



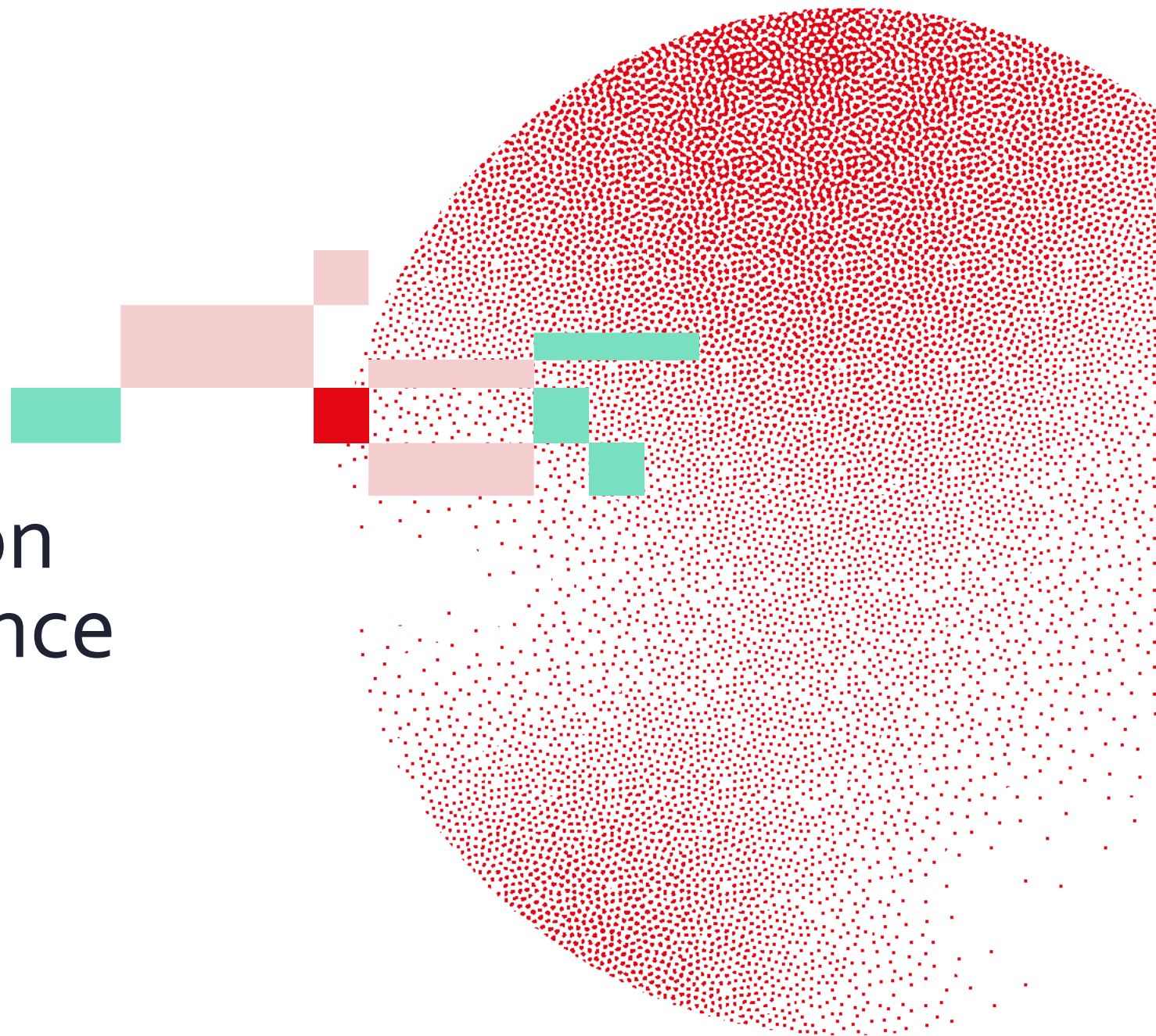
Swiss Institute of  
Bioinformatics

DAY 2, PART 1

# Structure comparison metrics and confidence metrics

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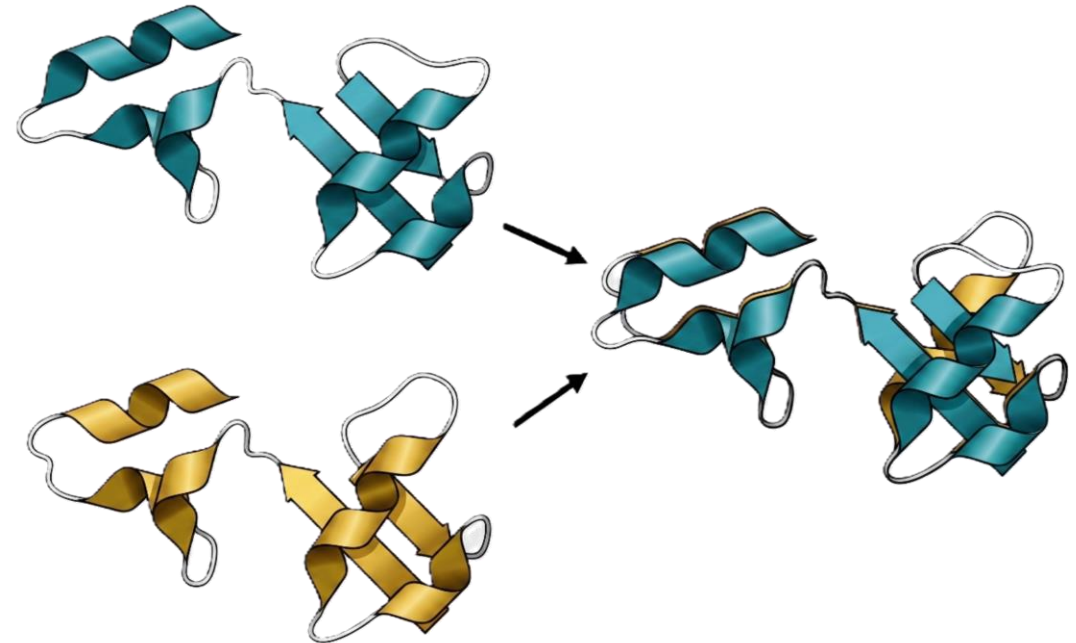
23-24 June 2026





# Structure comparison

- To determine prediction quality
  - Prediction vs experimental ground truth
- To determine structural diversity
  - Conformation 1 vs conformation 2

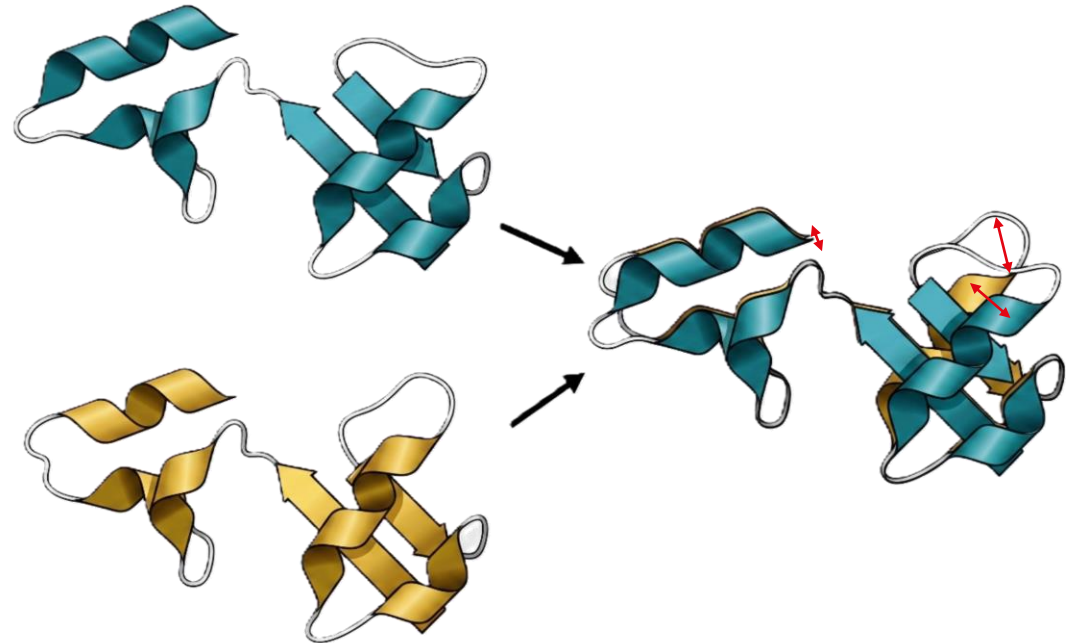


# RMSD (Root mean square deviation)

- Needs structural alignment
- Distances between atoms in structure 1 and structure 2

$$RMSD = \sqrt{\frac{1}{N} \sum_{i=1}^N d_i^2}$$

- Size dependent
- Lower is better



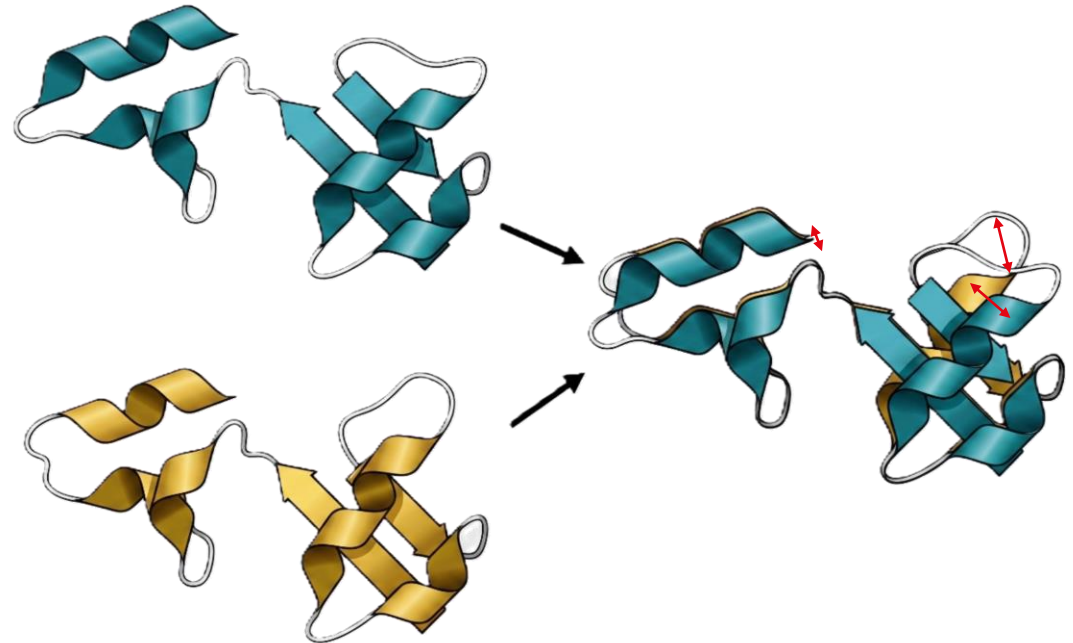


# TM (template modelling) score

- Needs structural alignment
- Distances between atoms in structure 1 and structure 2

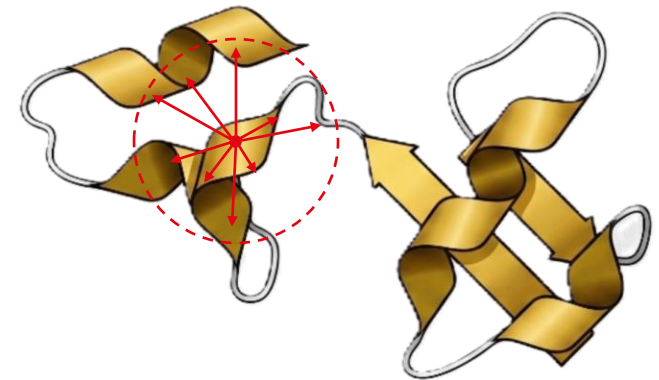
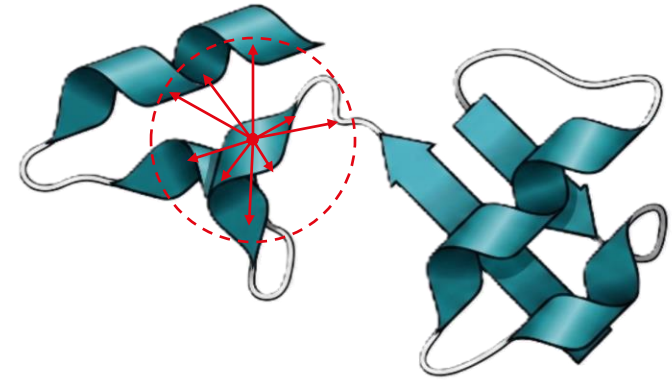
$$TM = \max \left( \frac{1}{L_N} \sum_{i=1}^{L_T} \frac{1}{1 + \left( \frac{d_i}{d_0} \right)^2} \right)$$
$$d_0 = 1.24 \times \sqrt[3]{L_N - 15} - 1.8$$

- Size independent
- Range: 0-1  
Higher is better



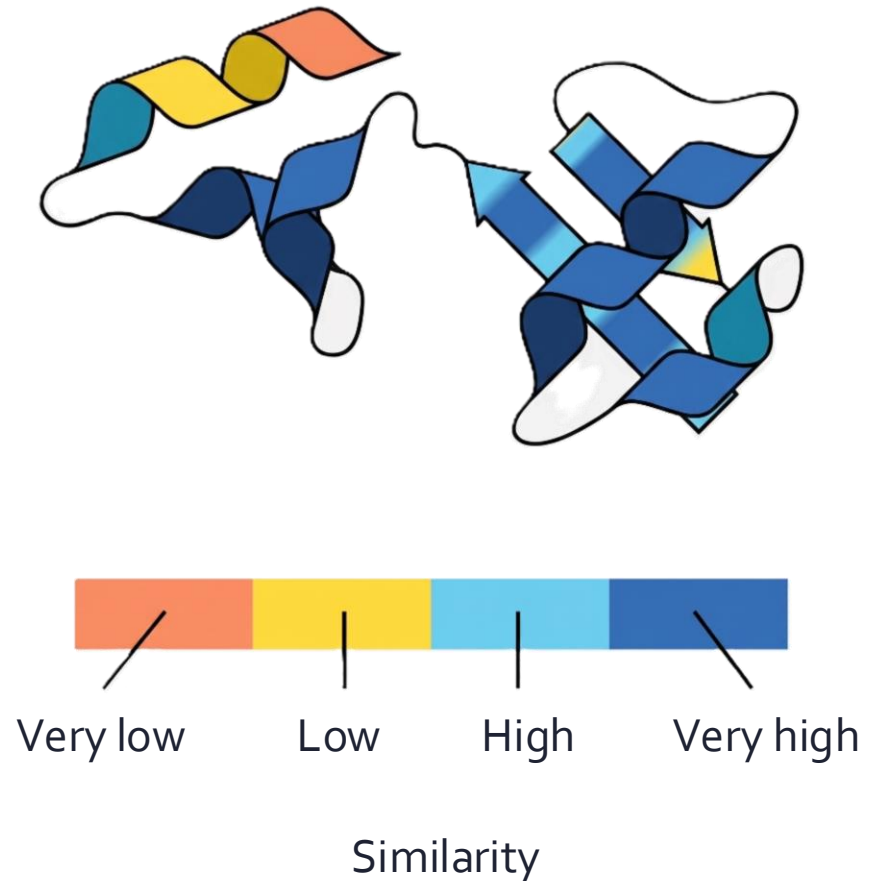
# LDDT (local distance difference test)

- Local metric, per residue
- Does not need alignment
- Fraction of conserved distances within a cutoff radius
- Range: 0-100  
Higher is better



# LDDT (local distance difference test)

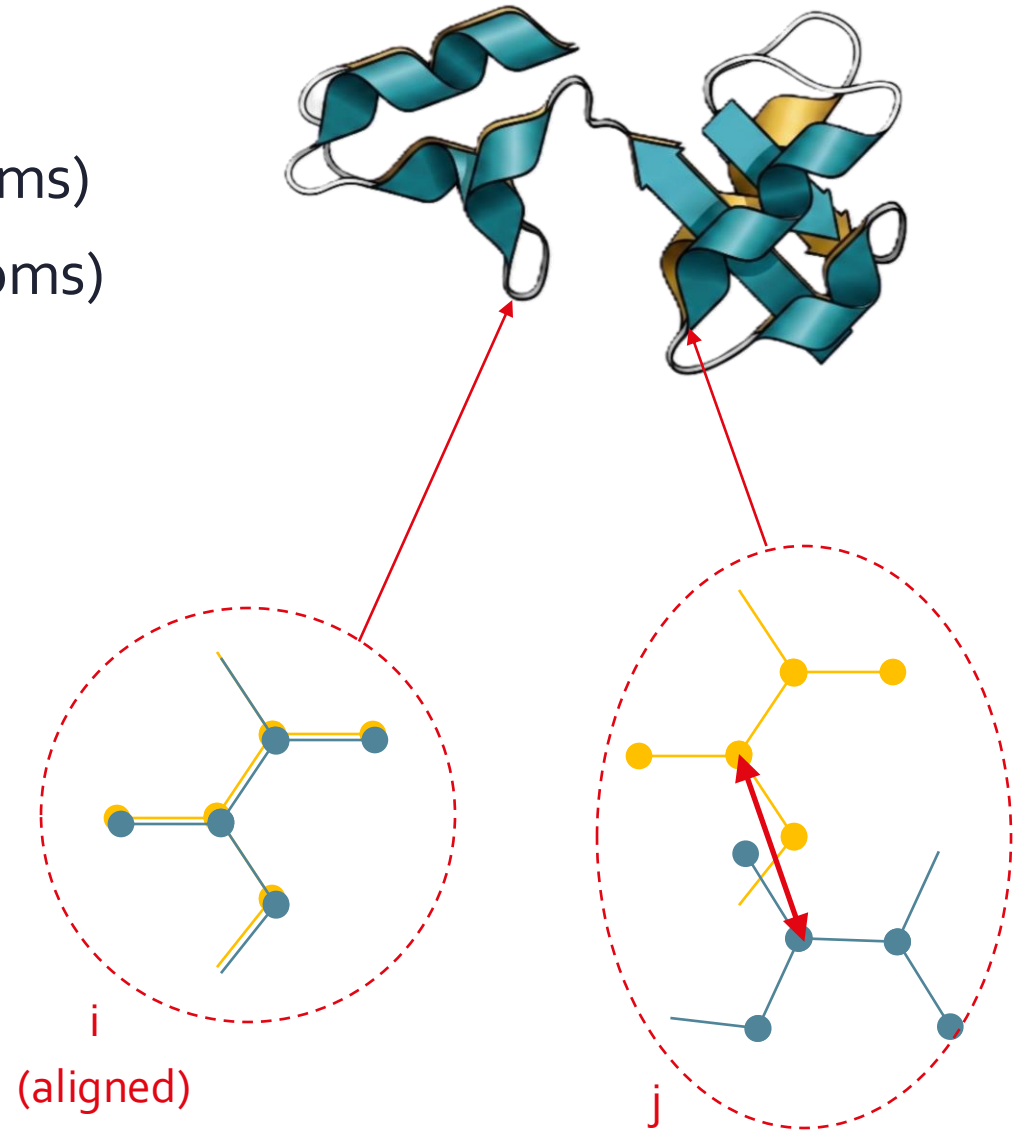
- Local metric, per residue
- Does not need alignment
- Fraction of conserved distances within a cutoff radius
- Range: 0-100  
Higher is better





# AE (aligned error)

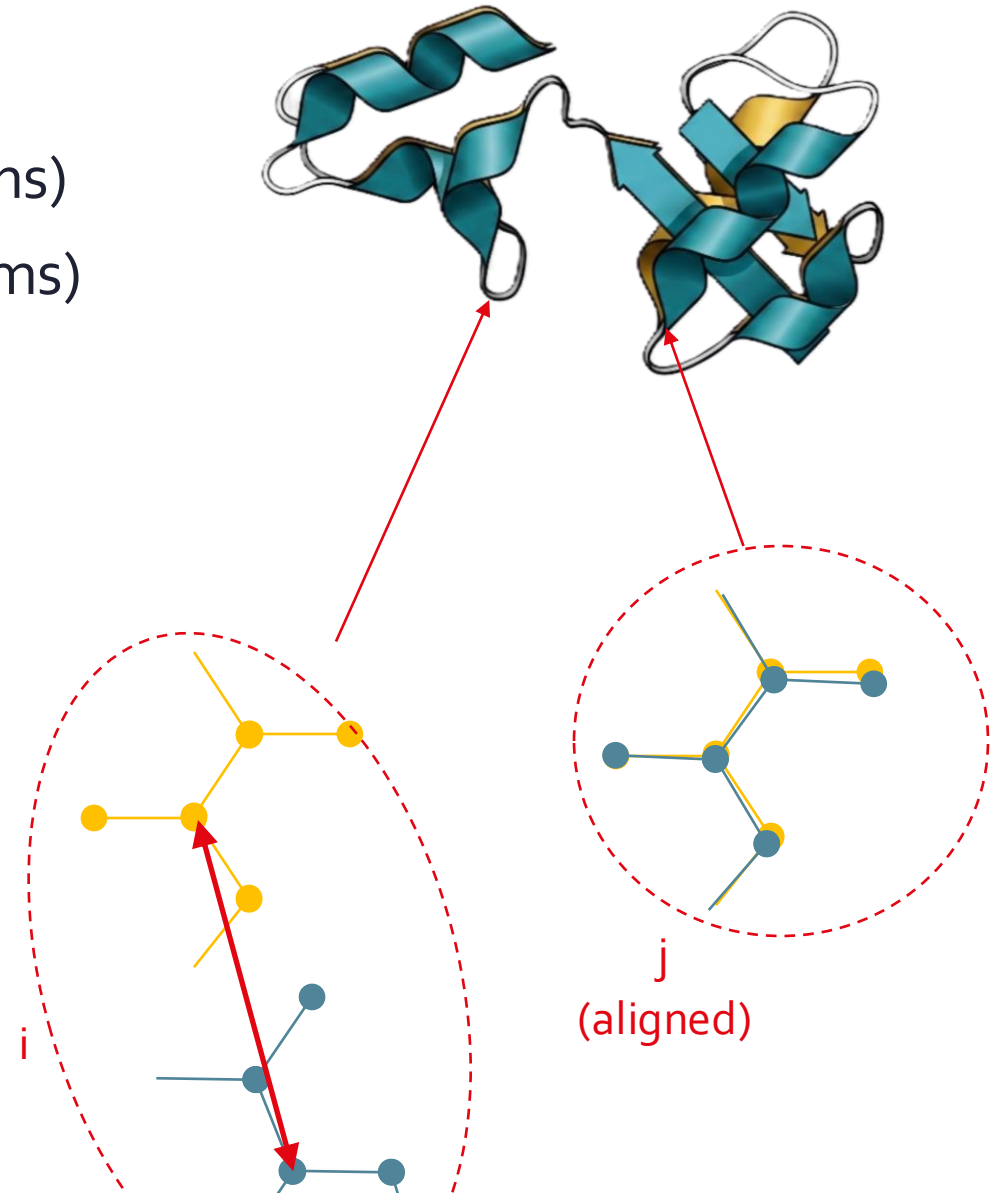
- For a pair of residues (i and j)
- Align around one residue (i) (N, C<sub>α</sub>, C atoms)
- Difference at the other residue (j) (C<sub>α</sub> atoms)
- Not symmetric





# AE (aligned error)

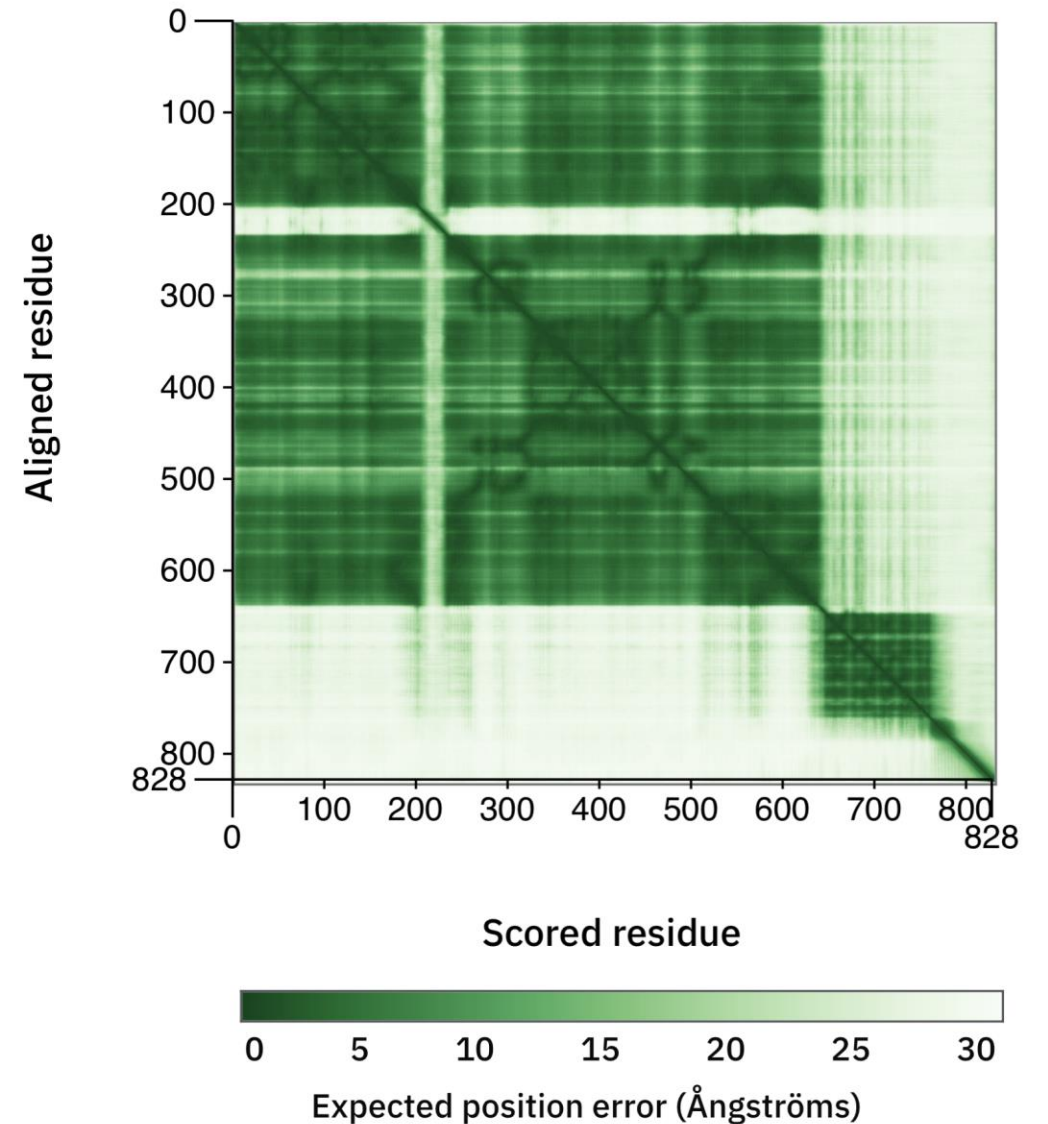
- For a pair of residues (i and j)
  - Align around one residue (i) (N,  $C_{\alpha}$ , C atoms)
  - Difference at the other residue (j) ( $C_{\alpha}$  atoms)
- 
- Not symmetric





# AE (aligned error)

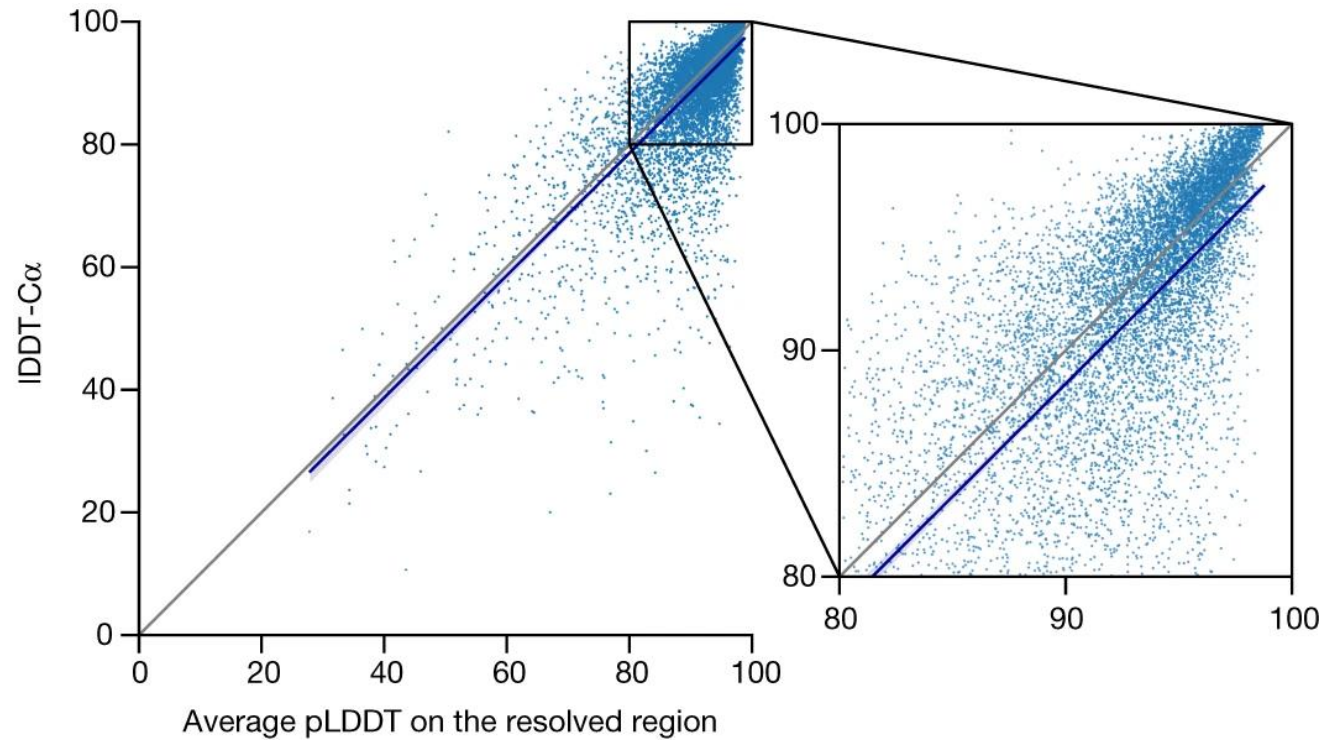
- For a pair of residues (i and j)
  - Align around one residue (i)
  - Difference at the other residue (j)
- 
- Not symmetric



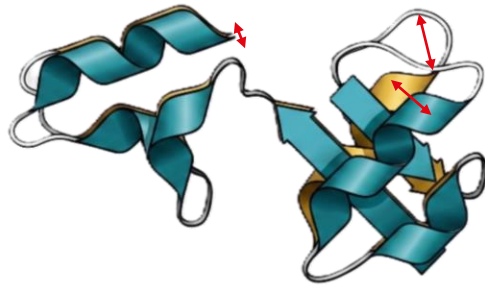


# Confidence metrics

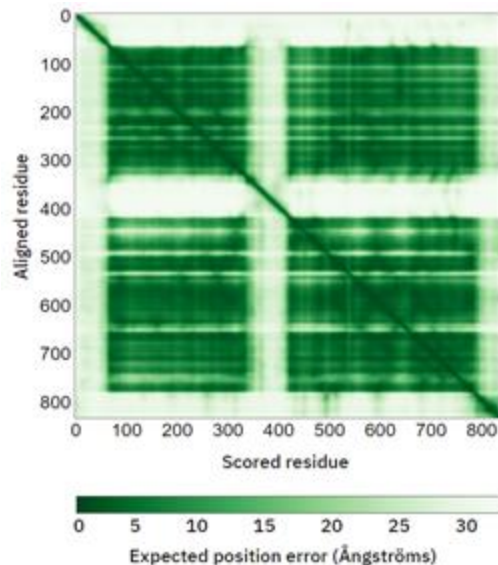
- Predicted
  - LDDT  $\rightarrow$  pLDDT
  - AE  $\rightarrow$  PAE
  - TM  $\rightarrow$  pTM
- Confidence of the model in the prediction
- Correlate with prediction quality (but not perfect)



- Can be thought of as being calculated from PAE (but isn't exactly in reality)



$$TM = \max \left( \frac{1}{L_N} \sum_{i=1}^{L_T} \frac{1}{1 + \left( \frac{d_i}{d_0} \right)^2} \right)$$

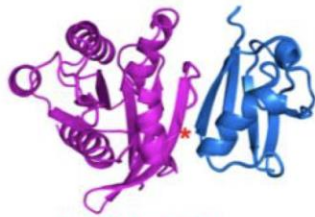


$$pTM = \max_i \left( \frac{1}{L} \sum_{j=1}^L \frac{1}{1 + \left( \frac{PAE_{ij}}{d_0} \right)^2} \right)$$

# ipTM (interface pTM)

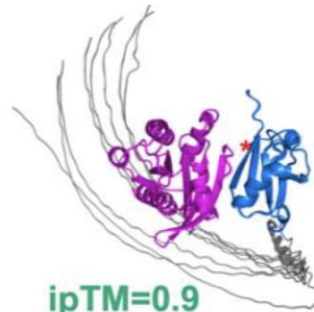
- pTM is calculated for the whole complex
- ipTM is calculated only for the interface
- Non-interacting domains and disordered regions can reduce it

**Example 1:**  
no disorder in  
either chain



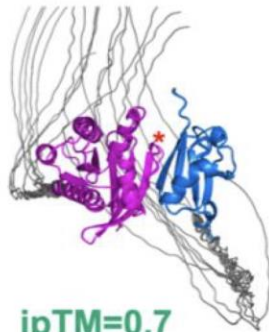
ipTM=0.9

**Example 2:**  
60 disordered  
residues in one  
chain



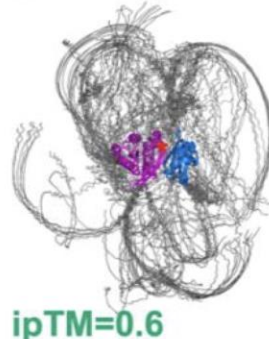
ipTM=0.9

**Example 3:**  
60 residues  
disorder in both  
chains

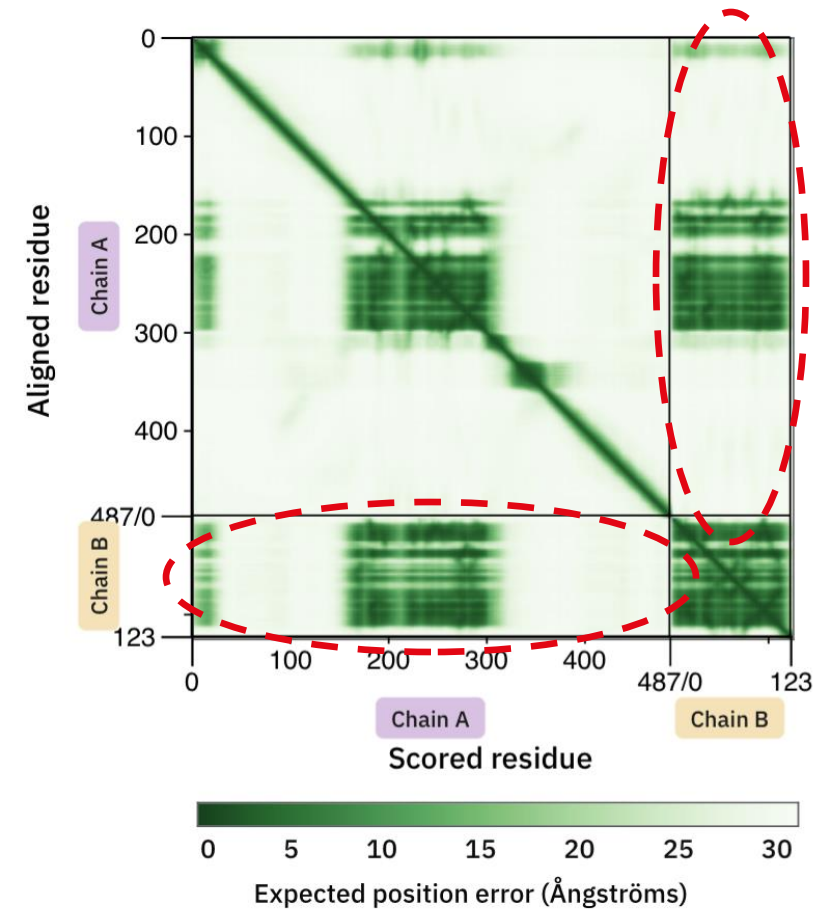


ipTM=0.7

**Example 4:**  
120 disordered  
residues in both  
chains



ipTM=0.6





# pTM and ipTM

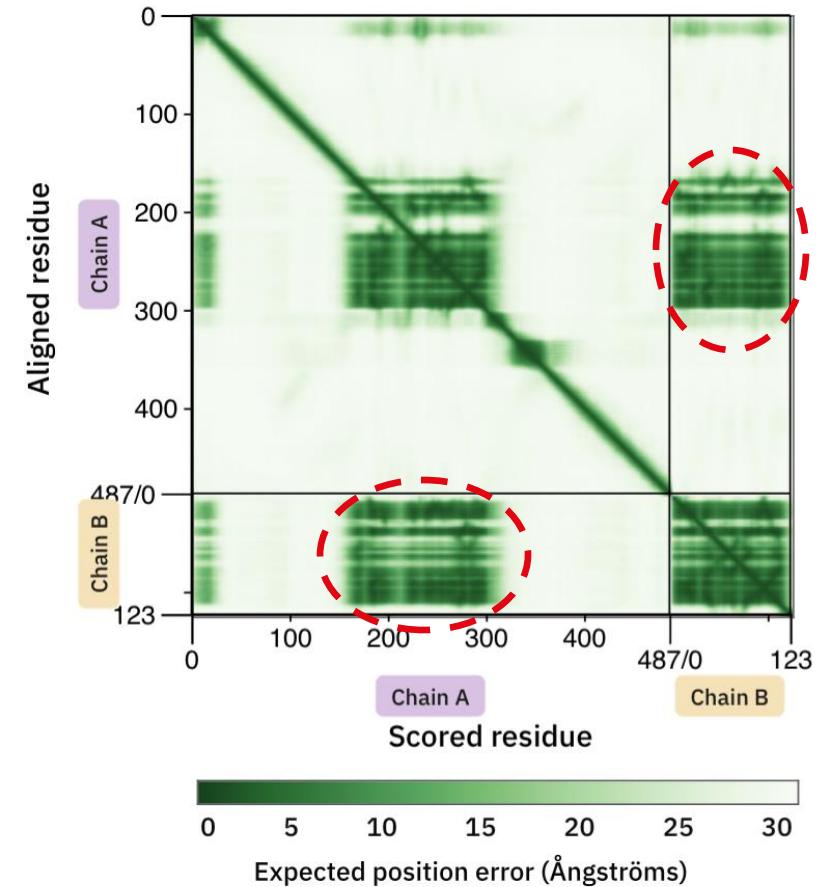
From AlphaFold3 GitHub:

- pTM above **0.5** suggests the complex fold may be broadly correct
- ipTM > **0.8**: confident; **0.6-0.8**: gray zone; < **0.6**: possibly failed
- The TM score is very strict for small structures or short chains:  
pTM assigns values less than 0.05 when fewer than 20 tokens are involved
- For these cases PAE/iLIS/ipSAE or pLDDT may be more indicative of prediction quality



# ipSAE (interaction prediction Score from Aligned Errors)

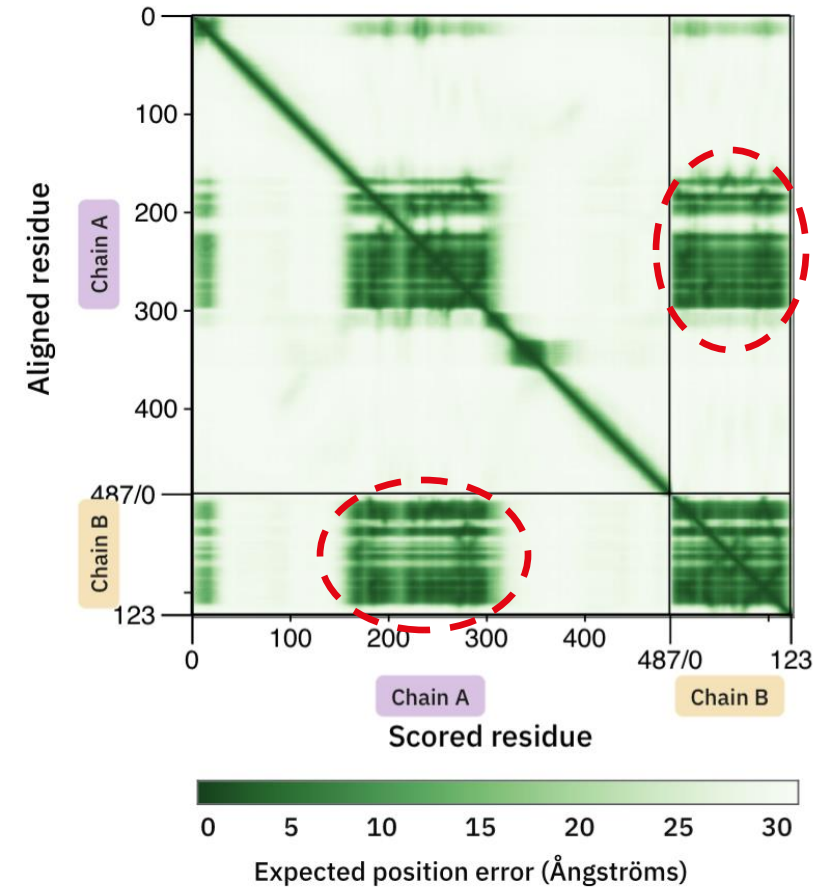
- Improved ipTM
- Includes only residue pairs with good PAE scores ( $PAE \leq 15 \text{ \AA}$ )
  - If both interacting chains contain a lot of disorder or accessory contacts that don't form the contact, it ignores those
- Calculated from PAE
  - Not calculated by AlphaFold
  - Calculated separately, from the AlphaFold outputs
- **ipSAE  $\geq 0.8$** : The predicted homodimer interface is modelled with high accuracy
- **0.7 - 0.8**: The predicted interface is likely correct with a well-arranged interaction
- **0.6 - 0.7**: The interaction may be correct but should be interpreted cautiously
- **< 0.6**: The predicted interaction is unlikely to represent a reliable complex





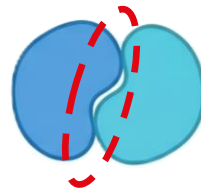
# LIS (local interaction score)

- conceptually similar to ipSAE
- Includes only residue pairs with good PAE scores ( $\text{PAE} \leq 12 \text{ \AA}$ )
- Calculated from PAE
  - Not calculated by AlphaFold
  - Calculated separately, from the AlphaFold outputs

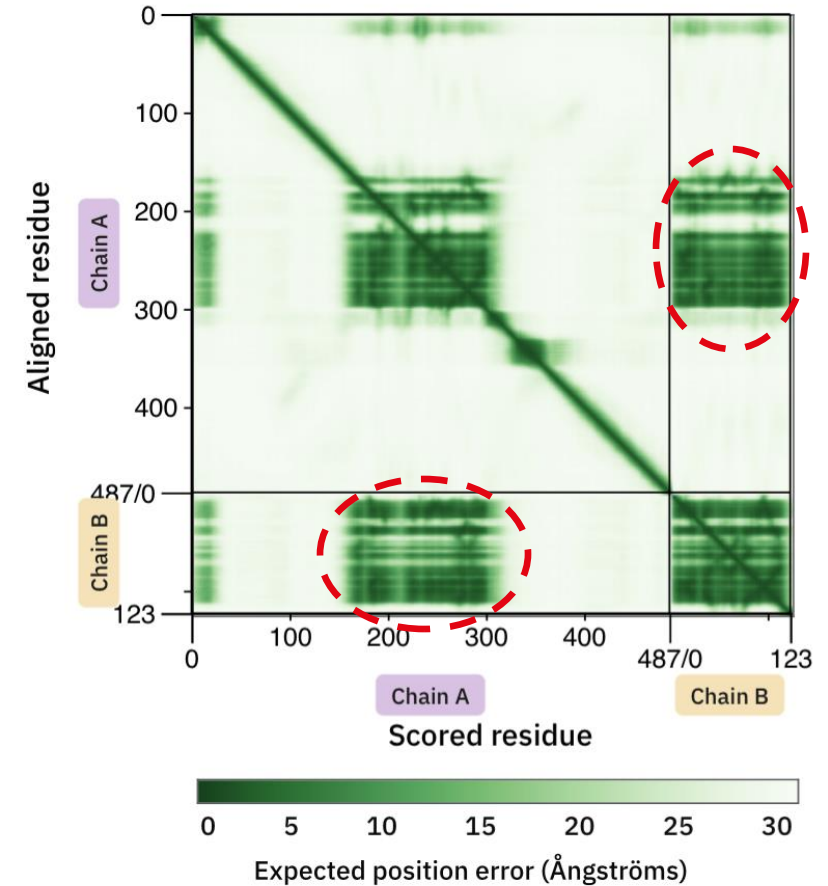


# cLIS (local interaction score)

- Includes only residue pairs with good PAE scores
- Includes only residue pairs that are physically close (PAE  $\leq 12$  Å and  $C_{\beta}$ - $C_{\beta}$  distance  $\leq 8$  Å)



- Calculated from PAE
  - Not calculated by AlphaFold
  - Calculated separately, from the AlphaFold outputs



- It is recommended to use iLIS score
- iLIS is a combination of LIS and cLIS:

$$iLIS = \sqrt{LIS \cdot cLIS}$$

- A high-confidence interaction is suggested if **iLIS  $\geq$  0.223**



# PAE Viewer

For interactive visualization of PAE matrix:

<https://pae-viewer.uni-goettingen.de/>

You can visualize dynamic PAE plots and match PAE data onto predicted structures stored in the `fold_<job_name>_model_<N>.cif` files.

## PAE VIEWER Interactive display of the Predicted Aligned Error

An error that occurs for some score JSON files which include the pLDDT has been fixed! ✕

If you're experiencing any display issues, please make sure you're using the newest version of your browser. The webpage was tested on Windows 10 with version 108 of Firefox, Chrome and Edge. On iOS, version 111 of Firefox and Chrome was used, as well as Safari 16.3 and Opera 97.0. ✕

Also, if the page is unresponsive, please make sure you're not using a browser plug-in blocking the cookie consent banner.

Examples   [Upload](#)   [Offline version](#)   [Citation](#)

Choose one of the following examples or use the 'Upload' tab to upload your own protein complex data. The examples are complexes predicted by AlphaFold-Multimer from O'Reilly et al. (2023).

[PdhA-PdhB-PdhI](#)   
 [SufD-SufC-SufB](#)   
 [FlIT-FlID](#)

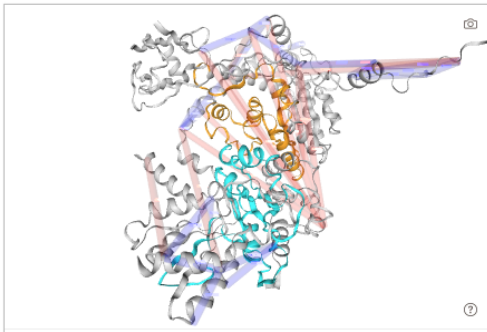
**PdhA (371 aa)**

MAAKTKKAIYEGKKQFDATKKQFFEQIINIKGEVNEAANFDLDDQIKKIMRRMVFTRVLDQRSISLH**QDRGLGYARTAGQASQIASHFALEKECFVLPYRDVFGIWHGLFLYSAFLTSGRHFGNCPDVAAS6PQI11GAGIITAGVAIGI**  
**KYRGRKAVASITGGGGI**SGDFYEGINRAGAYKAPALFVYNNRYAISTPVEKSAETIAQKVAAGIVGVQDGMPLAVYAATAEAKERAINGEGFTLLETITRYGPHMAGDOPTKYRKEIENEKQKDLVRFRAFLENKGLMSEEEAKVIE  
 DAKKEIKQAKKADAEPRQVTDLMKIMYKMPHNLLEQFIIYTKRESK

**PdhB (325 aa)**

MAQMTMIQAITDALRTELKBBENVLFGEDVGVNGGVFR&EGLQKFEKPRVDFDTFLA&SIPGGIAGL&SINGFRFVMB4DFF**GVVYEVNDVSVSGQAMARVRSGGRWISPVITRSFPF&GVHTPELH&SLEGLVAG&SIRKVVIPISPPDARGLLISA**  
**IRONDVVFLEHMKLYBSFRQEVVEEETIELGKADVREGTDLISITYG**AMVHESLKADELEKDGISAEEVDLRTVPLDITIIASVERTGRAIVVQEAQRQAGIAANVVAEINDRAILLSEAPVLRVAADPTVFFPSQAESVHFNKDVLETARKV  
 LEF

**Selected:** X: GLY 85 (PdhB) - GLY 211 (PdhB), Y: ARG 71 (PdhA) - ALA 179 (PdhA)



**PdhA - PdhB - PdhI**

**Confidence metrics:**  
 Mean pLDDT<sup>2</sup>: 95.4577  
 pTM<sup>1</sup>: 0.9229  
 ipTM<sup>1</sup>: 0.8940

**Structure color scheme:**  
 by subunit  
 model confidence (pLDDT)

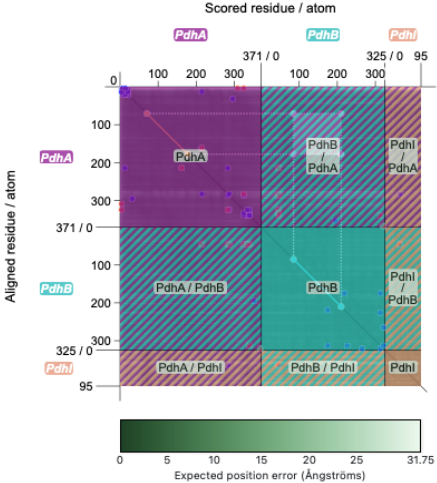
**Crosslink color scheme:**  
 satisfied / violated restraint  
 intra / inter protein links  
 hide all crosslinks

**Note:** a Ca-Ca distance ≥ 30 Å is considered a restraint violation.

**Scored residue / atom**

	PdhA	PdhB	PdhI
PdhA	0 / 0	371 / 0	325 / 0
PdhB	100 / 0	100 / 0	300 / 0
PdhI	300 / 0	300 / 0	95 / 0

**Aligned residue / atom**



Expected position error (Ångströms)

**Cursor:**

**Selection:**  
 X: GLY 85 (PdhB) - GLY 211 (PdhB), Y: ARG 71 (PdhA) - ALA 179 (PdhA)

**toggle region overlay**  
 (alternatively, hold the 'Shift' key when the cursor is within the PAE Viewer)

[Download PAE matrix image](#)  
[Download graph as SVG](#)



# LIVIA

Browser-based tools for analyzing protein-protein interactions from structure predictions. All analysis runs locally in your browser — no data leaves your device and no installation is needed.

Calculates iLIS, ipSAE etc.

Web: <https://flyark.github.io/LIVIA/>

GitHub: <https://github.com/flyark/LIVIA.git>

Preprint: [Kim & Perrimon 2026, bioRxiv](#)