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RESEARCH ARTICLE

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Modeling of protein complexes in CAPRI Round 37 using template-based approach combined with model selection

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Abstract

We participated in Round 37 of the Critical Assessment of PRediction of Interactions (CAPRI), held jointly with the 12th edition of the Critical Assessment of protein Structure Prediction (CASP12), having two major objectives. First, we intended to test the utility of our PPI3D web server in finding and selecting templates for comparative modeling of structures of protein complexes. Our second aim was to evaluate the ability of our model accuracy estimation method VoroMQA to score and rank structural models for protein-protein interactions. Using sequence search in PPI3D and HHpred servers we identified multimeric templates for 7 of 11 CAPRI targets, and models of at least acceptable quality were constructed for 6 of them. The clustering and visual analysis features implemented in the PPI3D software were instrumental in detecting alternative protein-protein interaction interfaces among the identified templates. When a single binding mode was observed for homologous proteins, the structural modeling of the protein complex was fairly straightforward, whereas choosing the correct interaction template from several alternatives turned out to be a difficult task requiring manual intervention. The combination of full structure and interaction interface VoroMQA scores effectively ranked structural models of protein complexes and selected models of better quality from the CAPRI Scoring sets. The overall results show possible uses of PPI3D and VoroMQA in structural modeling of protein-protein interactions and suggest ways for further improvements of both methods.

KEYWORDS

alternative interaction interfaces, homology modeling, model quality assessment, protein-protein interactions, template identification

1 | INTRODUCTION

The number of known protein sequences is noticeably higher than the number of experimentally determined protein structures. Due to the rapid accumulation of genome sequencing data the gap between these numbers only grows, but it can be in part bridged using computational protein structure prediction.[1,2] However, in order to understand molecular mechanisms the knowledge of the structures for individual proteins is not sufficient. Proteins usually interact with each other while performing their functions, and these interactions have to be analyzed in detail to understand the biological mechanisms. Obviously, the most comprehensive information on protein-protein interactions can be obtained from the structures of protein complexes, but their experimental determination is even more difficult than in the case of individual proteins. On the other hand, there are multiple experimental methods capable of promptly determining if specific proteins interact with each other. Thus there is another large gap between the number of known protein-protein interactions and the number of available corresponding structures. It is therefore not surprising that there are active efforts to develop computational methods also for structural modeling of protein complexes.[3]

Methods of protein structure prediction are tested every two years in the Critical Assessment of protein Structure Prediction (CASP) experiments.[4,5] The participants of CASP experiments are invited to generate structural models for proteins (called targets) given only their sequences. The produced models are then compared to the experimental protein structures that are unknown to the predictors at the time of modeling. Such blind testing is very useful in revealing strengths and weaknesses of the protein structure modeling methods. Similarly, the progress in computational modeling of protein interactions is continually

monitored in the Critical Assessment of PRediction of Interactions (CAPRI) experiments.[6,7] CAPRI started as a protein docking challenge: the participants had to assemble the protein complex from the supplied structures of individual subunits. Since 2014, CASP and CAPRI are collaborating,[8] and in recent years, similarly as in CASP, CAPRI participants are provided with only the sequences and oligomeric states of the proteins. The predictors may use any method of their choice to model the target protein complex. For example, they can generate models of individual subunits and then use protein-protein docking to model the interactions, or they can use template-based methods for protein complex structure modeling.

Comparative (template-based, homology) modeling is the most accurate and most widely used method for protein structure prediction.[9] It is based on the empirical observation that evolutionary related proteins tend to have similar structures. In view of the observation that protein complexes also tend to preserve their structures in the course of evolution,[10] comparative modeling is also feasible for protein-protein interactions.[11] Thus, we decided to participate in CAPRI Round 37, held alongside the CASP12 experiment, having the aim to test the usefulness of our newly developed template detection and structure assessment methods for comparative modeling of protein complexes.

One of the limiting steps in comparative modeling is the ability to identify structural templates. The extent to which templates for modeling protein complexes are available in the Protein Data Bank (PDB)[12] has been debated in several publications.[13,14] The disagreement on the number of available templates notwithstanding, perhaps one of the major problems is that there are few publicly available and easy-to-use tools for finding these templates. To facilitate this task we have developed the PPI3D web server, which allows searching, analyzing and modeling binary protein-protein interactions.[15] The utility of this software in finding templates for modeling structures of protein complexes has already been demonstrated by an independent research group in recent CAPRI rounds.[16] In CAPRI Round 37, we wished to test the usefulness of the PPI3D server for structural modeling of protein complexes more thoroughly.

If structural template(s) can be identified, it is fairly straightforward to generate multiple comparative models based on different sequence-structure alignment variants and/or different templates. However, the next step, selection of the most accurate model from the set of multiple models, presents a formidable challenge. Not surprisingly, CAPRI experiments even have a dedicated scoring category, the task being to select the best models from a pool of decoys.[17] To assess protein structure models we have recently developed a method called Voronoi tessellation-based Model Quality Assessment (VoroMQA).[18] It combines the idea of knowledge-based statistical potentials with the advanced use of the Voronoi tessellation of atomic balls.[19] VoroMQA uses contact areas instead of distances for describing and seamlessly integrating both the explicit interactions between protein atoms and the implicit interactions of protein atoms with the solvent. One of the attractive features of VoroMQA is its flexibility, since the method can produce scores at atomic, residue and global levels. Thus, although VoroMQA is not optimized to score interactions, it can derive an interaction interface quality score from the local scores of the atoms that participate in the interchain contacts. A previous large-scale evaluation has shown that some of the scores for protein structure assessment can successfully compete with docking-specific scoring functions.[20] This observation encouraged us to test the ability of VoroMQA to select good structural models of protein complexes.

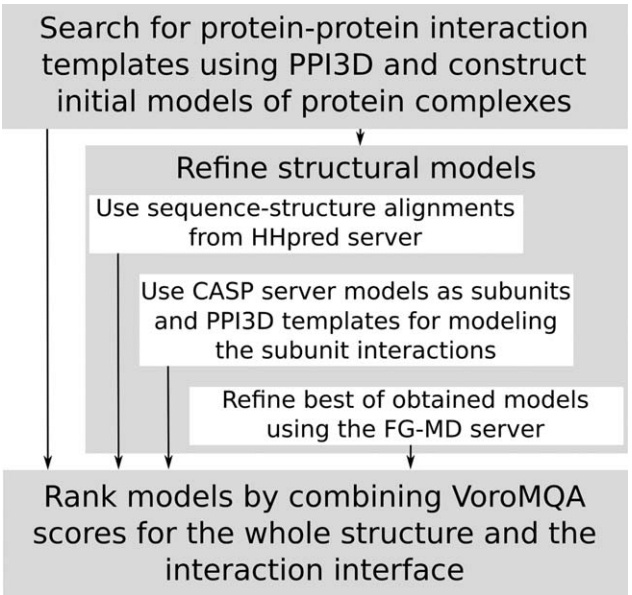

FIGURE 1 The workflow of comparative modeling of protein complexes

2 | METHODS

2.1 | Modeling outline

The outline of the modeling procedure that was used for modeling CAPRI targets is given in Figure 1. The search for templates was done using the PPI3D web server. If any templates were found, initial models were also generated using the PPI3D software. Next, the structural models were refined including the improvements in sequence alignment and the structure refinement. In the last step models were ranked using the VoroMQA ranking procedure that involved scoring both the entire structure of the complex and the interaction interfaces between protein subunits.

2.2 | Template search and construction of initial models

The initial search for templates was performed by the PPI3D web server.[15] Given the protein sequences, PPI3D searches for binary protein-protein interaction data for homologous proteins in the PDB. PPI3D sequence searches are performed using either BLAST or PSI-BLAST methods.[21] Using BLAST, the server searches directly in the database of sequences, associated with the structural data on protein interactions. In the case of PSI-BLAST, the sequence profile (position-specific scoring matrix) is initially generated using clustered NCBI

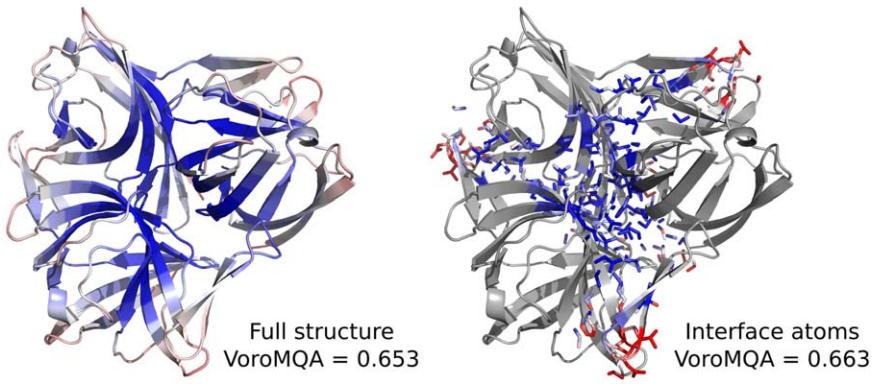

FIGURE 2 Evaluation of structures for protein complexes with VoroMQA using the full structure and the interface atoms. The experimental structure of T110/T0860 (PDB: 5FJL) is used for illustration

nonredundant sequence database, and then the resulting profile is utilized to find homologs having structural data on interactions.

The structural data in PPI3D web server are first clustered by the sequence similarity and then by the interaction interface similarity. As a result, if several homologous structures contain highly similar interaction interfaces, they all are assigned to a single cluster. Conversely, if the interaction interfaces differ, structures are split into different clusters even if interacting proteins are the same. Binary protein interactions that belong to different clusters may be further aligned with TM-align[22] and visually compared. These features allow identification of the protein-protein interaction templates without losing the alternative protein-protein interaction interfaces.

When PPI3D was able to find any homologs of the target sequences having structural data on protein interactions, the initial models of protein dimers were generated using the structure modeling implemented in the PPI3D web server. If necessary, the structures were subsequently remodeled to represent higher oligomeric states. All structural models were generated by MODELLER[23] using its multichain modeling feature, that is, the whole structure of the protein complex was modeled simultaneously.

2.3 | Refinement of the structural models

The PPI3D web server is designed primarily for the search and analysis of structural data on protein interactions. It provides the homology models only as an additional feature; therefore, no model refinement and optimization techniques are implemented. To improve the initial structural models we applied additional steps including the adjustment of sequence alignment and the structure refinement.

Sequence alignments that are based on sequence profile-profile comparison (like HHpred) have been shown to be superior to the alignments based on sequences comparison (like BLAST).[24] Therefore, after the initial search and modeling by PPI3D additional sequence-structure alignments were generated for the same templates using the HHpred server available from the MPI Bioinformatics Toolkit.[25,26] All the alignments were utilized to generate structural models for the target protein complex using MODELLER.

The previous CASP-CAPRI experiment showed that higher-quality models of individual subunits usually lead to higher-quality docking models of protein complexes.[8] In order to test this feature for homology modeling of interaction interfaces, for each target we selected one or two CASP server models for individual subunits that had the best VoroMQA scores. The structure of the protein complex was then generated using these server models and the oligomeric templates obtained from the PPI3D and HHpred results.

The results of previous CASP-CAPRI experiment have also pointed out the utility of structural refinement for the development of higher-quality models of protein complexes.[8] Therefore, we refined our models obtained using reliable templates by applying fragment-guided molecular dynamics implemented in the FG-MD server.[27]

2.4 | Evaluation of structural models of protein complexes using VoroMQA

Structural models were ranked using VoroMQA.[18] We calculated two scores for every considered model: a VoroMQA score of the full structure ($VoroMQA_{full}$) and a VoroMQA score of the atoms directly involved in interchain interactions ($VoroMQA_{interface}$) (Figure 2). The interchain interactions were defined at the level of atoms using the Voronoi tessellation of atomic balls.[19] The same procedure was utilized for ranking our structural models prior to submission and for selecting 10 best models from the CAPRI Scoring sets, except that for the scoring part of the CAPRI experiment, we disregarded models with the large number of clashes.

For every scoring set we ranked the models using tournament-based procedure as described below. Let us consider two models A and B of the same target. We say that A "wins" against B if $VoroMQA_{full}(A) > VoroMQA_{full}(B)$ and $VoroMQA_{interface}(A) > VoroMQA_{interface}(B)$. If $VoroMQA_{full}$ and $VoroMQA_{interface}$ scores do not agree on which model is better, then we call it a "draw." Given a set of models we record the numbers of all the possible wins and draws for every model. Then we rank the models by their wins and draws.

It is possible to consider more than two scores for the tournament-based ranking. While we did not do it during CAPRI Round

TABLE 1 Summary of the modeling results for CAPRI Round 37 targets

CAPRI target	CASP target	Oligomeric state	Interface template available	Template sequence identity (%)	CAPRI model evaluation (first/best)[a]
T110	T0860	3	Yes	20	Medium/medium
T111	T0867	3	Yes	50	High/high
T112	T0881	3	Yes	15–22	Acceptable/acceptable
T113	T0884-T0885	2	No	...	Incorrect/incorrect
T114	T0875	2	Partial	...	Incorrect/incorrect
T115	T0877	2 or 1	No	...	*Cancelled*
T116	T0893	2	Yes	24–30	Incorrect/incorrect
T117	T0903-T0904	4	Partial	...	Incorrect/incorrect
T118	T0906	8	Yes	45–46	High/high
T119	T0917	2	Yes	25–33	Medium/medium
T120	T0921-T0922	2	Yes	14–20, 27–35	Incorrect/medium

[a]According to the CAPRI evaluation scheme, the models are classified into the four quality categories: high, medium, acceptable, and incorrect.[28] The quality assignments are provided for the first and the best submitted models.

37, we tried an additional VoroMQA-based interface energy estimate afterwards. That estimate, denoted as $VoroMQA_{interface\_energy}$, was computed by applying the VoroMQA statistical potential function to calculate the unnormalized pseudo-energy of interface contacts. Therefore, $VoroMQA_{interface\_energy}$ is even more local than $VoroMQA_{interface}$. Also, unlike $VoroMQA_{full}$ and $VoroMQA_{interface}$, $VoroMQA_{interface\_energy}$ is heavily dependent on the total area of interface contacts.

3 | RESULTS AND DISCUSSION

3.1 | Availability of templates for CAPRI targets

The summary of our modeling results in CAPRI Round 37 is presented in Table 1, and the detailed results are given in the Supporting Information Table S1. The templates were identified for 7 of 11 targets, and models for these targets were generated as described in section 2. The detailed description of the successes and difficulties in modeling these targets is given in subsequent sections. It is worth noting that except for CAPRI T112/CASP T0881 the templates were identified using the PPI3D web server. However, in some cases the sequence alignments resulting from PSI-BLAST search in PPI3D were too short and had to be refined using remote homology detection methods like HHpred. This suggests that the PPI3D server could be further improved by integrating more sensitive sequence search methods.

Several CAPRI targets had only low reliability or partial templates. For example, monomeric templates were readily available for T114/T0875. However, the only identified template for the dimer interface had a domain swapped between two chains.[29] As a result, we did not produce any homodimer model of at least acceptable quality for this target according to the CASP and CAPRI model evaluation. Similarly, no protein-protein interaction templates could be found and thus no homology models were built for heteromeric targets T113/T0884-T0885 and T117/T0903-T0904.

3.2 | Modeling of CAPRI targets with templates representing only one interaction mode

In most cases when we found templates for comparative modeling of the protein complexes, only a single protein-protein interaction mode was observed among the template structures. This was true for the trimeric viral fiber head domains (T110/T0860, T111/T0867, and T112/T0881), the octameric fructose biphosphatase (T118/T0906) and the dimeric Red Sea protein (T119/T0917). For all these targets multiple PDB entries containing homologous proteins were found, but the identified binary interaction interfaces either were in the same PPI3D clusters or had highly similar structures despite being in different clusters. Consequently, several models were built for each of these targets using different PDB entries as templates, which were selected according to the quality of the protein structure (defined by higher resolution, better R factors, smaller number of residues with missing coordinates) and sequence alignment parameters (lower PSI-BLAST and HHpred E-value, higher HHpred probability, higher sequence identity).

Typical targets of this category had only one or two results in PPI3D, so the template selection was straightforward. The template selection was a bit more interesting for the Red Sea protein (T119/T0917), a member of the iron-containing alcohol dehydrogenase family (Pfam: PF00465). According to the PPI3D results, most of the closest homologs contained the same protein-protein interaction mode having the interface area of >1000 Å^2, yet there were also a few smaller alternative interfaces. One of these alternatives originated from the interaction between the primary dimers in the decameric complex of the 1,3-propanediol dehydrogenase (PDB: 3BFJ).[30] However, since T119 was annotated as a dimer, we considered this alternative interface irrelevant. Another small alternative interface (604 Å^2) originated from the only dimeric biological assembly of *Escherichia coli* lactaldehyde reductase FucO (PDB: 5BR4).[31] However, the analysis of this structure using PDBePISA[32] revealed an additional larger interface that was the same

as in other structures of the same protein (PDB: 1RRM or 2BI4[33]), suggesting that the biological assembly in the PDB entry 5BR4 is assigned incorrectly. Thus, a simple analysis ruled out smaller interaction interfaces as suitable modeling templates, leaving only one possible protein-protein interaction mode for modeling the T119 dimer.

After the selection of templates using the aforementioned procedures, models of protein complexes were built using BLAST or PSI-BLAST sequence alignments from the PPI3D server or corresponding HHpred sequence alignments. This already resulted in models of at least acceptable quality according to CAPRI criteria[28] for most of the targets (see Table 1 and Supporting Information Table S1). Models of even better quality were obtained for targets having templates with higher sequence identity such as T111 and T118. This clearly demonstrates the utility of comparative structural modeling for protein complexes, provided that multimeric templates are available.

Based on results of the previous CASP-CAPRI experiment two observations related to the accuracy of models of protein complexes were made.[8] First, using higher-quality monomer models usually resulted in better models of multimeric structures. Second, structural refinement of the complexes using protein-protein docking procedures helped to improve homology models. We tried to make use of both of these observations. We remodeled the structures of the complexes using selected best CASP server models as monomers and the identified protein interaction interfaces as templates for the assembly. Unfortunately, we cannot make definite conclusions regarding the utility of high-quality monomer models from CASP servers, as it turned out that most of the selected CASP models were already optimized for the multimeric protein structure. Following the second observation, we refined our initial models by fragment-guided molecular dynamics implemented in FG-MD server.[27] The structural refinement slightly improved the interaction interface in most of the models (Supporting Information Table S2), but as the number of targets in CAPRI Round 37 was small, the significance of this result is not clear.

3.3 | Difficulties in choosing templates from alternative interaction modes

The cases described above are examples of a simple modeling pipeline, not very different from homology modeling of individual proteins. However, two CAPRI targets (T116/T0893 and T120/T0921-T0922) had templates that contained alternative protein-protein interaction interfaces. Such alternative binding modes are quite frequent[34] and may even occur between the same proteins under different circumstances.[35] For both targets we could readily identify the alternative protein interaction interfaces among the template structures using the clustering and visual comparison features of the PPI3D web server. Despite that, in both cases we encountered difficulties in choosing the correct template for structural modeling.

The first of the targets having templates with alternative binding modes was T116 (PDB: 5IDJ), a homodimeric histidine kinase representing the core of the sensory protein CckA from *Caulobacter crescentus*.[36] At first it looked like an easy target, because multiple templates could be found by the PPI3D server using a simple BLAST search. However, the initial analysis of the identified homologs revealed that the interaction mode of subunits varied in different templates. At the time of modeling we had no clue which orientation of subunits might be correct; therefore, a number of models were generated quasi-automatically using different templates and sequence alignments. Ten best models were then selected using the VoroMQA scoring procedure. Considering the large number of available template structures, it was quite unexpected that all of the submitted models were incorrect according to CAPRI criteria.[28] Therefore, we decided to analyze these results in more detail.

Histidine kinases usually have two domains: the *D*imerization and *H*istidine *p*hosphotransfer (DHp) domain and the *C*atalytic and *A*TP binding (CA) domain connected to each other by a flexible linker (Figure 3).[37] CAPRI model evaluation procedure uses superposition-based criteria, ligand root-mean-square deviation (RMSD) and interface RMSD,[28] which are known to be sensitive to even tiny changes in domain orientation. Therefore we additionally evaluated our models using CAD-score, a versatile superposition-independent measure suitable for assessing the accuracy of structural models (including multidomain proteins and protein complexes) as well as interaction interfaces.[38,39] CAD-score produces values in the range [0;1], where CAD-score = 1 corresponds to identical structures. The summary of the evaluation is given in Table 2. The full-structure CAD-score of our models is 0.49-0.58, which is comparable to the CAD-scores of CASP server models. However, if we consider CAD-score values for the interchain interface, two distinct groups of models emerge. One group has CAD-score values <0.07, indicating that the interaction interface in these models is completely different from the target structure. Another group of models has the interface CAD-score in the 0.28-0.35 range, indicating that significant part of the interchain contacts are predicted correctly in model structures. A similar pattern may also be observed when analyzing the f_{nat} data from CAPRI criteria.

The DHp domain of a histidine kinase forms a four-helix bundle with DHp domain from another subunit upon dimerization.[37] Two distinct arrangements of helices have been observed for these kinases (Figure 3C) depending on whether the phosphorylation occurs in *cis* or in *trans*, that is, whether the CA domain phosphorylates the same or another subunit.[40] The interaction between DHp domains in the T116 dimer corresponds to one of the previously known interaction modes,[36] thus it could be modeled by choosing a suitable template.

Interestingly, the interaction between the DHp and CA domains in the T116 structure differs from the interaction observed in all previously solved structures of histidine kinases.[36] The CA domains are swapped between two protein chains (Figure 3C). Thus, despite the correctly predicted interchain interaction in some of our models, the CA domains are orientated differently compared to the target structure in all of them. Apparently, this particular domain orientation observed in T116 could not be modeled using the template-based techniques. Even so, the biological significance of this domain swap is unclear as it might well be a consequence of crystal contacts.[36] Moreover, the orientation between CA and DHp domains in histidine kinases has been shown to be highly flexible (Figure 3B) and may depend on ligand binding, histidine phosphorylation states, and so forth.[37,41,42] At the time of

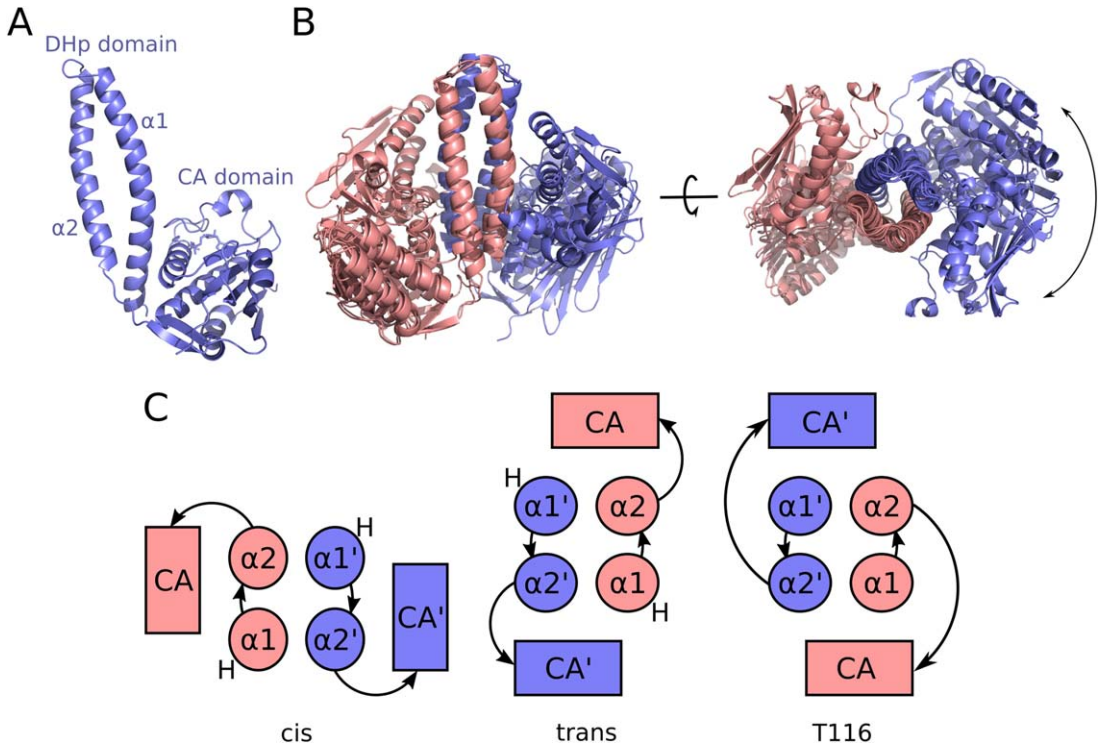

FIGURE 3 Interactions in the dimeric histidine kinases. A, The experimental structure of the monomer of histidine kinase CckA from *Caulobacter crescentus* (T116/T0893, PDB: 5IDJ). B, The flexibility of the interactions between DHp and CA domains in different structures of histidine kinases (PDB structures 4BIU, 4BIV, 4BIX and 4CB0 with four-helix bundles of DHp domains aligned). C, Previously observed arrangements of the four-helix bundles of histidine kinases having different autophosphorylation modes and the swapped CA domain orientation in the T116 structure

modeling it was not known that T116 was crystalized in complex with ADP. Therefore, it appears that in such circumstances the observed orientation of the CA domains could hardly be reproduced even by the most effective computational methods.

T120 (PDB: 5M2O), the cohesin-dockerin heterodimer from *Ruminococcus flavefaciens*, represents another case involving templates with alternative protein-protein interactions. Cohesins and dockerins have been shown to have dual binding modes: changing just a few residues

TABLE 2 Modeling results of T116/T0893 (homodimeric histidine kinase)

Model[a]	f_{nat}[b]	L-rms[c]	I-rms[d]	Interface CAD-score[e]	Template and the alignment method
1	0.13	43.89	15.34	0.07	4Q20, HHpred
2*	0.54	65.03	14.21	0.31	4BIV, BLAST
3	0.10	41.54	11.58	0.06	3D36, HHpred
4*	0.47	32.77	14.67	0.28	4CB0, HHpred
5*	0.52	60.51	16.77	0.34	4GCZ, HHpred
6*	0.51	29.63	14.43	0.33	4BIX, BLAST
7*	0.46	29.22	14.78	0.30	4CB0, BLAST
8*	0.46	61.39	17.47	0.32	4GCZ, BLAST
9*	0.56	64.71	14.56	0.35	4BIV, HHpred
10	0.00	43.04	13.93	0.01	4Q20, BLAST

[a]All models were classified as incorrect by CAPRI criteria. Models that are partially correct according to interchain contacts are marked with an asterisk.
[b]Fraction of native residue-residue contacts in the interface of the model. The residues from different chains are considered to be in contact if any of their atoms are within 5 Å distance.[28]
[c]Root-mean-square deviation (RMSD) of the ligand (the smaller protein) structure, calculated after the model ant the target structures of the receptor (the larger of the two proteins) are superimposed.[28]
[d]Backbone RMSD of the superimposed target and model interface residues, having at least one atom within 10 Å from the other protein molecule.[28]
[e]CAD-score, calculated for the interchain interface.[38]

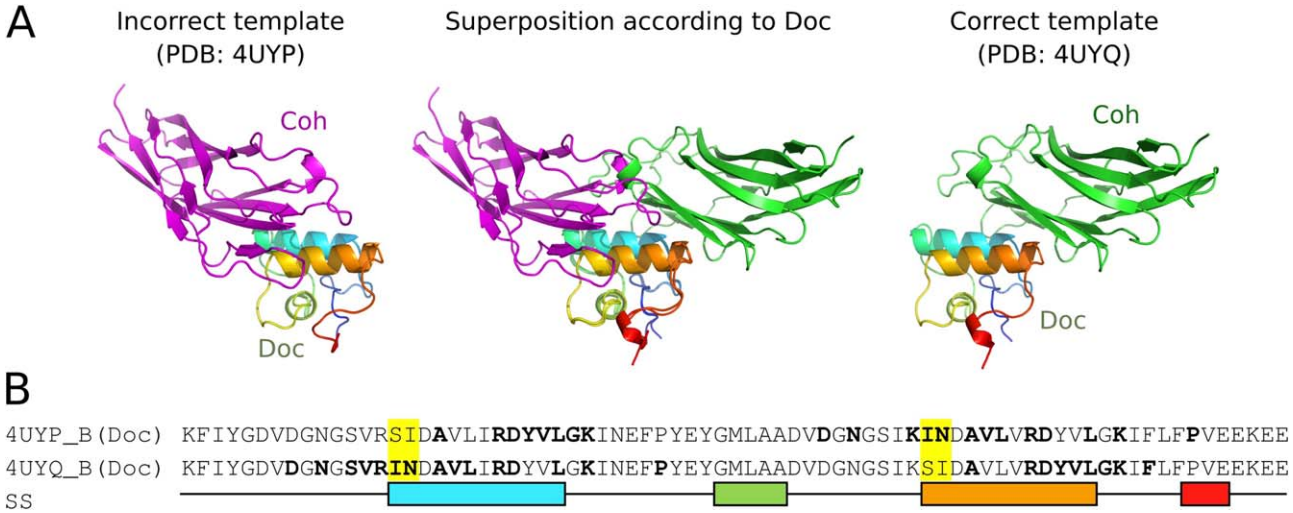

FIGURE 4 Alternative protein-protein interaction modes observed for cohesin-dockerin (Coh-Doc) complexes. A, Minimal sequence change rotates dockerin (rainbow colored) by 180° upon binding the same cohesin molecule (magenta and green). B, Alignment of mutant dockerins with sequence differences highlighted in yellow. The interface amino acids are shown in bold. The secondary structure is displayed below the alignment

in the dockerin molecule may change its orientation within the complex by 180° (Figure 4).[43–45] Such a dual binding mode appears to be unique in the molecular interaction field and its biological role is not yet understood.[46] Several exceptions having only single binding mode were also found.[47] Interestingly, the T120 structure is one of these exceptional cases.[48]

At the time of modeling T120 we could clearly identify the existing two alternative protein interaction modes using PPI3D server (Figure 4), but it was not obvious which one represents the correct template for modeling the heterodimer. As a result, we constructed a number of models using both interaction modes and selected a balanced set of 10 models: 6 of the submitted models corresponded to the orientation that had the best VoroMQA scores, and 4 models corresponded to the alternative one. Disappointingly, for this target VoroMQA failed to select the model having correct binding mode as the best, therefore we submitted 6 incorrect and 4 medium or acceptable models (Table 3). In the aftermath, we checked whether any additional criteria might have helped us to choose the right template. It turned out that the largest difference between incorrect and correct templates was the number of identical interface residues in the alignment of target and template dockerins (Table 3).

Taken together, those cases (T116 and T120), in which we had to choose from templates having different protein-protein interaction modes, illustrate that sometimes the modeling of protein complexes may not be straightforward and multiple aspects have to be considered.

TABLE 3 Modeling results of T120/T0921-T0922 (cohesin-dockerin heterodimer)

Model[a]	Template[b]	Template sequence identity Coh (%); Doc (%)	Interface sequence identity Coh (%); Doc (%)	CAPRI model evaluation	Interface CAD-score
1	3UL4	16; 27	12; 19	Incorrect	0.015
2	3UL4	20; 29	12; 19	Incorrect	0.043
3*	4DH2	16; 27	10; 29	Medium	0.204
4*	4DH2	16; 27	10; 29	Acceptable	0.195
5	3UL4	20; 29	12; 19	Incorrect	0.051
6*	4DH2	16; 27	10; 29	Medium	0.18
7*	4UYQ	17; 35	13; 38	Acceptable	0.183
8	4UYP	17; 33	15; 24	Incorrect	0.016
9	1OHZ	19; 24	18; 11	Incorrect	0.04
10	2VN6	14; 25	7; 6	Incorrect	0.015

[a]The models that were built using the correct interface templates are marked with an asterisk.
[b]Several models were generated using PDB structures 3UL4 and 4DH2 as templates, differing by sequence-structure alignments (PPI3D or HHpred) and/or the FG-MD structural refinement.

TABLE 4 Selection of best models from CAPRI Scoring sets using individual VoroMQA-based scores and their combinations

Selection method[a]	Sum[b]	T110	T111	T112	T113	T116	T119	T120
Best possible interface CAD-score	2.81	0.32	0.69	0.49	0.11	0.46	0.43	0.31
Tournament($VoroMQA_{full}$, $VoroMQA_{interface}$, $VoroMQA_{interface\_energy}$)	1.95	0.3	0.64	0.49	0.05	0.12	0.34	0
Tournament($VoroMQA_{full}$, $VoroMQA_{interface}$)	1.42	0.3	0.56	0.09	0	0.12	0.34	0
$VoroMQA_{full}$	1.35	0.29	0.67	0	0.02	0.04	0.34	0
$VoroMQA_{interface}$	1.25	0	0.54	0.29	0.04	0	0.38	0
$VoroMQA_{interface\_energy}$	0.76	0.29	0.01	0	0.01	0.46	0	0

[a]Selection of models based on either individual VoroMQA-based scores, the combination of two (used in CAPRI) or three (introduced after the CAPRI experiment) scores. Best possible CAD-score indicates what could be achieved in the ideal case.
[b]The sum of the interface CAD-score values of the selected best models. Only the targets for which at least one model in the CAPRI Scoring set had interface CAD-score >0 were considered. T118 was omitted because its structure was not known at the time of analysis.

3.4 | Scoring protein complexes using VoroMQA

One of the important components of our modeling pipeline was the ranking of models using VoroMQA scores of the full structure and of the interface. This procedure successfully ranked the structural models, favoring models of higher quality for most of the targets. We suppose that one of the reasons for the successful ranking of the models was the integration of global and local VoroMQA scores into a single scoring pipeline. This feature was tested in more detail during the CAPRI Scoring experiment.

After the CAPRI experiment, we decided to check how different VoroMQA-based scores, individually and in combinations, perform on the CAPRI Scoring sets and whether the scoring procedure used during CAPRI Round 37 was optimal. To this end, we first used CAD-score to identify the models from the scoring sets with the most accurately modeled interfaces. We then recorded CAD-score values of selections made by different VoroMQA-based scores or their combinations. The results are presented in Table 4. When used alone, the locally focused VoroMQA-based scores ($VoroMQA_{interface}$ and $VoroMQA_{interface\_energy}$) are more prone to making severe selection errors than $VoroMQA_{full}$. However, combining global and local VoroMQA-based scores turned out to be advantageous. Tournament-based selection methods performed better compared to those based on individual scores. In CAPRI Round 37, we used a combination of only two scores ($VoroMQA_{full}$ and $VoroMQA_{interface}$), but it turned out that the addition of the third score, $VoroMQA_{interface\_energy}$, would have further improved the model selection performance.

4 | CONCLUSIONS

Our results in CAPRI Round 37 demonstrated the utility of comparative modeling for the construction of models for protein complexes. If structural templates for the protein-protein interaction can be found, simple homology modeling may already yield models of acceptable quality for protein complexes. Nevertheless, the template search and selection is harder for multimeric proteins, because alternative modes of protein interactions may be a confounding factor. CAPRI revealed the usefulness of the PPI3D web server in detecting structural templates for modeling protein complexes and especially in its ability to identify alternative protein-protein interaction modes.

The VoroMQA method turned out to be instrumental in ranking and selection of structural models of protein complexes. Although this method is not optimized for scoring protein-protein interfaces, the combination of global (full structure) and local (interaction interface) VoroMQA scores was effective in selecting higher-quality structural models of most multimeric proteins. Still, VoroMQA had problems with the selection of best models when two alternative protein interaction modes were observed among template structures.

Taken together, our results demonstrate the strengths and weaknesses of PPI3D and VoroMQA in structural modeling of protein complexes and show the directions for further improvement of both methods. Last but not least, our analysis shows the necessity of a more comprehensive evaluation of model accuracy. In the cases when multidomain proteins interact through only one domain, the RMSD-based criteria are too sensitive to possibly insignificant changes in domain orientation and thus hinder the detection of models having nearly correct interchain interfaces. Moreover, the use of only global model structure evaluation does not allow identification of reasonably well-predicted local structure or interface regions. Thus, it would be beneficial to also evaluate the quality of structural models by superposition-free methods of structure comparison that can assess both global and local model accuracy.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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