# AlphaFold2 fails to predict protein fold switching

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## Abstract

AlphaFold2 has revolutionized protein structure prediction by leveraging sequence information to rapidly model protein folds with atomic-level accuracy. Nevertheless, previous work has shown that these predictions tend to be inaccurate for structurally heterogeneous proteins. To systematically assess factors that contribute to this inaccuracy, we tested AlphaFold2's performance on 98 fold-switching proteins, which assume at least two distinct-yet-stable secondary and tertiary structures. Topological similarities were quantified between five predicted and two experimentally determined structures of each fold-switching protein. Overall, 94% of AlphaFold2 predictions captured one experimentally determined conformation but not the other. Despite these biased results, AlphaFold2's estimated confidences were moderate-to-high for 74% of fold-switching residues, a result that contrasts with overall low confidences for intrinsically disordered proteins, which are also structurally heterogeneous. To investigate factors contributing to this disparity, we quantified sequence variation within the multiple sequence alignments used to generate AlphaFold2's predictions of fold-switching and intrinsically disordered proteins. Unlike intrinsically disordered regions, whose sequence alignments show low conservation, fold-switching regions had conservation rates statistically similar to canonical single-fold proteins. Furthermore, intrinsically disordered regions had systematically lower prediction confidences than either fold-switching or single-fold proteins, regardless of sequence conservation. These results suggest that AlphaFold2's high prediction confidences for fold switchers stem from the assumption that proteins assume one dominant fold. Our results emphasize the need to look at protein structure as an ensemble and suggest that systematic examination of fold-switching sequences may reveal propensities for multiple stable secondary and tertiary structures.

**Keywords** fold-switching, protein-folding, AlphaFold2, structural heterogeneity

## Introduction

AlphaFold2 has revolutionized protein structure prediction by using sequence information to rapidly model protein folds with atomic-level accuracy[1; 2]. Its predictions are generated by a deep neural network that identifies features of both multiple sequence alignments (MSAs) and experimentally determined protein structures. In other words, AlphaFold2 predictions are generated by a highly sophistcated deep-learning model that excels at recognizing correlations between protein sequence and structure.

AlphaFold2's approach to protein structure prediction is rooted in a large training set of experimentally determined protein structures. Indeed, without the Protein Data Bank (PDB), a publically available repository of nearly 200,000 protein structures[3], it would be impossible to predict protein structure through deep learning[4]. Consequently, deep-learning-based approaches are likely to miss protein properties that are not apparent from experimentally determined structures. For example, the conformations accessible to structurally heterogeneous proteins, whose overall secondary and tertiary structures are either unstable or change in response to their environment, cannot be captured in a single protein structure. Thus, it is not surprising that AlphaFold2 often fails to accurately predict the conformations of intrinsically disordered proteins[5-7], whose structures are highly heterogeneous. Specifically, AlphaFold2 predictions cover 98.5% of sequences in the human proteome (https://alphafold.ebi.ac.uk/), but only 58% of residues are modelled with high confidence[5; 7]. Many low-confidence predictions correspond to intrinsically disordered proteins/regions (IDPs/IDRs), often predicted to fold into long filaments[5; 6]. Furthermore, it remains unknown how accurately high-confidence predictions capture the structures of unchararacterized proteins, especially those either with few homologs in sequence databases or with sequences dissimilar to proteins represented in the PDB.

Here, we systematically assess whether AlphaFold2 captures the structural heterogeneity of fold-switching proteins. Contrasting IDPs/IDRs, which are natively unstructured, fold-switching proteins either assume distinct stable structures under different cellular conditions or populate two stable folds at equilibrium[8-10]. Thus, the primary sequences of fold-switching proteins encode more than one ordered state[9; 11]. As AlphaFold2 maps primary sequence to three-dimensional structure, we compared its predictions with experimentally determined protein

structures to explore whether it identifies the two stable structures encoded by fold-switching sequences, a single structure, or something else.

## Methods

### *Dataset of fold-switching proteins*

A set of 98 proteins[9; 12] that assumed at least two distinct-yet-stable secondary/tertiary structures (folds) was used for the analysis. Out of the 98 (**Table S1**), 93 had the alternate fold solved in PDB that were considered in this study; the remaining 5 were either homologs of fold switchers, expected to switch folds because their homologs do, or were shown to switch folds by methods other than crystallography, NMR or cryo-EM[12]. The sequences of the 5 proteins with only conformation solved were used mainly for generating predictions, followed by analyzing prediction scores after modeling (*Assessment of model quality*) and also for conservation scores from the MSA generated during prediction (*Conservation scores and rate of evolution using MSA*). They were not used for structural comparisons (using TM-scores and RMSD).

### *Dataset of intrinsically disordered proteins (IDP) / intrinsically disordered regions (IDR)*

A set of 99 proteins were randomly selected from DisProt (https://disprot.org/), a database of experimentally characterized intrinsically disordered proteins, with disordered regions manually curated from the literature[13]. The proteins chosen for the analysis (**Table S2**) had disordered regions ranging from 20-100 residues (to keep their average sizes similar to fold-switching regions); these regions were not located at termini. The set also included the three disordered proteins mentioned previously[6]: histone acetyltransferase p300 (Uniprot: Q09472, DisProt: DP00633), CREB-binding protein (Uniprot: Q92793, DisProt: DP02004) and the RNA-binding protein FUS (Uniprot: P35637, DisProt: DP01102).

### *AlphaFold2 model generation*

The FASTA sequences of fold-switching proteins were extracted from PDB SEQRES records and used as input to the AlphaFold2 structure prediction model[1], an open-source implementation of the inference pipeline of AlphaFold v2.0 (https://github.com/deepmind/alphafold) maintained on the NIH HPS Biowulf cluster (http://hpc.nih.gov). Ideally, the sequences for the two PDB pairs (corresponding to the two folds)

would be identical, but for 56 of the pairs the sequences were not identical, usually due to insertions or deletions. Hence we performed modeling for 154 proteins (**Table S3**). The template database contained PDB structures and sequences released until 2021-07-31. The 99 proteins from the DisProt database were modelled with the same parameters as the fold-switching proteins (**Table S2**).

***Assessment of model quality***

AlphaFold's top-scoring models are ranked from 1 to 5 by per-residue Local Distance Difference Test (pLDDT) scores, quantified by determining the fraction of predicted Cα distances that lie within their expected intervals[1]; they are compared to the original PDB structure using structural alignment as implemented in TM-align[14], and TM-score (ranging from 0 to 1) is reported as the measure of overall accuracy of prediction for the models. TM-score > 0.6 implies roughly the same fold[15], and a higher value indicates a better match. We also calculated root mean square deviations (RMSDs) followed by overall sequence alignments using ProFit (Martin, A. C. R, http://www.bioinf.org.uk/software/profit/) to augment TM-scores when the values were low (<0.5).

***Ordering conformations***

Conformations with higher TM-scores for at least 3/5 AlphaFold2 predictions were designated "Fold1." Two exceptional cases, 6z4u/5tpn, were designated "Fold1" because they had moderate/good TM-scores for 2/5 AlphaFold2 predictions, whereas the remaining 3 predictions had poor/moderate TM-scores for both folds.

***Clustering in Figure 1***

Figure 1 was clustered with k-means clustering, as implemented in the python module scikit-learn[16]. The number of clusters was determined by searching for the first value of k-means inertia whose second derivative with respect to cluster number was smaller than the second derivatives of its immediate neighbors (**Figure S1**). Predictions in Cluster 1 with either TM-score < 0.8 were assigned to Cluster 2. TM-scores of fold-switching regions of proteins from Cluster 1 were determined by excising fold-switching regions from both experimentally determined structures and the 5 AlphaFold models and comparing them with TMalign. Orders of Fold1 and

Fold2 were identical as in **Table S1** except for 5B3Z_A/5BMY_A, all of whose predictions were biased toward Fold2, and thus their ordering was switched for **Figure S3**. The fold-switching region of this pair was small compared with the whole protein (29/403 residues), explaining the switch in pordering. Predictions for Fold2 were considered significantly larger if their TM-scores exceeded the TM-scores of Fold1 by at least 0.05, ruling out cases where predictions were equally good for both folds but the TM-score was marginally better for Fold2.

***Fold-switching/single-folding regions***

As reported previously[17], we divided the alignments into fold-switching and single-folding regions, since fold-switching proteins are typically composed of both[9]. Sequences of fold-switching regions are reported in **Table S4**.

***Conservation scores and rate of evolution using MSA***

The multiple sequence alignments (MSAs) generated by AlphaFold2 to predict residue-residue distances and orientations were used to determine evolution rates, using Rate4Site (https://www.tau.ac.il/~itaymay/cp/rate4site.html)[18]. The program requires an MSA file to compute a phylogenetic tree and then calculates the relative conservation score for each column in the MSA. An empirical Bayesian method, which significantly improved the accuracy of conservation score estimates over the Maximum Likelihood method, was used to generate the rates[18]. The scores are represented as grades ranging from conserved-9 to variable-1.

***Statistical tests***

The distributions of pLDDT scores and Rate4Site grades in fold-switching regions were compared to the rest of the protein (the single-folding regions) and with a set of 99 IDP/IDRs (**Table S2**). Significance of differences in pLDDT distributions was calculated by employing the two-sample Kolmogorov-Smirnov and Epps-Singleton tests implemented in SciPy[19]. As Rate4Site scores yield a discrete distribution, only the Epps-Singleton test was used.

***Scripts and figures***

The scripts used for all analyses were written in Perl and Python; protein figures were generated in PyMOL[20] and plots in Matplotlib[21] and seaborn[22].

## Results

### *AlphaFold2 predictions are biased towards one conformation/fold*

The five top-scoring AlphaFold2 models of each fold-switching sequence were compared with two distinct, experimentally determined conformations using TMalign, which quantifies similarity of secondary structure elements by TM-score[14]. Because fold switching involves remodeling of secondary structure, we used TM-score as our primary metric for structural similarity (**Table S1, Methods**). Furthermore, we organized both experimentally determined conformations by systematically terming the most accurately predicted conformation "Fold1" and the alternate conformation "Fold2" (**Methods**).

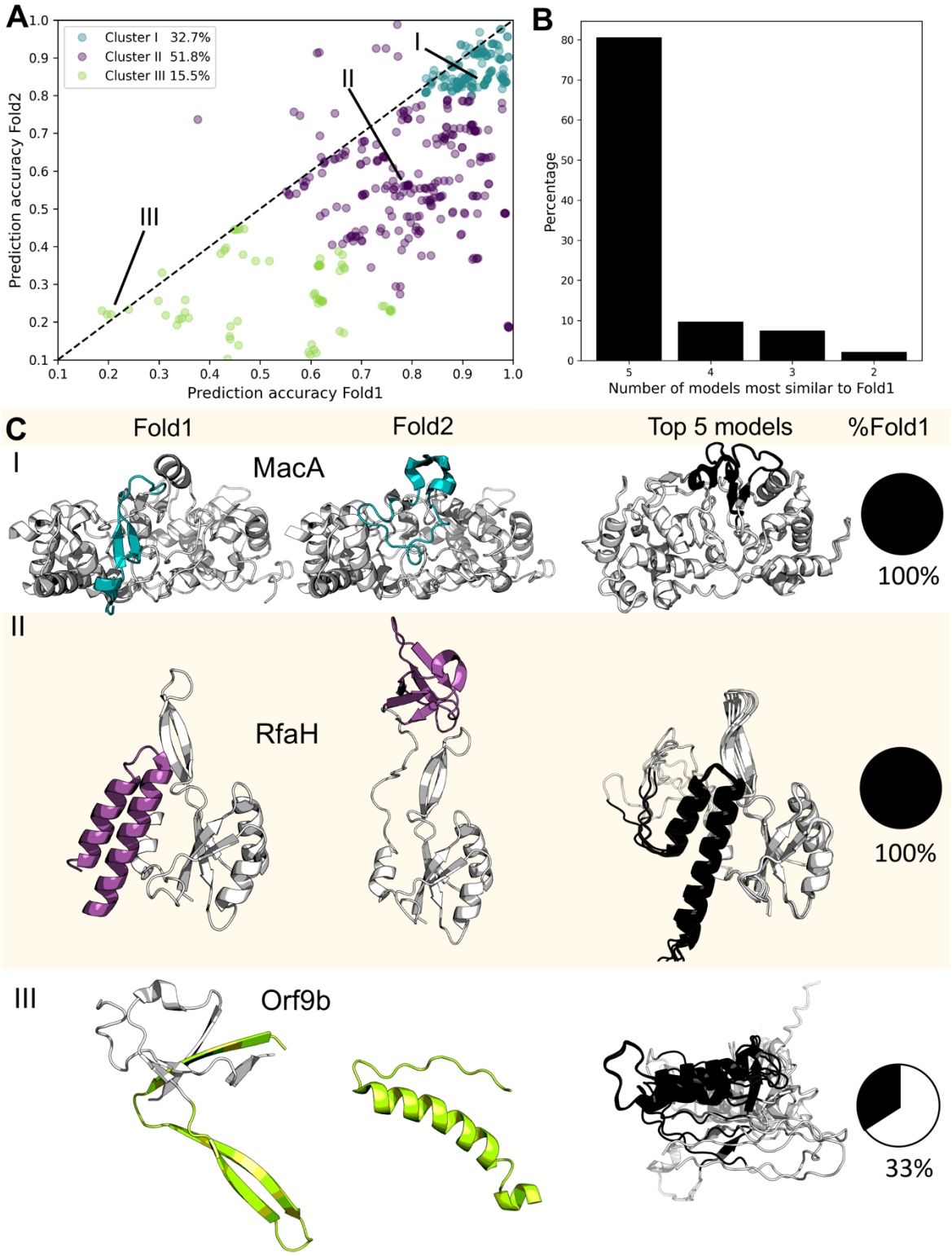
AlphaFold2 models were highly biased toward Fold1. A scatterplot of prediction accuracies, measured by TM-scores, indicates that nearly 94% of predictions fall below the identity line (**Figure 1A)** and are thus more similar to Fold1 than Fold2. The k-means algorithm was used to subdivide the scatterplot into three clusters, corresponding to the "elbow" of k-means inertia with respect to the number of clusters (**Figure 1A, Figure S1, Methods**). To simplify discussion, clusters were ordered by prediction quality rather than size. Cluster I comprised ~33% of all predictions, which were the most accurate for both conformations (TM-scores ≥ 0.8). Cluster II comprised ~52% of all predictions and generally paired either one good prediction (TM-score ≥ 0.8) and one moderate prediction (0.6 ≤ TM-score < 0.8) or two moderate predictions. Cluster III comprised the remaining 15.5% of predictions, all of which had at least one poor prediction (TM-score < 0.6).

Structural predictions tended to be conformationally homogeneous. Specifically, all 5 models were most similar to Fold1 in over 80% (75/93) of fold-switching sequences (**Figure 1B**). Additionally, TM-scores of Fold1 and Fold2 were very close (average difference in TM-scores 0.022 ± 0.017) in 13 of the remaining 18 cases, again indicating high levels of structural similarity among AlphaFold2-predicted models. The remaining 5 cases sample both conformations with moderate-to-good accuracy, and representatives are shown in **Figure S2**. Fold-switching proteins can assume a more stable "ground" state and a less stable "excited" state[23]. Specifically, only a few fold switchers have been shown to populate two folds simultaneously[10; 24-26]. The rest seem to populate a single "ground state" fold under physiological conditions and switch to an "excited state" in response to environmental changes, such as changes in redox potential[27] or addition of a

binding partner[28]. One might argue that if AlphaFold2 captured the ground state, its predictions could reasonably be considered correct. It does not always identify the ground state, however. For example, it predicts that KaiB assumes an "excited" thioredoxin fold[23] in 5/5 cases. Furthermore, though lymphotactin, Mad2, and PimA interconvert between two folds under physiological conditions[10; 25; 26], AlphaFold2 captures only one of their two conformations.

Examples of fold-switching proteins from all three clusters are shown in **Figure 1C**. In Cluster 1, a short region of MacA, a bacterial cytochrome C peroxidase, switches folds during reductive activation[29]. AlphaFold2 predicts that its fold-switching region assumes only the oxidized conformation in its 5 best models. Although all models in Cluster 1 had good TM scores (≥0.8) for both conformations, they were more similar to Fold1 than Fold2. Good scores for Fold2 result from shorter lengths of fold-switching regions compared to the lengths of the remainder of the protein (**Table S4**), which had good overall predictions except for the relatively short fold-switching regions. Indeed, TM-scores comparing predicted and experimentally determined fold-switching regions only (instead of the entire protein chain) were biased toward Fold1 (**Fig. S3**): only 10% of predictions (15/150) had better TM-scores for Fold2 (**Methods**). Prediction qualities for 14/15 of Fold2-favored predictions were poor for both experimentally determined folds (TM-score < 0.6), demonstrating AlphaFold2 did not capture either experimentally determined conformation well in most Fold2-favored cases. Representing Cluster 2, RfaH, a transcription factor that regulates the expression of virulence genes in *E. coli*, has a C-terminal domain (CTD) that completely switches between α-helix and β-sheet folds[24]. AlphaFold2 predicts that its CTD assumes only the autoinhibited α-helix conformation in its top 5 models. In Cluster 3, Orf9b, a protein encoded by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), assumes both a dimeric β-sheet form and a monomeric α-helical form that binds the mitochondrial host protein, Tom7[30]. In 2/5 cases, AlphaFold2 predicts a conformation similar to its β-sheet fold (TM-scores of ~0.66 for both models). The remaining three models are partially helical but mostly unstructured and do not correspond well to either experimentally determined structure as evidenced by TM-scores ranging from 0.17-0.23 (**Table S1**). These three poor predictions may result from Orf9b's shallow alignment of 6 sequences, an inadequate number for generating robust distance restraints[1; 31]. Furthermore, many amyloid-forming proteins, such as alpha-synuclein, amylin, and the αβ42 peptide, also fall into Cluster 3. This result corroborates previous work indicating that AlphaFold2's approach is not yet sensitive enough to robustly predict the

**Figure 1. AlphaFold2 fails to predict fold switching**. 94% of AlphaFold2 predictions fall below in the identity line (dashed line, **A**), indicating bias toward Fold1. Predictions were clustered by the quality of their correspondence with experiment (good predictions with TM ≥ 0.8 for both conformations were colored teal; moderate, purple; poor, green). Furthermore, all 5 of AlphaFold2's best models corresponded to Fold1 for 81% of fold-switching sequences **(B).** Experimentally determined structures representing all three clusters are shown in **C** and compared with the top 5 AlphaFold2 predictions for their sequences. Experimentally determined fold-switching regions are colored according to their cluster; predicted fold-switching regions are black; single-folding regions are gray.



conformations of fibril-forming proteins[32; 33].

TM-scores can sometimes mislead, especially when comparing mostly helical segments and/or highly dissimilar structures or sequences. To ensure the accuracy of this metric, TM-scores were plotted against sequence identities between the protein and the prediction (**Figure S4A**). For twilight cases with sequence alignments and TM-scores <0.5, the RMSD values were also large (mostly >10 Å), as seen in the bar plot inset. Hence, structural deviations in these twilight cases are corroborated by high values of RMSD. Additionally, as with TM-scores, RMSDs tended to be better for Fold1 than Fold2 (**Figure S4B**). Specifically, predictions where RMSD was ≤ 5Å for at least one structure were better for Fold1 in 83% of cases.

***AlphaFold2 prediction confidences are significantly higher for fold-switching sequences than for intrinsically disordered proteins (IDPs)***

As with fold-switching proteins, AlphaFold2 frequently mispredicts the conformations of IDPs[6; 7]. Mispredicted IDP conformations often have low confidence scores[5], calculated using the per-residue Local Distance Difference Test (pLDDT), which quantifies the fraction of predicted Cα distances that lie within their expected distance intervals[1]. Higher pLDDT scores indicate good agreement between prediction and expectation; pLDDT scores >90, 70, 50, 0 are considered very high, high, low, and very low, respectively.

AlphaFold2's prediction confidences were compared for fold-switching, single-folding, and intrinsically disordered sequences. AlphaFold2 was run on 99 IDP sequences randomly selected from the DisProt database[13] (**Methods**). The pLDDT scores of predicted IDPs were compared with those of the fold-switching and single-folding protein regions determined previously (**Methods**). **Figure 2** shows that IDPs have lower average pLDDT scores (54.5 ± 24.1) than fold-switching (80.3 ± 17.7) and single-folding (86.5 ± 15.5) sequences. Furthermore, 74.0%/86.9% of fold-switching/single-folding residues had good pLDDT scores (≥70), compared with only 30.3% for IDPs. Finally, the overall distributions of all three sets of sequences were statistically dissimilar ($p \sim 0$, Kolmogorov-Smirnov and Epps-Singleton tests). Together, these results demonstrate that, in contrast to IDPs, AlphaFold2 predictions of fold-switching sequences have relatively high confidences, though not quite as high as single-folding protein regions.

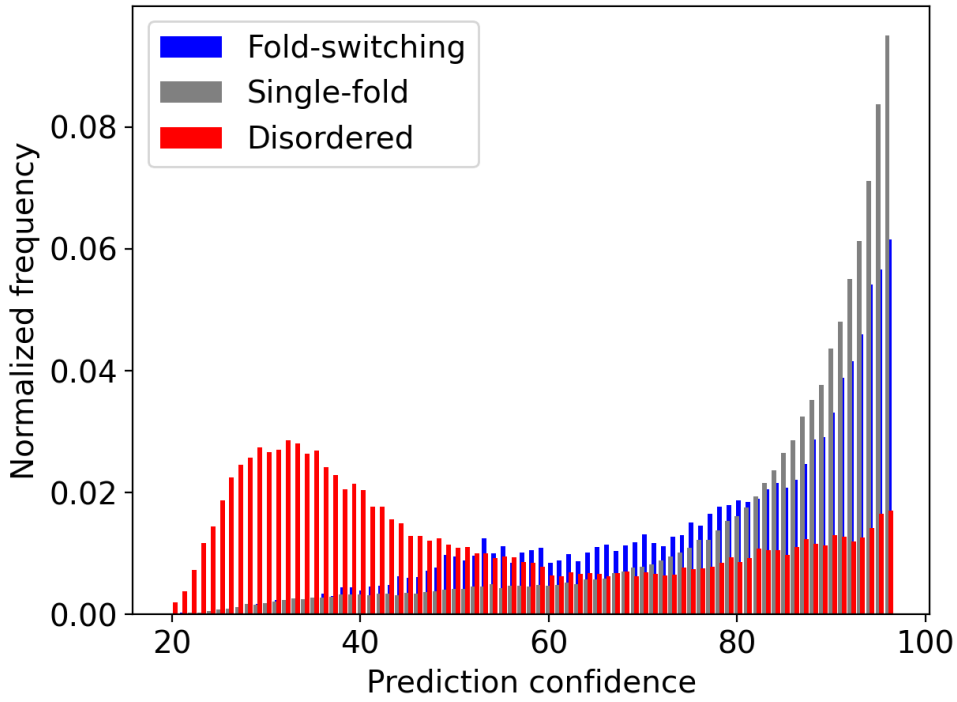



**Figure 2.** Distributions of AlphaFold2 predictions, measured by pLDDT scores, differ between fold-switching (blue), single-fold (gray), and intrinsically disordered (red) protein sequences. Lower pLDDT scores indicate lower prediction confidences. Thus, AlphaFold2 is generally less confident in its predictions of IDPs than fold-switching or single-folding proteins.

***Fold-switching sequences tend to be more conserved than IDPs***

AlphaFold2 does not capture the conformational heterogeneity of IDPs or fold switchers particularly well. While its prediction confidences are generally low for IDPs, they are higher for fold switchers. We investigated whether the low prediction confidences of IDPs resulted from their rapid rates of sequence evolution[34]. This often confounds construction of statistically robust multiple sequence alignments (MSAs), necessary inputs for generating accurate distance restraints for protein structure prediction[31]. Thus, stronger conservation of fold-switching sequences could be a possible explanation for higher pLDDT scores.

We calculated the evolutionary rates of the sequences from our set of 98 fold switchers and compared them with 99 IDPs. Specifically, we ran Rate4Site[18] on MSAs generated and used by AlphaFold2 for predictions of fold-switching proteins and IDPs (**Methods**). Distributions of conservation scores, with lower numbers implying less conservation, are shown in **Figure 3**. Rates of single-folding and fold-switching regions were narrowly within the realm of statistical similarity $p < 0.074$ (Epps-Singleton test, Methods). By contrast, evolutionary rates of IDP sequences were significantly faster than either fold-switching or single-folding sequences as evidenced by statistically dissimilar distributions with $p < 1.1*10^{-46}$ and $4.4*10^{-111}$ (Epps-Singleton test, **Methods**) for fold switchers and single folders, respectively. These results suggest that

AlphaFold2 predictions of fold-switching proteins may have higher confidences because their sequences are more highly conserved than for IDPs.

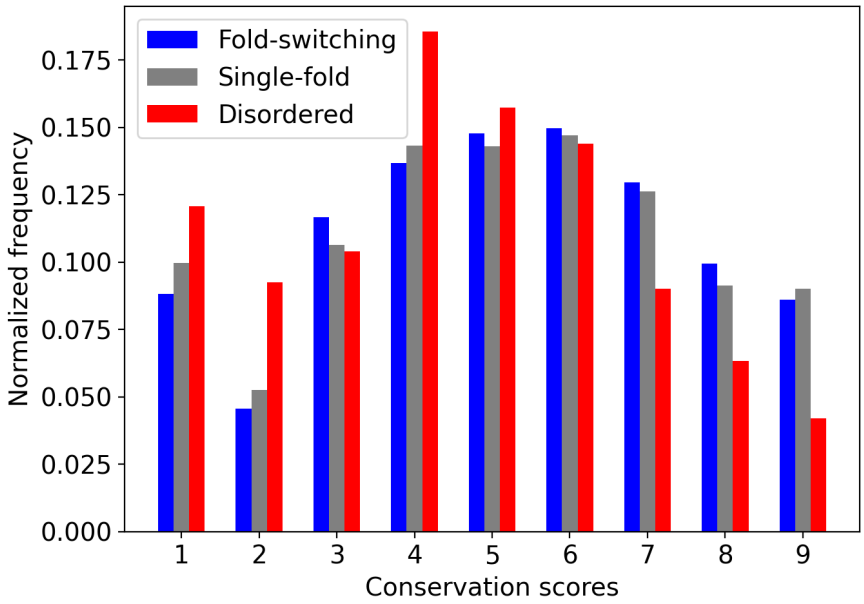



**Figure 3.** Evolutionary rates of IDPs, as indicated by Consurf grades (1=rapid evolution; 9= high conservation) differ between fold-switching (blue), single-fold (gray), and intrinsically disordered (red) protein sequences. Sequences fold-switching and single-fold proteins tend to be more conserved than IDP sequences.

***Higher prediction confidences suggest that AlphaFold2 assumes fold-switching proteins have a single dominant fold***

Frequencies of good AlphaFold2 prediction confidences (pLDDT scores ≥ 70) increase with residue conservation for fold-switching, single-folding and disordered proteins (**Figure 4A**). Thus, rapid evolutionary rates, specificaly conservation scores of 1-3, are associated with lower prediction confidences. This is likely explained by rapidly evolving sequences having shallower and/or poorly aligned MSAs[1].

Nevertheless, shallow MSAs do not fully explain why AlphaFold2 prediction confidences are higher for fold switchers than for IDPs. Good pLDDT scores level off at around a conservation level of 4 for both fold switchers and single folders, whereas pLDDT scores continue to increase with conservation level for disordered proteins (**Figure 4A**). Furthermore, for all conservation levels, distributions of prediction confidences for disordered residues were skewed systematically lower than corresponding distributions of fold-switching and single-folding residues (**Figure 4B**). Based on this result, it is likely that AlphaFold2 was developed to search for one "most probable"

fold instead instead of an ensemble of possible conformations. This supposition is consistent with 3 additional observations:

(1) AlphaFold2 was trained on the PDB, which contains mostly single-fold proteins[9].

(2) AlphaFold2 tends to settle on a protein's fold early in the prediction process. Specifically, it predicts protein structure using a pairwise representation of amino acid distances in addition to MSAs. These distances are typically determined early in the prediction process and fluctuate minmally with increasing iterations, even for difficult targets[1].

(3) Most structure prediction algorithms assume that proteins assume one stable fold.

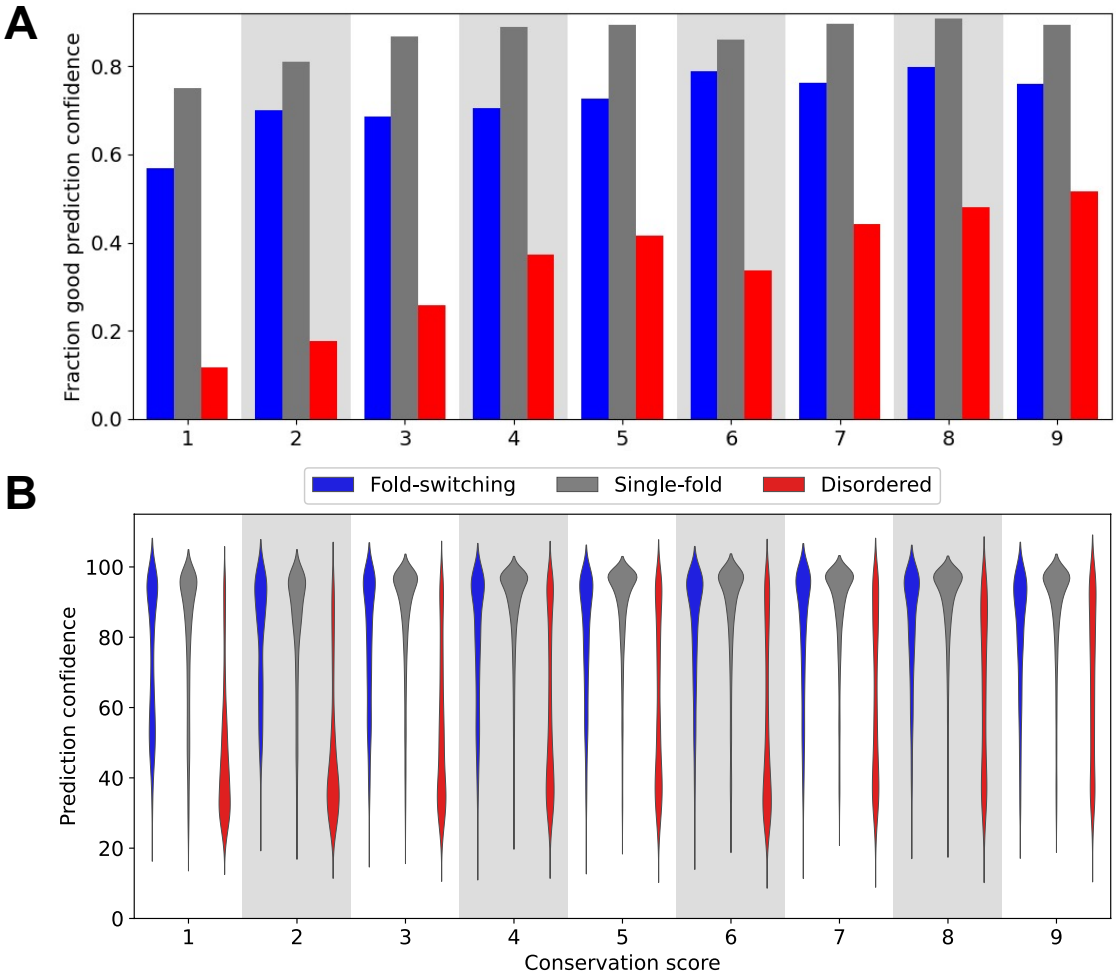



**Figure 4.** The fraction of AlphaFold2 predictions with pLDDT scores ≥ 70 (considered high-confidence predictions) increases as sequence conservation increases (**A**). Distributions of prediction confidences (quantified by pLDDT scores) are skewed lower for disordered proteins (red) than for single-fold (gray) and fold-switching proteins (blue, **B**). Wider regions correspond to more populated prediction confidences. In both cases, conservation score was determined using Rate4Site; higher scores correspond to more conserved sequences. Gray/white backgrounds group protein regions with the same conservation score.

***Discussion***

AlphaFold2 is a major advance in protein structure prediction[1], particularly for single-fold proteins[2]. Nevertheless, it is based more on pattern recognition than biophysical principles[4]. Specifically, its deep-learning model is trained on protein sequences and experimentally determined protein structures, neither of which reveal folding mechanisms. Without other fundamental information about protein structure, such as thermodynamics (what balance of forces favor the folded state?) and kinetics (what pathways do proteins traverse between unfolded and folded states?)[4; 35], AlphaFold2's deep learning approach can only reveal apparent properties of experimentally determined protein structures. Thus, it is not surprising that its predictions often fail for proteins whose properties are not fully apparent from solved protein structures, such as IDPs[5-7].

Here we show that AlphaFold2 consistently fails for fold-switching proteins, which remodel their secondary structures and change their functions in response to celluar stimuli[9]. AlphaFold2 failed to predict fold switching in its top 5 models for 88/93 proteins. Instead, it consistently predicted that fold switchers assume one dominant fold. Since proteins are typically assumed to have one fold, this result is not surprising, especially because AlphaFold2's training set, the Protein Data Bank, contains relatively few fold-switching proteins[8; 9]. It is notable, however, that AlphaFold2's predictions do not always correspond to "ground state" structures of fold switchers, such as KaiB and Lymphotactin. This is further evidence that its predictions are primarily rooted in sophisticated pattern recognition, not protein biophysics.

Unlike IDPs, prediction confidences for fold-switching sequences are relatively high (74.0% have pLDDT scores > 70 compared with 30.3% for IDPs). This result, combined with the weak relationship between pLDDT distributions and conservation scores for fold-switching proteins, suggests that AlphaFold2 assumes that stably folded proteins assume one dominant structure. This assumption leads it to miss biologically relevant structural information for some proteins, despite high-confidence predictions. It also raises the question of how much of the full picture AlphaFold2's full-genome predictions[7] capture.

The dramatic structural rearrangements of fold-switching proteins regulate biological processes[36] and are associated with numerous diseases, including COVID-19[30], cancer[37], Alzheimer's[38], and malaria[25]. Thus, predicting fold-switching proteins is an important problem. While some progress has been made[12; 39; 40], much work remains to identify features unique to fold-

switching proteins. Furthermore, detailed biophysical characterization of fold-switching proteins[41; 42] is needed. These challenges present an opportunity to improve predictive methods and possibly identify fundamental biophysical principles that are not yet well understood. Such discoveries could help to advance the field of protein structure prediction from sophisticated pattern recognition to methods based fully on protein biophysics.

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**Supplementary Information**

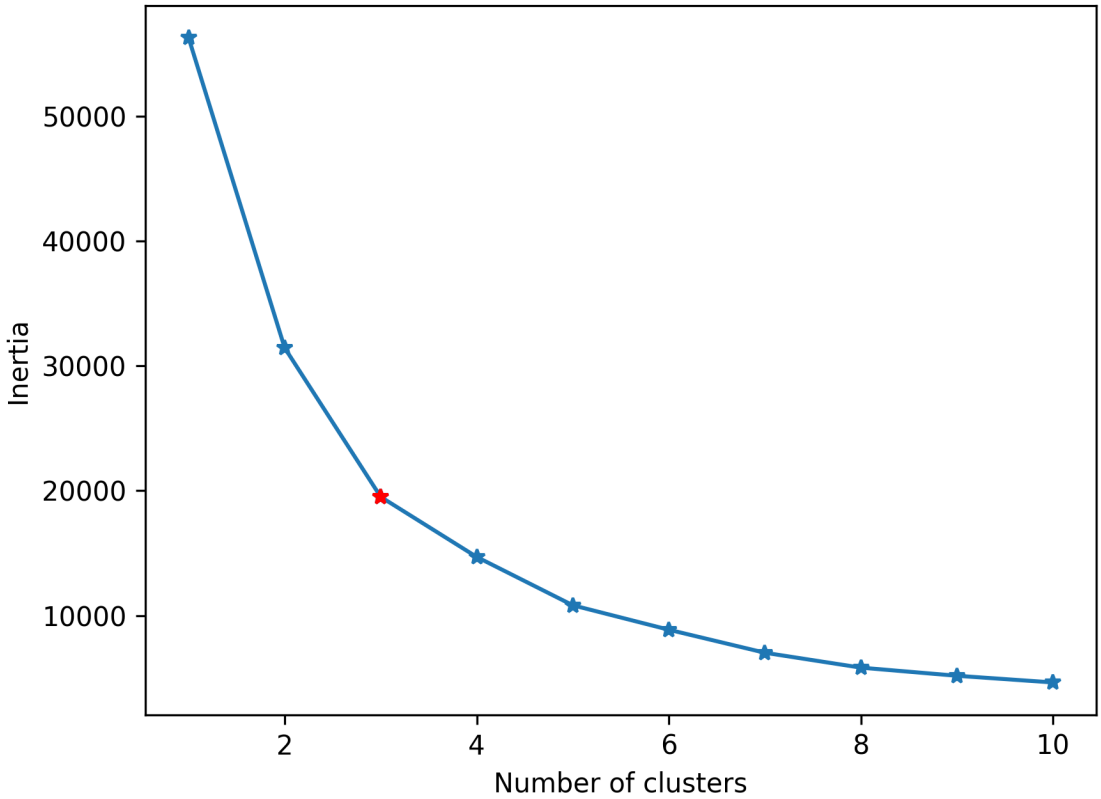



**Fig. S1** K-means inertia versus number of clusters for determining the optimal number of clusters to use in **Figure 1A**. The optimal number, determined by finding the minimal number of clusters whose second derivative is less than that of its immediate neighbors, is shown in red.

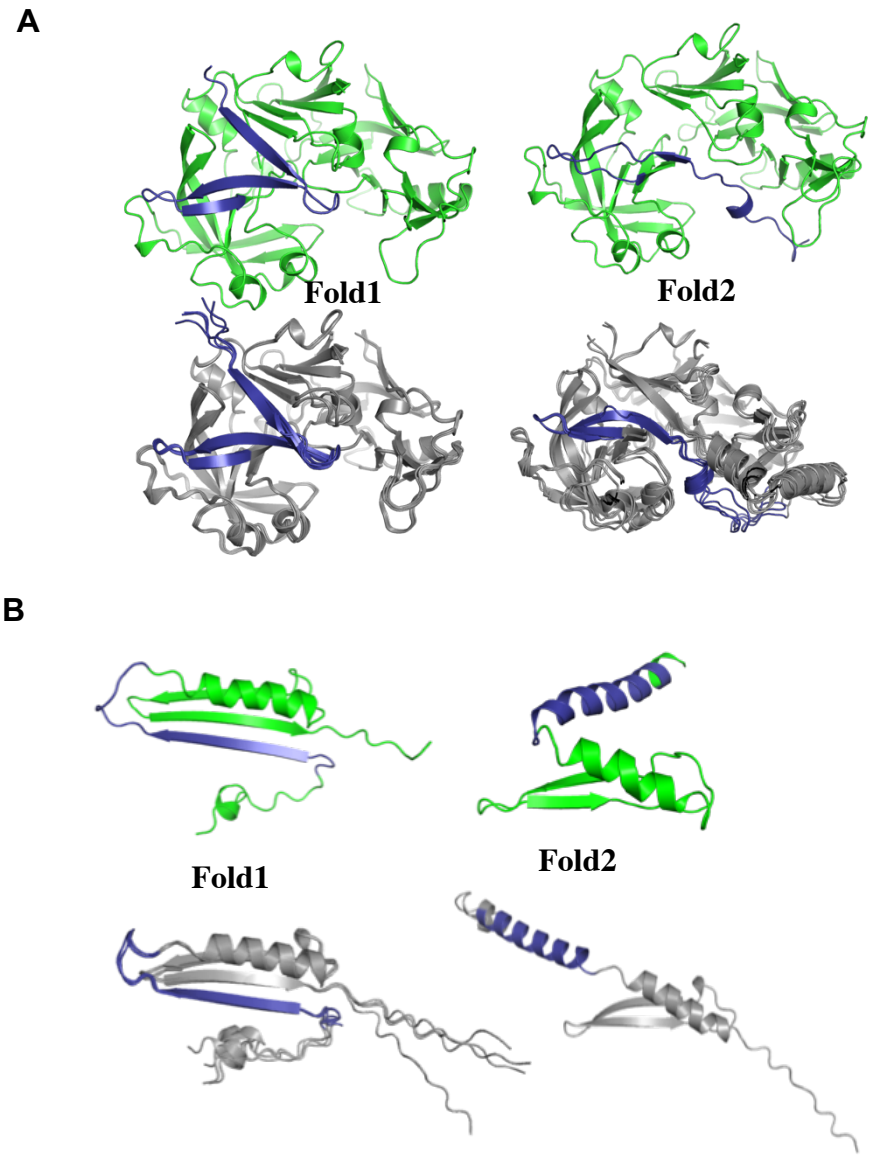



**Fig. S2** Examples when both folds are present among AlphaFold2 predictions**.** Fold-switching regions are blue in both experimentally determined and predicted folds (single-fold regions green and gray, respectively). The PDBIDs, chains and average TM-scores are as follows: **(A)** Plasmepsin. 1qs8B/1miqB: 0.95/0.93 and **(B)** MinE. 2kxoA/3rj9C: 0.77/0.51. In (**A**), each sequence of the pair was used to generate 5 models, all 5 predictions are shown for both Fold1 and Fold2. In (**B**), only one sequence was used to generate 5 models, Fold2 is seen in the model ranked 3; models 1-2 and 4-5 correspond to Fold1.

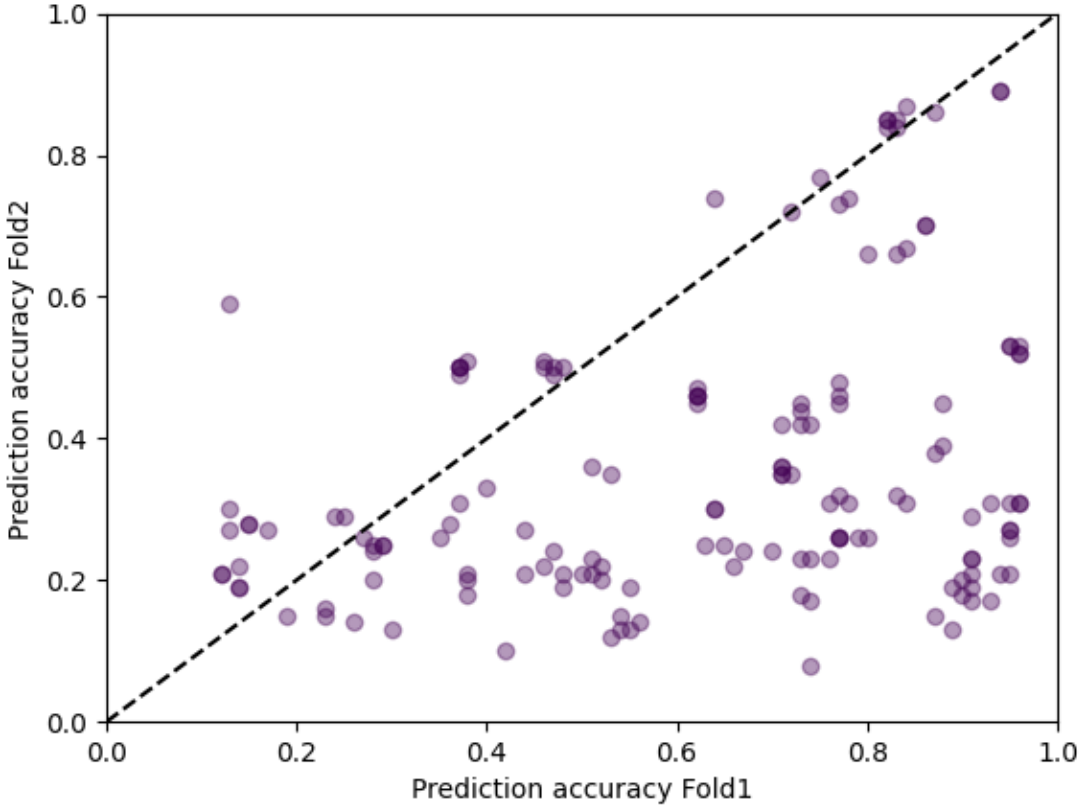
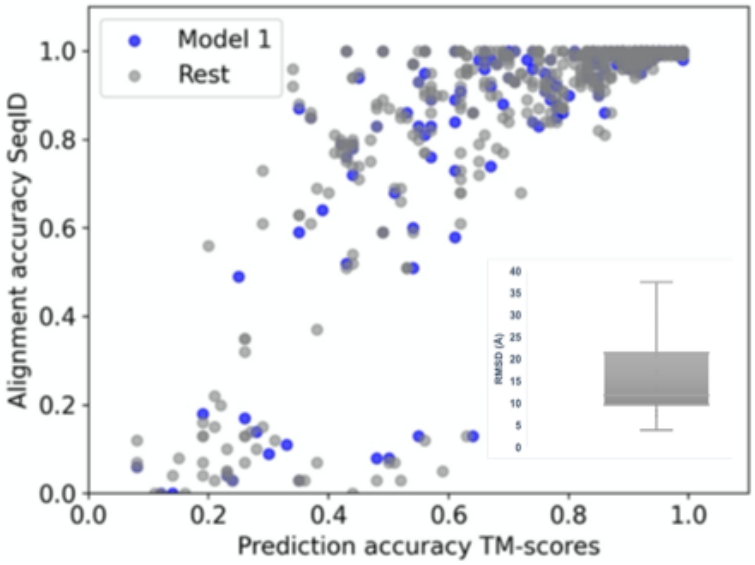



**Fig. S3**. AlphaFold2 predictions of only fold-switching regions of proteins from Cluster 1, whose overall folds are predicted well (TM-scores ≥ 0.8, **Figure 1A**), are more accurate for Fold1 than Fold2. Prediction accuracies were quantified using TM-scores, and 41% of predictions were innacurate (TM-score < 0.6) for both experimentally determined conformations of fold-switching regions.

**A**




**B**

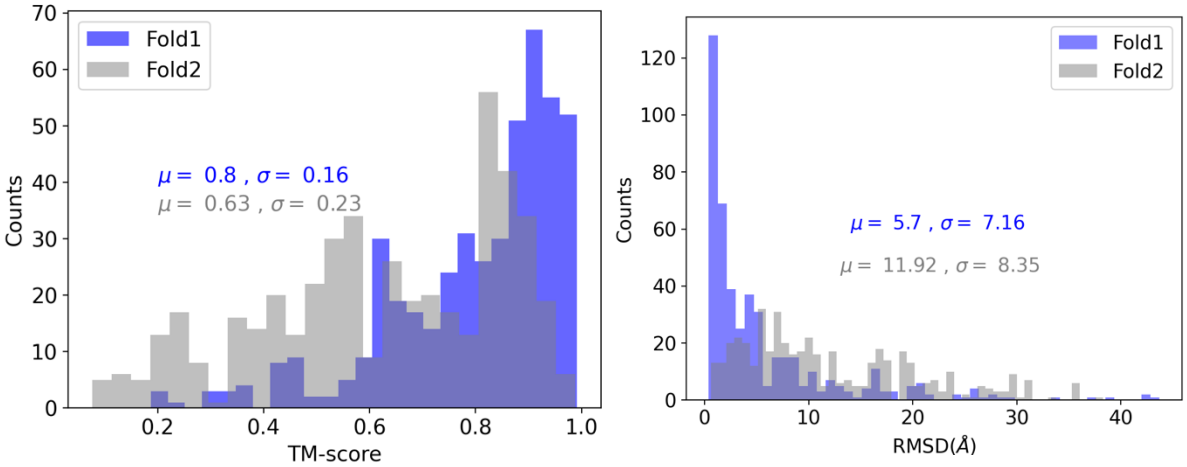



**Fig. S4** (**A**) Comparing alignments methods used by TMalign (structural alignment) vs. RMSD (sequence alignment), for cases having sequence alignment <0.5; RMSD values are presented as box and whiskers plot in inset. **(B)** Distribution of prediction accuracy of Fold1 and Fold2 of all 5 prediction for 93 protein pairs as TM-scores (left) and RMSD (right).

**Table S1.** List of fold-switching protein pairs (PDBID and chain) used for the analysis; first column corresponds to Fold1 and second to Fold2. Those without an experimentally determined structure for one of the pair are only listed in the first column. TM-scores for each of the 5 models are shown in subsequent columns. Scores are identical if sequences of both PDBs were identical. Scores of the first 5 and second 5 models differ if sequences were not identical. Then models of corresponding sequences were compared with corresponding PDBs. Table attached separately.

**Table S2.** Disordered proteins analyzed. Columns correspond to Disprot IDs, Uniprot IDs, and disordered region. Attached separately.

**Table S3.** List of pairs in which sequences are not identical in length and sequence identity, structure prediction was performed using both sequences for the pair. Attached separately.

**Table S4.** List of pairs (PDBIDs), lengths of fold-switching regions, and the sequence of the fold-switching region. (For those pairs not having the second fold solved in PDB, only the first PDB is reported.