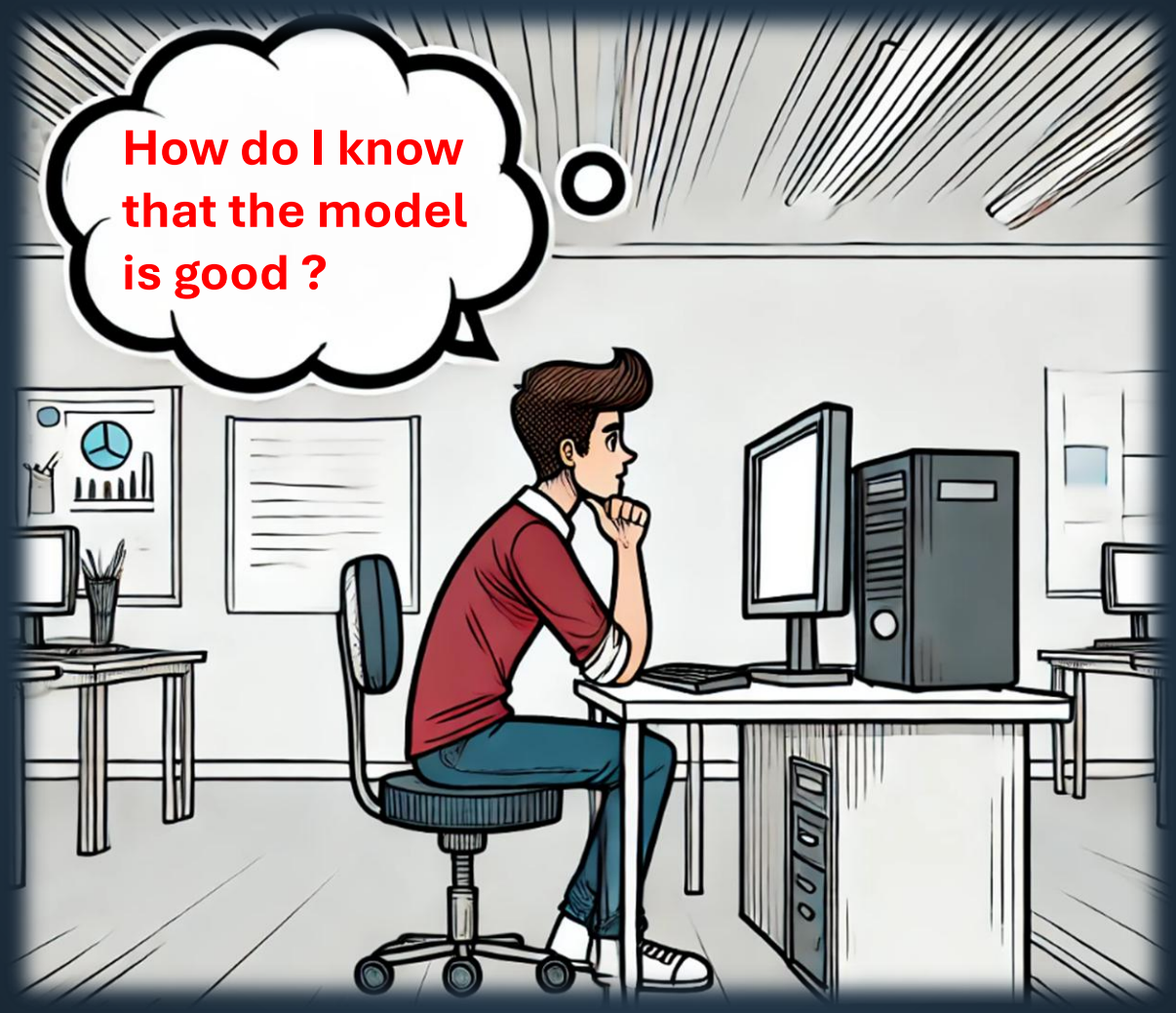


AlphaFold is good for model prediction, but it's also good to predict a ***CONFIDENCE SCORE***

SCORING

AlphaFold predict good scoring fonction, that **YOU CAN TRUST!**

- If a score is good : then it’s good.
- If a score is bad : then it’s bad.
- If a score is average ? *Meh.. Let’s see*

Different kind of scores

Scoring

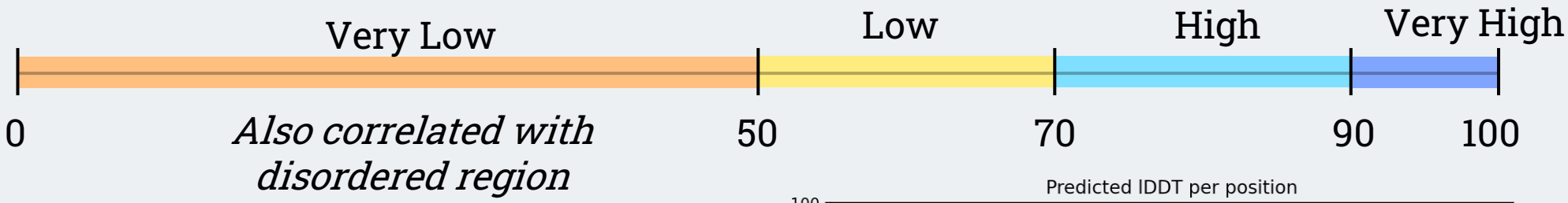
Score	Description	Scale	Single Protein	Complex	Comments
pLDDT	Predicted Local Distance Difference Test: Confidence in the local structure at the residue level.	Local (residue) + Global (if averaged)	Yes	No	Provides an estimation of the quality of the structure around each residue. Widely used for single proteins.
PAE	Predicted Aligned Error Provides per-residue error estimates for pairs of residues in the model.	Local (residue pair) Global (if averaged)	Yes	Yes	Useful for assessing the reliability of relative positioning between residues or domains in bot single proteins and complexes.
pTM	Predicted Template Modeling Score: Evaluates the global accuracy of the models.	Global (model)	Yes	Yes	Suitable for complexes in AlphaFold-Multimer. Reflects how well the global structures match expected models.
ipSAE	Score evaluating interaction confidence using PAE filtered on reliable residue pairs and a length-adjusted d0	Local (Interface Residue -residue) Global (complex, via max over chains)	No	Yes	More robust than ipTM for distinguishing true vs false interactions. Ignores disordered regions via PAE cutoff and adjusts d0 to avoid inflated scores. Suitable for complexes with flexible or long accessory regions.
iPTM	Inter-chain Predicted TM-score: Evaluates interactions between chains in a complex.	Global (complex)	No	Yes	Complements pTM for multi-chain complexes by capturing the quality of intermolecular interfaces.
actifPTM	Score assessing the presence and quality of interfaces in complexes	Global (model)	No	Yes	Indicates the relevance of intermolecular interfaces in multi-protein complexes.
LIS (Local Interaction Score)	Score evaluating the local quality of inter-residue interactions.	Local (interaction)	No	Yes	Often used to check the consistency of local interactions in models.
pdockQ	Predictive score for docking quality in complexes (AlphaFold-Multimer).	Global (complex)	No	Yes	Strongly correlates with interface quality in binary complexes. Based on the average pLDDT of the interfaces.
pdockQ2	Improved version of pdockQ, integrating more criteria for complex systems.	Global (complex)	No	Yes	Used to refine the quality prediction of complexes with multiple chains or interfaces.
Interface pLDDT	Average pLDDT score for residues in the interfaces.	Local (interface residues)	No	Yes	Indicates confidence in specific residues within binding interfaces.

Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

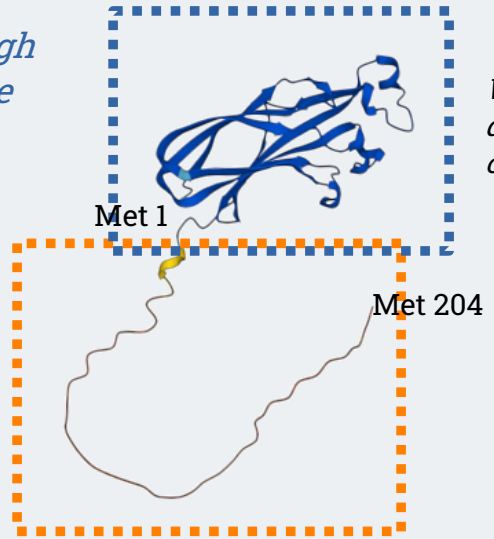
Predicted Local Distance Difference Test:

→ Confidence in the local structure at the residue level.

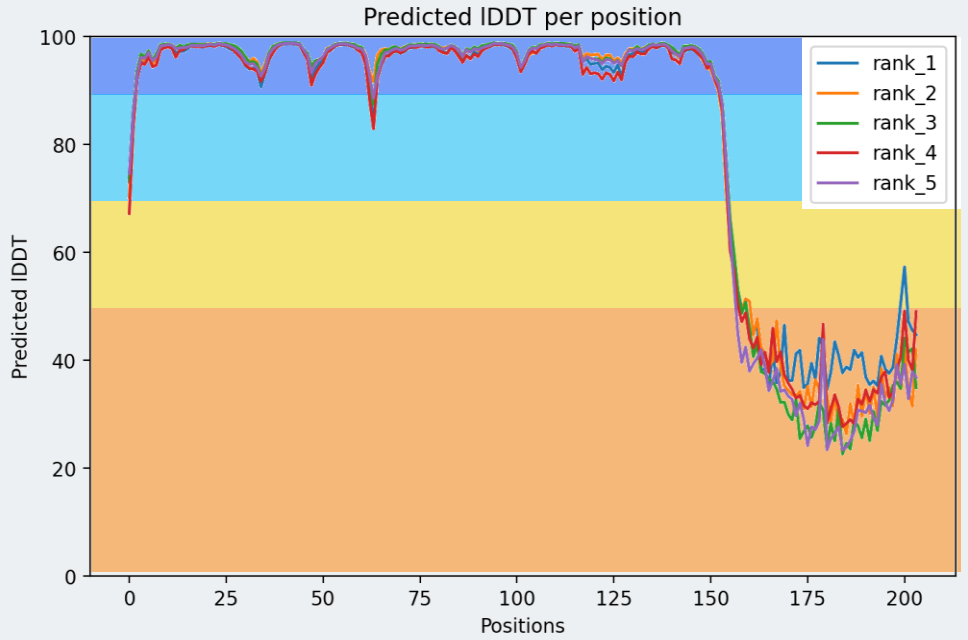


Zone of high confidence

Well structured domain with high confidence scores



Zone of low confidence
Disorded



Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

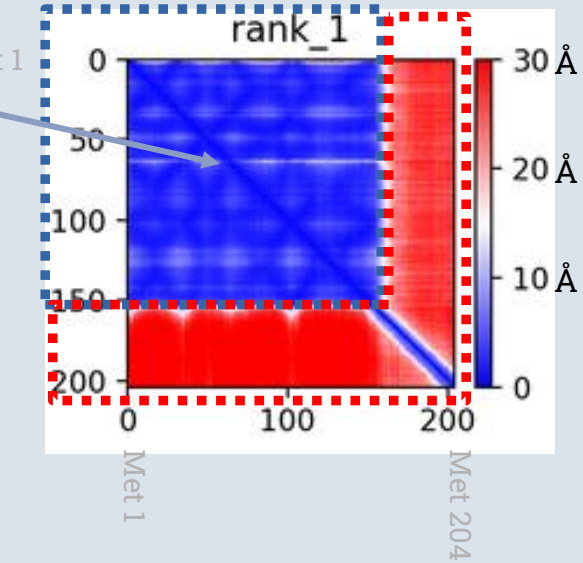
Predicted Aligned Error :

Error on relative 3D position between 2 residues

High confidence in relative positions between these residues

Low confidence in relative positions

You can easily spot predicted domains
= squares along the diagonal



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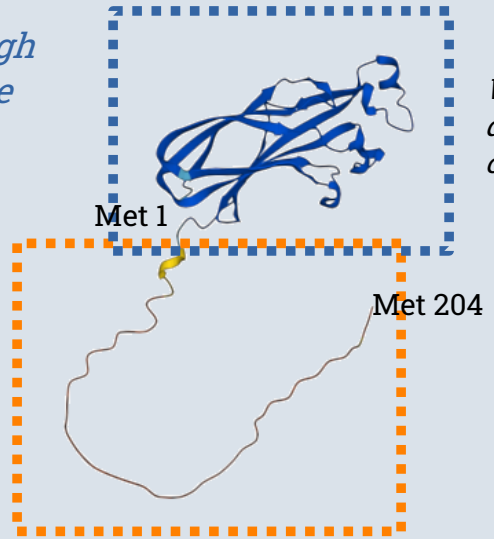


Similar to a contact map, except it doesn't give a distance (D) between 2 residues but the estimated error (E) on that distance
=> distance between residues x & y is equal to D ± E

Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

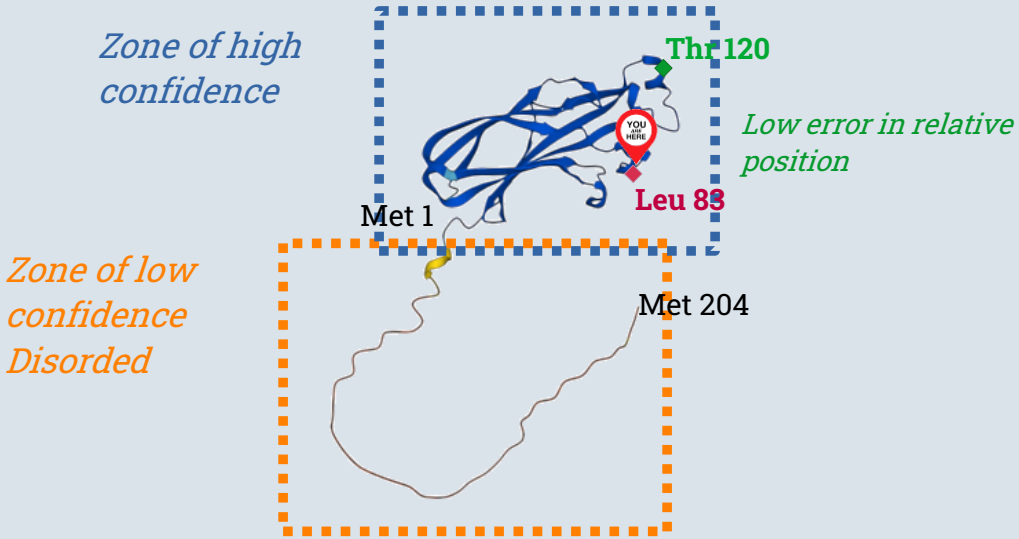
Predicted Aligned Error :

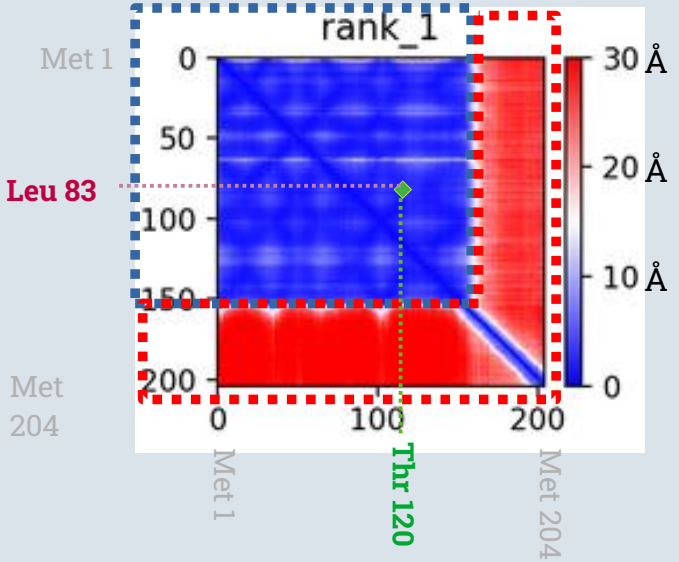
Error on relative 3D position between 2 residues

Estimated error on the distance of Thr 120 relative to Leu 83 is very low (blue)

→ *high confidence in relative positions*

→ *linked to Thr 120 and Leu 83 being within a same domain on the protein*





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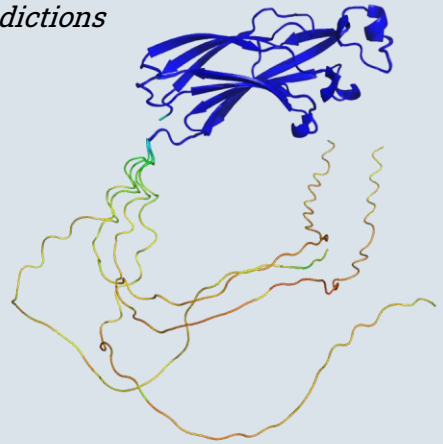
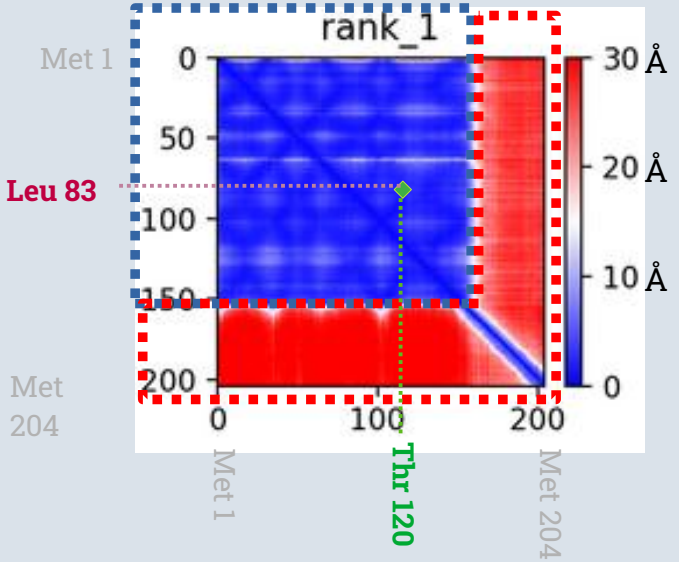
Zone of high confidence

Zone of low confidence Disorded

The low confidence region is even more obvious when comparing the different predictions





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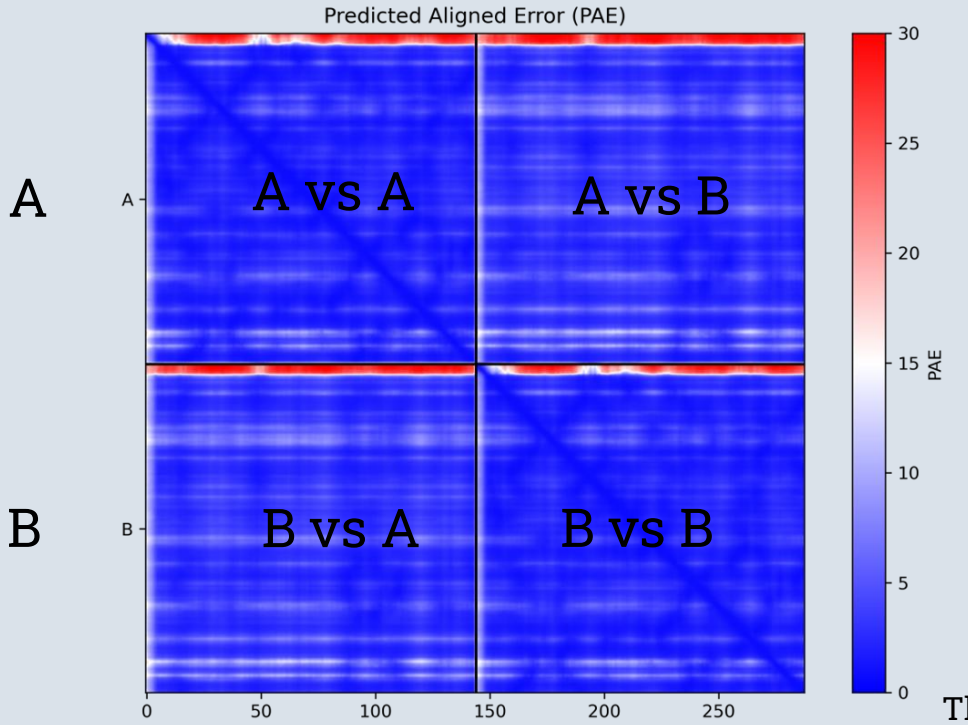
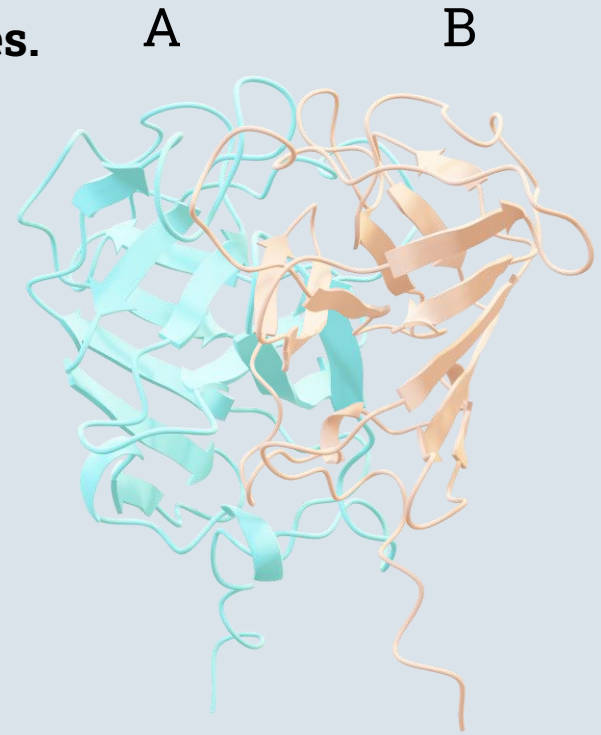
PAE can also be used to evaluate protein complexes.





The two monomers are well predicted (A-A and B-B PAE is low)
The protein complex, dimer, is well predicted (A-B and B-B is low)

Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

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Error on relative 3D position between 2 residues

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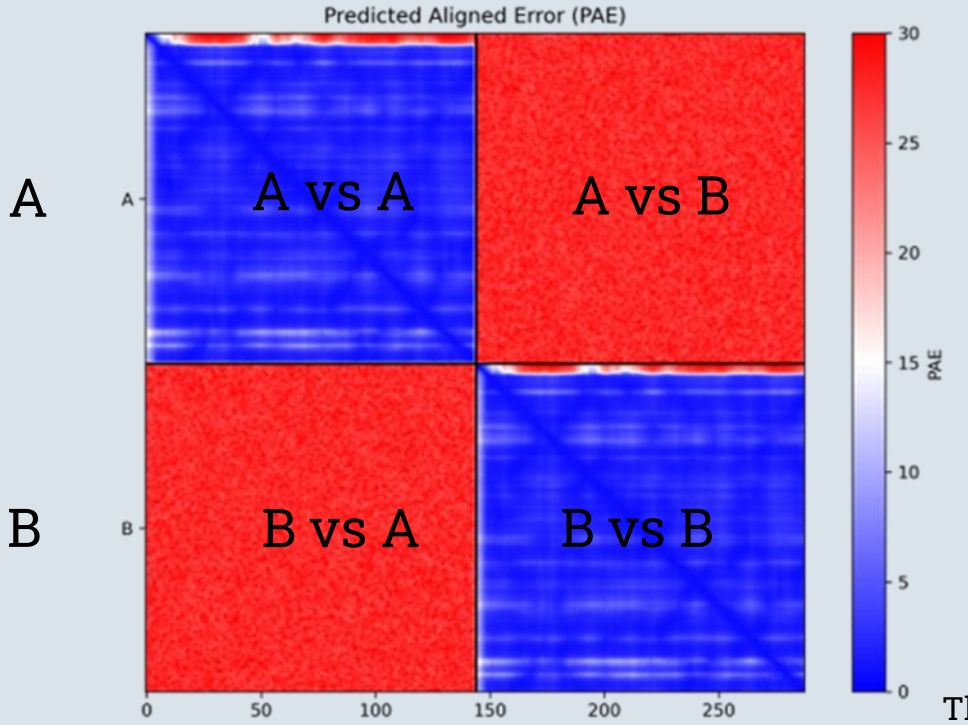

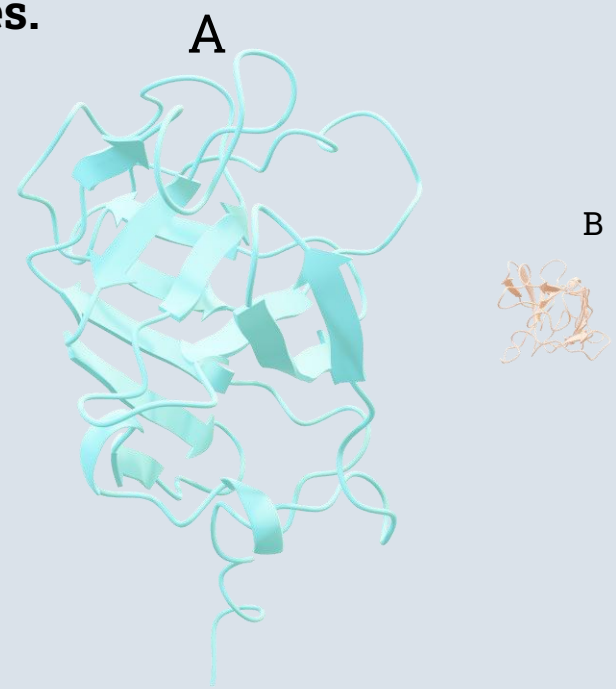

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Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

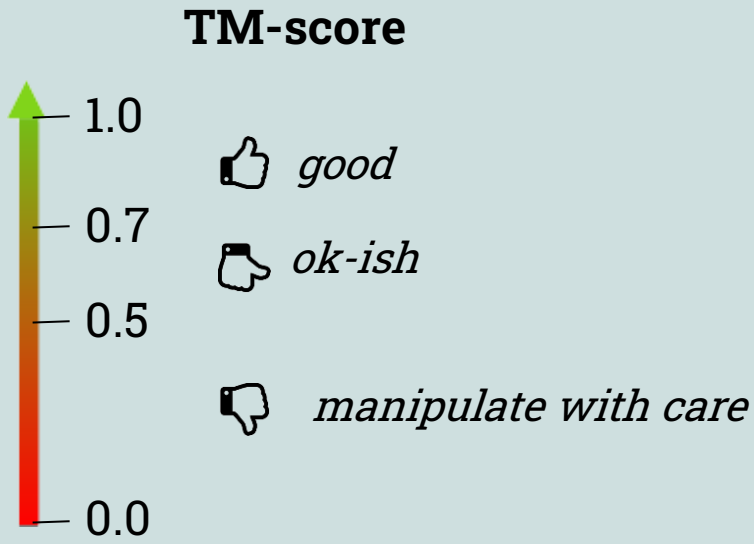
Predicted Template Modeling score :
Evaluates the global accuracy of the models compared to a hypothetical true experimental structure.

pTM

- predicted TM-score - *a similarity metric between your model and the theoretical true structure* - estimated from the PAE

ipTM

- pTM-score – the pTM score calculated between Chains (when multimers)



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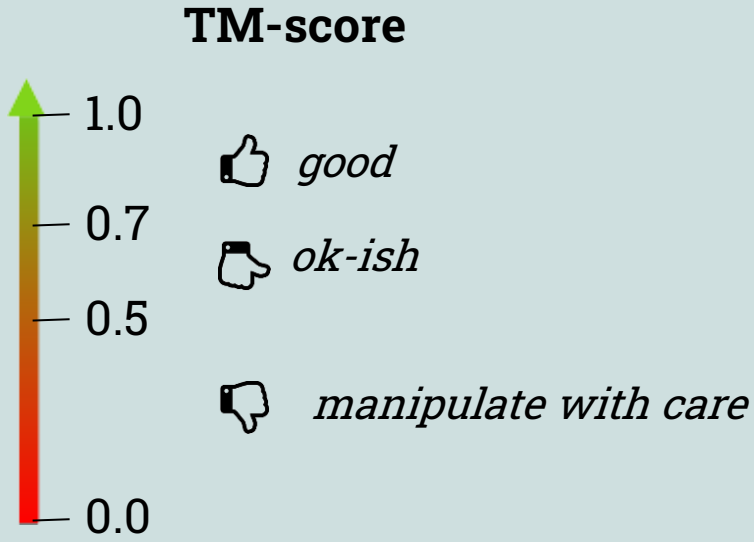


Scoring

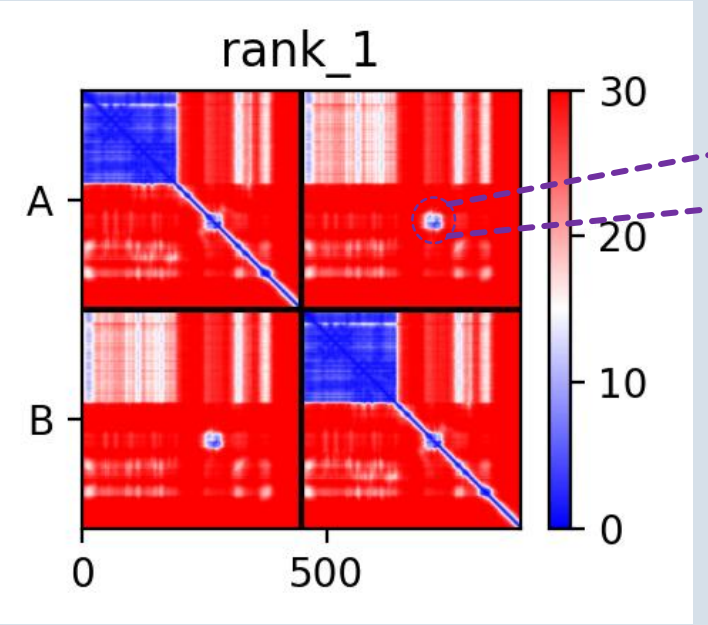
pLDDT | PAE | pTM / ipTM | actifpTM | Examples

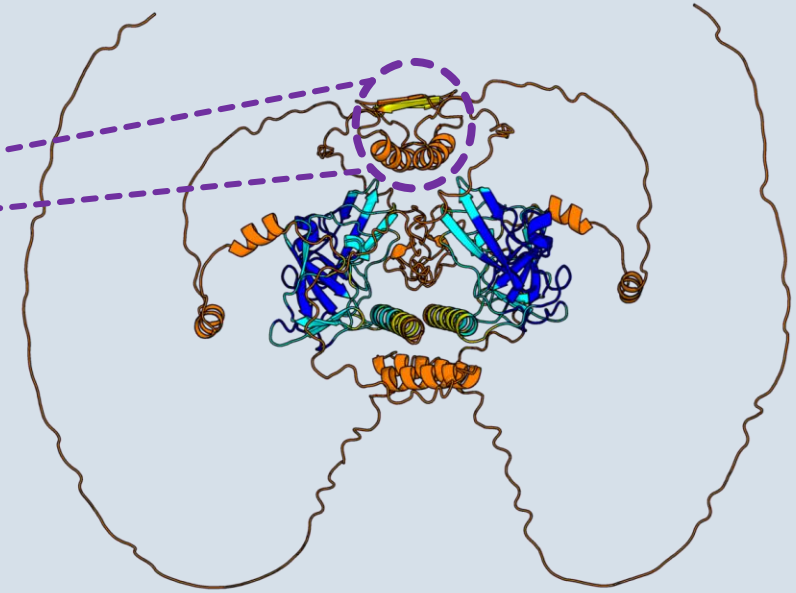
ACTual InterFace Predicted Template Modeling score :

Very similar to ipTM, but more precise when only a sub-region of the complexes are interacting.

Problem with pTM / ipTM → It is based on the whole PAE matrix.
Not sensible to LOCAL prediction, and iPTM is actually an *interchain* pTM.

Let's take an example :





pLDDT = 50.5
pTM = 0.39
ipTM = 0.27

GOOD OR BAD MODEL ?

actifPTM = 0.743

Good, but not everywhere!

Scoring

pLDDT | PAE | pTM / ipTM | ipSAE | Examples

ipSAE

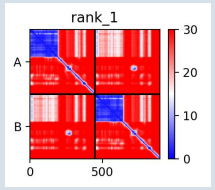
interaction prediction Score from Aligned Errors

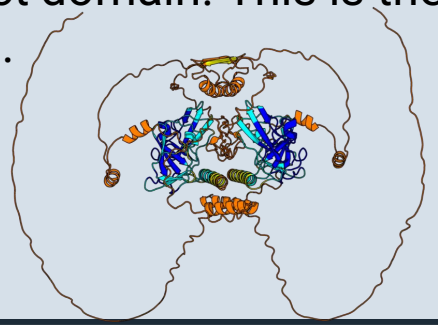
- Score derived from PAE matrix
- Aims : Correct the biais introduced by the length of the models (especially if the protein has a disordered region)
- Start from the PAE matrix with adjustable cutoff to ignore region that can be close in the models, but with high PAE.

ipSAE : ipSAE value for given PAE cutoff and d0 determined by number of residues in 2nd chain with PAE<cutoff

ipSAE_d0chn: Normalized over the total length of the chain (may still be skewed by long flexible tails).

ipSAE_d0dom : Normalized as if the protein were a compact domain. This is the most reliable score for validating a specific biological interaction.



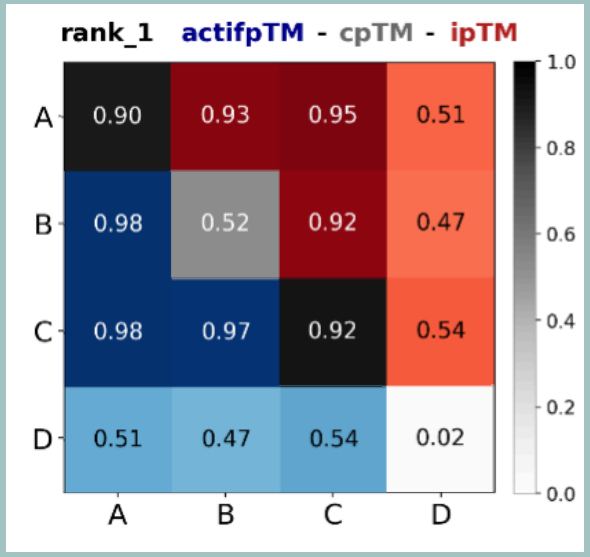


pLDDT = 50.5
pTM = 0.39
ipTM = 0.27
actifPTM = 0.743
ipSAE = 0.043
ipSAE d0chain = 0.73
ipSAE d0dom = 0.37

Scoring

All in one score matrix

(output from colabfold)



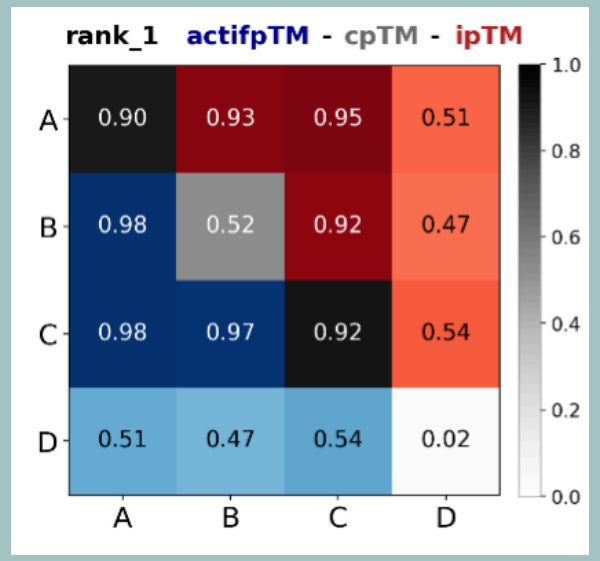
Number of chain in the complex : 4

Scoring

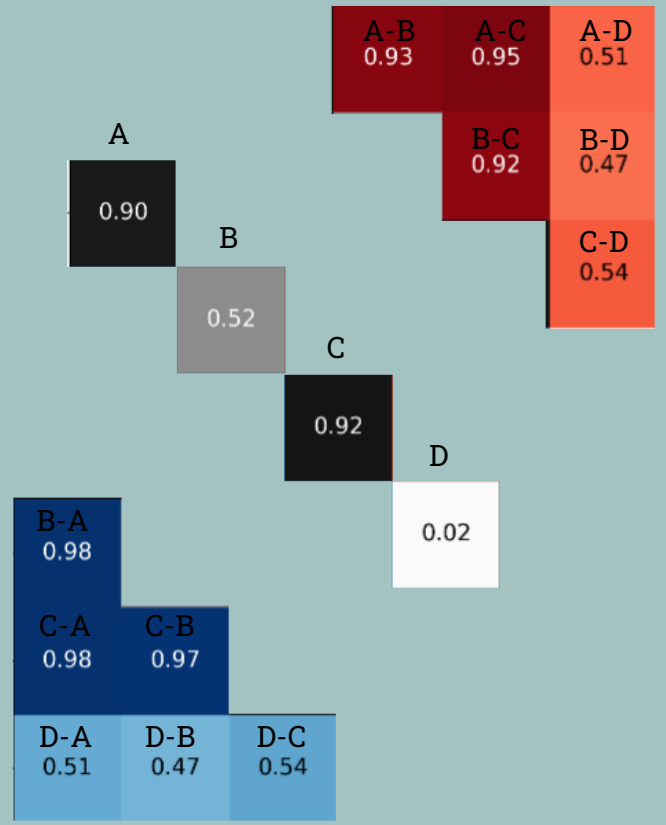
pLDDT | PAE | pTM / ipTM | actifpTM | Examples

All in one score matrix

(output from colabfold)



Number of chain in the complex : 4



iPTM score between chains

0 1

pTM score per chain

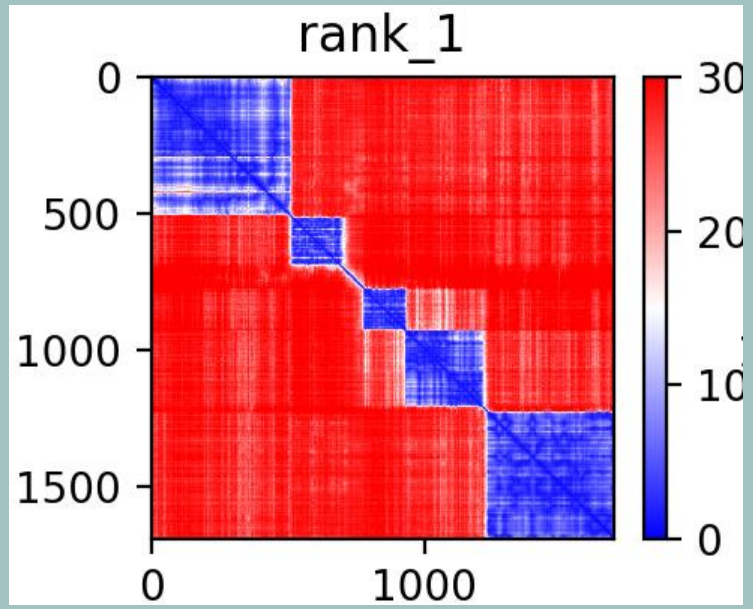
0 1

actifPTM score between chains

0 1

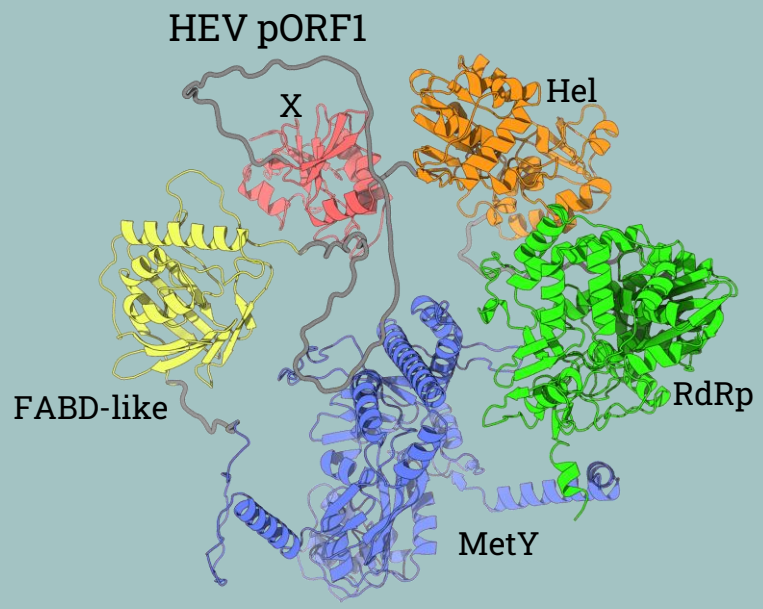
Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples



How many domains ?

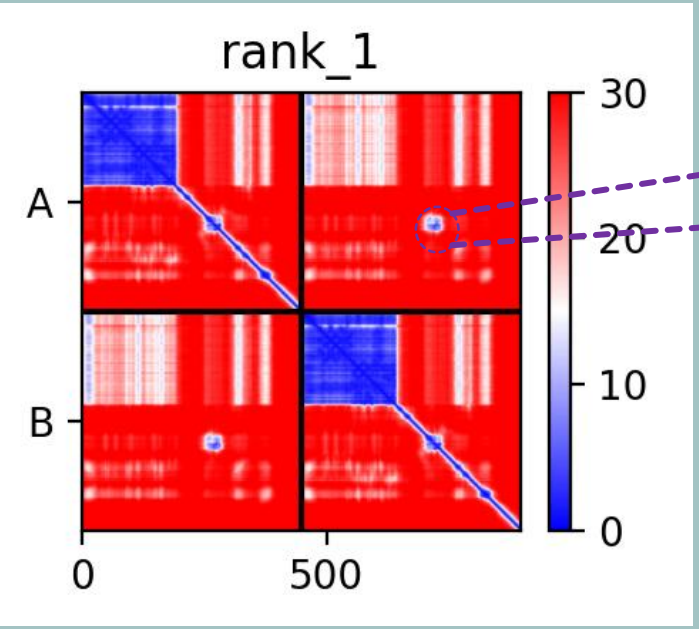
→ **5**

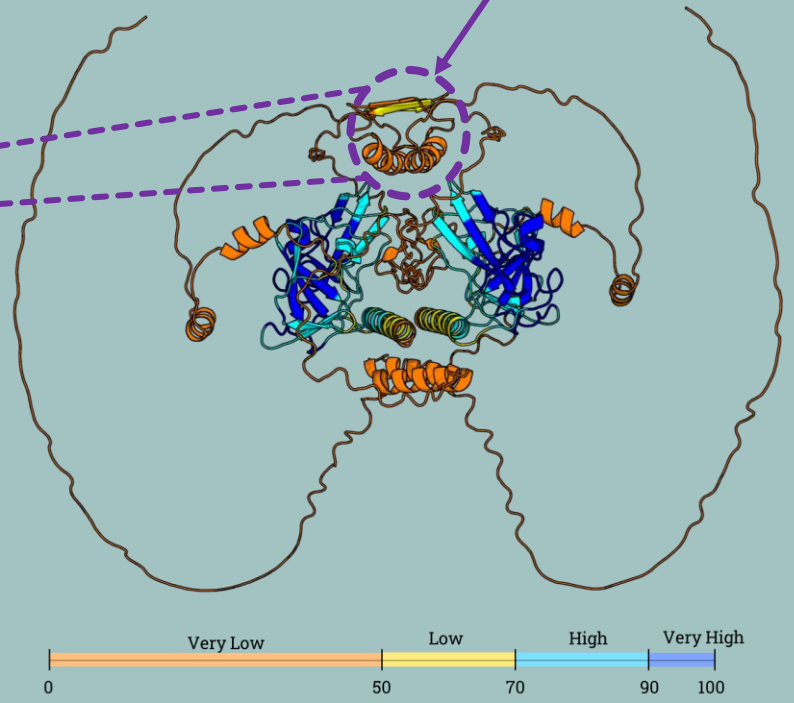


Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

What do you think about the pLDDT of this regions ?





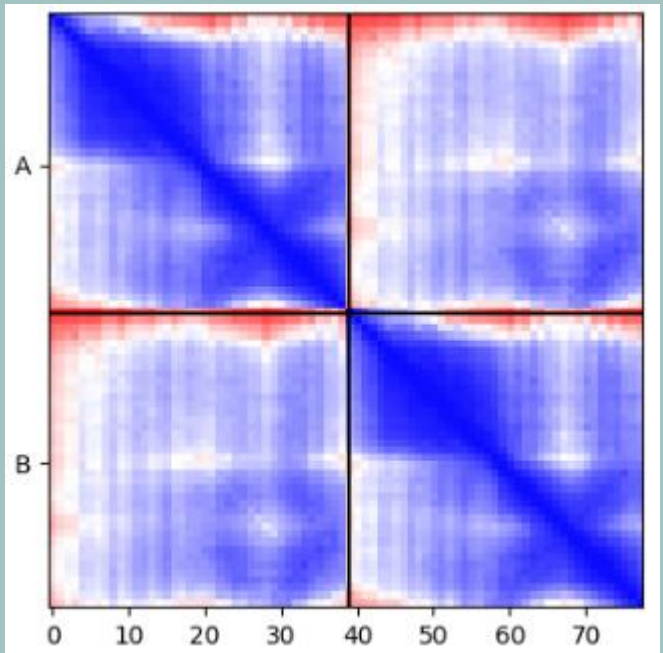
The pLDDT is low/very low. However the PAE for this area is good.
Strange isn't it ?

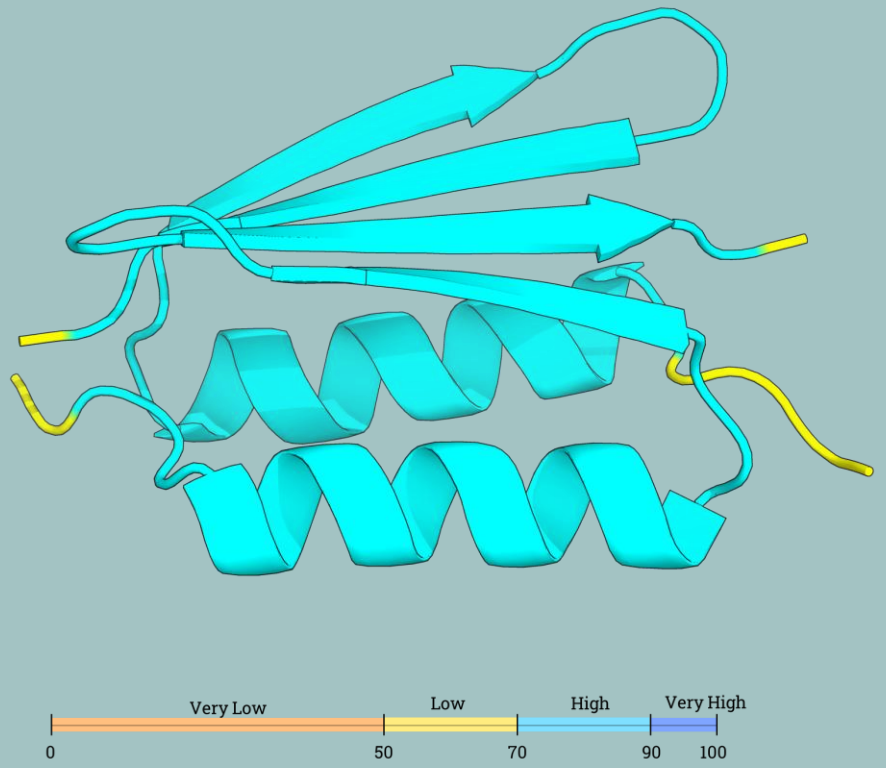
Let's isolate this region only…

Scoring

pLDDT | PAE | pTM / ipTM | actifpTM | Examples

What do you think about the pLDDT of this regions ?





Smaller is Better!

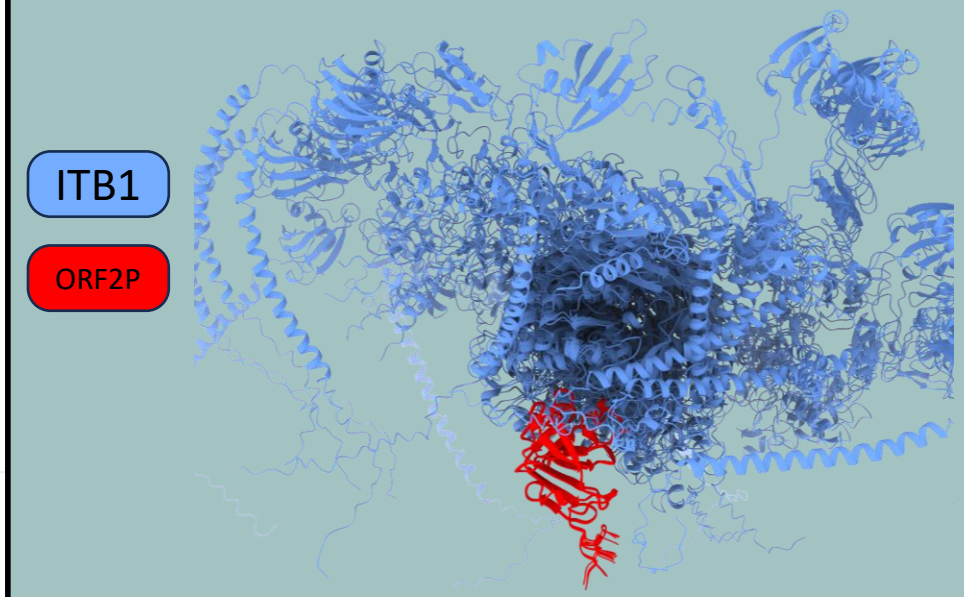
Scoring

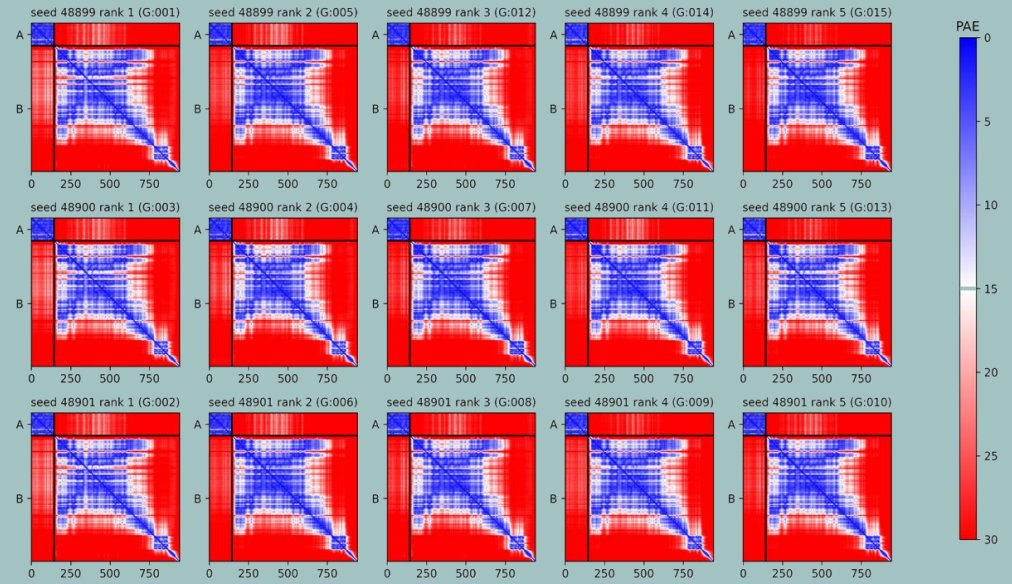
pLDDT | PAE | pTM / ipTM | actifpTM | Examples

2 small tips :

- Smaller is better!
- Alphafold has better prediction when the biological context is respected.

Predicting the interaction between the P domain of the Hepatitis E Virus ORF2 protein with Human ITB1





No good clear good PAE between ORF2P and ITB1

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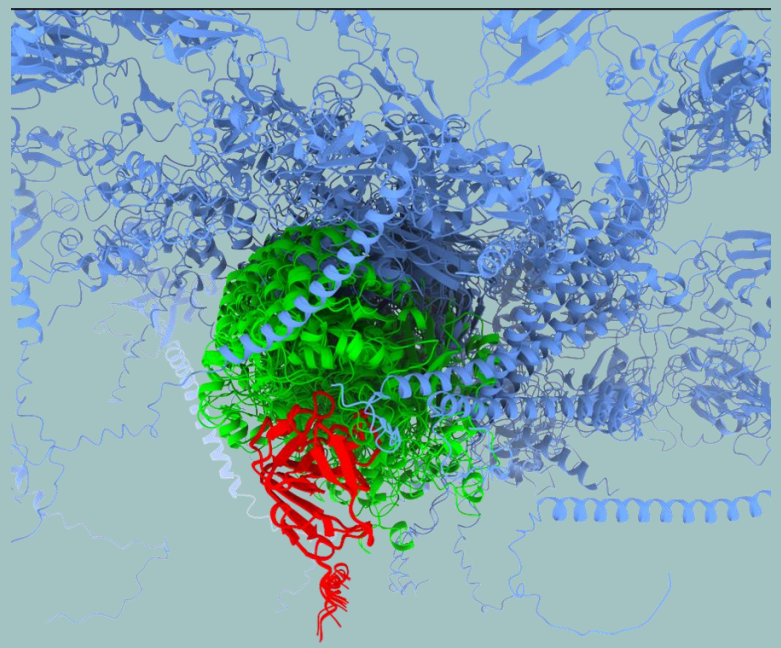

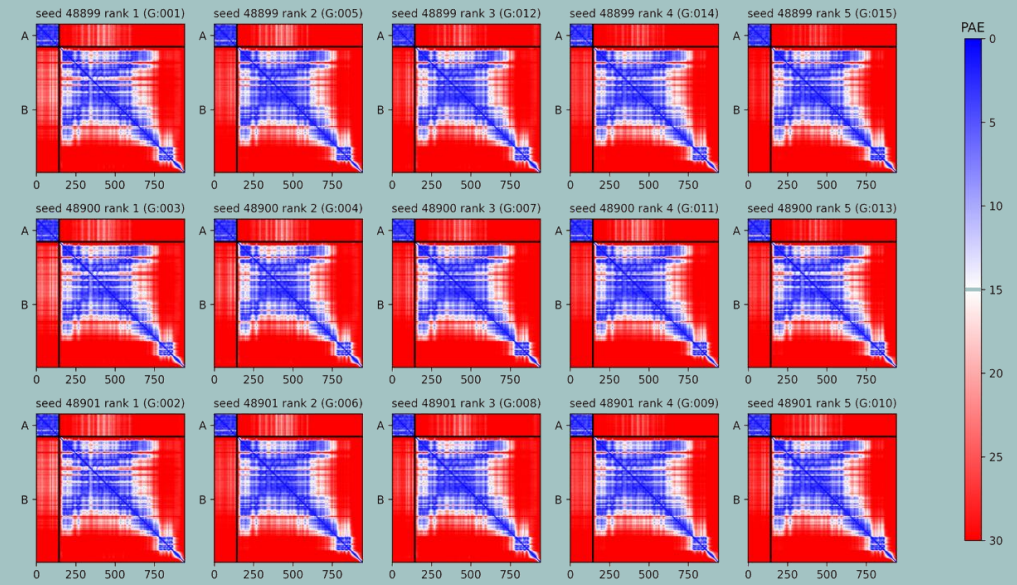

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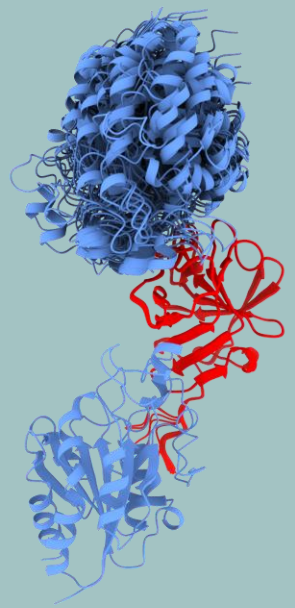
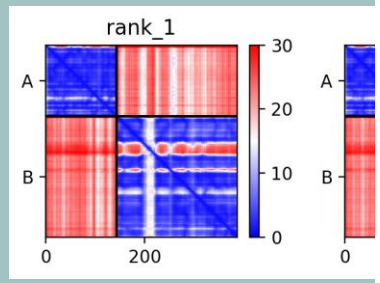
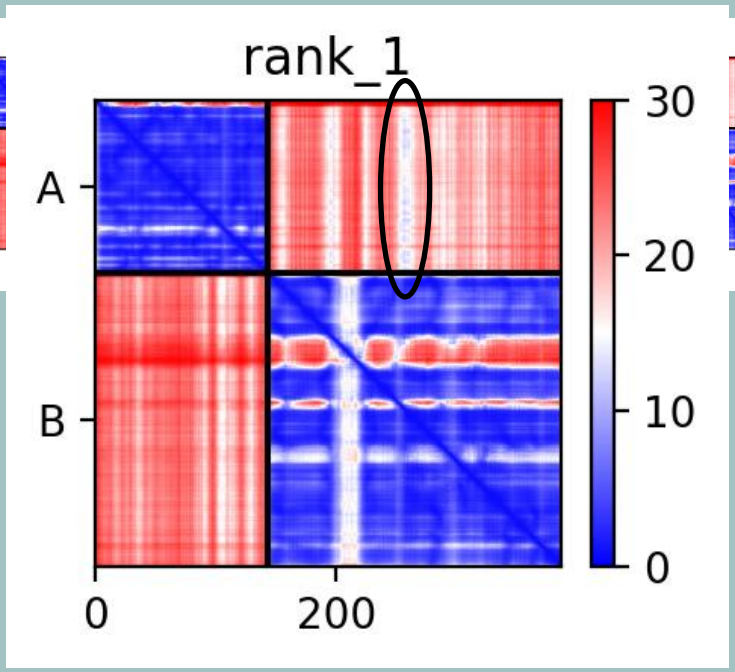
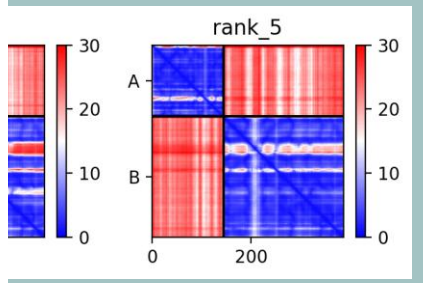
ITB1
139-380

ORF2P









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pLDDT | PAE | pTM / ipTM | actifpTM | Examples

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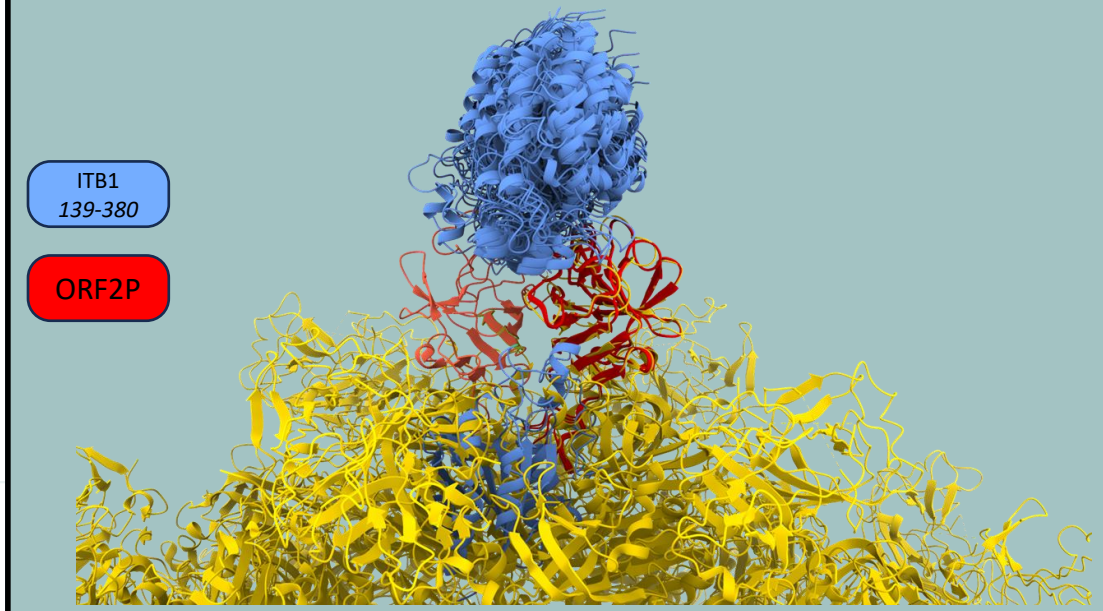

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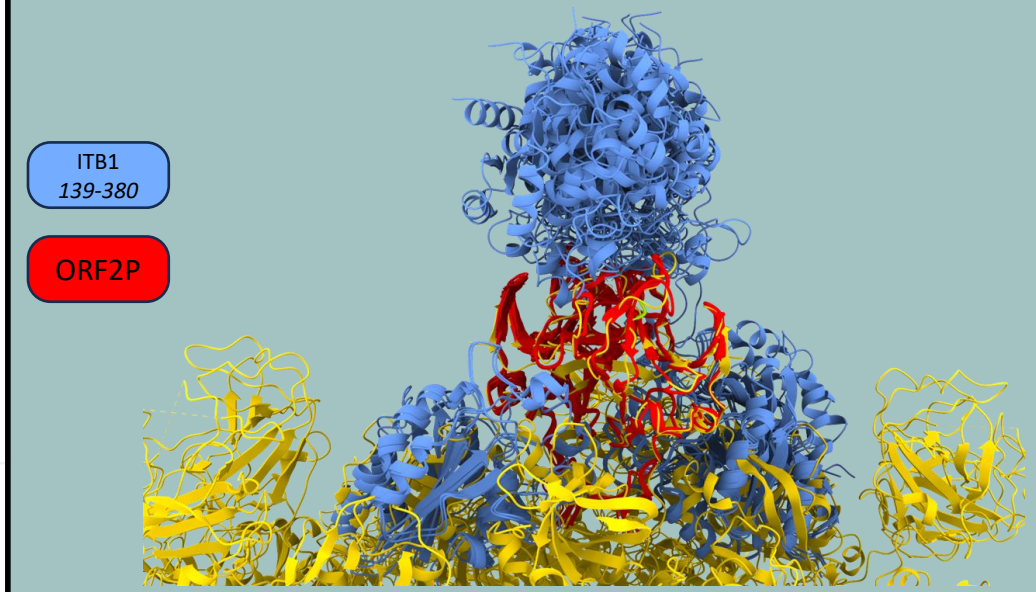

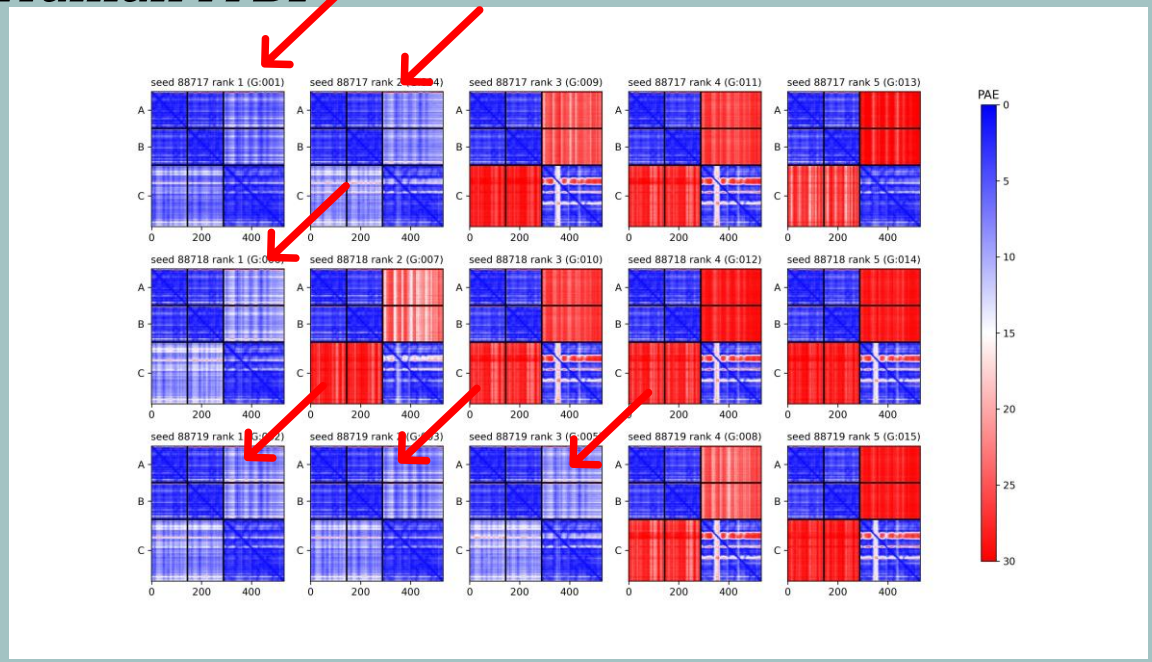

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PAE	Predicted Aligned Error Provides per-residue error estimates for pairs of residues in the model.	Local (residue pair) Global (if averaged)	Yes	Yes	Useful for assessing the reliability of relative positioning between residues or domains in bot single proteins and complexes.
pTM	Predicted Template Modeling Score: Evaluates the global accuracy of the models.	Global (model)	Yes	Yes	Suitable for complexes in AlphaFold-Multimer. Reflects how well the global structures match expected models.
ipSAE	Score evaluating interaction confidence using PAE filtered on reliable residue pairs and a length-adjusted d0	Local (Interface Residue -residue) Global (complex, via max over chains)	No	Yes	More robust than ipTM for distinguishing true vs false interactions. Ignores disordered regions via PAE cutoff and adjusts d0 to avoid inflated scores. Suitable for complexes with flexible or long accessory regions.
iPTM	Inter-chain Predicted TM-score: Evaluates interactions between chains in a complex.	Global (complex)	No	Yes	Complements pTM for multi-chain complexes by capturing the quality of intermolecular interfaces.
actifPTM	Score assessing the presence and quality of interfaces in complexes	Global (model)	No	Yes	Indicates the relevance of intermolecular interfaces in multi-protein complexes.
LIS (Local Interaction Score)	Score evaluating the local quality of inter-residue interactions.	Local (interaction)	No	Yes	Often used to check the consistency of local interactions in models.
pdockQ	Predictive score for docking quality in complexes (AlphaFold-Multimer).	Global (complex)	No	Yes	Strongly correlates with interface quality in binary complexes. Based on the average pLDDT of the interfaces.
pdockQ2	Improved version of pdockQ, integrating more criteria for complex systems.	Global (complex)	No	Yes	Used to refine the quality prediction of complexes with multiple chains or interfaces.
Interface pLDDT	Average pLDDT score for residues in the interfaces.	Local (interface residues)	No	Yes	Indicates confidence in specific residues within binding interfaces.