Rēs ipSAE loquunt: What's wrong with AlphaFold's *ipTM* score and how to fix it

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Abstract

AlphaFold's *ipTM* metric is used to predict the accuracy of structural predictions of protein-protein interactions (PPIs) and the probability that two proteins interact. Many AF2/AF3 users have experienced the phenomenon that if they trim full-length sequence constructs (e.g. from UniProt) to the interacting domains (or domain+peptide), their *ipTM* scores go up, even though the structure prediction of the interaction is unchanged. The reason this happens is due to the mathematical formulation of *ipTM* in AF2/AF3, which scores the interactions of whole chains. If both chains in a PPI complex contain large amounts of disorder or accessory domains that do not form the primary domain-domain or domain/peptide interaction, the *ipTM* score can be lowered significantly. The score then does not accurately represent the accuracy of the structure prediction nor whether the two proteins actually interact. We have solved this problem by: 1) including only residue pairs in the *ipTM* metric that have good predicted aligned error (*PAE*) scores; 2) by adjusting the d_0 parameter (a function of the length of the query sequences) in the TM score equation to include only the number of residues with good interchain *PAE*s to the aligned residue; and 3) using the *PAE* value itself and not the probability distributions over the aligned error to calculate the pairwise residue-residue *pTM* values that go into the *ipTM* calculation. The first two are crucial in calculating high *ipTM*s for domain-domain and domain-peptide interactions even in the presence of many hundreds of residues in disordered regions and/or accessory domains. The third allows us to require only the common output json files of AF2 and AF3 (including the server output) without having to change the AlphaFold code and without affecting the accuracy. We show in a benchmark that the new score, called *ipSAE* (interaction prediction Score from Aligned Errors), is able to separate true from false complexes more efficiently than AlphaFold2's *ipTM* score. The resulting program is freely available at https://github.com/dunbracklab/IPSAE.

Introduction

The AlphaFold programs (1-3) have had a profound impact on the structure prediction of proteins and protein complexes. AlphaFold-Multimer (v2.3) has enjoyed the widest use in predicting the structures of protein-protein interactions (PPIs), which are critical to essentially all biological processes. Since AlphaFold-Multimer code has been available for download since late 2021 (and v2.3 since December 2022), these programs have been extensively benchmarked for their ability to predict the structures of protein complexes accurately and their ability to predict whether two proteins interact. These benchmarks have utilized the scoring output from the AlphaFold programs, including residue-specific predicted local distance difference tests (*pLDDT*s), predicted aligned errors (*PAE*s) for residue pairs, and predicted template-modeling scores (*pTM*) and interface predicted template modeling scores (*ipTM*s) for the whole modeled system.

Typically, benchmarks have been constructed from Protein Data Bank (PDB) structures, and use the sequences provided for each PDB entry (e.g., the CASP competitions (4, 5) and others (6)). That is, they do not use the full UniProt sequences, which may contain disordered sequences and domains that do not form part of the interaction. PDB constructs are mostly fully ordered, save for some loops or short N and C terminal tails. In these cases, the *ipTM* score generally works well in assessing the accuracy of the structure prediction (7). However, in real-world situations where the interacting regions may not be known, structure predictions usually start with full-length protein sequences from UniProt. Then after observing which domains interact in the model with good *PAE* scores, it can be productive to input shorter sequence constructs to AlphaFold.

Many studies have noted that different sequence constructs produce different *ipTM* scores, even though the predicted interface contacts are unchanged (8-12). For example, Danneskiold-Samsøe et al. compared AlphaFold-Multimer v2.2 models produced from either full-length sequences of single-pass transmembrane receptors and their full-length unprocessed ligands, or various truncations of the proteins (e.g., the extracellular domains only and the proteolytically processed secreted ligand proteins) (13). *ipTM* scores were higher and more predictive for the shorter constructs comprising only the interacting domains. In a comprehensive study, Lee et al. found shorter fragments of peptides binding to protein domains often scored better than longer fragments or full-length proteins (14). Bret et al. developed a scanning approach to search through disordered sequence regions for protein domain binders, because the *ipTM* score was not successful on full-length sequences (8). Some reports have shown that *ipTM*s, which are calculated over whole chains, are less predictive than other measures. These measures include *pLDDT* values of interface residues (as in the pDockQ score) (15, 16), interface *PAE* values (*iPAE*'s) calculated over only interchain residue pairs within various cutoff distances (17, 18), or combinations of AlphaFold metrics and energy functions to evaluate interfaces (6).

In this paper, we investigate the origin of the behavior of AlphaFold's *pTM* and *ipTM* scores based on their mathematical descriptions in the AlphaFold papers. We then use this analysis to identify alternative formulations that are not sensitive to disordered regions or non-interacting accessory domains in either or both chains of pairwise interactions in AlphaFold models. We show that using only interchain residue pairs with good *PAE* scores in the evaluation of *ipTM* and evaluating the TM formula's d_0 parameter (which is based on sequence length) accordingly, we can produce good *ipTM* values for true interactions even in the presence of large amounts of disorder and accessory domains. The resulting code, which is freely available, works only on the *PAE* matrix provided in the default output of both AlphaFold2 and AlphaFold3. We have named the metric *ipSAE* for "interaction prediction score from aligned errors." The word is a play on the Latin phrase "*Rēs* ***ipsae*** *loquuntur*," meaning "The things speak for **themselves**," referring to the AlphaFold output scores.

Derivation of the *ipTM* and *ipSAE* scores

The TM score was developed by Zhang and Skolnick to assess the accuracy of predicted models of protein structures compared to experimental structures of the same proteins (19). It is defined as:

$$TM = \max_{alignments}\left[\frac{1}{L_{target}}\sum_{\substack{j\in common\\ residues}}^{L_{common}}\frac{1}{1+\left(\frac{d_j}{d_0}\right)^2}\right] \quad (1)$$

Each d_j is a distance between the predicted position of the Cα atom of residue *j* in the model and residue *j* in the experimental structure for a given superposition. A model of a protein can be superimposed in various ways on an experimental structure, and the maximum is taken over all possible alignments. In practice, the maximum is taken over only a subset of such alignments (e.g., by running different structure alignment programs or with different parameters). d_0 is a scaling factor that reduces or eliminates the length dependence of the TM score for alignments of unrelated proteins (19). It has a fitted value of

$$d_0 = 1.24\sqrt[3]{L_{target} - 15} - 1.8 \quad (2)$$

If L_{target} is 500 residues, d_0 has a value of about 8 (Figure 1). The original TM score was used in development of protein structure prediction methods, when the sequence length of the experimental structure (the target) might be longer than the sequence length of the model (e.g., if only a single domain of the target could be modeled using templates). Thus, a partial model (or template) was heavily penalized. In some cases, the experimental structure might be missing some residues due to poor electron density. The sum is therefore over the number of residues the model and experimental structure have in common (L_{common}).

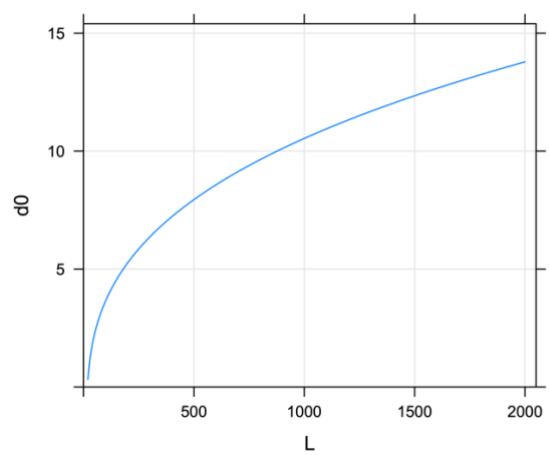

Figure 1. The d_0 parameter in the TM score equation as a function of sequence length *L*

AlphaFold2 and AlphaFold3 use the concept of "aligned error" to generate predicted accuracy metrics for output models (Figure 2). After superposing the N, Cα, and C atoms of residue *i* of a model onto the N, Cα, and C atoms of the same residue *i* in the experimental structure, the aligned

error (AE) of residue *j* is the distance between the Cα atom of residue *j* in the model and Cα of residue *j* in the experimental structure. During training, the experimental structure is known, and the network is trained to predict a probability distribution over the aligned error distance when the experimental structure is not known (i.e., during inference). The probability distribution is defined over the AE_{ij} distance in 64 bins of width 0.5 Å (0Å-0.5Å, 0.5Å-1.0Å, …, 31.5-32Å), where the last bin also includes distances larger than 32 Å. The predicted aligned error, PAE_{ij}, for each pair of residues is calculated from the predicted probability distribution over the aligned error with the equation (Eq. 11 in AF3 paper supplemental):

$$PAE_{ij} = \sum_{b=1}^{64} p_{ij}^{b} \Delta_b \tag{3}$$

where $\Delta_b = (b - 0.5)/2$ is the center of each bin (0.25Å, 0.75Å, …, 31.75Å), p_{ij}^{b} is the probability of bin *b*, and $\sum_{b=1,64} p_{ij}^{b} = 1$.

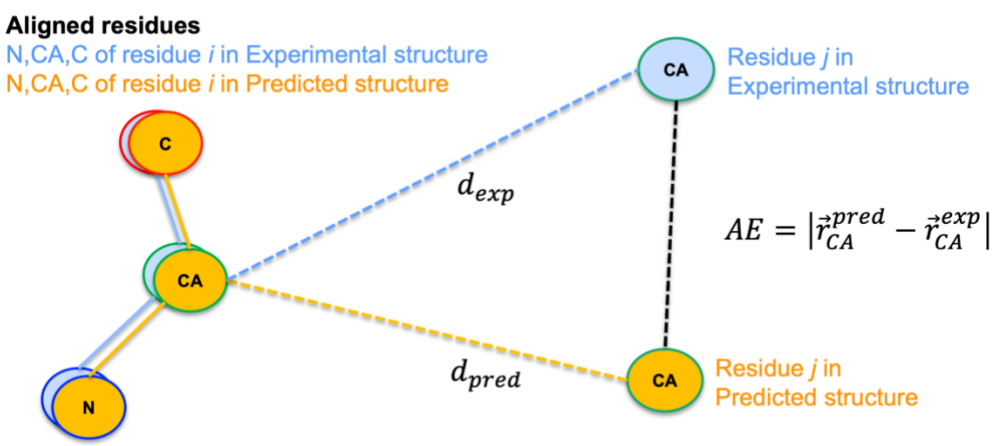

Figure 2. Definition of aligned error (AE) in AlphaFold2 and AlphaFold3

For a single chain (or a whole protein complex), the PAE_{ij} values can be substituted into Equation 1 for the TM score to provide an equation for the *pTM* score (predicted template modeling score):

$$pTM = \max_i \left[\frac{1}{L} \sum_{j=1}^{L} \frac{1}{1 + \left(\frac{PAE_{ij}}{d_0} \right)^2} \right] \tag{4}$$

The role of residue *i* in this equation is to create a set of alignments used to calculate the TM score, one for each residue in the chain (or complex). The value of *pTM* is then calculated from the highest scoring of these alignments, just as in Equation 1 for the original TM score

In the AlphaFold papers, the expression under the sum is instead calculated as an expectation value from the probability distribution of the aligned error used in Eq. 3 (AF3 paper supplemental

Eq 17):

$$pTM = \max_i \left[\frac{1}{L} \sum_{j=1}^{L} \sum_{b=1}^{64} p_{ij}^{b} \frac{1}{1 + \left(\frac{\Delta_b}{d_0}\right)^2} \right] \tag{5}$$

For simplicity, we define the pairwise *pTM* matrix from the aligned error probability distribution as:

$$pTM_{ij} = \sum_{b=1}^{64} p_{ij}^{b} \left(\frac{1}{1 + \left(\frac{\Delta_b}{d_0}\right)^2} \right) \tag{6}$$

or alternatively (as an approximation) from the *PAE* value.

$$pSAE_{ij} = \frac{1}{\left(1 + \left(\frac{PAE_{ij}}{d_0}\right)^2\right)} \cong pTM_{ij} \tag{7}$$

The $pSAE_{ij}$ can be used anywhere pTM_{ij} can be used.

The residue-specific mean value of pTM_{ij}, based on the alignment of residue *i* is given by:

$$pTM_i = \frac{1}{L} \sum_{j=1}^{L} pTM_{ij} \tag{8}$$

From these equations, we can generalize the expression for *pTM* by specifying the residue sets for the alignments (set S_1, residues *i*) and those for the residue displacements between modeled structure and experimental structure, if it were known (S_2: residues *j*):

$$pTM = \max_{i \in S_1} [pTM_i] = \max_{i \in S_1} \left[\operatorname*{mean}_{j \in S_2} pTM_{ij} \right] \tag{9}$$

For a complex of two protein chains, A and B, we can perform the residue-residue structure superpositions over one chain (e.g., S_1=chain A) and calculate the TM score over the other chain (S_2=chain B), which would then contain a rotation-translation component as well as the accuracy of the structural model of chain B. So

$$ipTM(A \rightarrow B) = \max_{i \in A} \left[\operatorname*{mean}_{j \in B} \left(pTM_{ij} \right) \right] \tag{10}$$

When AlphaFold2 or AlphaFold3 provides a value of $ipTM$ for a pair of chains, it provides a single value which is the maximum of the two asymmetric values (or equivalently the maximum over all residues in both chains of the interchain pTM_i):

$$ipTM = \max[ipTM(A \rightarrow B), ipTM(B \rightarrow A)] \tag{11}$$

AlphaFold3 provides an *ipTM* for each chain where the maximum is taken over all residues *i* in that chain and the mean is over all residues in all other chains.

$$ipTM(chain\ X) = \max_{i \in X}\left[\operatorname*{mean}_{j \in notX} pTM_{ij}\right] \tag{12}$$

In AlphaFold2 and AlphaFold3, the overall *ipTM* of any multiprotein complex is calculated from the maximum over all residues of the mean $ipTM_{ij}$, where the mean is taken over all residues in all other chains that do not contain residue *i*. The value of d_0 is the sum of all protein chain lengths in the model.

$$ipTM = \max_{i}\left[\operatorname*{mean}_{j \in notChain(i)} pTM_{ij}\right] \tag{13}$$

Our experience, and that of many others (13), demonstrates a problem in the calculation of *ipTM* in the presence of disordered residues and other domains in the sequence constructs that do not interact between the chains. Frequently, users of AlphaFold2 and AlphaFold3 have to repeat calculations with different protein constructs that remove the disordered regions and observe an increase in *ipTM*, even though the interacting domain-domain or domain-peptide complex structure remains the same. This occurs especially when there is disorder in both chains of a complex, rather than just one of them.

The reason is clear from the equations presented above. For a protein-protein complex, *ipTM* is a mean value of pTM_{ij} over all residues *j* in one of the chains, after superposition on one residue *i* in the other chain (after taking the maximum over all residues *i*). It therefore includes pTM_{ij} values between ordered and disordered residues, which are almost always very poor. Any mobile domains that do not interact also lower the score.

As an example, we take the interaction between KRAS and the RAS-binding domain of RAF1 (Figure 3). When only the ordered domain sequences are input to AlphaFold-Multimer (v2.3), the *ipTM* is 0.9. When disorder is added to only one chain (the blue RAF1 in Example 2), the *ipTM* is still 0.9. This occurs because a residue in RAF1 (marked by a red asterisk) has high pTM_{ij} values with all residues in the fully ordered KRAS chain (magenta). But when disorder is present in both chains (Examples 3 and 4 in Fig. 3), the *ipTM* is decreased because every residue in each chain has some low pTM_{ij} values with residues in the other chain. The decrease is proportional to the relative amount of disorder to order in the chain with *less* disorder. For example, with 120 disordered residues in each chain (Example 4), RAF1-RBD and KRAS are 61% and 41% disordered respectively. The *ipTM* value is due to residue T68 of RAF1, which sits in the interface

with KRAS. Its pairwise pTM_{ij} values with KRAS residues are 59% ordered (at ~0.9 each) and 41% disordered (at ~0.2 each), or approximately 0.59*0.9 + 0.41*0.2 = 0.61 (the AF2 output value is 0.59).

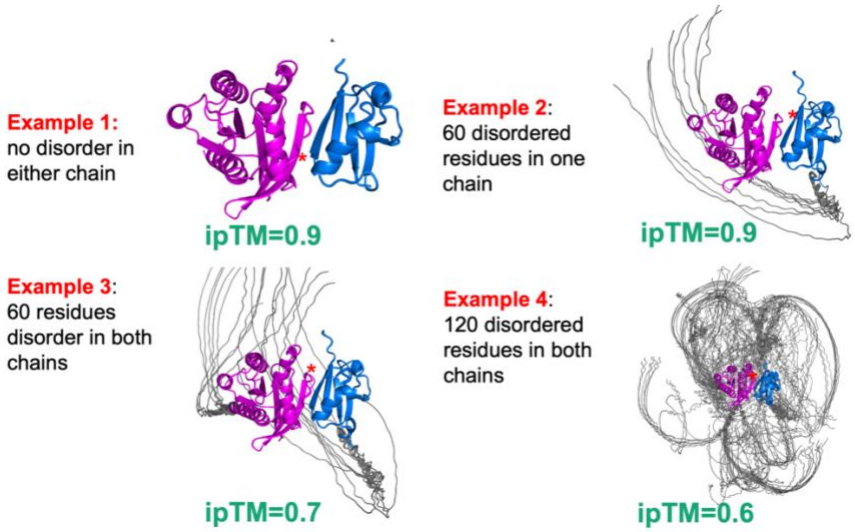

Figure 3. AlphaFold2 models of the complex of KRAS (magenta) and the RAS-binding domain of RAF1 (blue). Disordered residues (15 repeats of the sequence GGGS) were added to the N or C terminus (or both) of one or both chains, which residues the $ipTM$ when this occurs in both chains (Examples 3 and 4).

There are several ways of dealing with this. In the *ipTM* expressions, we could skip residue pairs where one (or both) residues have *pLDDT* values less than some cutoff value (e.g. *pLDDT*<50). This does not always work: auxiliary domains in one or both of the proteins that do not contribute to the protein-protein interaction will have good *pLDDT*s but poor intermolecular pTM_{ij}, thus lowering the *ipTM*.

The *ipTM* could be calculated over only contacting residues in the model within some cutoff distance. This can also be a problem because disordered residues or auxiliary domains in one or both chains can contact the other chain and contribute poor pTM_{ij} to the *ipTM* evaluation,

Varga et al recently proposed using the predicted distance distograms produced by the AlphaFold2 network to restrict the calculation of *ipTM* to interchain residue pairs that are predicted to be in contact (20). This method excludes disordered regions and auxiliary domains that do not have a strongly predicted interaction, even if they are in contact in the models. Their score, called *actifpTM,* is calculated over the subsets of residues that make up the interface of two chains but only those that AlphaFold2 is confident about. *actifpTM* is now implemented within the ColabFold framework (21).

We propose another alternative, where we use the PAE_{ij} values to restrict the *ipTM* calculation to interchain residue pairs that have well predicted aligned error distances, regardless of whether they are in or near the protein-protein interface. In contrast to *actifpTM*, we adjust the value of d_0 in the asymmetric *ipTM* expression to the number of residues in the chain under the mean expression with good interchain *PAE* values (Equation 10). This is critical, because a small number of interchain residue pairs with spuriously good PAE_{ij} and consequently good pTM_{ij} values may produce an unrealistically *ipTM* if d_0 is not adjusted.

We define the *ipSAE* score (*i*nterface *p*redicted TM *S*core based on *A*ligned Errors) for two chains (A and B) as follows:

$$ipSAE(A \rightarrow B) = \max_{i \in A}\left[\underset{\substack{j \in B \\ PAE_{ij} < cutoff}}{\text{mean}}\left(\frac{1}{1+\left(\frac{\text{PAE}_{ij}}{d_0}\right)^2}\right)\right] \tag{14}$$

and

$$\begin{aligned} d_0 &= 1.24\sqrt[3]{L_{PAE<cutoff} - 15} - 1.8 \qquad & L \geq 27 \\ d_0 &= 1 & L < 27 \end{aligned} \tag{15}$$

Here, $L_{PAE<cutoff}$ is the number of unique residues in chain B that have $PAE_{ij} < cutoff$ given the identity of the aligned residue *i*. We use a minimum value of 1 for d_0, since Yang and Skolnick did not test the fit for proteins shorter than 30 amino acids (d_0=1 for L~26.5), and the denominator in Eq. 14 starts to blow up for values << 1.0, which may not be realistic or helpful. In the AlphaFold code, the minimum value is set to 19, since *L*=18 produces a negative number.

For a given chain pair, the *ipSAE* score is the maximum of the two asymmetric values:

$$ipSAE(A,B) = \max[ipSAE(A \rightarrow B), ipSAE(B \rightarrow A)] \tag{16}$$

ipSAE can be calculated for every pair of chains in a multi-chain complex from the *PAE* matrices from the AlphaFold2 or AlphaFold3 output json files.

Results

RAF1 complexes

The TKL family kinase, RAF1, contains three domains: a RAS-binding domain (RBD: residues 56-131), an immediately adjacent cysteine-rich domain (CRD: residues 138-184), and a protein kinase domain (PK: residues 340-614). The rest of the chain of length 648 residues is intrinsically disordered (residues 1-55, 185-339, and 615-648).

As noted above, AlphaFold-Multimer models of the RAF1-RBD with KRAS result in *ipTM* values that are lowered in the presence of artificial disordered sequences when they are present in both chains (Figure 3). The *ipSAE* value of 0.8 for Example 4 eliminates most of the disorder effect.

RAF1 interacts with the TKL family pseudokinase KSR1, facilitating the activation of RAF1 and its translocation to the membrane (22). KSR1 also contains three domains: the coiled-coil/Sterile-α-motif domain (CC-SAM: residues 30-172), a cysteine-rich domain, homologous to the CRD of RAF1 (CRD: residues 347-391), and a protein pseudokinase domain (pPK: residues 599-833).

There is no experimental structure of a RAF1-KSR1 complex, but it has been hypothesized that the two kinase domains bind in a mode similar to the well-known BRAF homodimer (23).

AlphaFold-Multimer models of the heterodimer sequences of full-length RAF1 and full-length KSR1 show a kinase/pseudokinase heterodimer that is very similar to the BRAF homodimer (23) with *ipTM* values between 0.38 and 0.41 across 25 models (5 seeds by all 5 sets of AF2 model weights, without templates). The other folded domains and disordered regions of both proteins are not in fixed position relative to the kinase domains across the 25 models, and do not show any key interprotein interactions (Figure 4A).

As described above, we can use the *PAE* values to calculate *ipTM*-like scores over specified interprotein residue pairs. AF2 calculates the full *pTM* matrix with a value of d_0 that is the combined length of the two proteins. If we calculate *pTM* with the *PAE* values instead and use the same d_0, the interchain $pSAE_{ij}$ and pTM_{ij} are highly correlated (Figure 4B).

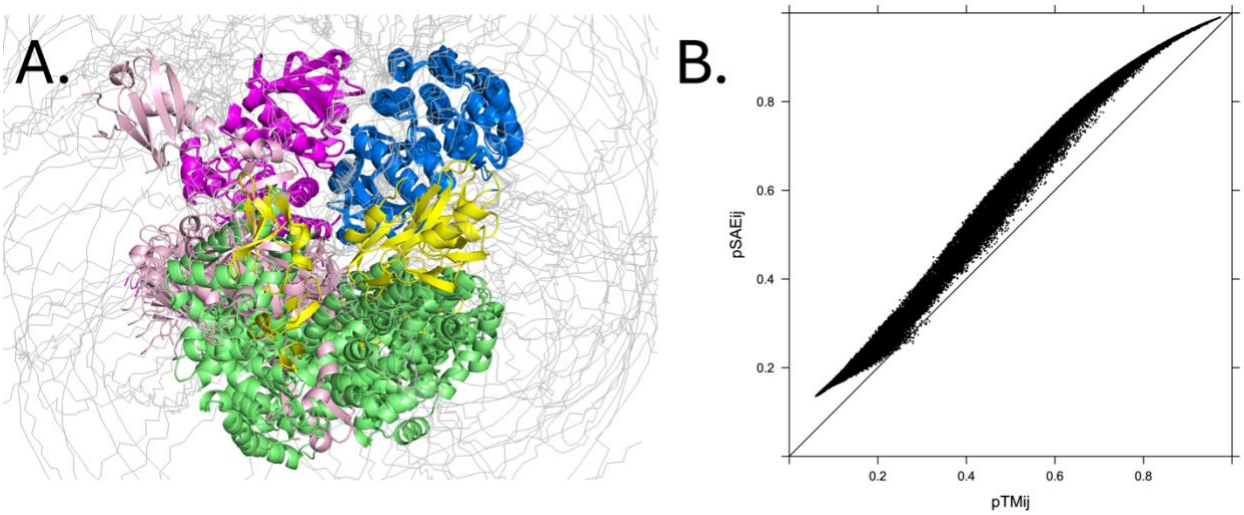

Figure 4. RAF1-KSR1 models. A. AlphaFold2 models of full-length RAF1 and KSR1. RAF1 RBD-CRD domains in pink and kinase domain in magenta. KSR1 CC-SAM domains in green, CRD in yellow, and pseudokinase domain in blue. The 25 models are aligned on the kinase domain of RAF1. The non-kinase domains are not fixed relative to the two kinase domains. **B. Scatterplot of interchain $pSAE_{ij}$ vs pTM_{ij} values for RAF1-KSR1 complex.** pTM_{ij} was calculated from the *pTM* matrix output by AlphaFold2 (from a modified version of ColabFold). It uses a d_0 from the combined length of both proteins. $pSAE_{ij}$ was calculated with no *PAE* cutoff and with a d_0 also based on the combined length of both protein chains.

AlphaFold-Multimer calculates the *ipTM* via Equations 10 and 11 by calculating the pTM_i for each residue in both chains (Figure 5). The two kinase domains are responsible for the high scoring regions, while the accessory domains in each protein are visible as small bumps in the plots. The maximum value in the curve in Figure 5 occurs for residue W632 of KSR1, which is in the interface between the kinase domains. If we use the $pSAE_{ij}$ value to calculate $pSAE_i$ for each aligned residue *i* of the complex and adjust d_0 for the number of residues that have a good *PAE* for the aligned, we see higher scores for the kinase domains in RAF1 and KSR1 and zero for the accessory domains. AlphaFold2's *ipTM* for the top-ranking complex is 0.41, while the *ipSAE* score is 0.73.

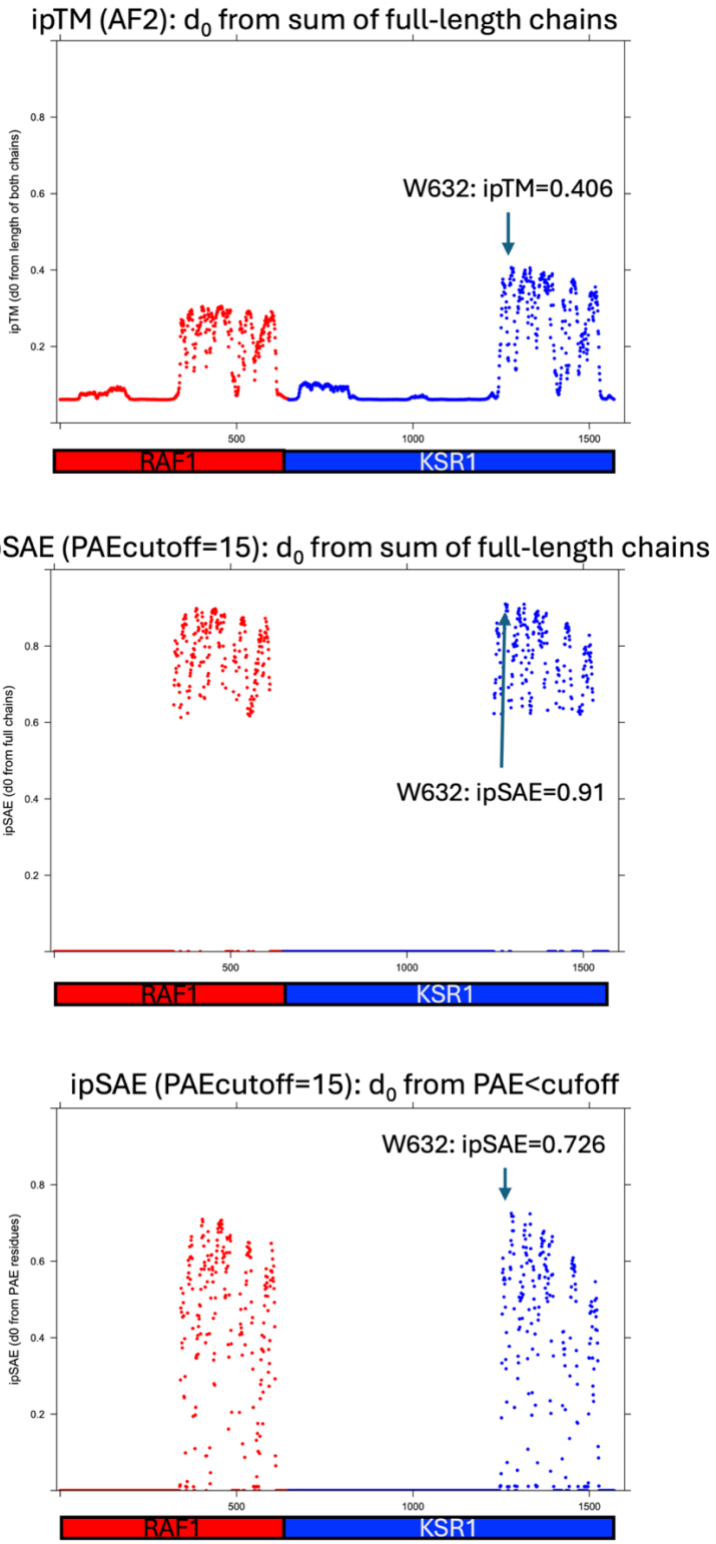

Figure 5. Per-residue *ipTM* and *ipSAE* scores for the RAF1-KSR1 complex. *ipTM* (top) was calculated from the pairwise *pTM* matrix from AlphaFold-Multimer v2.3 (ColabFold). It uses a d_0 from the combined length of the two proteins (1571 amino acids; d_0=12.57). *ipSAE* with a *PAE* cutoff of 15.0 and the same value of d_0 (middle figure). A cutoff for the *PAE* score of 15.0 Å was used to produce the *ipSAE* scores (bottom figure). The maximum occurs for W632 of KSR1 for both scores (green arrows) with a value of d_0 of 6.32 (286 residues in RAF1 with *PAE*<15 Å).

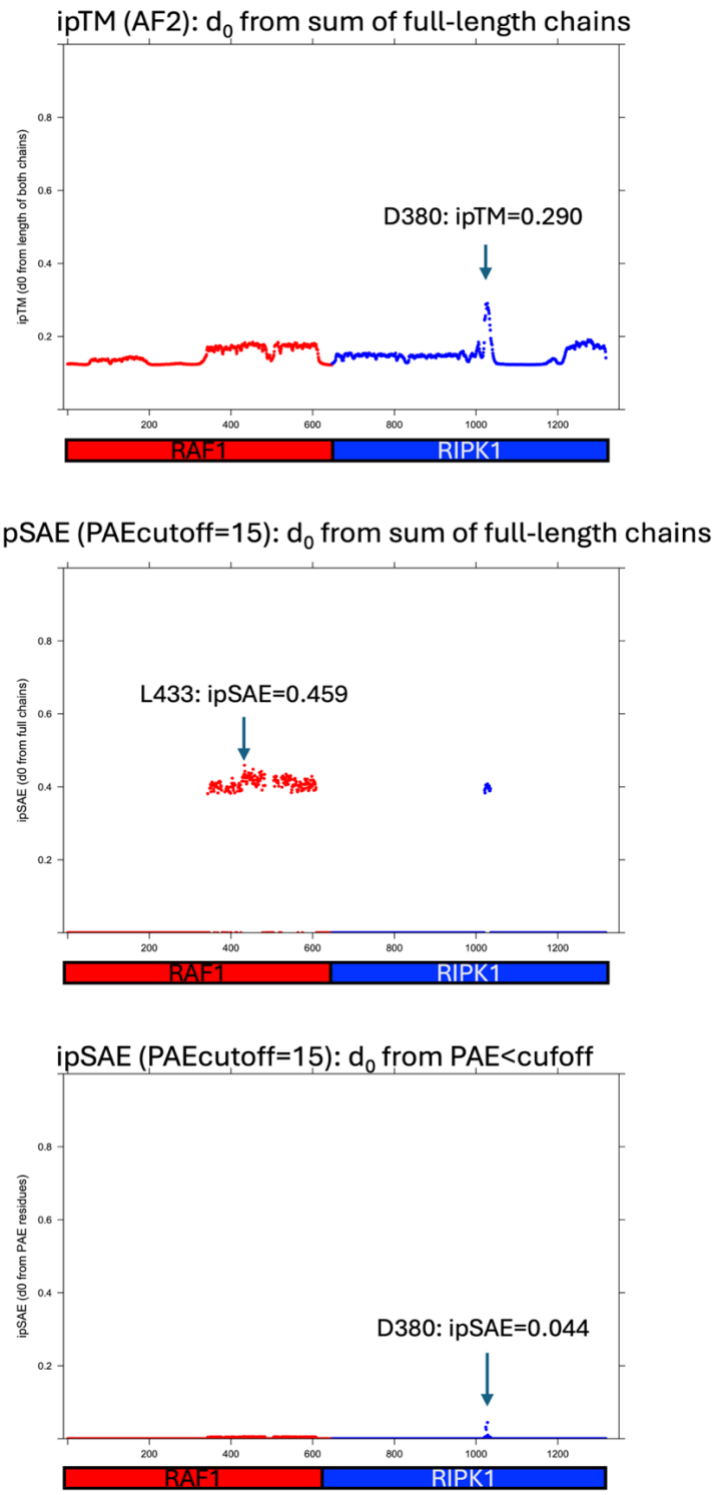

Figure 6. Per-residue *ipTM* (top) and *ipSAE* scores (middle and bottom) for the RAF1-RIPK1 pseudocomplex. A cutoff for the *PAE* score of 15.0 Å was used to produce the *ipSAE* scores. The *ipTM* score per residue scores (top plot) show a modest interaction between the chains with a maximum value at residue D380 of RIPK1, *ipTM*=0.290. AF2 uses d_0 from the sum of the full chain lengths. A *PAE* cutoff using the same d_0 (middle plot) (from the sum of both chain lengths) raises the *ipSAE* values compared to the *ipTM* values. But adjusting d_0 to account for the number of residues in the mean pTM_{ij} calculation (e.g. for each residue in RAF1, this is the number of residues in RIPK1 that have *PAE*<*PAEcutoff*) significantly lowers the score of the non-interacting proteins to a value of 0.044.

The TKL family kinase, RIPK1, is not known to bind to RAF1. RIPK1 has a kinase domain (PK: residues 8-324), a RIP homotypic interaction motif (RHIM: residues 531-547), and a Death domain (DD: residues 567-671). *ipTM* and *ipSAE* plots by residue are shown in Figure 6, demonstrating the effects of using the *PAE* cutoff and the evaluation of d_0 based on the number of residues with *PAE* less than a cutoff of 15 Å. The top plot shows the per-residue *ipTM* scores from AlphaFold2 (by averaging each row of the interchain pTM_{ij} values output from a modified version of ColabFold). AF2 uses a d_0 based on the sum of the two chain lengths (in this case 648 + 671 = 1319 residues, d_0=11.75). In the middle plot, the *PAE* matrix is used to limit the number of pTM_{ij} used for each residue. It uses the same value of d_0 (11.75) as in the top plot. The resulting residue-specific *ipSAE* values are much higher than the *ipTM* values, with an overall *ipSAE* value of 0.459 from the alignment on residue L433. This is expected because residue pairs with good *PAE* values will have high pairwise *ipTM* (or *ipSAE*) values. But the number of such pairs in truly non-interacting proteins is quite low, if AlphaFold is working as expected. In the bottom plot, the combined effect of the *PAE* cutoff of 15 Å and the residue-specific d_0 values bring the residue *ipSAE* values way down. The overall value of *ipSAE* is now 0.044, indicating that the proteins are not likely to interact. The value of d_0 was 3.05 from 75 residues below the *PAE* cutoff.

Benchmark of recent PDB entries

We identified a set of 40 PDB entries that share at most 40% identity with any chain present in the PDB prior to the AlphaFold-Multimer v2.3 cutoff date of Sept. 30, 2021. The entries had to have exactly two unique sequences and have a biological assembly consistent with a pairwise interaction of the two unique sequences (e.g., we excluded assemblies larger than octamers and chose entries where the shorter sequence interacted with only one copy of the longer sequence). Each sequence had to have at least 12 amino acids in the coordinates of the PDB file. Sequence identities were obtained from the PISCES webserver (24). We ran AlphaFold-Multimer v2.3 on the PDB sequences themselves and from the full-length Uniprot sequences, as identified from SIFTS (25) (as given in the PISCES sequence files). We also created a set of 70 AlphaFold jobs by randomly creating heterodimer pairs by mixing sequences from different entries in the set of 40 PDB entries. These were run with the full-length Uniprot sequences only.

The results of the *ipTM* and *ipSAE* scores are shown in Figure 7. The top left panel shows the *ipTM* values calculated from the *pTM* matrix in ColabFold. AF2 uses a value of d_0 calculated from the sum of the lengths of the two sequences in each query. Our values of *ipTM* agree exactly with the *ipTM* values present in the AF2 json output files. If we use the *PAE* values in the *ipTM* expression (but no *PAE* cutoff), instead of the AF2 *pTM* matrix, we get quite similar distributions (top right panel). In both panels, there is overlap in the density between values of *ipTM* or *ipSAE* from 0.3 to 0.7 for the true dimers (full-length Uniprot sequences, blue curves and data points) and false dimers (full-length Uniprot sequences, magenta curves and data points).

In the next three rows, kernel density plots of *ipSAE* values are shown for all three sets of targets with different values of the *PAE* cutoff in descending order (32 = no cutoff, 25, 20, 15, 10, and 5 Å). As the *PAE* cutoff decreases, the density in the mid-range of *ipSAE* decreases, separating true from false dimers more effectively than the *ipTM* values from AlphaFold (top left panel). The true dimers with Uniprot sequences have significantly improved *ipSAE* values at lower cutoffs, because they contain disorder and accessory domains that do not form part of the interaction between the two proteins. The PDB sequences (green curves), conversely, do not change that much with the *PAE* cutoff since they do not usually contain disordered regions or mobile domains that do not form part of the interaction. The overall results indicate that the *ipSAE* score may be better at separating true from false interactions even in the presence of disordered sequences and/or accessory domains in both sequences. **Cutoffs of 10 or 15 Å may be most suitable.**

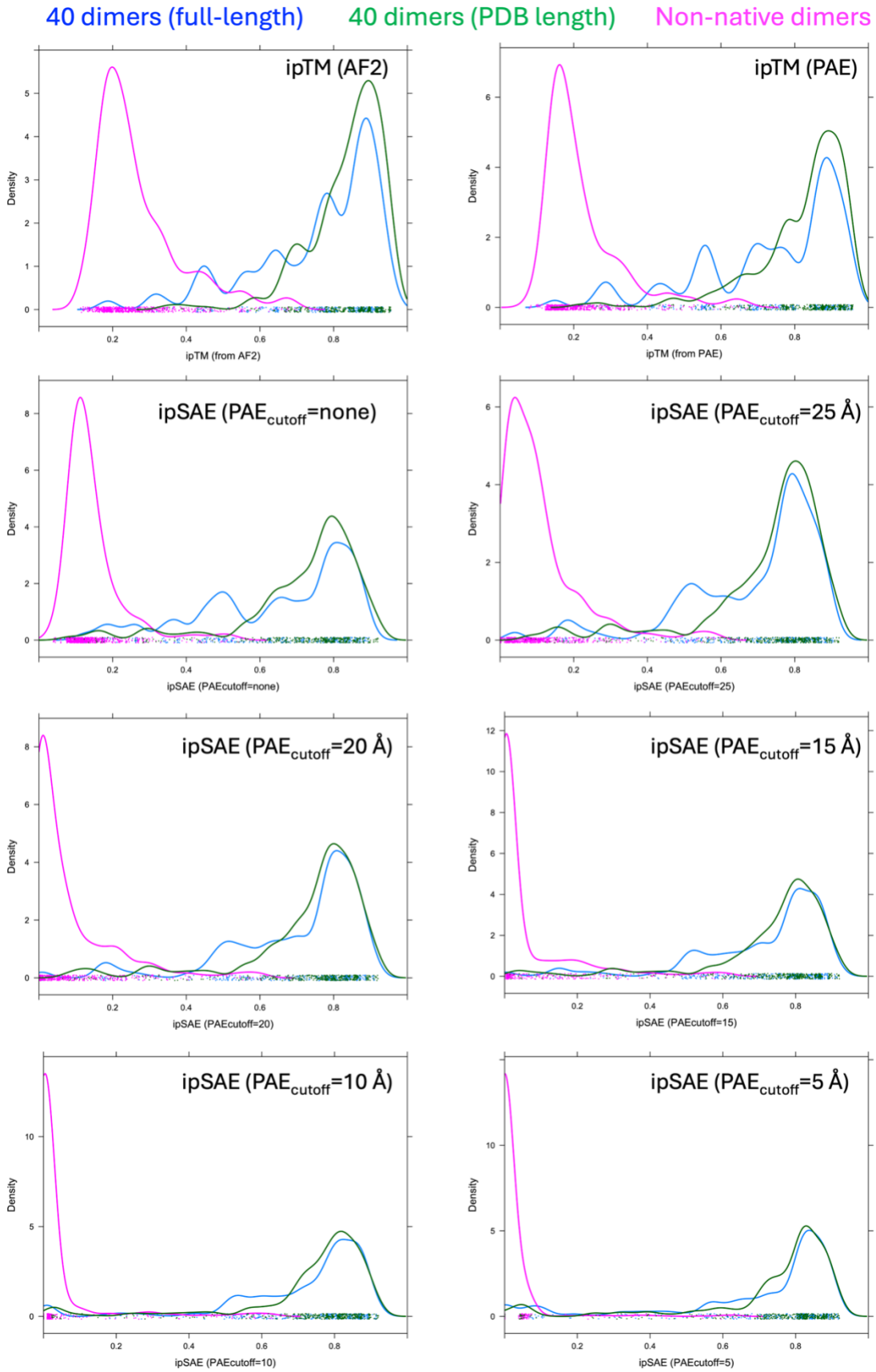
40 dimers (full-length)
40 dimers (PDB length)
Non-native dimers
ipTM (AF2)
ipTM (from AF2)
ipTM (PAE)
ipTM (from PAE)
ipSAE (PAE$_{cutoff}$=none)
ipSAE (PAEcutoff=none)
ipSAE (PAE$_{cutoff}$=25 Å)
ipSAE (PAEcutoff=25)
ipSAE (PAE$_{cutoff}$=20 Å)
ipSAE (PAEcutoff=20)
ipSAE (PAE$_{cutoff}$=15 Å)
ipSAE (PAEcutoff=15)
ipSAE (PAE$_{cutoff}$=10 Å)
ipSAE (PAEcutoff=10)
ipSAE (PAE$_{cutoff}$=5 Å)
ipSAE (PAEcutoff=5)
Density

Figure 7. Benchmark of recent PDB heterodimers. A set of 40 heterodimer PDB entries with less than 40% sequence identity to any sequences in the PDB prior to October 1, 2021 were identified. The PDB sequences were used as queries to AlphaFold-Multimer v2.3 (green curves). The full-length Uniprot sequences for these chains were also used as a second set of 40 target complexes for AF-Multimer v2.3 (blue curves). A third set of 70 targets was built from mixing the Uniprot sequences from different entries (magenta curves). The plots show kernel density estimates of *ipTM* and *ipSAE* for the top 10 ranked complexes (AF2 ranking based on 0.8**ipTM* + 0.2**pTM*) out of 25 models (5 seeds x 5 AF2 weight-sets with no templates used). The top left panel shows *ipTM* based on the *pTM* matrix from AF2. The top right panel shows *ipTM* calculated from the *PAE* matrix instead of the *pTM* matrix (the *PAE* value is used in the denominator of the *pTM* expression instead of the sum over probabilities). The remaining rows show *ipSAE* values with different *PAE* cutoffs used in the mean value calculation. d_0 for these calculations was based on the *PAE* cutoff. The set of 40 PDB entries is: 7f4p, 7qii, 7sck, 7t5p, 7tj4, 7wmv, 7wwq, 7ytu, 7zd5, 8a51, 8a82, 8bfj, 8blw, 8cdp, 8dqv, 8fbd, 8fzz, 8g0p, 8gs1, 8guo, 8hi7, 8hk0, 8ir4, 8jj9, 8jmq, 8jzd, 8orn, 8ows, 8q4h, 8qvc, 8r5i, 8s2m, 8vjl, 8vx9, 8wx5, 8xfb, 8y2n, 8ypu, 8zlz, 9dk1.

Comparison with actifpTM

Varga et al. (20) identified the same problem with the *ipTM* score as we have discussed above – that disordered regions depress the *ipTM* score when they are not part of the binding interface between two proteins. They gave four example systems of protein-peptide complexes: PDB entries 1ycr (MDM2 and P53 peptide), 2a25 (E3 ubiquitin ligase SIAH1 and Calcyclin binding protein peptide), 3zgc (KEAP1 and NF2L2 peptide), and 4h3b (MAPK10 and SH3 domain-binding protein 5 peptide). We ran AlphaFold-Multimer v2.3 on the Colabfold Jupyter notebook, which calculates the *actifpTM* values, using the full-length Uniprot sequences of both chains (instead of the PDB constructs or short elongations of these, used in the *actifpTM* preprint). The calculations were performed with two seeds, no templates, and 3 recycles. We calculated *ipSAE* at different *PAE* cutoffs on the rank001 models from Colabfold. The results are shown in Table 1. For three of the targets, AlphaFold produces good models where the binding peptide is correctly placed on the folded domain, even though the full-length Uniprot sequence was provided to Colabfold. After superposition onto the folded domain from the PDB structure (chain A in all cases), the RMSDs were 1.32, 0.72, and 1.12 Å for entries 1ycr, 2a25, and 3zgc. For these three entries, the *actifpTM* values were quite high, ranging from 0.93 to 0.97. The *ipSAE* values were lower with values around 0.68, 0.55, and 0.73 Å respectively (at *PAE* cutoff 10 Å).

Table 1. Comparison of *actifpTM* targets with their *ipTM* and *ipSAE* values

	1ycr	2a25	3zgc	4h3b	RAF1kd/LysC	RAF1/RIPK1
RMSD	1.324 Å	0.715 Å	1.191 Å	99.948 Å	---	---
ipTM	0.298	0.669	0.719	0.443	0.388	0.277
actifpTM	0.943	0.928	0.972	0.690	0.467	0.462
***ipSAE* (5 Å)**	0.702	0.547	0.801	0.000	0.000	0.000
***ipSAE* (10 Å)**	0.684	0.551	0.733	0.019	0.012	0.000
***ipSAE* (15 Å)**	0.661	0.519	0.694	0.155	0.058	0.006
***ipSAE* (20 Å)**	0.641	0.516	0.650	0.198	0.084	0.113
***ipSAE* (25 Å)**	0.610	0.512	0.642	0.198	0.086	0.117
***ipSAE* (32 Å)**	0.230	0.491	0.614	0.197	0.087	0.137

Full-length Uniprot sequences were used for all complexes, except RAF1kd/LysC where only the kinase domain was used for RAF1. RAF1kd/LysC and RAF1/RIPK1 are not known to be true complexes and are not in the PDB. Values are given for the rank001 model (out of 10) from the Colabfold Jupyter notebook with no templates, two seeds, and 3 recycles.

The 4h3b structure is quite different. AlphaFold places the wrong peptide from SH3BP5 into the inhibitory binding site on the kinase domain MAPK10. In the PDB structure, residues 341-350 bind to the kinase domain. But in the model from full-length Uniprot sequences, the 341-350 segment is 100 Å away. Instead, residues 425-439 bind to the kinase domain in the SH3BP5 binding site. The *ipTM* from AlphaFold is 0.443, and the *actifpTM* value is 0.690, while the *ipSAE* values range from 0.0 (no *PAE* pairs less then 5 Å) to 0.20 (*PAE* cutoff 25 Å).

We also ran Colabfold calculations of the RAF1 kinase domain with a presumably non-interacting protein, chicken lysozyme C (LYSC_HUMAN), and full-length RAF1 with RIPK1. For LYSC, The *ipTM* value was 0.388 and the *actifpTM* was 0.467. The *ipSAE* scores successfully identify the non-interaction with *ipSAE* values from 0.0 to 0.1 (Table 1, last column). For RIPK1, *ipTM* was 0.277, *actifpTM* was 0.462, and *ipSAE* was 0.0 (*PAE* cutoffs ≤ 15 Å).

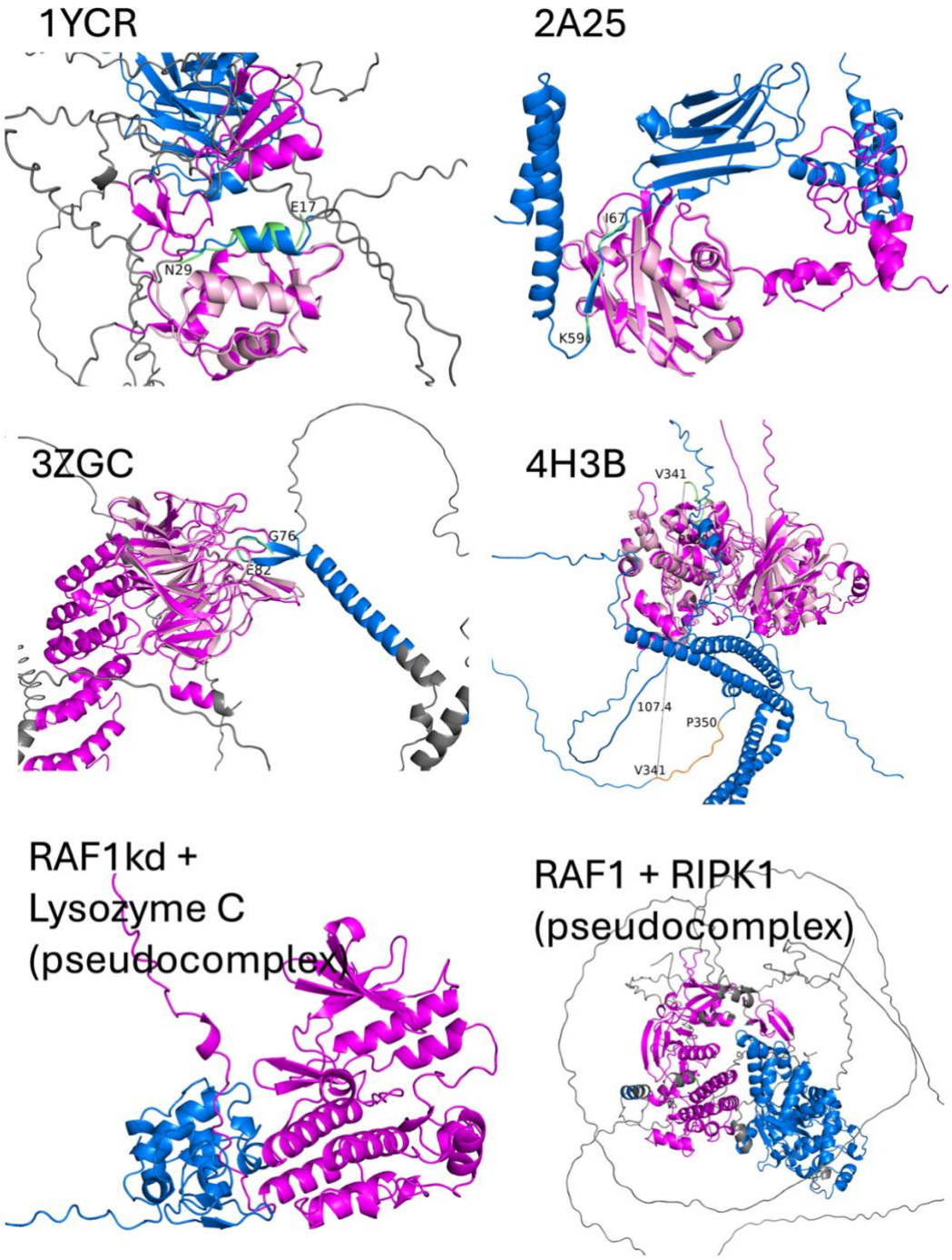

Figure 8. Top-ranked ColabFold AlphaFold-Multimer v2.3 models of protein complexes from full-length Uniprot sequences. PDB entries 1YCR, 2A25, 3ZGC, and 4H3B were used as examples in the preprint of Varga et al. RAF1 kinase domain plus chicken Lysozyme C and full-length RAF1 with RIPK1 are examples of non-interacting proteins and their resulting scores.

Discussion

We have proposed an *ipTM*-like score based on the output of AlphaFold2. The *ipSAE* score is calculated over interchain residue pairs that pass a *PAE* cutoff, thus eliminating the effect of disordered regions in both chains and/or accessory domains that AlphaFold2 does not predict to be part of the binding interface. On a benchmark of 40 heterodimer complexes in the PDB not very similar (at 40% sequence identity) in the AlphaFold2 training set and 70 non-interacting sequence pairs from the same set, models based on full-length Uniprot sequences showed greater discrimination between true and false dimers with the *ipSAE* score compared to *ipTM*. We also showed that in some cases, our score behaves better at discrimination true than false interactions than the recently proposed *actifpTM* score of Varga et al. A true comparison would require a much larger set of targets.

Like *ipTM* in AlphaFold3 output and the *actifpTM* score, the *ipSAE* score can be calculated for every pair of chains in a multi-chain complex from AlphaFold2 output. We calculate the asymmetric values (A→B is different from B→A, where the first chain contains the aligned residues and the second chain contains the scored residues in the *PAE* values), as well as the maximum over all residues in both chains. It is possible there is insight to be gained in considering both values, rather than just the maximum, particularly for protein-peptide complexes.

While we have shown that *ipSAE* is able to distinguish true from false interacting pairs, even in the presence of substantial disorder and non-interacting accessory domains, additional benchmarking is certainly required to demonstrate that the *ipSAE* metric is able to rank the structural accuracy of models of a given complex.

Further comparison is needed to other scores presented in the literature that account for the flaws in *ipTM* in various ways. We made a few comparisons to the *actifpTM* score (20), which like *ipSAE* limits (and weights) the contribution of pairwise *ipTM* matrix elements to the resulting score. Kim et al. presented to the Local Interaction Score (26), which is obtained from the by converting *PAE* scores to a score from 0 to 1.0 and averaging over all interchain residue pairs with *PAE* ≤ 12 Å. The pDockQ (27) and pDockQ2 (17) scores are based on the *pLDDT* and *PAE* scores of interface residues, and also attempt to improve on the *ipTM* score from AlphaFold.

The d_0 parameter in the TM expressions presents challenges for short peptides. In the original TM score paper, no individual structures were compared that were shorter than 40 amino acids. d_0 becomes negative when the protein length is less than 19 amino acids, with a resulting d_0 value of 0.17 when the length is 19. But in that case, the denominator of the *pTM* expression blows up and becomes very low, not matter how accurately the position of a peptide bound to a folded domain is predicted. To avoid this, we chose to set a minimum value of d_0 to 1.0, which is a peptide length of approximately 27 amino acids. But this is somewhat arbitrary and needs to be investigated further.

Finally, the method for calculating the *ipTM* in the AlphaFold programs relies on the maximum *ipTM* over the residues in both chains. But many protein pairs have multiple domain-domain interactions separated by disordered regions. In these cases, the *ipTM* only scores one domain-domain pair (which ever scores highest) and the other(s) do not contribute. Examination of the *PAE* plot is helpful in identifying such cases. Models can then be produced with shorter constructs to estimate the *ipTM*s of each domain-domain interaction. Outputting *ipSAE* values from different aligned residues (not just the maximum value) may be useful in deriving a more useful metric than the methods described here and elsewhere. Our script, described below, outputs a file with the by-residue values of *ipSAE* which may be used for this purpose.

Usage and Output

The code is written in Python3 and takes as input a json file from AlphaFold2 or AlphaFold3 and corresponding PDB-format or mmCIF-format files for the coordinates respectively. The commands to use are:

```
python ipsae.py  <path_to_json_file>  <path_to_af2_pdb_file>  <pae_cutoff> <dist_cutoff>
python ipsae.py  <path_to_json_file>  <path_to_af3_cif_file>  <pae_cutoff> <dist_cutoff>
```

For example:

```
python ipsae.py RAF1_KSR1_scores_rank_001_alphafold2_multimer_v3_model_4_seed_003.json  \
RAF1_KSR1_unrelaxed_rank_001_alphafold2_multimer_v3_model_4_seed_003.pdb 15 15

python ipsae.py fold_raf1_ksr1_mek1_full_data_0.json fold_raf1_ksr1_mek1_model_0.cif 15 15
```

The output from the second command is given in Figure 9.

Chn1	Chn2	PAE	Dist	Type	ipSAE	ipSAE_d0chn	ipSAE_d0dom	ipTM_af	ipTM_d0chn	n0res	n0chn	n0dom	d0res	d0chn	d0dom	nres1	nres2	dist1	dist2	Model
A	B	15	15	asym	0.563173	0.824097	0.678527	0.460	0.362276	292	1571	541	6.28	12.57	8.21	249	292	90	83	fold_raf1_ksr1_mek1_model_0
B	A	15	15	asym	0.546718	0.818835	0.671409	0.460	0.442774	278	1571	539	6.14	12.57	8.20	259	280	83	92	fold_raf1_ksr1_mek1_model_0
A	**B**	**15**	**15**	**max**	**0.563173**	**0.824097**	**0.678527**	**0.460**	**0.442774**	**292**	**1571**	**541**	**6.28**	**12.57**	**8.21**	**280**	**292**	**92**	**83**	**fold_raf1_ksr1_mek1_model_0**
A	C	15	15	asym	0.261359	0.484143	0.338764	0.510	0.428632	319	1041	491	6.54	10.71	7.88	171	320	0	0	fold_raf1_ksr1_mek1_model_0
C	A	15	15	asym	0.232004	0.486093	0.350874	0.510	0.270827	260	1041	513	5.96	10.71	8.03	250	263	0	0	fold_raf1_ksr1_mek1_model_0
A	**C**	**15**	**15**	**max**	**0.261359**	**0.486093**	**0.350874**	**0.510**	**0.428632**	**319**	**1041**	**513**	**6.54**	**10.71**	**8.03**	**263**	**320**	**0**	**0**	**fold_raf1_ksr1_mek1_model_0**
B	C	15	15	asym	0.636110	0.829537	0.732184	0.770	0.751586	344	1316	607	6.76	11.74	8.61	262	345	69	72	fold_raf1_ksr1_mek1_model_0
C	B	15	15	asym	0.559980	0.802212	0.692571	0.770	0.343057	291	1316	598	6.27	11.74	8.56	307	291	71	69	fold_raf1_ksr1_mek1_model_0
B	**C**	**15**	**15**	**max**	**0.636110**	**0.829537**	**0.732184**	**0.770**	**0.751586**	**344**	**1316**	**607**	**6.76**	**11.74**	**8.61**	**291**	**345**	**69**	**72**	**fold_raf1_ksr1_mek1_model_0**

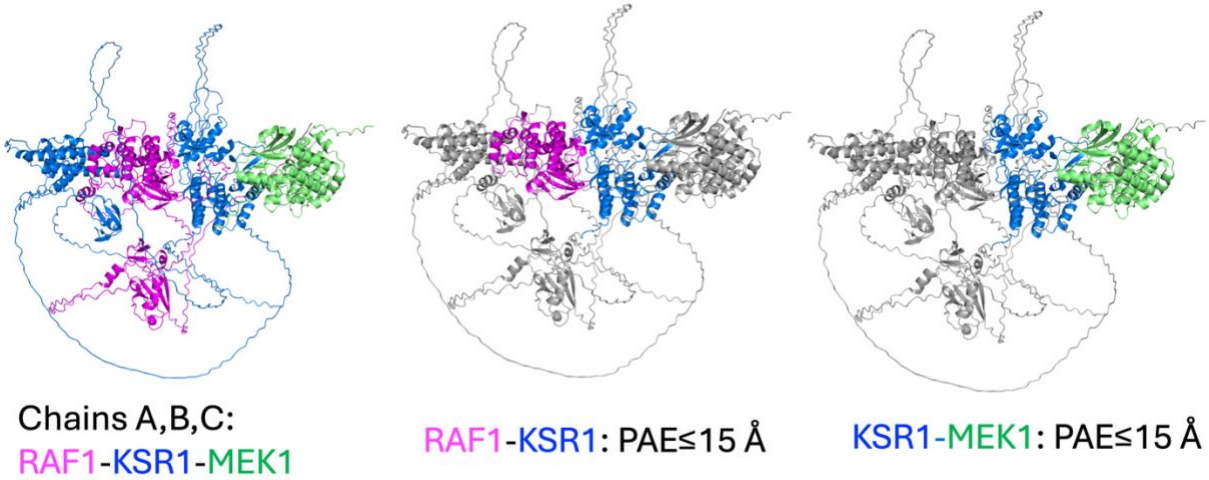

Figure 9. Output of `ipsae.py` on an AlphaFold3 model of a ternary complex of full-length human RAF1, KSR1, and MEK1 (Uniprots: RAF1_HUMAN, KSR1_HUMAN, MP2K1_HUMAN). The asymmetric values of the *ipSAE* metrics are given in rows with type equal to "asym." The maximum value of each metric (over X→Y and Y→X asymmetric values) is given in the row labeled "max" (shown in bold type). Bottom: (left) top ranked AlphaFold3 model with chains labeled by color: RAF1 (chain A: magenta), KSR1 (chain B: blue), MEK1 (chain C: green). Middle: After coloring all three chains gray, PyMOL script alias "color_A_B" colors magenta and blue all residues in chains A and B respectively that have one or more interchain *PAE* values less than the cutoff (15 Å). Right: color B_C colors residues blue and green if they have interchain *PAE* values less than the same cutoff.

The code reads the overall *ipTM* from the AlphaFold2 json file, which has one value for any size protein complex. Given the name of the AlphaFold3 "full_data" json file, the code will read the *chain_pair_iptm* from the corresponding "summary_confidences" json file, if it exists. In this example, that would be named `fold_raf1_ksr1_mek1_summary_confidences_0.json`. In the output, these scores are called *ipTM_af*. For AlphaFold2, all chain pairs have the same value of *ipTM_af*. For the example in Figure 9, AlphaFold3 calculate *ipTM* pairwise values for 0.46 for RAF1-KSR1

(chains A and B), 0.51 for RAF1-MEK1 (chains A and C), and 0.77 for KSR1-MEK1 (chains B and C).

To calculate the *ipSAE* and other metrices, the code reads the *PAE* values from the respective json files. AlphaFold2 provides a square *PAE* matrix with row and column dimensions of the length of the combined protein sequences. The rows are aligned residues and the columns are scored residues. AlphaFold3 structure predictions may include post-translationally modified amino acids as well as ligands. The standard amino acids have single tokens and therefore single rows or columns in the *PAE* matrix in the json file. Modified amino acids, however, have one token per atom (e.g., phosphoserine, residue type SEP, has 10 tokens). We use the Cα atom as the appropriate token for the *PAE* matrix, so we can construct a square *PAE* matrix covering only one row or column per amino acid (whether modified or not). Ligands are excluded (label_seq_id=".").

Since AlphaFold2 does not calculate pairwise *ipTM* scores for multi-protein complexes and AlphaFold3 provides only the symmetric (maximum) pairwise *ipTM* scores, we use the *PAE* matrix to calculate pairwise *ipTM* scores. To calculate d_0 for the *ipTM* calculation, we use the sum of the full-length protein sequences for each sequence pair, as AlphaFold2 does (for dimer complexes) and AlphaFold3 does for all chain pairs. This metric is called *ipTM_d0chn*, where *d0chn* indicates that d_0 is calculated from the chain lengths. The values for the complex in Figure 9 are 0.443, 0.429, and 0.752 respectively (compare the *ipTM_af* values of 0.46, 0.51, 0.77 respectively).The small differences arise from using the *PAE* values in the pairwise *pTM* matrix (Equation 7), instead of the expectation value over the probability distribution of *PAE* (Equation 6).

With the *PAE* matrix and *PAEcutoff* value, we can calculate the asymmetric *ipSAE* scores (Equation 14) and the overall *ipSAE* score (Equation 16), which is the maximum value of the two asymmetric scores for each chain pair. For the regular *ipSAE* score, we use d_0 based on the number of residues in the scored chain that have *PAE*<*PAEcutoff*, given the aligned residue in the aligned chain. The number of residues (*n0res*) and the value of d_0 (*d0res*) are given in the output. For the example in Figure 9, the *ipSAE* values are: 0.563, 0.261, and 0.636. The columns *nres1* and *nres2* provide the number of residues in the first and second chains that have interchain *PAE* values (for the same pair of chains) less than the cutoff (15 Å in this case). In the asym lines, these are for the aligned residues and scored residues respectively. In the "max" lines, they are the maximum of the two asymmetric values. Thus, RAF1 and KSR1 have maximum values (scored or aligned) with *PAE* less than the cutoff of 280 and 292 residues respectively.

The next two columns, *dist1* and *dist2*, provide the number of residues with *PAE* less than the cutoff and Cα-Cα distance less than the distance cutoff set by the user (15 Å in this case). RAF1 does not contact MEK1 in the model, and the *dist1* and *dist2* values are both 0 for chains A+C. The *ipSAE* value is correspondingly only 0.261, while the *ipTM_af* value is 0.51 (probably because RAF1 can also interact with MEK1 but does not do so in this model).

The `ipsae.py` script outputs a PyMOL script with aliases to color residues in each pair of chains with *PAE* less than the cutoff. These residues are highlighted in magenta and blue in the middle structural figure in Figure 9 for RAF1+KSR1 and the right-side structural figure in blue and green for KSR1+MEK1.

The script also calculates two other forms of *ipSAE* for comparison purposes: *ipSAE_d0chn* and *ipSAE_d0dom*. *ipSAE_d0chn* uses the same *PAE* cutoff as *ipSAE* but calculates d_0 from the sum of the two full-length sequence lengths (*n0chn*, *d0chn*). *ipSAE_d0dom* uses a value of d_0 from the number of residues in the two chains that have any interchain *PAE* values less than the *PAEcutoff* (*nres1*, *nres2*).

Finally, for plotting figures like Figures 5 and 6 (e.g., the residue- and chain-pair specific values for *ipSAE*), the script outputs a file with name like:

```
fold_raf1_ksr1_mek1_model_0_15_15_byres.txt
```

with columns:

```
i, AlignChn, ScoredChain, AlignResNum, AlignResType, AlignRespLDDT, n0chn, n0dom,
n0res, d0chn, d0dom, d0res, ipTM_pae, ipSAE_d0chn, ipSAE_d0dom, ipSAE.
```

The value *i* is the residue number across all chains (from 1 to total number of residues in model). The aligned chain refers to the chain with residues *i* in the pTM expressions, the scored chain covers residues *j*. *n0res* and *d0res* are residue-specific values for the number of residues with *PAE* less than the chosen cutoff and the corresponding d_0 value. The other values are all chain-pair specific.

Code availability: A python3 script is available at github.com/dunbracklab/IPSAE.

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Statement:

I support efforts to increase diversity, equity, inclusion, and accessibility in scientific research.
I support the right of transgender individuals to live their lives free of discrimination.

References

1. R. Evans *et al.* Protein complex prediction with AlphaFold-Multimer. *BioRxiv* (2021).
2. J. Jumper *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583-589 (2021).
3. J. Abramson *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493-500 (2024).
4. R. D. Schaeffer, L. Kinch, A. Kryshtafovych, N. V. Grishin. Assessment of domain interactions in the fourteenth round of the Critical Assessment of Structure Prediction (CASP14). *Proteins* **89**, 1700-1710 (2021).
5. L. T. Alexander *et al.* Protein target highlights in CASP15: Analysis of models by structure providers. *Proteins* **91**, 1571-1599 (2023).
6. V. Mischley, J. Maier, J. Chen, J. Karanicolas. PPIscreenML: Structure-based screening for protein-protein interactions using AlphaFold. *bioRxiv* (2024).
7. R. Yin, B. Y. Feng, A. Varshney, B. G. Pierce. Benchmarking AlphaFold for protein complex modeling reveals accuracy determinants. *Protein Sci.* **31**, e4379 (2022).
8. H. Bret, J. Gao, D. J. Zea, J. Andreani, R. Guerois. From interaction networks to interfaces, scanning intrinsically disordered regions using AlphaFold2. *Nature communications* **15**, 597 (2024).
9. G. Pándy-Szekeres, L. P. Taracena Herrera, J. Caroli, A. A. Kermani, Y. Kulkarni, G. M. Keserű, D. E. Gloriam. GproteinDb in 2024: new G protein-GPCR couplings, AlphaFold2-multimer models and interface interactions. *Nucleic Acids Res.* **52**, D466-D475 (2024).
10. F. Homma, J. Lyu, R. A. van der Hoorn. Using AlphaFold Multimer to discover interkingdom protein–protein interactions. *The Plant Journal* **120**, 19-28 (2024).
11. I. Mitic, K. A. Michie, D. A. Jacques. Assessing the Validity of Leucine Zipper Constructs Predicted in AlphaFold2. *bioRxiv*, 2024.2010. 2014.618350 (2024).
12. J. Martin. AlphaFold2 predicts whether proteins interact amidst confounding structural compatibility. *Journal of Chemical Information and Modeling* **64**, 1473-1480 (2024).
13. N. Banhos Danneskiold-Samsoe *et al.* AlphaFold2 enables accurate deorphanization of ligands to single-pass receptors. *Cell Syst* **15**, 1046-1060 e1043 (2024).
14. C. Y. Lee *et al.* Systematic discovery of protein interaction interfaces using AlphaFold and experimental validation. *Mol. Syst. Biol.* **20**, 75-97 (2024).
15. R. Yin, B. G. Pierce. Evaluation of AlphaFold antibody–antigen modeling with implications for improving predictive accuracy. *Protein Sci.* **33**, e4865 (2024).
16. G. Bellinzona, D. Sassera, A. M. Bonvin. Accelerating protein–protein interaction screens with reduced AlphaFold-Multimer sampling. *Bioinformatics Advances* **4**, vbae153 (2024).
17. W. Zhu, A. Shenoy, P. Kundrotas, A. Elofsson. Evaluation of AlphaFold-Multimer prediction on multi-chain protein complexes. *Bioinformatics* **39** (2023).
18. E. W. Schmid, J. C. Walter. Predictomes: A classifier-curated database of AlphaFold-modeled protein-protein interactions. *bioRxiv* (2024).

19. Y. Zhang, J. Skolnick. Scoring function for automated assessment of protein structure template quality. *Proteins: Structure, Function and Genetics* **57**, 702-710 (2004).
20. J. K. Varga, S. Ovchinnikov, O. Schueler-Furman. actifpTM: a refined confidence metric of AlphaFold2 predictions involving flexible regions. *arXiv preprint arXiv:2412.15970* (2024).
21. M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov, M. Steinegger. ColabFold: making protein folding accessible to all. *Nat. Methods* **19**, 679-682 (2022).
22. N. R. Michaud, M. Therrien, A. Cacace, L. C. Edsall, S. Spiegel, G. M. Rubin, D. K. Morrison. KSR stimulates Raf-1 activity in a kinase-independent manner. *Proceedings of the National Academy of Sciences* **94**, 12792-12796 (1997).
23. T. Rajakulendran, M. Sahmi, M. Lefrançois, F. Sicheri, M. Therrien. A dimerization-dependent mechanism drives RAF catalytic activation. *Nature* **461**, 542-545 (2009).
24. G. Wang, R. L. Dunbrack, Jr. PISCES: recent improvements to a PDB sequence culling server. *Nucleic Acids Res.* **33**, W94-98 (2005).
25. J. M. Dana, A. Gutmanas, N. Tyagi, G. Qi, C. O'Donovan, M. Martin, S. Velankar. SIFTS: updated Structure Integration with Function, Taxonomy and Sequences resource allows 40-fold increase in coverage of structure-based annotations for proteins. *Nucleic Acids Res.* **47**, D482-D489 (2019).
26. A.-R. Kim, Y. Hu, A. Comjean, J. Rodiger, S. E. Mohr, N. Perrimon. Enhanced protein-protein interaction discovery via AlphaFold-Multimer. *bioRxiv*, 2024.2002.2019.580970 (2024).
27. P. Bryant, G. Pozzati, A. Elofsson. Improved prediction of protein-protein interactions using AlphaFold2. *Nature communications* **13**, 1265 (2022).