

CAPRI: A Critical Assessment of PRedicted Interactions

Joël Janin,^{1*} Kim Henrick,² John Moult,³ Lynn Ten Eyck,⁴ Michael J. E. Sternberg,⁵ Sandor Vajda,⁶ Ilya Vakser,⁷ and Shoshana J. Wodak⁸

¹Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France

²European Bioinformatics Institute (EBI-EMBL), Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom

³Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland

⁴San Diego Supercomputer Center, UCSD 0505, La Jolla, California

⁵Structural Bioinformatics, Biochemistry Building, Department of Biological Sciences, Imperial College, London, United Kingdom

⁶Biomedical Engineering, Boston University, Boston, Massachusetts

⁷Bioinformatics Laboratory, Department of Applied Mathematics and Statistics, SUNY at Stony Brook, Stony Brook, New York

⁸Service de Conformation de Macromolécules Biologiques et Bioinformatique, Campus Plaine-BC6, Université Libre de Bruxelles, Brussels, Belgium

ABSTRACT CAPRI is a communitywide experiment to assess the capacity of protein-docking methods to predict protein–protein interactions. Nineteen groups participated in rounds 1 and 2 of CAPRI and submitted blind structure predictions for seven protein–protein complexes based on the known structure of the component proteins. The predictions were compared to the unpublished X-ray structures of the complexes. We describe here the motivations for launching CAPRI, the rules that we applied to select targets and run the experiment, and some conclusions that can already be drawn. The results stress the need for new scoring functions and for methods handling the conformation changes that were observed in some of the target systems. CAPRI has already been a powerful drive for the community of computational biologists who develop docking algorithms. We hope that this issue of *Proteins* will also be of interest to the community of structural biologists, which we call upon to provide new targets for future rounds of CAPRI, and to all molecular biologists who view protein–protein recognition as an essential process. *Proteins* 2003;52: 2–9. © 2003 Wiley-Liss, Inc.

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INTRODUCTION

This issue of *Proteins* describes CAPRI, a Critical Assessment of PRedicted Interactions, and the first communitywide experiment devoted to predicting protein–protein interaction and protein docking.¹ In 2001–2002, 19 participating groups tested their docking procedures in the blind prediction of seven target protein–protein complexes (Tables I and II). Five atomic models submitted by each group to independent assessors were compared to newly obtained X-ray structures of the complexes, which crystallographers had made available for the evaluation. The outcome of the experiment was discussed at the First

CAPRI Evaluation Meeting held in La Londe des Maures in Southern France, on September 19–21, 2002.

CAPRI was modeled on CASP, the Critical Assessment of Methods of Protein Structure Prediction, which began in 1992 at the initiative of John Moult. Four CASP evaluation meetings were held between 1994 and 2000 and a fifth at the end of 2002. The results were reported in special issues of *Proteins*.^{2–5} CASP stimulated the entire field of protein structure prediction and helped it develop by testing methods in blind predictions. Protein docking and structure prediction substantially overlap, and many groups in the docking community recognized the potential gains from a CASP-like experiment.⁶ A blind docking prediction should start from the known X-ray or NMR structures of two proteins and end with a comparison to a structure of the complex to which the predictors did not have access. Before CAPRI, this had been attempted only twice: in 1994 on the initiative of N. Strynadka and her colleagues of the University of Alberta⁷ and in 1996 as part of CASP2.⁸ In each case, the number of participating groups was small and there was a single target: the β -lactamase/BLIP complex for the Alberta challenge, a viral antigen-antibody complex in CASP2.

ORGANIZATION OF THE EXPERIMENT

The idea of launching a communitywide docking experiment was brought forward in June 2001 at the Conference on Modeling Protein Interactions in Genomes held in Charleston, South Carolina⁹ and was strongly supported. Because of the likely difficulty in finding suitable targets, it was proposed that CAPRI would be data driven. Unlike CASP, which has a fixed time schedule, the experiment would start whenever an experimentalist offers an adequate target and end 6–8 weeks later with the submission of the predicted structures and their assessment.¹

*Correspondence to: Joël Janin, Laboratoire d'Enzymologie et Biochimie Structurales-CNRS, 91198 Gif-sur-Yvette, France. E-mail: janin@lebs.cnrs-gif.fr

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TABLE I. Targets of Rounds 1 and 2

Target	Complex	Receptor	Ligand	Interface		Residue contacts ^c	Nonpolar/polar/charged composition ^d	Biochemical information ^e	Ref.
				RMSD (Å) ^a	area (Å ²) ^b				
T01	HPr kinase-HPr 1kkl	kinase 1jbl	HPr lsph, 1ptf	2.0	1690	56	49/24/28	P-loop of kinase near HPr Ser46	23
T02	Rotavirus VP6-Fab	VP6 lqhd	bound Fab	0.6	1575	52	44/46/9	CDRs EM of complex	^f
T03	Flu hemagglutinin-Fab 1ken	hemagg. 1hgh	bound Fab	1.0	1960	63	35/57/8	CDRs	21
T04	α-amylase-camelid Ab AM-D10 1kxv	amylase 1pif	bound VHH	0.4	1820	58	38/25/38	CDRs	22
T05	α-amylase-camelid Ab AM-07 1kxt	amylase 1pif	bound VHH	0.4	1705	64	43/35/22	CDRs	22
T06	α-amylase-camelid Ab AM-D9 1kxq	amylase 1pif	bound VHH	0.4	2310	65	36/42/21	CDRs, enzyme inhibition	22
T07	SpeA superantigen-TCRβ 110x	TCRβ 1bec	SpeA 1blz	1.1	1225	37	28/44/28	Homolog in PDB 1jck	26

^aRoot-mean-square displacement of main-chain atoms due to conformation changes between the free proteins and the complex. The RMSD is calculated on a monomer in targets 1–3. It excludes the bound antibody moieties of targets 2–6.

^bThe interface area is the sum of the solvent accessible surface area of the ligand and the receptor less that of the complex. Only one ligand molecule is considered in targets 1–3.

^cPairs of receptor and ligand residues with at least one atom 5 Å apart.

^dPercent of the interface residues bearing nonpolar, neutral polar or charged side-chains.

^eAvailable in the literature at the time of prediction.

^fM.C. Vaney and F. Rey, to be published.

TABLE II. CAPRI Participant Groups

Group	Institution	Predictions
Abagyan	Scripps Research Institute, La Jolla, CA	All targets
Camacho	Boston University, Boston, MA	All targets
Eisenstein	Weizmann Institute, Rehovot, Israel	All targets
Fitzjohn-Bates	Cancer Research UK, London, UK	Targets 4–7
Gardiner	Sheffield University, UK	Targets 1–3
Gray-Baker	University of Washington, Seattle, WA	All targets
Mustard	University of Aberdeen, UK	Target 7
Norel	Columbia University, New York, NY	All targets
Olson	Scripps Research Institute, La Jolla, CA	Targets 1–3
Palma-Krippahl	Universidade Nova, Lisboa, Portugal	Targets 2, 4–7
Ritchie	University of Aberdeen, UK	All targets
Shoichet	Northwestern University, Chicago, IL	Target 1
Sternberg-Smith	Imperial College, London, UK	All targets
Ten Eyck	UCSD, La Jolla, CA	All targets
Umemaya	Kitasato University, Kitasato, Japan	Targets 4–6
Vakser	SUNY, Stony Brook, NY	Targets 1–3
Valencia	Universidad Autonoma, Madrid, Spain	Target 1
Wang	Beijing Polytechnic University, Beijing, China	Targets 4–7 ^a
Weng	Boston University, Boston, MA	All targets
Wolfson-Nussinov	Tel Aviv University, Israel-NCI, Frederick, MD	All targets

^aSubmissions made after the deadline for round 2.

Round 1 of CAPRI began in July 2001, with three target protein–protein complexes and 19 predictor groups. It was followed in January 2002 by round 2, with four new targets and 14 participating groups (a 15th group submitted predictions after the deadline). A total of 557 atomic models were submitted by the predictors in these two rounds. All have now been assessed and fully evaluated. The authors of this article are the members of the CAPRI Management Group, who were responsible for the organization of the experiment. As the primary contact person with the experimentalists, Joël Janin was in charge of

collecting targets, a crucial step for the entire experiment. Kim Henrick created and maintained the CAPRI Web site (<http://capri.ebi.ac.uk/>) hosted by the European Bioinformatics Institute. The Web site has been the major method of communication between the management group and the predictors. All the information on the targets was given through a passworded section of the Web site, and all predictions were deposited there. Shoshana Wodak and her coworkers accepted the role of assessors. This included collecting the predictions, establishing and calculating all quality measures used in the evaluation, selecting the best

predictions for each target, and finally assessing the current state of the art. In this issue, the assessors' report is followed by contributions from individual prediction groups giving insight into their methodology. Taken together, the articles included in this issue of *Proteins* should provide an accurate and fairly complete overview of the present state-of-the-art in predicting protein-protein interaction by structure-based methods.

DOCKING IN THE POSTGENOMIC AGE

Interest in protein-protein interaction has grown fast during the last few years, largely as the outcome of proteomics studies such as genome-wide yeast two-hybrid assays^{10,11} or high-throughput mass spectrometry.^{12,13} These studies show that most if not all proteins have interacting partners in the cell, and often more than one. They provide long lists of putative complexes, which beg biochemical and structural characterization. The emerging map of protein-protein interactions is a major challenge to experimentalists and also to computational biologists.¹⁴ On the applied side, potential drugs are being developed to control the interaction between two proteins. Every new X-ray or NMR structure of a complex provides a level of understanding that nonstructural methods complement efficiently but cannot replace. Many of the suggested complexes may be weak and transient and, even after the protein components have been isolated and their structures determined, the fraction that will be amenable to direct analysis by X-ray crystallography or NMR will remain small. Thus, computational methods that can elucidate the details of specific protein-protein interaction at the atomic level are becoming of greater value as more structures of individual proteins are determined while the protein-protein interaction map expands, but they must be assessed first.

Unlike small molecule docking, which has become almost a routine computational tool in rational drug design,¹⁵ protein-protein docking has remained largely an academic exercise up to now. The few programs that are available on the Web have rarely been used outside the laboratories that directly participate in method development, and their capacity to produce meaningful biological information remains to be shown. A communitywide blind prediction test seemed a necessary step toward proving the value of the prediction methods and assessing their reliability, before transferring the technology to a wider circle of users. For protein folding, CASP and its equivalent CAFASP for Web servers¹⁶ have proved that some technologies have achieved maturity, particularly for homology modeling, and detected others that are promising, yet still need development.¹⁷ Over the years, CASP has pushed predictors to provide fully automated services and make protein structure prediction servers accessible to a much broader community. We hope that CAPRI will promote the same progress in the area of protein-protein docking and recognize that, on the way, some of the findings may be uncomfortable.

WHAT IS A TARGET FOR CAPRI?

Like CASP, CAPRI relies on the generosity of experimentalists willing to communicate unpublished atomic coordinates on a confidential basis. Any soon-to-be-released protein structure can be a CASP target, but not all soon-to-be-released protein complexes are valid targets for CAPRI: the component structures must be known independently. Taking a complex apart and reconstructing it in the computer is known as "bound" docking. It is a useful computational exercise to test programs but not a realistic problem. In bound docking, the two components are frozen in their bound conformation. A lock-and-key fit is achieved in the right orientation and position and only there, which very much biases the prediction. "Unbound" docking starts from the two independently determined protein structures. Therefore, it must handle the conformation changes that are inevitable on association and make docking a far more difficult task than just searching for an exact fit.

The Protein Data Bank¹⁸ contains a few dozen systems for which the complex and its two component proteins have had their structure independently determined, enabling unbound docking. Systems in which only one of the two structures is known allow for "bound-unbound" docking. There, one component is from the independent structure, and the second component is taken from the complex. Conformation changes in the unbound component generally suffice to prevent an exact fit at the interface. Although less scarce than for unbound docking, these systems still represent <1% of the PDB. A list of entries that can be used as a benchmark for unbound and bound-unbound docking is given in this issue.¹⁹

THE TARGETS OF ROUNDS 1 AND 2

Given the paucity of suitable structures, it was a major achievement and a very important contribution from the crystallographic community that seven targets, two unbound and five bound-unbound, were made available for CAPRI predictions within a few months (Table I and Fig. 1). The seven targets covered different biological systems and offered a variety of degrees of difficulty. All the complexes had interfaces burying areas in the range of 1200–2300 Å², similar to most antigen-antibodies or enzyme-inhibitor complexes. These interfaces implicated 17–37 residues on each component proteins, and these residues formed 37–64 pairwise contacts. Judged by the type of the residues involved, the targets represented the whole range of hydrophobic and electrostatic interactions that is commonly observed in protein-protein complexes.²⁰

The five bound-unbound targets were antigen-antibody complexes in which the antigen structure was known in advance. Coordinates for the antibody moieties, taken from the complex and randomly reoriented, were kindly communicated by the crystallographers at the beginning of each round. We owe target T02, a complex of the rotavirus VP6 protein with Fab fragments of a monoclonal antibody, to Dr. M.C. Vaney and F. Rey (LVMS-CNRS, Gif-sur-Yvette, France); Target T03, a complex between the influenza virus hemagglutinin and a Fab fragment that pre-

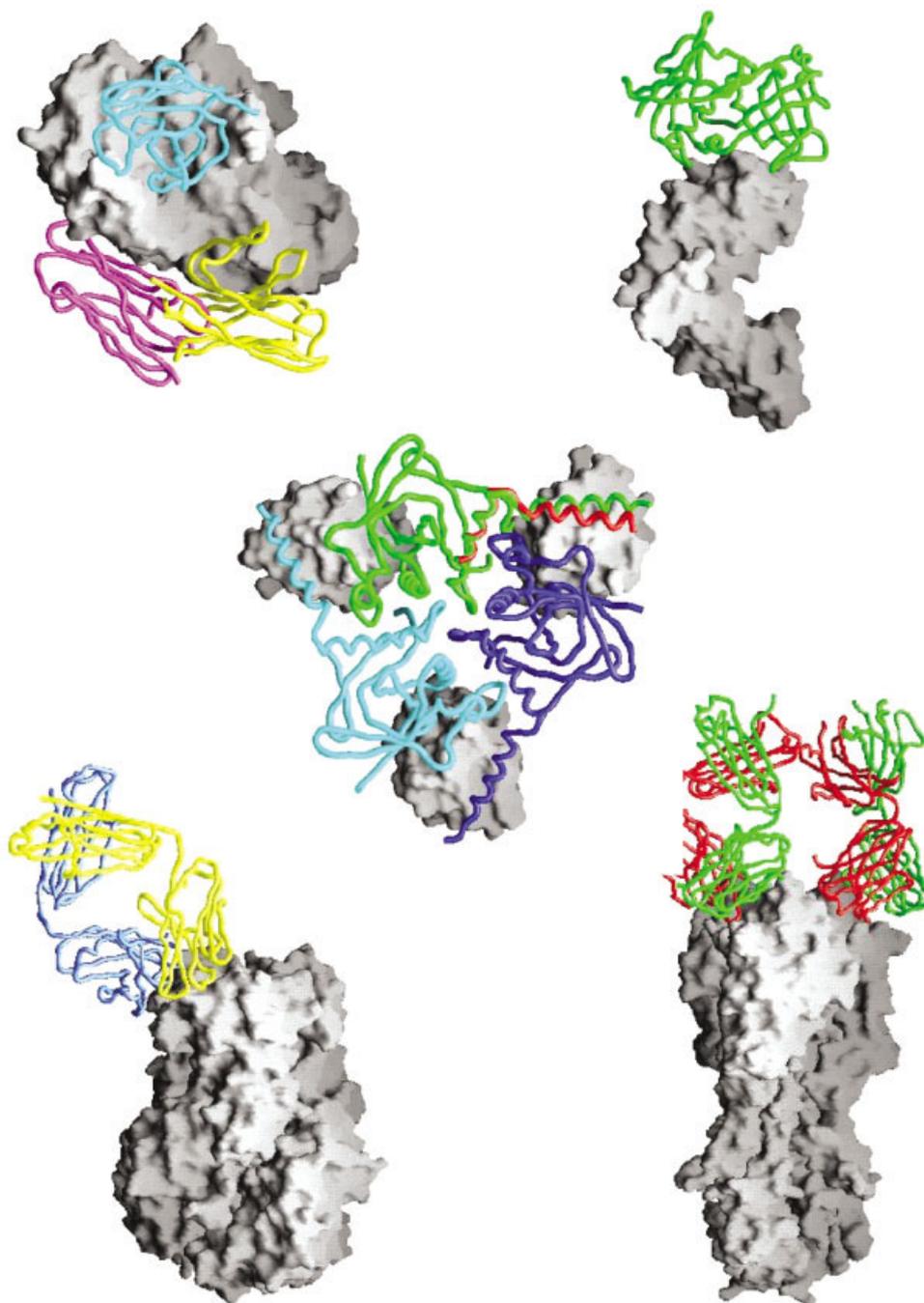


Fig. 1. The seven targets of CAPRI. **Top left:** The camelid VHH domains (drawn as backbone tubes) of T04, T05, and T06 bound to pig α -amylase²² (drawn as a molecular surface). The blue domain of AM-D9 in T06 faces the active site of the enzyme and binds through its CDRs. AM-D10 in T04 and AM-B7 in T05 bind in an unusual lateral way through framework as well as CDR residues. **Top right:** The streptococcal superantigen toxin (tube) bound to TCR β in T07.²⁶ **Center:** Half the HPr kinase hexamer (tubes) with three bound HPr molecules (surface). The C-terminal helix in red is from the free kinase. It moves to the position in green in the complex.²³ **Bottom left:** One of the three symmetry-related Fab molecules (tubes) bound to the VP6 trimer (surface) in T02. **Bottom right:** The flu hemagglutinin trimer (surface) binds two Fab molecules (tubes) in T03.²¹ Drawn with GRASP.³⁸

vents the hemagglutinin low pH transition,²¹ to Dr. C. Barbey and M. Knossow (LEBS-CNRS, Gif-sur-Yvette, France); and targets T04, T05, and T06, three complexes of the same pig α -amylase with different VHH domains of

camelid antibodies,²² to Dr. C. Cambillau and colleagues (AFMB-CNRS, Marseilles, France). The two viral antigens were large trimeric proteins with some 10,000 atoms, a challenge to the capacity of existing docking software. The

single-domain camelid antibodies illustrated original—and largely unexpected—features of antigen-antibody recognition. The antibody VHH domains of targets T04 and T05 interacted with their respective epitopes through framework residues as well as the hypervariable loops of the complementarity determining regions (CDRs). Together, the five targets offered the opportunity of running a major exercise, and a fairly successful one, in epitope prediction and antibody docking with little interference of conformational changes.

Targets T01 and T07 were for unbound docking. Target T01, a gift of Dr. S. Fieulaine and S. Nessler in J. Janin's group at LEBS-CNRS (Gif-sur-Yvette, France), was a complex between a protein kinase from *Lactobacillus casei* and its protein substrate, the small protein HPr.²³ The enzyme is a hexamer with a fold unrelated to eukaryotic protein kinase. Because it also catalyzes HPr dephosphorylation by phosphorolysis, it is known as HPr kinase-phosphorylase.²⁴ Biochemical information was available in the literature on the site of phosphorylation (Ser46 of HPr) and on the location of the enzyme-active site. Nevertheless, the HPr-HPr kinase complex proved to be a difficult target. In the complex, the kinase undergoes a conformation change that causes a root-mean-square main-chain displacement (RMSD) of 2.0 Å between the free and bound subunit (Table I). This compares with the RMSDs of 0.4–1.0 Å, which are observed in the antigen moieties of targets T02–06 (the RMSD is 0 in the bound antibody moieties). The change seen in the kinase is mostly a rotation of the C-terminal helix, which makes contacts with HPr. Such changes should be expected in unbound docking, yet they are encountered in few of the systems on which most docking software is tested. Ironically, the X-ray structure of a closely related kinase,²⁵ released a few months after round 1 was completed, has the C-terminal helix in a position close to that seen in the complex. Target T01 is likely to promote new developments in the way docking methods handle flexibility and conformation changes. Target T07, a gift of Dr. R. Mariuzza and E.J. Sundberg (CARB, Rockville, MD) involved a streptococcal superantigen toxin binding to the β -chain of the T-cell receptor.²⁶ Its rather small interface also made it a difficult problem for straight computational docking, but a homology search would lead to a related structure already deposited in the PDB. Most predictors submitted at least one model that was close to that structure, which proved to be the right choice.

THE DOCKING PROCEDURES

Docking procedures have been reviewed recently,^{27,28} and those that have been used for CAPRI predictions are described by their authors in specific articles of this issue. Some of the algorithms are well established, others are essentially novel, and all underwent quick evolution during the last 2 years, if only because they had to meet demands posed by the CAPRI targets. Although the first attempt to computationally reconstitute a complex by docking two proteins together dates from the late 1970s,²⁹ algorithms (and computers) that could systematically

search the six-dimensional space of rigid body docking became available only in the early 1990s.^{31–36} The representation of the protein surface on a cubic grid^{31,34} and the fast Fourier transform search algorithm,³³ which several CAPRI participants used are from this period. Other very interesting algorithms have appeared since but, in the last 10 years, the bulk of the effort has been directed toward solving two major problems: introducing flexibility or softness in the rigid body search³¹ and developing a scoring function that discriminates efficiently between the correct docking solution and the many false positives that the search brings up. Both problems are grounded in the physical chemistry of proteins: they demand an accurate representation of the protein conformation and of all the free energy terms that govern folding and assembly. In practice, the contributions of conformation changes, electrostatics, and the solvent have to be approximated. The procedures that were used in CAPRI explore some of the many ways to do these approximations. Because no definitive solution has yet emerged, improving the scoring functions and handling flexibility will be once again top priorities on the agenda of the predictors.

USING BIOLOGICAL INFORMATION IN DOCKING

Incorporating nonstructural information in docking procedures is another field of active research. For each target of CAPRI, some literature data were available to the participants. Most predictors used this information, either to restrict the six-dimensional search and gain computer time or to eliminate false positives after the search.

In target T01, the phosphate-binding loop (P-loop) at the active site of HPr kinase had to be near Ser46 of HPr, which the kinase phosphorylates. This assumption was made by most participants. In addition, at least two groups used as a guide the conservation of other residues in the vicinity of the active site. This information was correct, but it proved incapable to drive the search toward the right solution in face of the conformation change in the kinase. In contrast, sequence information alone led to the correct solution for target T07: the superantigen toxin-TCR β complex had a homologue in the PDB, and the mode of binding was conserved. The same reasoning could not be used in predicting targets T02–06, but in antigen-antibody complexes, the CDRs of the antibodies could be assumed to be part of the set of interacting residues. Although founded, this assumption turned out to be misleading in the case of targets 4 and 5. In these complexes with α -amylase, the two camelid VHH domains adopt an unusual orientation relative to their epitope, and their CDRs provide only one fraction of the contact.²² Most prediction procedures failed by unduly restricting the range of positions and orientations allowed to the VHH domains during the search. Thus, there were some good predictions of the region of α -amylase that forms the epitope in target T05, but none of the structures was correct. In target T06, the VHH domain has a more usual mode of binding, with the CDRs pointing toward the α -amylase. It forms a large interface with the enzyme and blocks its active site. The native solution was

easily found by a variety of docking methods. It is of interest that the camelid antibody of target T06 has a higher affinity for α -amylase than the other two,³⁶ and there was a correlation between the affinity and the rate of success of the predictions on these three targets.

THE EVALUATION CRITERIA

The value of a prediction depends first on what it will be used for. The structure of a complex predicted by docking may find many applications, each with its particular demands. Site-directed mutagenesis, for instance, relies on interface residues being (mostly) correctly identified, but it ignores the geometry of the complex. Conversely, fitting an all-atom model in a medium-resolution electron microscopy image is sensitive to the geometry, not to the nature of the residues. Thus, we had to develop for CAPRI evaluation criteria that could meet different requirements. This was done first while assessing round 1, and the procedure was reviewed at the evaluation meeting. The criteria are briefly described below, and we refer to the article by Mendez et al.³⁷ in this issue for further details.

To describe the geometric fit, the root-mean-square displacement of the ligand (ligand RMSD) has been commonly used in the past. It is obtained by comparing the positions of the ligand C α or main-chain atoms in the predicted model and the X-ray structure, after a least-square superposition of the receptors in the two models. The fit of the predicted to the experimental ligand position can be described by two other numbers: the misorientation angle, which is the angle of the residual rotation that superimposes the two ligand positions after superposing the receptors, and the misplacement distance between the centers of mass of these two positions.

With a large ligand, the RMSD may give a poor impression of the fit at the interface. For instance, an elongated molecule binding to the receptor at one end may yield a high value even though most of the interface residues and their contacts are correctly predicted. This was taken into account by measuring the RMSD only on the ligand residues that contribute to the interface in the X-ray structure. The interface RMSD is a geometric test of the quality of the prediction of the regions that interact on the two molecules and of the ligand-receptor contacts. These features could be directly evaluated in the prediction and the X-ray structure by comparing: (a) the sets of receptor residues in contact with the ligand; (b) the sets of ligand residues in contact with the receptor; and (c) the sets of pairwise residue-residue contacts in the two proteins. (a) and (c) involved 17–34 residues in the CAPRI targets. The ratio of correctly predicted to native contact pairs should correlate with the interface RMSD, unless it is artificially increased by pushing the ligand into the receptor. This was checked by monitoring the number of bad contacts (steric overlaps) between atoms of the two molecules and rejecting predictions where that number was unacceptably high. Despite their limitations, we thought that there was valuable information in both the geometric measure of fit and the fractions of correctly predicted to native residues or pairs and used both as a basis for evaluation.

ACCURATE AND USEFUL PREDICTIONS

What then, is a good prediction? The experience on test systems, confirmed by the results of rounds 1 and 2 of CAPRI, is that a structural model with an interface RMSD <2 Å typically predicts $>30\%$ of the native residue-residue pairwise contacts. This requires at least 50% of the contact residues to be correctly identified. Then, the ligand RMSD is <5 Å and the misorientation $<20^\circ$, depending on the shape and size of the molecules and of the interface. Although the latter limits may seem generous, the accuracy of such a prediction should be sufficient for most applications. Achieving a 30% correct prediction of the pairs in contact is highly demanding and a very satisfying achievement for the predictors. In CAPRI rounds 1 and 2, this degree of accuracy was obtained in one prediction of target T02, two of T03, seven of T06, and seven of T07.³⁷ Although the success on T07 may have relied on the presence of an homologue in the PDB, the accurate prediction of T02, T03, and T06 is genuine and impressive. Remarkably, the epitopes recognized by the antibodies of these three targets were identified with $>80\%$ accuracy in all these predictions.

Lesser quality predictions may certainly be useful in some applications. We considered as acceptable all docking models that had $>10\%$ of the correct contact pairs and an interface RMSD <4 Å. In most of these, half of the interface residues or more were correctly predicted on both the ligand and the receptor. Thus, the models could serve to guide site-directed mutagenesis and generate biochemical experiments that would eventually identify the correct contacts. Nevertheless, the geometric fit to the X-ray structures was often poor, and the ligand was misoriented by up to 60° in some of the models. Seven predictions of T01 were in that category, five of T02, but none of T03–06. In T01, a few ($<20\%$) of the residues involved in contacts on HPr and in the kinase active site were known or could be guessed from the structure in advance. The best models of that target correctly predicted 50–80% of the contact residues and 20–33% of the pairs. In the antigen-antibody complexes, epitope prediction was either accurate (as in T02, T03, and T06) or it failed completely (in T04 and T05), probably due to assumptions made by the predictors concerning the role of the CDRs and the mode of antibody binding, rather than to the docking procedures themselves.

PROGRESS IN DOCKING

The only blind protein-docking experiments before CAPRI were performed on the single targets offered at the Alberta Challenge in 1994 and at CASP2 in 1996. In 1994, six groups submitted predictions of the β -lactamase-inhibitor complex. All came reasonably close to the crystal structure and reproduced most of the native residue-residue contacts.⁷ Thus, they were accurate predictions as defined above. In the best case, the main-chain RMSD between the inhibitor in the prediction and the X-ray structure was only 3.5 Å. In 1996, four groups submitted a total of 13 models of an antibody bound to hemagglutinin.⁸ The best submission contained only 8 of 59 native residue-

residue contacts and had an interface RMSD of 8.5 Å, well above the limit we set for acceptable predictions. Thus, the two tests gave opposite impressions of the predictive capacity of docking methods. However, the success on one target and failure on the other clearly had more to do with the nature of the targets than with the performances of the methods. All algorithms did well on the β -lactamase-inhibitor complex, presumably because of large complementary surfaces forming an interface burying 2560 Å², whereas the antibody-hemagglutinin complex was entirely beyond their reach.

With seven protein-protein complexes as targets, the two rounds of CAPRI offered a much better opportunity to evaluate the performances of the prediction procedures under a variety of conditions. The evaluation meeting suggested that substantial progress has been made since CASP2 in 1996. Collectively, the 19 participant groups submitted accurate predictions of four of the seven targets and acceptable ones of five. This included the antibody-hemagglutinin complex of target 3, which was comparable in difficulty to the single CASP2 target, and the α -amylase-camelid VHH domain complex of T06, which was correctly predicted by half of the groups. Thus, docking is on its way to becoming a practical approach that can contribute to our understanding of protein-protein recognition.

THE FUTURE OF CAPRI

The management group intends rounds 1 and 2 of CAPRI to be followed by others. We are ready to organize new challenges on a continuous basis, depending on the availability of targets, and to call regular meetings in which the predictors can collectively evaluate their achievements. We hope that new groups with novel algorithms will participate in these challenges. More than ever, the organization of future rounds of CAPRI will depend on the goodwill of crystallographers and NMR scientists who can provide the targets. The management team calls on all structural biologists who reckon that prediction is a useful complement to experiment and that the models generated by docking can guide genetic and biochemical experiments, once the methods and algorithms have been extensively tested and their validity assessed.

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REFERENCES

- Janin J. Welcome to CAPRI: a critical assessment of predicted interactions. *Proteins* 2001;47:257.
- Proteins* 1995;23:Suppl 3.
- Proteins* 1997;Suppl 1.
- Proteins* 1999;Suppl 3.
- Proteins* 2001;Suppl 5.
- Moult J, Fidelis K, Zemla A, Hubbard T. Critical assessment of methods of protein structure prediction (CASP): round VI. *Proteins* 2001;Suppl 5:2–7.
- Strynadka NCJ, Eisenstein M, Katchalski-Katzir E, Shoichet B, Kuntz I, Abagyan R, Totrov M, Janin J, Cherfils J, Zimmermann F, Olson A, Duncan B, Rao M, Jackson R, Sternberg M, James MNG. Molecular docking programs successfully determine the binding of β -lactamase inhibitory protein to TEM-1 β -lactamase. *Nat Struct Biol* 1996;3:233–239.
- Dixon JS. Evaluation of the CASP2 docking section. *Proteins* 1997;Suppl 1:198–204.
- Vajda S, Vakser IA, Sternberg MJE, Janin J. Modeling of protein interactions in genomes: meeting report. *Proteins* 2002;47:444–446.
- Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, et al. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 2000;403:623–627.
- Ito T, Chiba T, Osawa R, Yoshida M, Hattori M., Sasaki Y. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci USA* 2000;98:4569–4574.
- Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edlmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 2002;415:141–147.
- Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutillier K, Yang L, Wolting C, Donaldson I, Schandorff S, Shewnarane J, Vo M, Taggart J, Goudreau M, Muskat B, Alfaro C, Dewar D, Lin Z, Michalickova K, Willems AR, Sassi H, Nielsen PA, Rasmussen KJ, Andersen JR, Johansen LE, Hansen LH, Jespersen H, Podtelejnikov A, Nielsen E, Crawford J, Poulsen V, Sorensen BD, Matthiesen J, Hendrickson RC, Gleeson F, Pawson T, Moran MF, Durocher D, Mann M, Hogue CW, Figeys D, Tyers M. Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 2002;415:180–183.
- Janin J, Wodak SJ. Protein modules and protein-protein interaction: toward a global view. *Adv Protein Chem* 2002;61:1–8.
- Gane PJ, Dean PM. Recent advances in structure-based rational drug design. *Curr Opin Struct Biol* 2000;10:401–404.
- Fischer D, Elofsson A, Rychlewski L, Pazos F, Valencia A, Rost B, Ortiz AR, Dunbrack RL Jr. CAFASP2: the second critical assessment of fully automated structure prediction methods. *Proteins* 2001;Suppl 5:171–183.
- Venclovas C, Zemla A, Fidelis K, Moult J. Comparison of performance in successive CASP experiments. *Proteins* 2001;Suppl 5:163–170.
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Res* 2000;28:235–242.
- Chen R, Mintseris J, Janin J, Weng Z. A protein-protein docking benchmark. *Proteins* 2003;(this issue).
- Lo Conte L, Chothia C, Janin J. The atomic structure of protein-protein recognition sites. *J Mol Biol* 1999;285:2177–2198.
- Barbey-Martin C, Gigant B, Bizet T, Calder LJ, Wharton SA, Skehel JJ, Knossow M. An antibody that prevents the hemagglutinin low pH transition. *Virology* 2002;294:70–74.
- Desmyter A, Spinelli S, Payan F, Lauwereys M, Wyns L, Muylder-

- mans S, Cambillau C. Three camelid VHH domains in complex with porcine pancreatic α -amylase. *J Biol Chem* 2002;77:23645–23650.
23. Fieulaine S, Morera S, Poncet S, Mijakovic I, Galinier A, Janin J, Deutscher J, Nessler S. X-ray structure of a bifunctional protein kinase in complex with its protein substrate HPr. *Proc Natl Acad Sci USA* 2002;99:13437–13441.
 24. Mijakovic I, Poncet S, Galinier A, Monedero V, Fieulaine S, Janin J, Nessler S, Marquez JA, Scheffzek K, Hasenbein S, Hengstenberg W, Deutscher J. Pyrophosphate-producing protein dephosphorylation by HPr kinase/phosphorylase: a relic of early life? *Proc Natl Acad Sci USA* 2002;99:13442–13447.
 25. Marquez JA, Hasenbein S, Koch B, Fieulains S, Nessler S, Russell RB, Hengstenberg W, Scheffzek K. Structure of full-length HPr kinase/phosphatase from *Staphylococcus xylosus* at 1.95 Å resolution: mimicking the product/substrate of the phospho transfer reactions. *Proc Natl Acad Sci USA* 2002;99:3458–3463.
 26. Sundberg EJ, Li H, Llera AS, McCormick JK, Tormo J, Schlievert PM, Karjalainen K, Mariuzza RA. Streptococcal superantigens bound to TCR β chains reveal diversity in the architecture of T cell signaling complexes. *Structure* 2002;10:687–699.
 27. Halperin I, Ma B, Wolfson H, Nussinov R. Principles of docking: an overview of search algorithms and a guide to scoring functions. *Proteins* 2002;47:409–443.
 28. Wodak SJ, Janin J. Structural basis of molecular recognition. *Adv Protein Chem* 2002;61:9–73.
 29. Wodak SJ, Janin J. Computer analysis of protein-protein interactions. *J Mol Biol* 1978;124:323–342.
 30. Cherfils J, Duquerroy S, Janin J. Protein-protein recognition analyzed by docking simulation. *Proteins* 1991;11:271–280.
 31. Jiang F, Kim SH. Soft docking: matching of molecular surface cubes. *J Mol Biol* 1991;219:79–102.
 32. Shoichet BK, Kuntz ID. Protein docking and complementarity. *J Mol Biol* 1991;221:327–346.
 33. Katchalski-Katzir E, Shariv I, Eisenstein M, Friesem AA, Aflalo C, Vakser IA. Molecular surface recognition: determination of geometric fit between proteins and their ligands by correlation techniques. *Proc Natl Acad Sci USA* 1992;89:2195–2199.
 34. Wang H. Grid-search molecular accessible surface algorithm for solving the protein docking problem. *J Comp Chem* 1991;12:746–750.
 35. Fischer D, Norel R, Wolfson H, Nussinov R. Surface motifs by a computer vision technique: searches, detection, and implications for protein-ligand recognition. *Proteins* 1993;16:278–292.
 36. Lauwereys M, Arbabi Gharoudi M, Desmyter A, Kinne J, Holzerr W, De Genst E, Wyns L, Muyldermans S. *EMBO J* 1998;17:3512–3520.
 37. Mendez R, Leplae R, de Maria L, Wodak SJ. Assessment of blind predictions of protein-protein interactions: current status of docking methods. *Proteins* 2003;52:51–67.
 38. Nicholls A, Sharp K, Honig B. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* 1991;11:281–296.