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Improving Protein Structure Prediction with Extended Sequence Similarity Searches and Deep-Learning-Based Refinement in CASP15

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Abstract

The human predictor team PEZYFoldings got third place with GDT-TS (First place with the Assessor's formulae) in the single-domain category and tenth place in the multimer category in CASP15. In this paper, I describe the exact method used by PEZYFoldings in competitions. As AlphaFold2 and AlphaFold-Multimer, developed by DeepMind, are state-of-the-art structure prediction tools, it was assumed that enhancing the input and output of the tools was an effective strategy to obtain the highest accuracy for structure prediction. Therefore, I used additional tools and databases to collect evolutionarily related sequences and introduced a deep-learning-based model in the refinement step. In addition to these modifications, manual interventions were performed to address various tasks. Detailed analyses were performed after the competition to identify the main contributors to performance. Comparing the number of evolutionarily related sequences I used with those of the other teams that provided AlphaFold2's baseline predictions revealed that an extensive sequence similarity search was one of the main contributors. The impact of the refinement model was minimal ($p < 0.05$ for the TM score). In addition, I noticed that I had gained large Z-scores with the subunits of H1137, for which I performed manual domain parsing considering the interfaces between the subunits. This finding implies that the manual intervention contributed to my performance. The prediction performance was low when I could not identify the evolutionarily related sequences. T1130 is an example; however, other teams can model better structures. Based on the discussions from the CASP15 conference, the two teams that ranked higher than PEZYFoldings had some hits for T1130. This may be because T1130 is a eukaryotic protein, whereas the additional databases used were mainly from metagenomic sequences, which primarily consist of prokaryotic proteins. These results highlight the opportunities for improvement in 1) multimer prediction, 2) building larger and more diverse databases, and 3) developing tools to predict structures from primary sequences alone. In addition, transferring the manual intervention process to automation is a future concern.

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A SHORT RUNNING TITLE

Improving protein structure prediction

THE FULL NAME OF THE AUTHORS

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The codes for the refinement model are available at https://github.com/t-oda-ic/afm_refiner under the Apache License, Version 2.0. The model parameters are available from the URL link on the same page under the Creative Commons Attribution 4.0 International Public License.

ABSTRACT

The human predictor team PEZYFoldings got third place with GDT-TS (First place with the Assessor's formulae) in the single-domain category and tenth place in the multimer category in CASP15. In this paper, I describe the exact method used by PEZYFoldings in competitions.

As AlphaFold2 and AlphaFold-Multimer, developed by DeepMind, are state-of-the-art structure prediction tools, it was assumed that enhancing the input and output of the tools was an effective strategy to obtain the highest accuracy for structure prediction. Therefore, I used additional tools and databases to collect evolutionarily related sequences and introduced a deep-learning-based model in the refinement step. In addition to these modifications, manual interventions were performed to address various tasks.

Detailed analyses were performed after the competition to identify the main contributors to performance. Comparing the number of evolutionarily related sequences I used with those of the other teams that provided AlphaFold2's baseline predictions revealed that an extensive sequence similarity search was one of the main contributors. The impact of the refinement model was minimal ($p < 0.05$ for the TM score). In addition, I noticed that I had gained large Z-scores with the subunits of H1137, for which I performed manual domain parsing considering the interfaces between the subunits. This finding implies that the manual intervention contributed to my performance.

The prediction performance was low when I could not identify the evolutionarily related sequences. T1130 is an example; however, other teams can model better structures. Based on the discussions from the CASP15 conference, the two teams that ranked higher than PEZYFoldings had some hits for T1130. This may be

because T1130 is a eukaryotic protein, whereas the additional databases used were mainly from metagenomic sequences, which primarily consist of prokaryotic proteins.

These results highlight the opportunities for improvement in 1) multimer prediction, 2) building larger and more diverse databases, and 3) developing tools to predict structures from primary sequences alone. In addition, transferring the manual intervention process to automation is a future concern.

KEYWORDS

tertiary protein structure, quaternary protein structure, protein structure prediction, deep learning, sequence similarity search, metagenomes

INTRODUCTION

The AlphaFold2 algorithm, developed by DeepMind, has demonstrated remarkable performance in protein structure prediction in Critical Assessment of Structure Prediction (CASP) 14[1-3]. This was followed by the development of the AlphaFold-Multimer algorithm[4], which can predict multimeric structures with high accuracy. (Hereafter, I call both AlphaFold2 and AlphaFold-Multimer AF2 unless there is a specific need to differentiate them.) Other protein structure prediction programs have emerged following the success of AF2[5-7]. However, AF2 demonstrated comparable or even better performance than the newer programs. Hence, optimizing AF2 is considered one of the most promising strategies for achieving the highest accuracy in protein structure prediction tasks.

Therefore, the challenges in CASP15 were as follows: (1) collecting a sufficient number of evolutionarily related sequences for input into AF2. (2) improving the structures generated by AF2.

Protein structure prediction tools are known to exhibit poor performance when there is a limited number of evolutionarily related sequences. Although AF2 exhibits reduced sensitivity to this problem, it remains a concern[1,8]. As a result, the collection of evolutionarily related sequences is a crucial step in the process. Utilizing large metagenomic databases is a prominent strategy for addressing this challenge[9]. Therefore, in addition to the databases employed in the official AF2 pipeline, I used PZLAST[10,11] to collect more metagenomic sequences. Furthermore, an in-house database was constructed using NCBI assembly[12] data to obtain sequences with taxonomic information because it was considered to be necessary to predict multimeric structures[4,13]. The nr database[14], a widely used extensive collection of sequences, was included and searched using a customized version of PSI-BLAST[15,16].

To accomplish the second objective, a deep learning model was constructed to improve the accuracy of the predicted structures. Additionally, it was assumed that AF2 (and other structure-prediction software using Multiple Sequence Alignments [MSAs]) required MSAs for high-quality prediction. However, they can be disrupted by the MSAs at the same time. For example, antibody complementary-determining regions are sequence-specific; therefore, the amino acids in MSA should not be considered. The details of this model have been described in the independent paper for the model[17]. Although the model was primarily designed to refine multimeric structures, it was considered to be useful to refine monomeric structures because the underlying principles must be similar.

For the CASP15 project, I devised a semi-automatic pipeline with several issues that need to be rectified. For example, AF2 can handle up to approximately 2200 amino acids (aa) in my environment. Therefore, if the number of amino acids was large, the sequences were cut into small pieces for prediction. In addition, the conserved domains had many hits, then the number of hits covering other regions was relatively small. In this case, sequences were sampled to flatten the MSA depth. Furthermore, many target-specific interventions exist because of various targets, including mutated proteins and targets required for predicting ensemble structures.

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As a result, my team got third place with GDT-TS, first place with Assessor's formulae in the single-domain category, and tenth place in the multimer category, which showed that my approach could achieve state-of-the-art performance. However, several problems have resulted in poor predictions, as described in this manuscript.

MATERIALS AND METHODS

PEZYFoldings in CASP15

Overall pipeline

A schematic representation of the pipeline is shown in Fig. 1A. The default AF2 pipeline process can be broadly divided into the following steps: MSA construction, structure prediction, and relaxation using OpenMM[18]. The main differences between the default AF2 pipeline and the PEZYFoldings pipeline include a more extensive sequence similarity search in the MSA construction step and the introduction of refinement steps. Details of each step are described in the following sections.

Sequence similarity search and MSA construction

The MSAs constructed in the pipeline are summarized below. In addition, the URLs and data downloaded from the databases are listed in Table S1.

PZLAST-MSA : Query sequences were submitted to the PZLAST[10,11] web API service with option "max_-out=10000." Because hits from PZLAST are fragmented sequences directly translated from sequencer reads, I aligned them with jackhmmer[19,20] and assembled them using a simple script; if aligned regions of two sequences were longer than 20 aa and the regions had an identity > 95 %, the sequences were merged.

PSIBLAST-MSA : PZLAST-MSA was inputted to PSI-BLAST[15] version 2.13.0 with PSI-BLASTexB[16] customization with the -in_msa option and options "-evalue 0.00001 -outfmt \"6 qseqid sallacc evalue pident nident qlen staxids sseq\" -max_target_seqs 100000 -num_threads 128." nr[14]and an in-house metagenomic database (described later) were searched simultaneously. In the early season, the number of iterations was set to two. In the later season (from T1173), it was changed to search iteratively up to three times using Position Specific Scoring Matrix (PSSM) checkpoint files; when the number of hit sequences was greater than 10,000, the iteration was terminated. If the final number of hit sequences was small (<10,000), PZLAST-MSA was merged. Sequences were aligned using jackhmmer. The taxonomy IDs of the sequences were added to a TaxID tag, which was used for sequence pairing in a later step.

HHBLITS-UNIREF-MSA : PSIBLAST-MSA was inputted to hhblits[21] (hhsuite[22] v3.3.0) using the UniRef30[23] database. With options "-all -n 3 -cpu 128."

HHBLITS-BFD-MSA : PSIBLAST-MSA was inputted to hhblits using the BFD[24] database with options "-all -n 2 -cpu 6." If the number of sequences in the MSA was larger than 10000, the MSA was filtered using hhfilter with options "-cov 30 -id 100 -diff 10000."

JACKHMMER-UNIPROT-MSA : A query sequence was inputted to jackhammer (hmmer[19,20] suite 3.3.2) using the Uniprot[25] database with options "–cpu 128 -E 0.00001 -N 3."

JACKHMMER-MGNIFY-MSA : A query sequence was inputted to jackhmmer using the MGnify[26] database with options "–cpu 128 -E 0.00001 -N 3." If the number of sequences in the MSA was larger than 10000, the MSA was filtered using hhfilter with options "-cov 30 -id 100 -diff 10000."

Final input MSA : PSIBLAST-MSA, HHBLITS-UNIREF-MSA, HHBLITS-BFD-MSA, JACKHMMER-UNIPROT-MSA, and JACKHMMER-MGNIFY-MSA were concatenated and filtered using hhfilter with options "-id 100 -cov 30 -maxseq 500000."

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Construction procedure of the in-house metagenomic database

The metadata of the assembly entries was downloaded from the NCBI FTP site on 2022-03-28. e entries that had "metagenome" in their description were extracted. The entries were checked to see whether they had translated_cds.faa, protein.faa.gz, cds_from_genomic.fna.gz, rna_from_genomic.fna.gz, or genomic.fna.gz in this order of priority. If the sequence data were nucleotides, they were translated using prodigal[27] with the default settings. If the prodigal could not be processed using the default settings, the "-p meta" option was used. A unique ID was generated for each entry and considered a taxonomy ID.

MSA filtering and feature building

After constructing the MSAs, I filtered them using several criteria and created variations of the MSAs according to the sequence identities: 1) clustered with sequence identity 95 %, 2) clustered with sequence identity 90 %, 3) filtered out if sequence identity with the query was less than 80 %, 4) filtered out if sequence identity with the query was less than 60 %, and 5) no identity filters were applied. Filtering was performed using hhfilter. I used the "-cov 30" option; however, in the middle of the season, I noticed that all unpaired sequences of a subunit were filtered out if the subunit length was less than 30 % of the total length of the multimeric structures. Therefore, the coverage values changed arbitrarily during the season. The input features for the AF2 networks are created in this step. This step allows flexible manipulation of the input features for AF2; for example, one can deliberately pair or unpair sequences, such as the AF2Complex[28] , and provide sparse residue indices to generate partial structures. I added extra gaps (the residue index) between subunits to predict multimer structures with the monomer version of AF2 [13,28]. TaxID tags or OX tags in the headers of the FASTA entries were used to pair sequences in the MSAs. TaxID tags were added to the headers of the sequences extracted from the nr and in-house metagenomic database. When sufficient computational resources were available, features were also created with skipping the pairing step. For antibody-antigen complexes, the paring step was always skipped (the sequences for H1140 were paired because of my error). In addition, I provided a3m files to the official feature-building pipeline and created input features for the network considering the possibility that I had bugs in my scripts.

Structure prediction by AlphaFold2 or AlphaFold-Multimer

The prediction was made with normal AlphaFold2 (model_1~5) and AlphaFold-Multimer parameters (model_1~5_multimer_v2) downloaded from https://storage.googleapis.com/alphafold/alphafold_params_-2022-03-02.tar on 2022-03-11. The number of recycling steps was typically set from 5 to 30, considering the time and computer resources. Intermediate structures were produced during recycling. Therefore, the pipeline produced approximately 1000-2000 structures in standard cases.

Model ranking and selection

The process of model ranking and selection involved utilizing the self-confidence metrics generated by AF2 as the criteria. For monomer targets, a sum of per-residue plDDTs higher than 70 was used because of the possibility of disordered regions. For multimer targets, the weighted sum of the predicted TM-score[29] (iptm $\times$ 0.8 + ptm $\times$ 0.2)[4] was used. When I predicted multimer structures with the monomer version, as it did not produce multimer metrics, all the unrelaxed structures were processed with the refinement model (see below). The top models were typically selected. For the rest of the submission, the TM-score software or MM-align[30]was used to maintain the variation in the structures (e.g., highly similar structures were not selected), considering ensembles, alternative forms, or mispredictions. Various human interventions were utilized in this step due to numerous issues that needed to be addressed. For example, models in which subunits did not interact with other subunits often had low TM-scores with other models and were selected in the semi-automatic pipeline. However, such models were avoided, as it was evident that the prediction was incomplete.

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Refinement

I constructed a deep-learning model that refined the predicted structures by fine-tuning the official AlphaFold-Multimer weight (model_1_multimer_v2). It uses a predicted structure and its amino acid sequence as the input and output refined structures. Further details on this model were provided in the independent paper[17]. The training conditions employed for the model used in CASP15 are listed in Table S2. The five structures selected as submission candidates were input into the refinement model. When sufficient time and resources were available, all predicted structures except the intermediate ones were fed into the refinement model.

Manual interventions

Domain parsing

Structures were usually predicted using full-length sequences of all subunits. However, when the total number of amino acids was large and could not be handled with my GPU, I performed domain parsing and MSA cropping or predicted the entire structure using the CPU mode. Domain parsing is divided into several steps. First, the sequences were split into fragments of lengths ranging from 500 to 1000 aa and selected with a random guess. In addition, I sometimes used the results of domain prediction using SMART[31]. Next, the structures were predicted using AF2, and the regions or subunits that interacted with them were visually inspected. Then, I decided on new boundaries to avoid disturbing the interface. Subsequently, the structures were predicted again, and the resulting models were assessed. The boundary decision and partial structure building steps were repeated until the quality of the partial structures was satisfactory. They were then concatenated with simple scripts, which performed structural alignment using the overlapped regions.

MSA depth arrangement

In cases where the targets encompassed markedly conserved domains, the resulting MSAs sometimes displayed considerable depth imbalances (Fig. 1B). If the depth of the MSA was highly skewed, sequences with amino acids in the sharrow regions were retained, and other sequences were randomly selected to flatten the depth (Fig. 1C). When the depth was insufficient, additional searches were performed to obtain additional sequences around the sharrow regions.

Visual inspections of the refined structures

Because the refinement model was trained with globular proteins[17], it sometimes produced globular structures (Fig. 1D, 1E) or many atom clashes. Therefore, I visually inspected the models, and if I observed any problems in the refined models, I did not use them.

Comparison with other teams' models

As ColabFold[32] team, NBIS-AF2-standard team, and NBIS-AF2-multimer team provided publicly available prediction results, I compared their models with my models and assessed whether the protocols worked well. If I perceived my model's quality as inferior to that of other teams, I undertook protocol revision by conducting extra sequence similarity searches or augmenting the number of recycling steps.

Docking or *de novo*-like structure prediction by the refinement model

When I could not build good structures using my basic pipeline, I performed docking or *de novo* -like structure prediction using the refinement model. The process for this approach was straightforward. When the predicted chains were randomly moved and fed into the refinement model, the model created complexes from the chains. Similarly, by feeding a structure with randomly placed atoms into the refinement model resulted in the generation of a reasonable structure.

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Target-specific process

Some other interventions such as point mutations on T1109 were conducted. A concise summary of target-specific processes can be found in Supplementary Text 1.

Assessment of the impact of individual element

Impact of extended sequence similarity search

To investigate the impact of the MSA construction protocol without manual intervention, I compared the MSAs with the baseline MSAs generated using the default settings of the AF2 pipeline. Targets less than or equal to 1,200 aa were considered because long sequences require manual intervention to avoid out-of-memory errors. Baseline MSAs provided by the NBIS-AF2-standard and NBIS-AF2-multimer teams were downloaded from http://duffman.it.liu.se/casp15 on 2022-12-27. The subunits of the assembly targets were predicted using AlphaFold-Multimer as the assembly entries. The number of sequences (Nseq) in MSA was calculated as the number of clusters using cd-hit[33]with the option "-c 1.0 -G 0 -n 5 -aS 0.9 -M 64000 -T 8." Feature building was performed without identity filtering. The structures were predicted using AF2 by setting the number of recycling steps to 15. Z-M1-GDT (Z-scores of MODEL 1 based on GDT-TS) were extracted from TSV files downloaded from the CASP15 website.

Impact of the refinement model

To evaluate the effect of the refinement model, the precision of the intermediate structures was measured by comparing their accuracy before and after refinement. The intermediate structures of the submitted models were collected from the backup files.

RESULTS AND DISCUSSIONS

Overall performance

My team, PEZYFoldings got third place with GDT-TS (First place with the Assessor's formulae) in the single-domain category and tenth place in the multimer category. Looking at the ranking on the all submitted models, PEZYFoldings got fourth place with GDT-TS (First place with the Assessor's formulae) in the single-domain category and fourth place in the multimer category. The improved ranking in the multimer category, considering all submitted models, suggests that there is room for enhancement in my ranking and selection process for multimeric structures.

After the competition, all generated models, including unsubmitted ones, were assessed based on their TM-scores[29] (Fig. S1, S2). Optimal chain mapping for the multimer targets were obtained using US-align[34,35] or MM-align[30]. TM-scores were calculated using TM-score software. Among the 93 single-domain targets with available ground truth structures, 10 targets had superior models displaying significant TM-score differences (>0.1) compared to the submitted models. Likewise, among the 36 multimer targets with available ground truth structures, three targets possessed better models exhibiting substantial TM-score differences (>0.1) compared to the submitted models. It is important to note that these results cannot be directly attributed to the inadequacy of my model ranking and selection procedure, as some models were not processed in the ranking and selection step. Instead, the results suggest the full potential of the structure prediction components.

Notable targets

In this section, I will discuss specific targets that are likely to be of particular interest to readers.

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T1130

For T1130, I could obtain hits only from the MGnify[26]database. However, the identities between the hits and the query was low. In addition, the target was described as an aphid protein; therefore, the hits were suspicious. Furthermore, confidence scores of the resulting models were poor. I performed *de novo* -like structure prediction using the refinement model and built approximately 5,000 models. And structures with relatively high self-confidence scores were submitted; however, these scores were lower than those of the usual targets. The plDDT of the top structure was 68.99. This did not reach the level often observed in successful predictions, which tend to have plDDT above 80. Assessment after the competition showed that all the produced structures had an insufficient TM-score (Fig. S1). According to discussions during the CASP15 conference, the two teams with the highest ranks in the single-domain category had hits for T1130. Therefore, my poor performance on this target was caused by a deficiency in the sequence similarity search conditions.

H1137

Due to the large size of H1137, domain parsing was performed through visual inspections. Initially, the features derived from the MSA were divided into multiple segments and concatenated, resulting in approximately 1000-2000 amino acids in total and the predicted partial structures (step 1, Table S3). Utilizing the outcomes of step 1, I constructed additional partial structures (step 1.5, Table S3) to verify the accuracy of my assumptions regarding subunit interactions. These predictions suggested that the N-terminal regions of s1-s6 interacted with s8 and s9, while s7 interacted with s8 and s9. Consequently, I constructed partial structures using: 1) N-terminal regions of s1-s6 and full-length s8 and full-length s9; 2) middle part of s1-s6; 3) C-terminal regions of s1-s6; 4) N-terminal regions of s1-s6, N-terminal regions of s7, full-length s8, and full-length s9; 5) full-length s7; and 6) GFP domain of s7 (step 2, Table S3). The predicted partial structures in step 2 were concatenated, and the subunit structures were extracted. Note that because complete structures of H1137 were intended to be built at the submission date of H1137, structures submitted as independent subunit structures were from partially concatenated structures.

Similar to the usual monomer targets, the sum of plDDTs was used as the selection criterion. The refinement was not performed because the performance of the refinement model on a partial structure was considered poor and the entire assembly structure was too large to process.

According to the single-domain category results in CASP15, I achieved Z-scores greater than 2.0 for six targets. Five of these six targets were the helical domains of H1137 subunits (D2 domains of s1, s2, s3, s4, and s5), which were challenging to predict as monomers. Hence, domain parsing that considers the interface was essential for my high performance.

T1173-D2

Regarding T1173, the semi-automatic protocol did not yield structures that displayed promising results when compared to the ColabFold[32] results (Fig. 2C, first panel and forth panel). Using Quick BLASTP[15,36,37] search, T1173 was observed to be part of a longer sequence. Therefore, I extended 196 aa from the N-terminus using a longer sequence and predicted the structures again. After constructing several structures, I noticed that the quality of the C-terminal region (based on visual inspection, Fig. 2C, second panel) was inferior compared to the ColabFold result (Fig. 2C, fourth panel). I examined the depth of the MSA and observed a highly skewed distribution; the deepest part had more than 400,000 sequences, while the last ten aa region had fewer than 1,000 sequences (Fig. 2A). Consequently, I selected hits in the final ten aa, randomly selected 500 sequences from the original MSA, and flattened their distribution (Figs. 2A, 2B). The resulting models appeared satisfactory (Fig. 2C, third panel). Nevertheless, it is important to note that other groups submitted more accurate structures. The N-terminal region of D2 (positions 63-113 in the full-length sequence) in my MODEL 1 could not be aligned with the ground truth structure using TM-score software. Enhanced structures might have been achieved if I had included more sequences from positions 63-113.

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Assessment of the impact of individual elements

Impact of the extended sequence similarity search

To examine the impact of the extended sequence similarity search process, I conducted an assessment of predictions after the competition, focusing on the differences in input MSAs. I employed two types of MSAs for predicting target structures. The first MSA set comprised MSAs utilized by PEZYFoldings (PEZY-MSA), while the second set was generated using the default settings of the AlphaFold2 or AlphaFold-Multimer pipeline provided by the NBIS-AF2-standard and NBIS-AF2-multimer teams (NBIS-MSA). I examined targets with a total length of 1200 aa or less. However, out-of-memory errors occurred for T1124, T1132, and T1174. Consequently, 54 single-domain targets were investigated. PEZY-MSA had at least one more sequences than NBIS-MSA, except for the targets T1133-D1, T1131-D1, T1122-D1, and T1119-D1 (Table S4) . Thus, I can confirm that I obtained more evolutionarily related sequences than the default settings in over 90% of cases. The number of sequences in PEZY-MSA for specific targets could be smaller than those of NBIS-MSA due to: 1) running hhblits[21] against UniRef30[23] and BFD[24] separately; 2) not using the UniRef90[38] database; 3) the number of iterations against BFD was changed to two from three; 4) using a more stringent e-value (0.00001) for jackhmmer[19,20]compared to the default settings (0.0001); and 5) applying hhfilter[22] to hits from BFD and MGnify. When clustering sequences with a sequence identity threshold of 62%, a criterion for effective sequence counts used in previous studies[13,39], I obtained larger values than the default settings in 43 of the 54 cases (Table S4).

The ΔTM-score (TM-score of structures with PEZY-MSA minus the TM-score of structures with NBIS-MSA) as a function of Nseq-NBIS-MSA (number of sequences in NBIS-MSA) is illustrated in Figs. 3C and 3D. Seven and five targets demonstrated a ΔTM-score >0.05 for MODEL 1 (the model with the highest confidence) and the best model among the five generated models, respectively (Fig. 3C, 3D). All targets with a ΔTM-score >0.05 had an Nseq-NBIS-MSA of less than 1000. The ΔTM-score for targets with Nseq-NBIS-MSA greater than 1000 was minimal, which is consistent with the results in the original publication; the quality of predictions by AlphaFold2 increases until the number of sequences or Neff is approximately 100-1000[1]. This trend was also observed in the CASP15 results. Among the 53 targets, I had nine targets with a Z-score greater than 1.0, and seven out of those nine targets had an Nseq-NBIS-MSA of less than 1000 (Figure 4E, Table S4).

Impact of the deep-learning-based refinement model

The TM-scores of the models submitted to the competition website were collected to investigate the refinement model's effectiveness. The TM-scores before and after the last refinement are summarized in Fig. 4. Models subjected to docking or *de novo* -like structure predictions were excluded. The refinement model improved the quality of some predicted structures (Figs. 4A, 4D); however, from the point of view of the performance in the competition, the differences in the TM-score were indistinguishable (Figs. 4B, 4C, 4E, 4F). In other words, although the refined structures had better accuracy than the original structures, the other structures achieved the same or better levels of accuracy without refinement. In CASP15, there were seven conventional antibody-antigen or nanobody-antigen targets. I could build three out of seven targets with an average DockQ[40] score >0.49, which meets the medium-quality threshold in CAPRI[41] criteria. As mentioned in the introduction, the refinement model was anticipated to perform well with antibodies. However, the results obtained from the model indicate that further efforts are required to reach the desired level of success.

CONCLUSION

The factors that would have significantly contributed to the results of the CASP15 were explored. The structure-building step was performed using AlphaFold2 and AlphaFold-multimer, indicating that the AF2 series is a state-of-the-art structure prediction tool. A detailed protocol analysis suggests that extensive MSA

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construction and manual interventions considering interfaces were the main factors for the high performance of the CASP15. However, there was at least one target in which other teams received more sequences for MSA; thus, there is still room for improvement in database construction or sequence similarity search steps. The performance could have been higher in the multimer categories, and I could not predict a sufficient model in most cases when no evolutionarily related sequences were observed. Therefore, developing a more effective protocol to address these issues is required. In addition, transporting the manual intervention process to automation will be an upcoming issue because manual intervention should be minimal.

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FIGURES

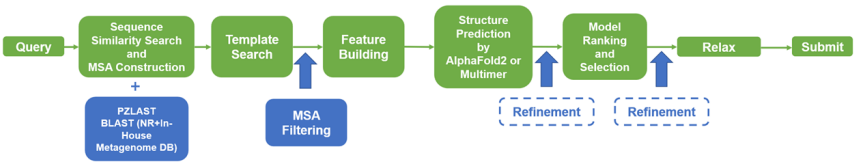

Figure 1A

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Figure 1B

Figure 1C

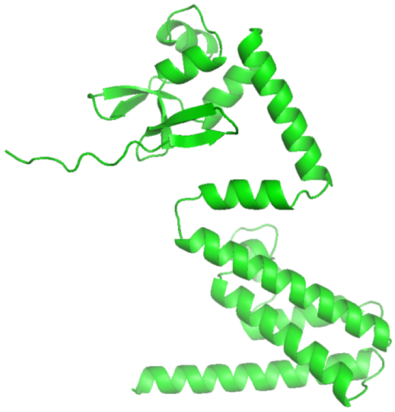

Figure 1D

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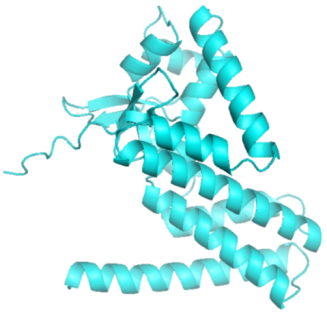

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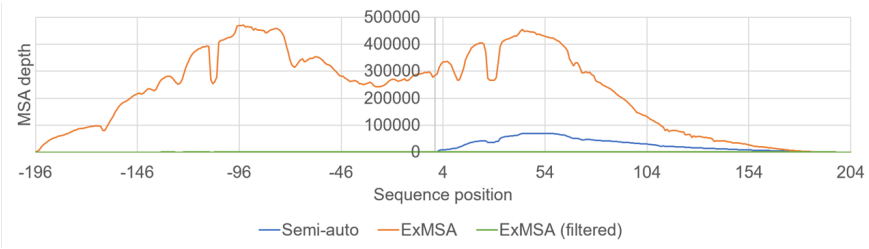

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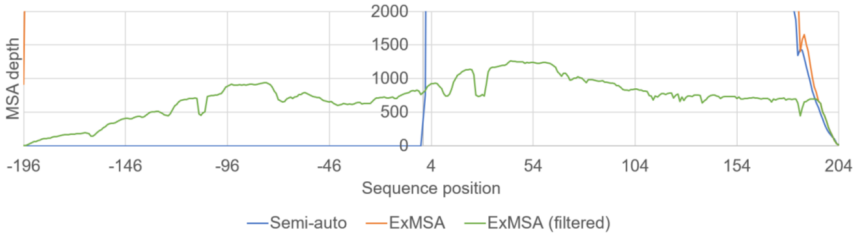

Figure 2B

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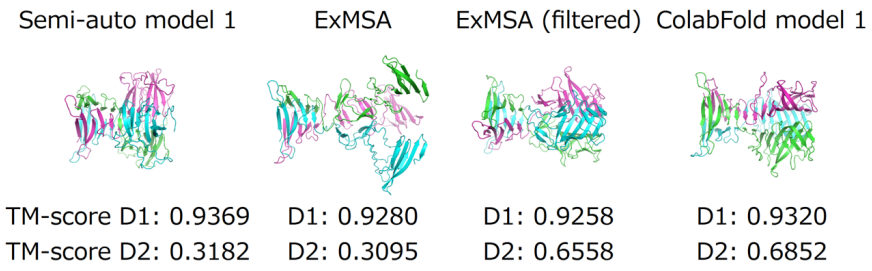

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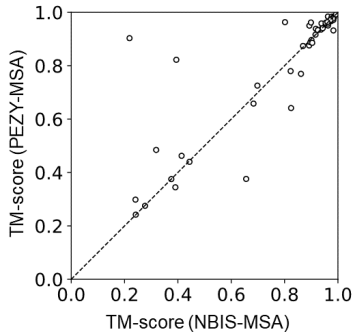

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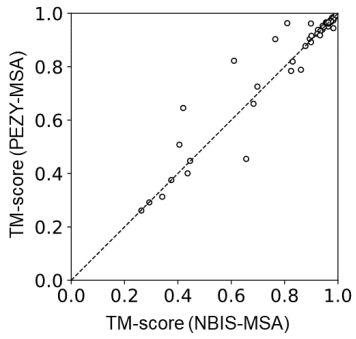

Figure 3B

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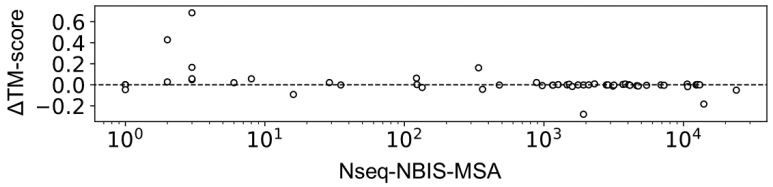

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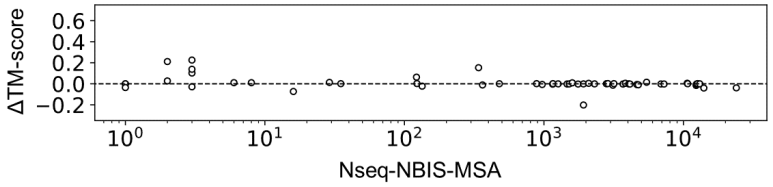

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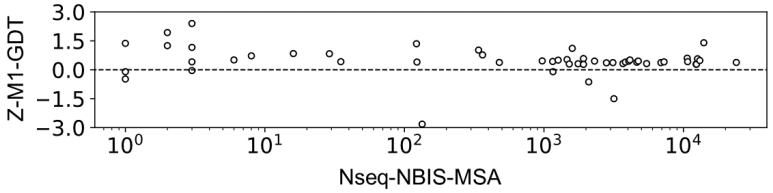

Figure 3E

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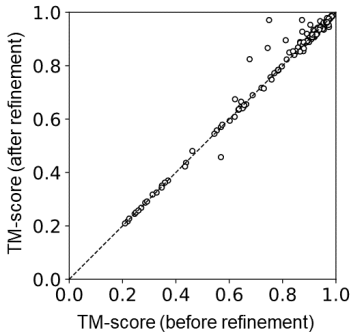

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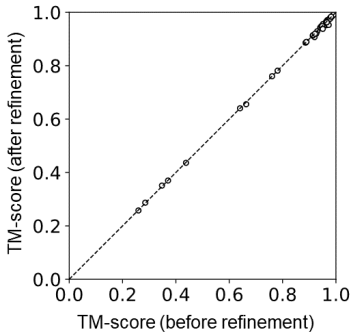

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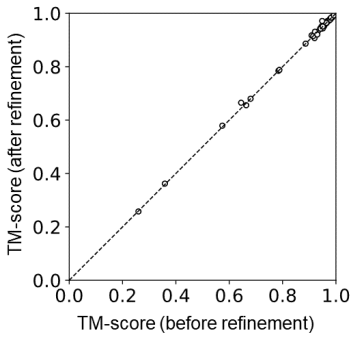

Figure 4C

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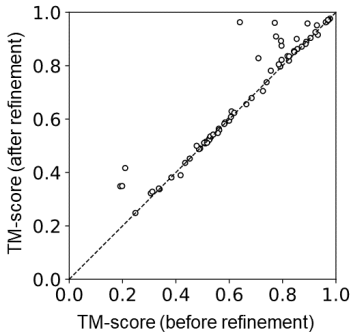

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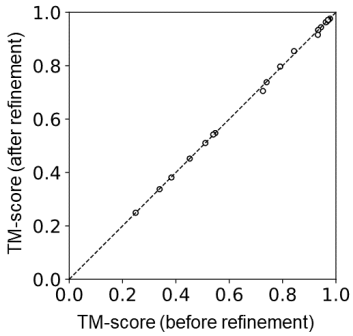

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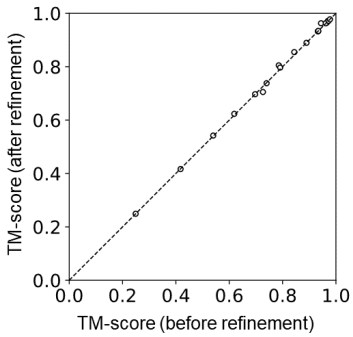

Figure 4F

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FIGURE LEGENDS

Figure 1. Prediction pipeline schematic and example targets for manual interventions. (A) A schematic representation of the prediction pipeline. The green blocks represent steps used in the default AF2 pipeline. Blocks with other colors represent modifications to the pipeline. (B) An example of targets for which MSA arrangements were performed. The y-axis represents MSA depth, and the x-axis represents sequence position. (C) The MSA shown in B after the manual arrangement. (D) An example of targets exhibiting a globular structure after the refinement. (E) The refined result of the structure shown in D. MSA, multiple sequence alignment.

Figure 2. Prediction conditions for T1173. "Semi-auto" represents MSA results from the standard pipeline outlined in the Materials and Methods section. "ExMSA" refers to MSA generated with an added 196 aa at the N-terminus. "ExMSA (filtered)" denotes the ExMSA after the MSA arrangement process detailed in the main text. (A) MSA-depth pre- and post-manual interventions. (B) MSA depth with a focus on y-axis values below 2000. (C) Predicted structure examples. Note that the TM score was unavailable during the prediction season. "ColabFold" represents the model downloaded from the ColabFold website (https://wwwuser.gwdg.de/˜mmirdit/casp15/). MSA, multiple sequence alignment; aa, amino acid.

Figure 3. Accuracy differences between structures predicted using MSAs generated by the PEZYFoldings pipeline (PEZY-MSA) and those provided by the NBIS-AF2-standard and NBIS-AF2-multimer teams (NBIS-MSA). (A) TM-scores of MODEL 1 (the model with the highest confidence) for each single-domain target. (B) TM-scores of the best structures for each single-domain target. (C) ΔTM-score (TM-score of models using PEZY-MSA minus TM-score of models using NBIS-MSA) of MODEL 1 as a function of Nseq-NBIS-MSA (number of sequences in NBIS-MSA). (D) ΔTM-score of the best models as a function of Nseq-NBIS-MSA. (E) CASP15 official Z-M1-GDT (Z-scores of MODEL 1 based on GDT-TS) for the single-domain category submitted by PEZYFoldings as a function of Nseq-NBIS-MSA. The results of the 52 targets used in this analysis were plotted. The results of T1145-D1 and T1145-D2 were not plotted in E because I did not submit MODEL 1 for this target.

Figure 4. Comparison of TM-scores for predicted structures before and after refinement. (A) TM-scores for all submitted structures for single-domain targets processed by the refinement model. (B) MODEL 1 structures' TM-scores for single-domain targets. (C) Highest TM-score among five submitted structures for single-domain targets. (D) TM-scores for all submitted structures for multimer targets processed by the refinement model. (E) MODEL 1 structures' TM-scores for multimer targets (F) Highest TM-score among five submitted structures for multimer targets.

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