data that help narrow the conformational space search in such cases.

Table 2. List of interface prediction methods available for docking predictions. For a complete overview of the available methods for protein–protein interface prediction and their performance refer to more in-depth reviews, e.g. Ref. [114] or [115].

Name	Type of prediction	Public Web Server
ConSurf [89]	Identifies close sequence homologues using (PSI-)BLAST, builds a multiple sequence alignment and then a phylogenetic tree. Position-specific conservation scores are then calculated using a empirical Bayesian or maximum-likelihood algorithm and divided into a discrete scale of nine grades.	http://consurf.tau.ac.il/
CPORT [93]	Consensus sequence-/structure-based predictor that combines WHISCY, PIER, ProMate, cons-PPISP, SPPIDER and PINUP.	http://haddock.chem.uu.nl/services/CPORT
InterProSurf [90]	Uses the solvent-accessible area of the monomers, together with a propensity scale for interface residues, and a clustering algorithm to identify high-scoring patches on the protein surface.	http://curie.utmb.edu/prosurf.html
Meta-PPISP [95]	Consensus structure-based predictor that combines cons-PPISP, PINUP and ProMate.	http://pipe.scs.fsu.edu/meta-ppisp.html
PredUs [88]	Potential interface residues are identified by iteratively mapping interaction sites of close and remote structural neighbors to individual residues on the query protein.	http://bhapp.c2b2.columbia.edu/PredUs/
PresCont [94]	Uses four residues properties – solvent-accessible area, hydrophobicity, sequence conservation and local environment of the amino acid in the protein – in a support vector machine classifier.	http://www-bioinf.uni-regensburg.de/php/prescont.php
ProMate [92]	Analyses the chemical character of surface residues, such as clustered hydrophobic and polar amino acids, as well as the B-factor of the residues in the unbound state.	http://bioinfo.weizmann.ac.il/promate/
PS-HomPPI [113]	Partner-specific interface predictor based on the *k* nearest interologues.	http://einstein.cs.iastate.edu/PSHOMPPI/
RCF [100]	Uses ZDOCK to generate models of the interaction and then analyses the contact frequency of each residue for each partner.	None
SPPIDER [91]	Integrates enhanced relative solvent accessibility predictions, evolutionary information, high-resolution structural data and physicochemical properties in a machine learning approach.	http://sppider.cchmc.org/
WHISCY [87]	Sequence conservation aided by surface structural information and the propensity scale for interface residues.	http://nmr.chem.uu.nl/Software/whiscy/

Another class of approaches to model protein complexes uses existing structures of homologous complexes to define the binding mode. Although modeling individual monomers by homology has been widely used as a step prior to docking [14], there is also a wealth of information in experimentally determined structures of homologous protein complexes. PRISM was developed to search and find, by rigid-body structural comparisons, template protein–protein interfaces that match a target protein, and then refine it using flexible docking protocols [101]. COTH is a powerful iterative threading approach that has been developed for protein–protein complex prediction [102]: it first queries a database of complex sequences and finds the 10 best matches, proceeds to model the sequence of the individual monomers, and finally superimposes these on the 10 original complex matches. Both methods were benchmarked against ZDOCK and a novel homology-based method (ZTEM) in a recent publication [105]. Kundrotas *et al*. claimed that 'there are enough templates to model nearly all protein complexes' [103] and developed a method to rigidly superimpose the interacting proteins on homologous complexes. Finally, KBDOCK [104] was also recently developed and uses domain–domain homology to model protein complexes.

Yet, sometimes even homologues interact differently [106] or show completely different specificities, even for as minute a change as an ASP to GLU mutation [107]. Homology-based methods will never accurately describe such effects, or allow the modeling of novel interaction modes. In addition, the template libraries used in these approaches should be expanded to include multidomain proteins, often the evolutionary forefathers of protein complexes, as demonstrated by the only successful prediction of the complex between the methyltransferase MTq2 and an activator protein (Trm12) from *Encephalitozoon cuniculi* (PDB 3Q87) [108] by the HADDOCK group [14].

Concluding remarks

Protein docking emerged in the late 1970s after a decade of methodological leaps in computational biology [109,110]. It has since grown, come of age and established itself as a pivotal method in structural

biology. The accuracy of current docking methods is far from perfect, but the inclusion of experimental information has benefitted them immensely. These so-called data-driven or integrative modeling methods can nowadays provide useful hints or even definite answers to biological problems. Their ability to deal with several sources of data and tackle evermore-complex systems is growing steadily. Plenty of challenges remain [111], e.g. genome-wide predictions, large conformational changes and binding-affinity prediction, but progress is being made in all fronts with quite satisfactory results. Although it is unreasonable to state that perfect predictions are around the corner, it is perfectly realistic to wonder if the next generation of scientists will be, much like their methods, computational/experimental hybrids and if these hold the key to unravel the mysteries of cellular and molecular biology.

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