Supplemental Material for:

AlphaFold2 fails to predict protein fold switching

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Supplementary Information

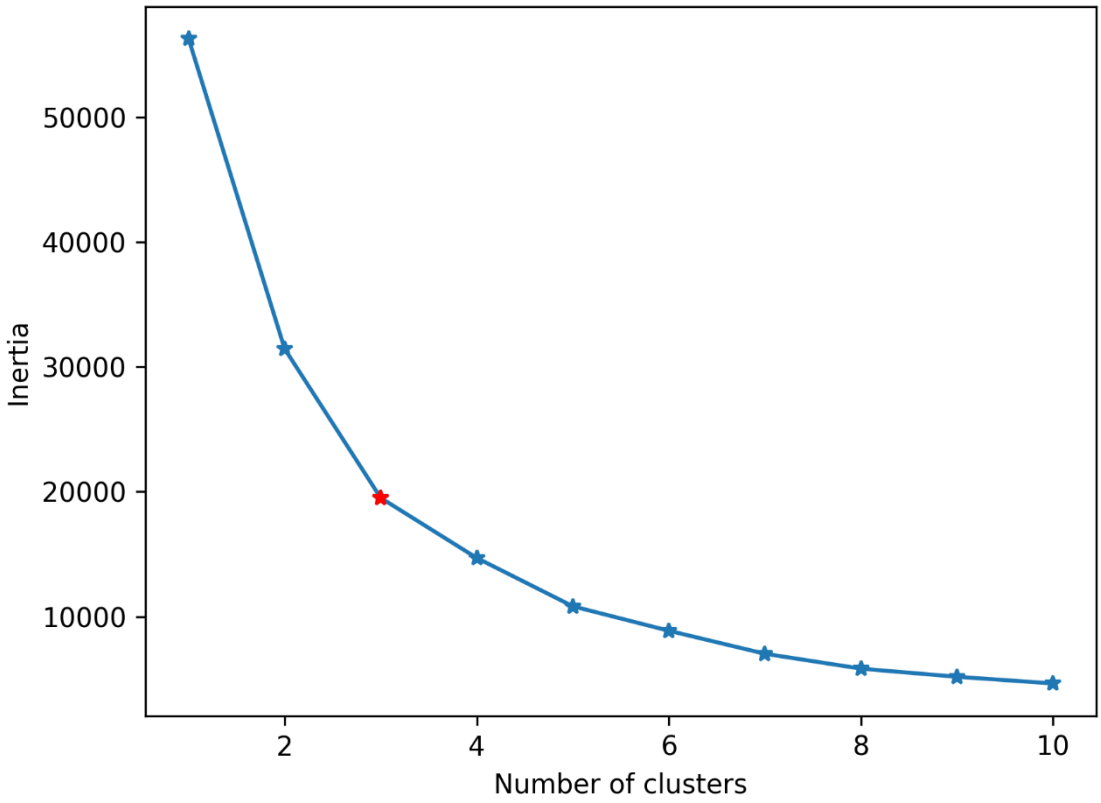
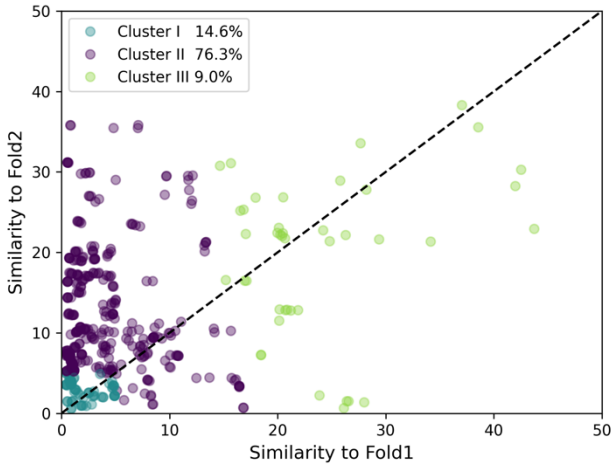
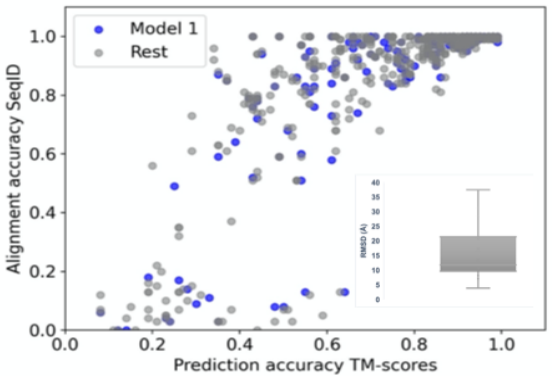


Fig. S1 K-means inertia versus number of clusters. The optimal value, determined by finding the minimal number of clusters whose second derivative is less than that of its immediate neighbors, is shown in red.

A



B



C

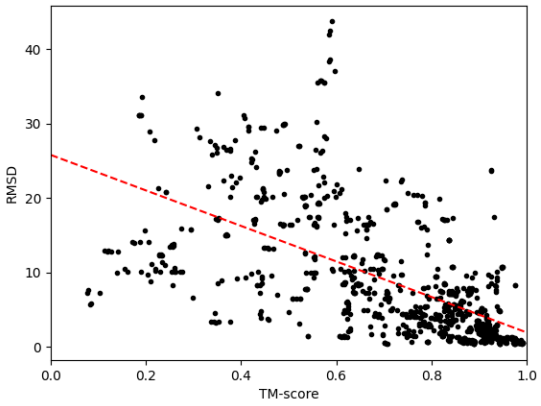


Fig. S2 (**A**). Prediction accuracies of fold-switching proteins, assessed by RMSD, are biased toward one fold. Of the accurately modelled proteins with RMSDs <5Å, 83% are more similar to Fold1 (above the identity line, implying lower RMSD). (**B**) Comparing alignments methods used by TMalign (structural alignment) vs. RMSD (sequence alignment by ProFit followed by structural superposition), for cases having sequence alignment <0.5; RMSD values are presented as box and whiskers plot in inset. (**C**). TM-scores and RMSDs of AlphaFold models and experimentally determined fold-switchers are correlated (r = -0.62). The negative slope is expected since high TM-scores and low RMSDs imply high prediction accuracies.

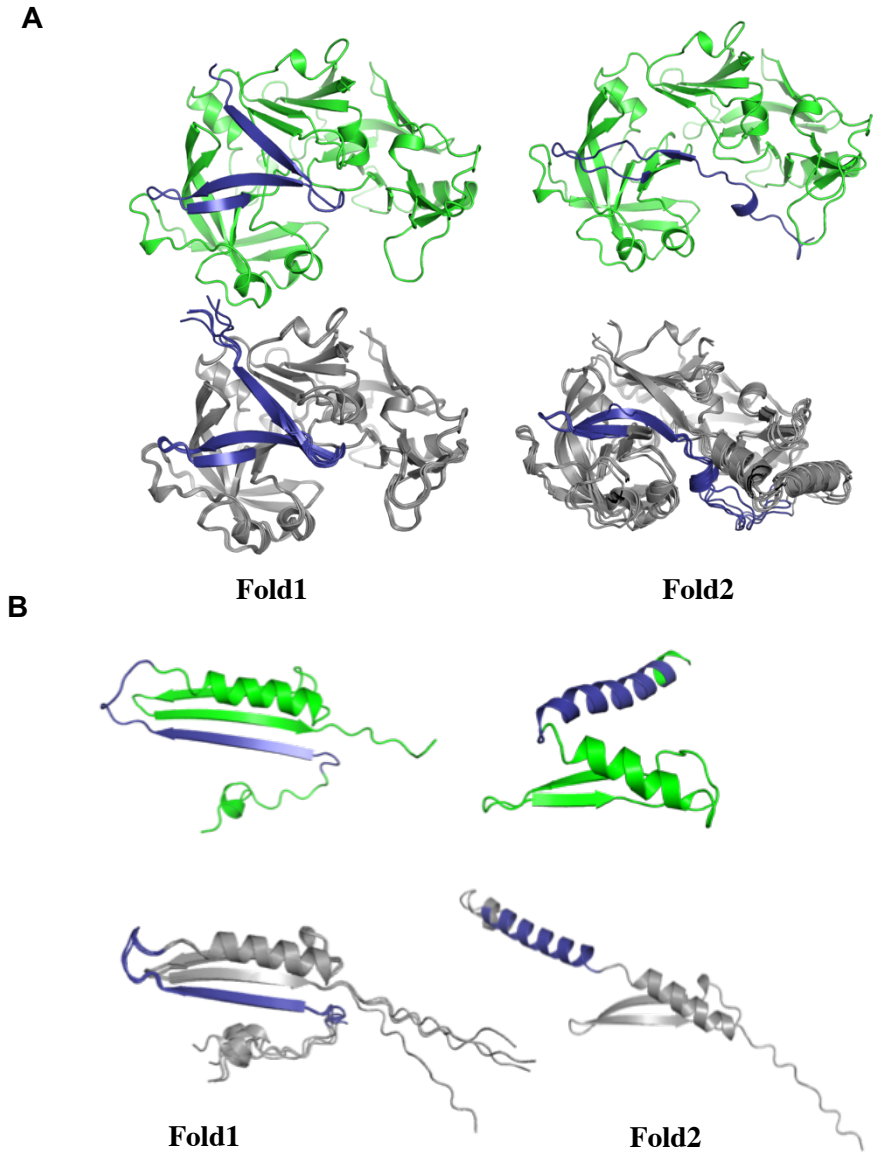
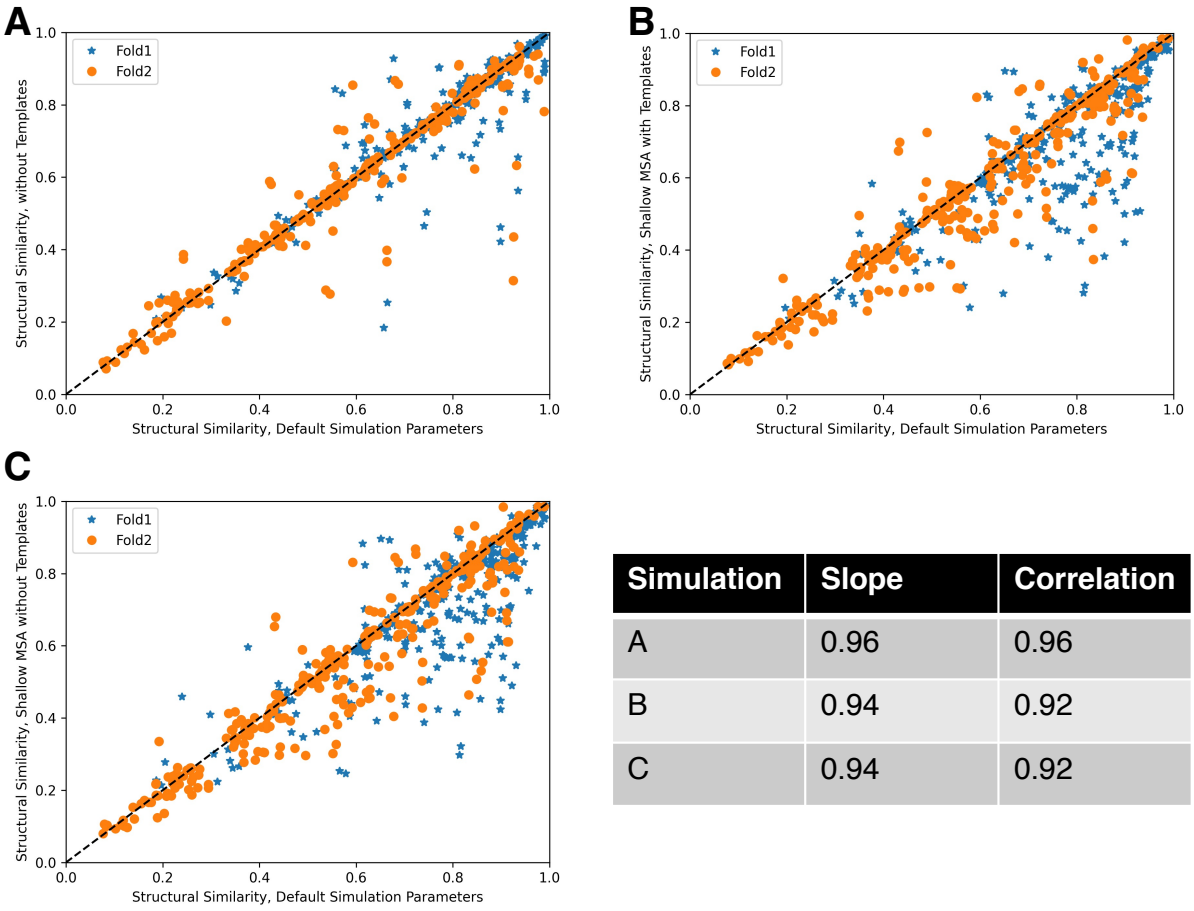


Fig. S3 Examples when both folds are present among AlphaFold2 predictions**.** Fold-switching regions are blue in both experimentally-determined and predicted folds (green and gray, respectively). The PDBIDs, chains and TM-scores, RMSDs are as follows: **(A)** Plasmepsin. 1qs8B/1miqB: 0.95/0.93, 1.23/1.46 Å and **(B)** MinE. 2kxoA/3rj9C: 0.77/0.51, 2.4/10.5 Å. **Table S4** reports all instances in which AlphaFold2 captures both Fold1 and Fold2.



Simulation	Slope	Correlation
A	0.96	0.96
B	0.94	0.92
C	0.94	0.92

Fig. S4. Varying simulation parameters does not appreciably enhance accurate sampling of Fold2. Overall, structural similarities (TM-scores) of Fold1 (blue stars) and Fold2 (orange circles) are slightly better when using default simulation parameters (full MSAs and including templates; all x-axes) than when excluding templates and using full MSAs (y-axis of **A**), including templates and using shallow MSAs (y-axis of **B**), and excluding templates and using shallow MSAs (y-axis of **C**). The slightly higher accuracy of default simulation parameters is evidenced by slopes <1 for all 3 comparisons; reported correlations are Pearson correlation coefficients. Identity lines are shown in black dashed lines. TM-scores of the top 5 models from all 93 fold switchers are shown in each plot. The four Fold2 conformations newly captured by varying simulation parameters are reported in **Table S4**.

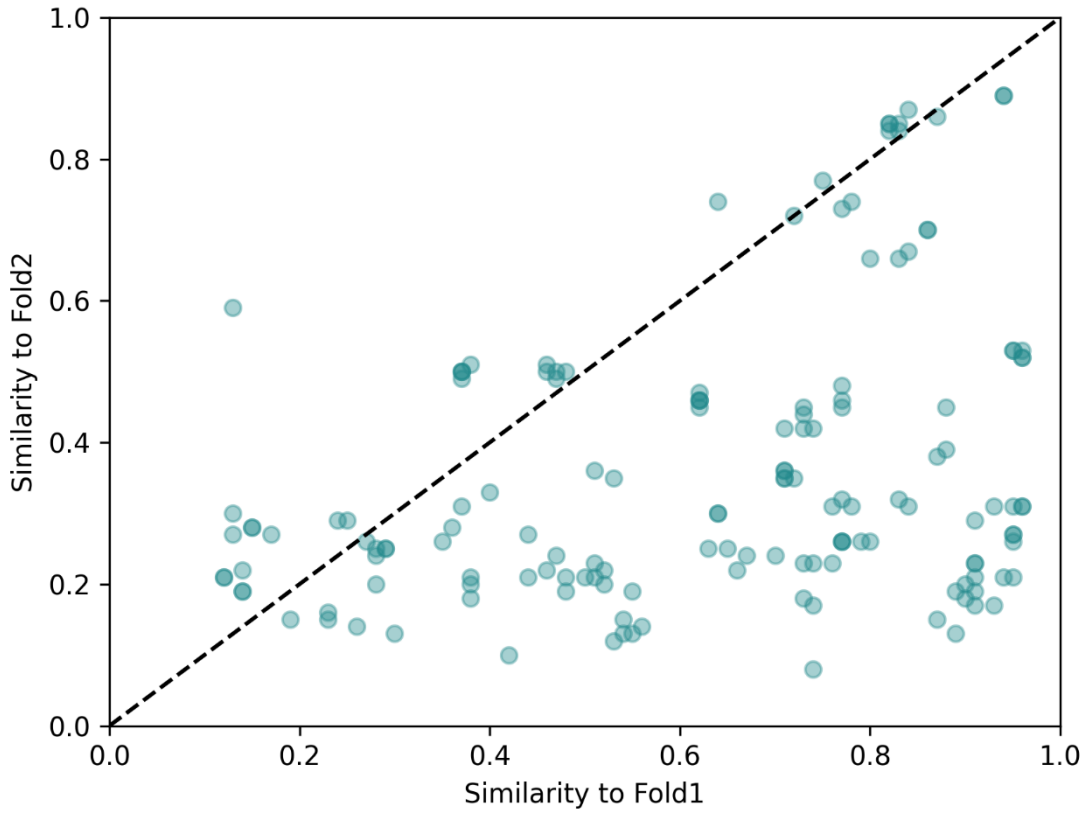


Fig. S5. AlphaFold2 predictions of fold-switching regions of proteins from Cluster 1, whose overall folds are predicted well (TM-scores ≥ 0.8, **Figure 1A**), are more accurate for one fold. Prediction accuracies were quantified using TM-scores, and 41% of predictions were inaccurate (TM-score < 0.6) for both experimentally determined conformations of fold-switching regions.

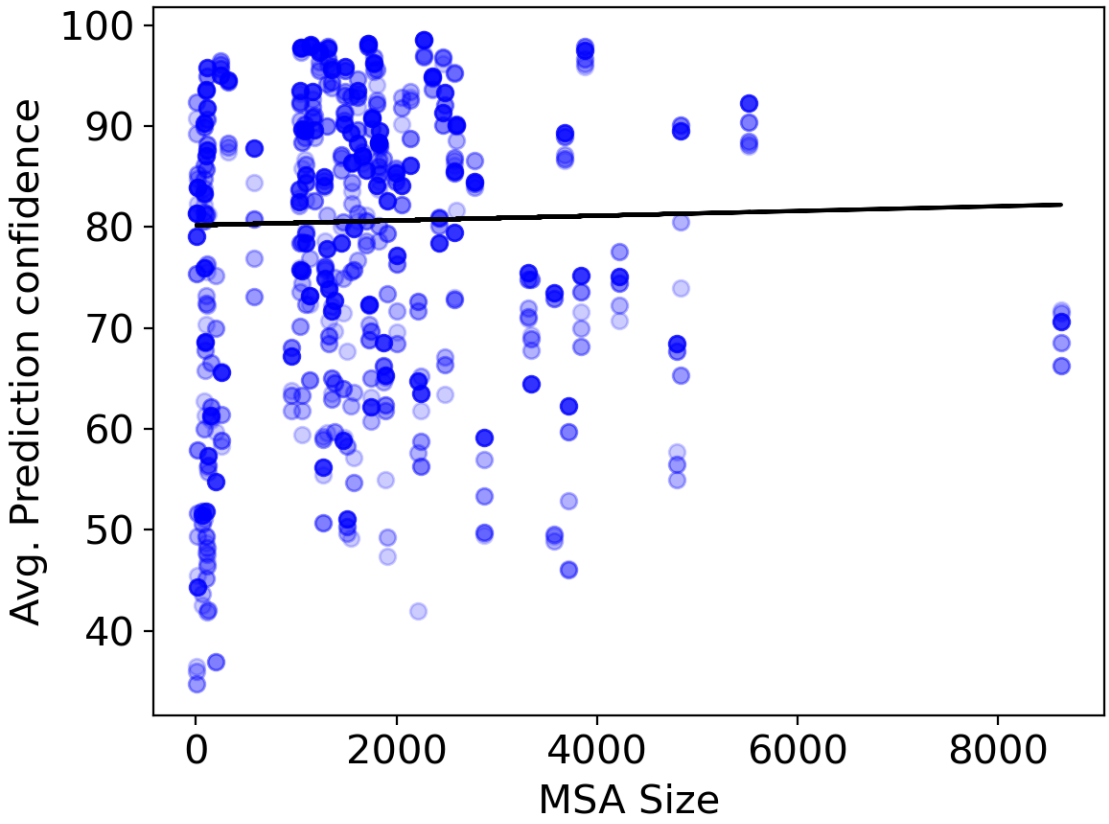


Figure S6. AlphaFold2 prediction confidences (pLDDT scores) are uncorrelated with MSA size (number of sequences in multiple sequence alignment), as evidenced by a Pearson correlation of 0.06 (slope of black line).