Analysis of AlphaFold2 Predictions for MPOX-22 Proteins

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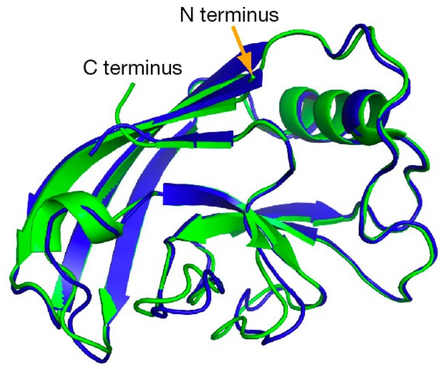
Norman Borlaug Endowed Research Scholar

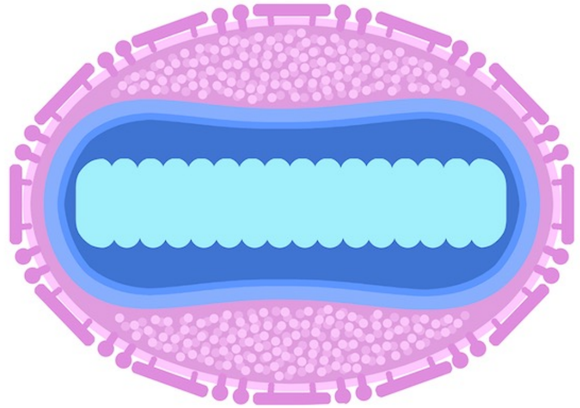
Review So Far

- **We used AF2 to predict protein 3D structure**
- **Each assigned a protein from the recently assembled MPOX-22' outbreak strain**
- **Today we are going to analyze our predicted structures and even infer the expected function**

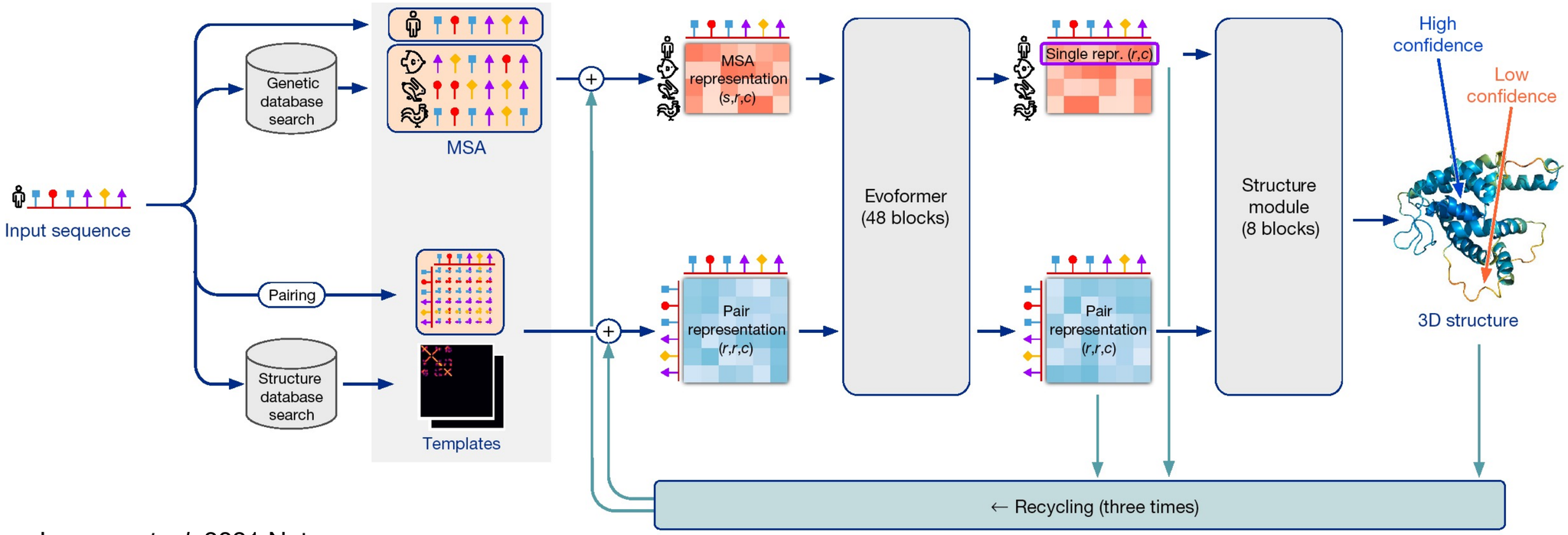


AlphaFold Experiment
r.m.s.d.$_{95}$ = 0.8 Å; TM-score = 0.93

Jumper, *et. al.* 2021 Nature.



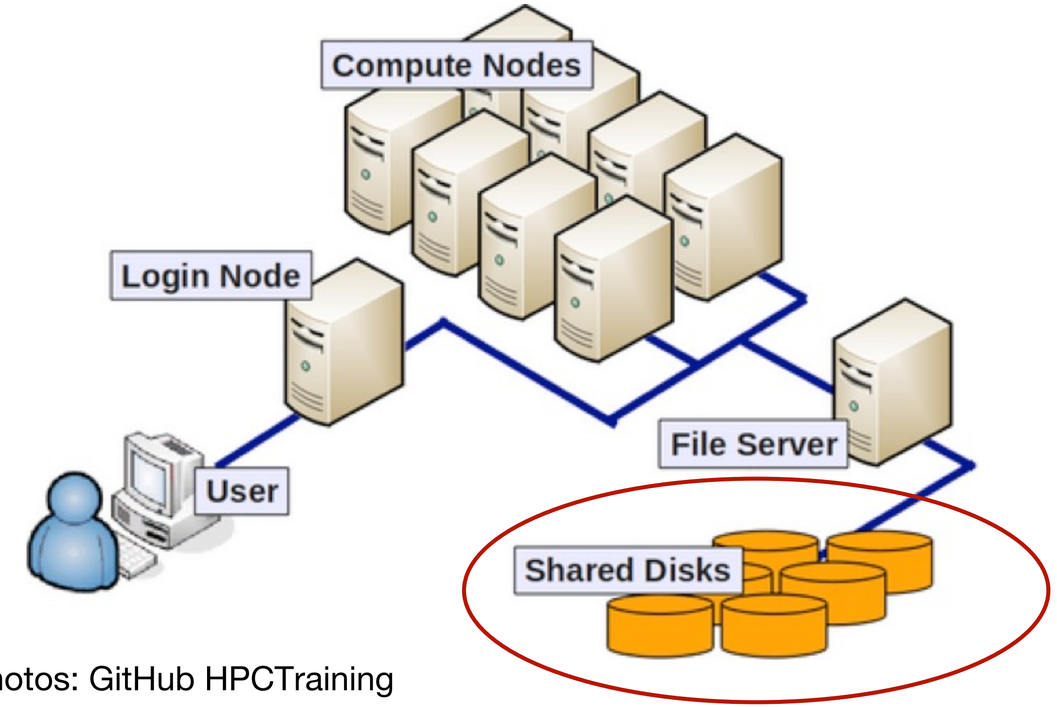
https://en.wikipedia.org/wiki/Mpox



Jumper, *et. al.* 2021 Nature.

Extracting Our Output For Analysis

- Our data is stored here, we need to offload it so that we can analyze it
  - You will download the files ranked_0.pdb, ranked_debug.json
  - and any file ending in the .pkl extension
  - You will upload the entire output folder to the class drive folder
  - You also must install ChimeraX for us to view the structures



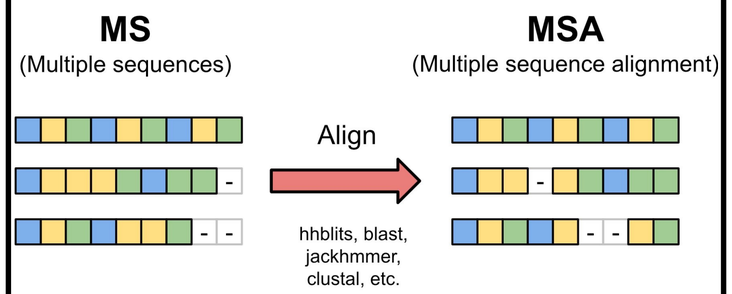
Photos: GitHub HPCTraining

Output of AlphaFold2

https://github.com/deepmind/alphafold#alphafold-output

How Can We Evaluate Our Confidence In The Model?

Sequence Homology

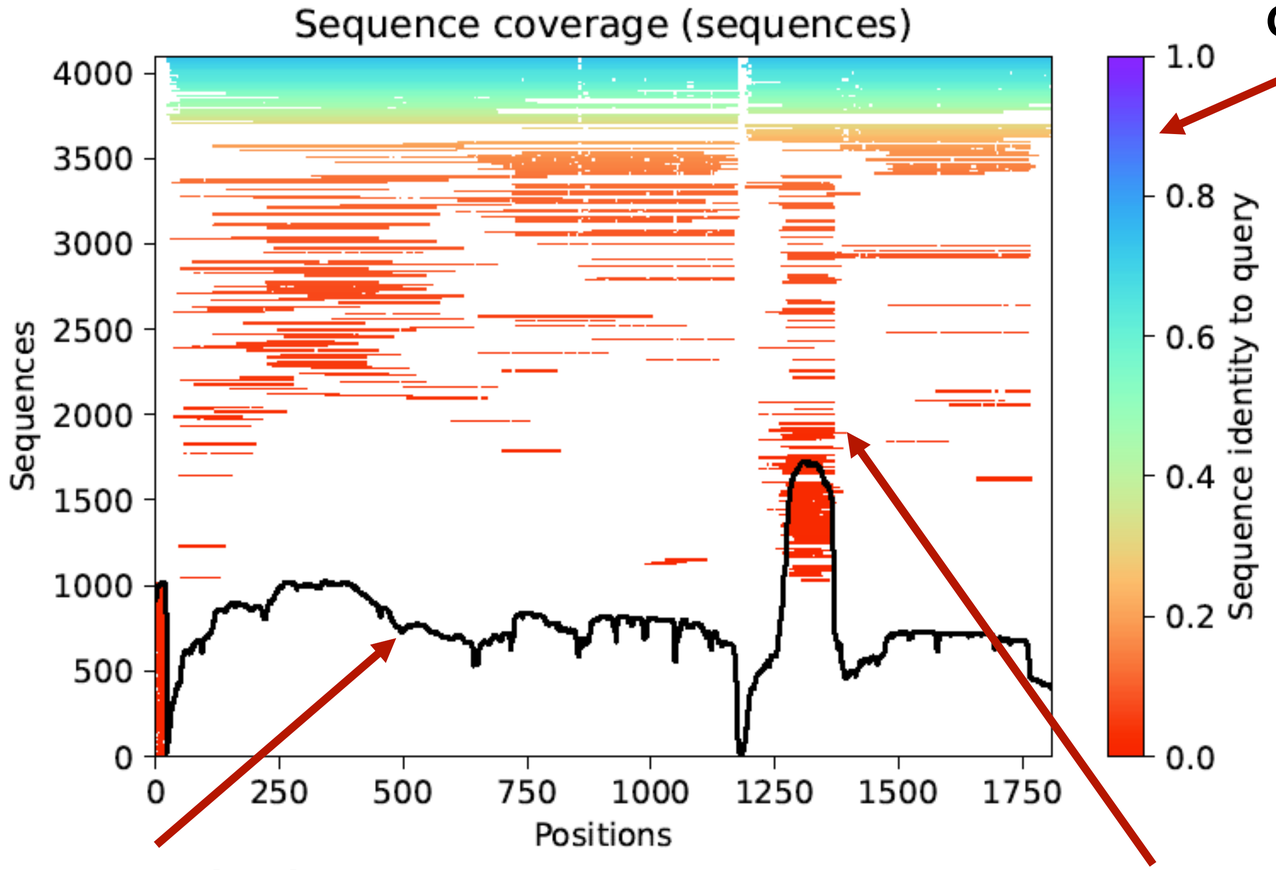


Petti S, *et. al. Bioinformatics*. 2023;39(1)

- **THE limiting factor**
  - **sequence coverage depth**
  - **>30 sequences/residue**
- **Typically areas of low coverage:**
  - **random disordered coils**
  - **low pLDDT**
  - **High pAE**

Sequence Coverage Plot

Sequence coverage plot from FLS2-BAK1-flg22 Receptor Complex - From AlphaFold 2.1.3



Color indicates how similar the protein is to our sequence

Black line shows coverage depth (i.e. how many homologous sequences at given residue)

Each line here represents a single sequence (protein)

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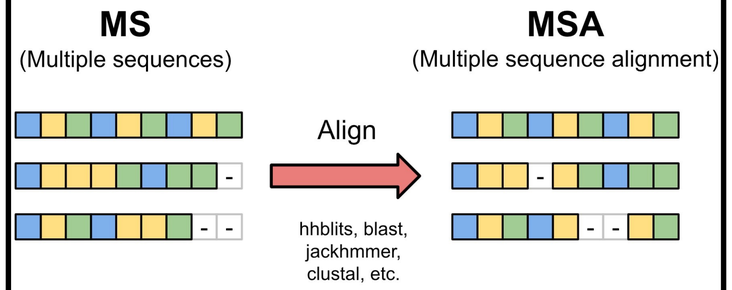

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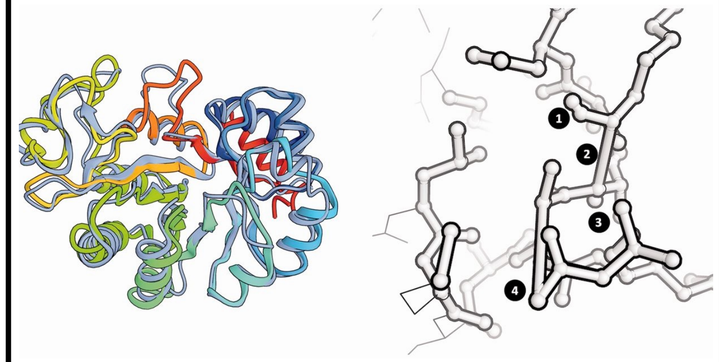
Side-Chain Cα Confidence



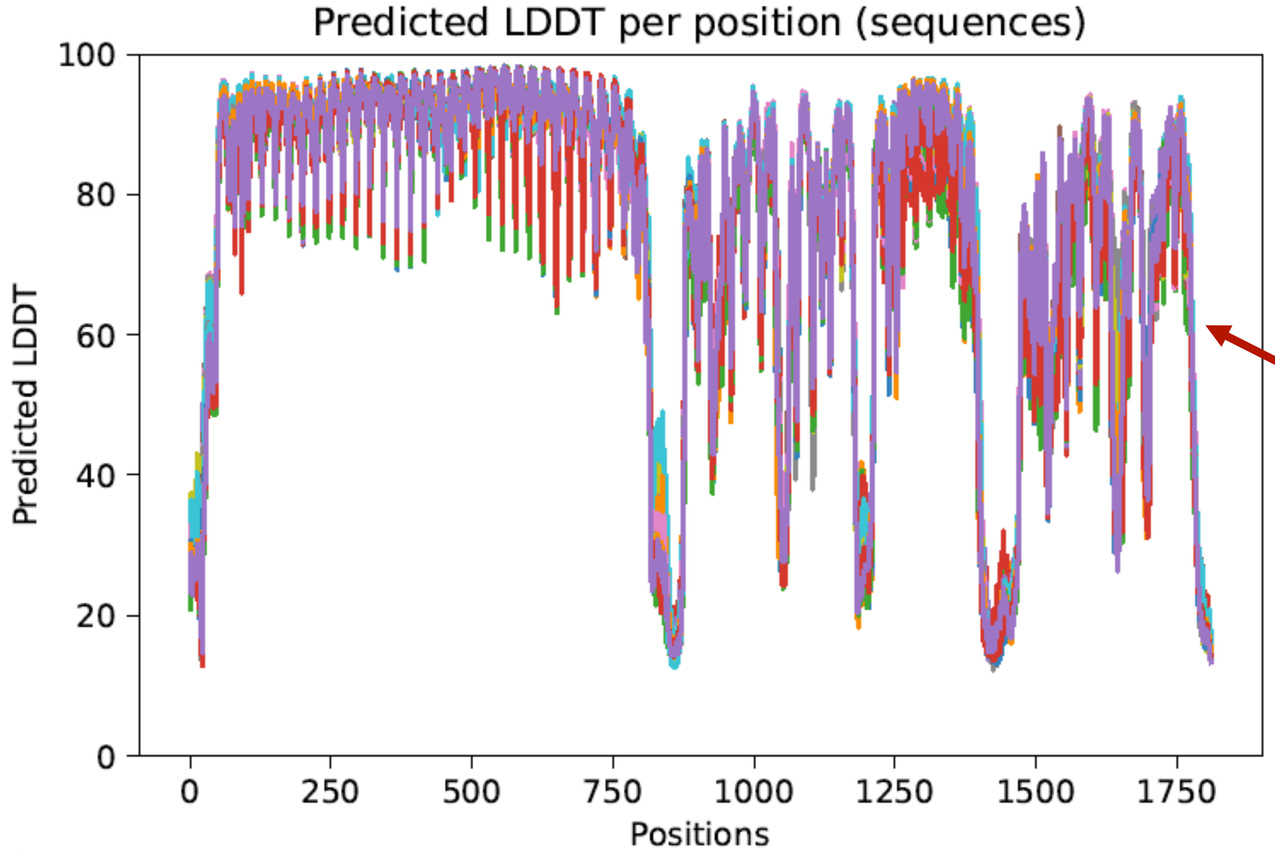
Mariani V, *et. al., Bioinformatics*. 2013;29(21)

- **Local Distance Difference Test**
  - **3D-structure depedent**
  - **(LDDT)**
- **R-group "feasibility"**
  - **Very High (pLDDT > 90)**
  - **Confident (90 > pLDDT < 70)**
  - **Low (70 > pLDDT > 50)**
  - **Very Low (pLDDT < 50)**

***suitable for experimental design**

Predicted Local Distance Differance Plot

pLDDT plot for 25 models of FLS2-BAK1-flg22 Receptor Complex - From AlphaFold 2.1.3



Each line represents a different predicted model for the structure (Your's will only have 5)

Remember we want this value to be as >90 for experimental design

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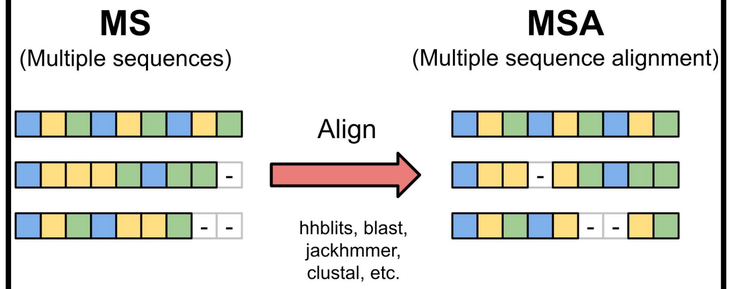
Sequence Homology



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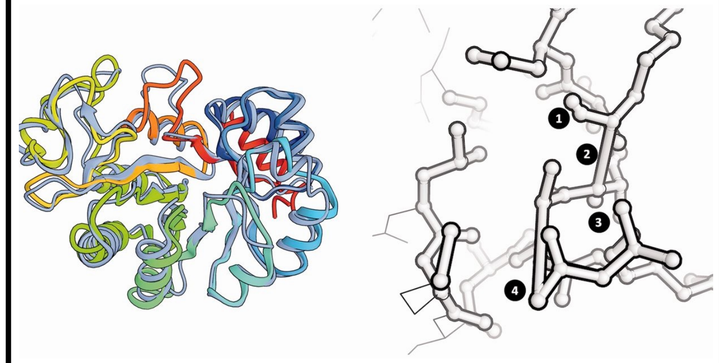

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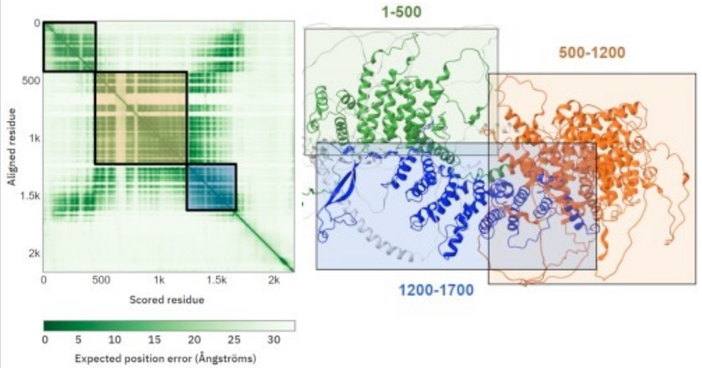
Inter-domain Accuracy

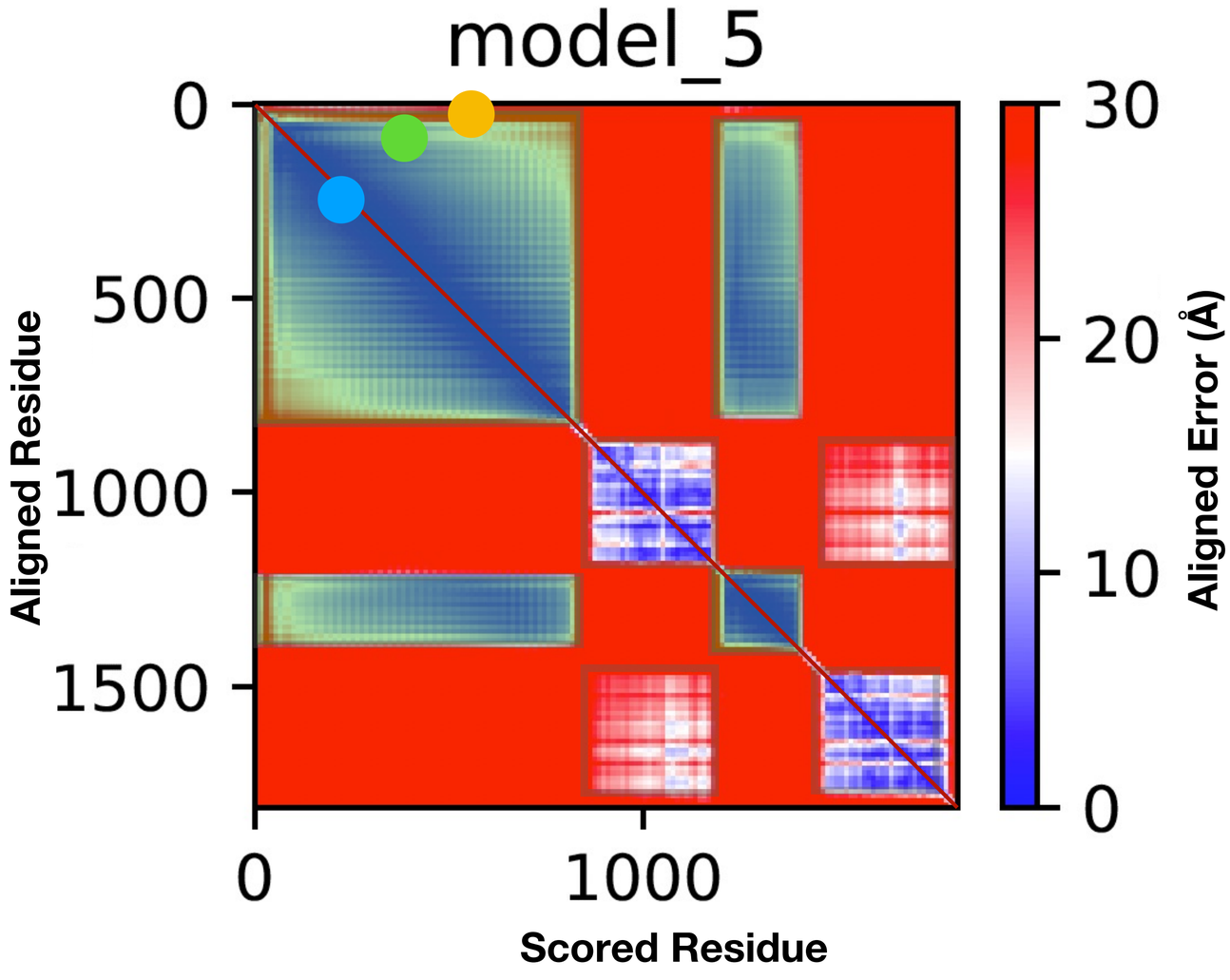


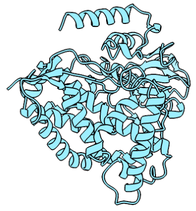
Varadi M, *et. al., Nucleic Acids Res*.2022;50

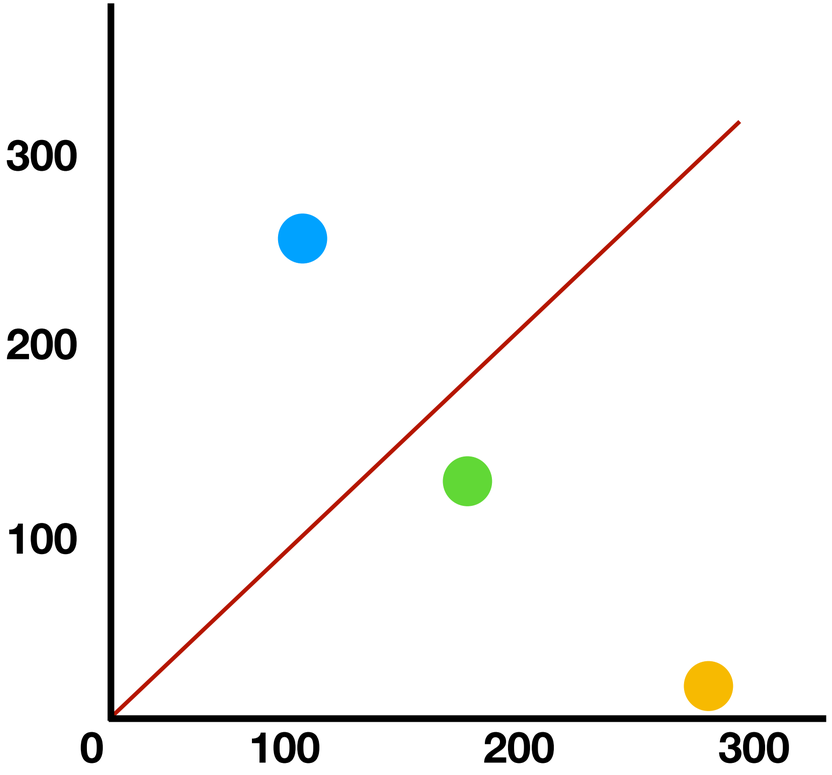
- **Aligned Error**
  - **3D-structure indepedent**
  - **(AE)**
- **Relative position of domains**
- **Mutual location of domains**

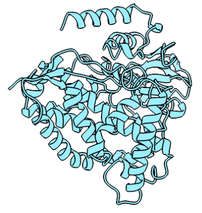
Predicted Aligned Error Plot

pAE plot for model 5 of FLS2-BAK1-flg22 Receptor Complex - From AlphaFold 2.1.3



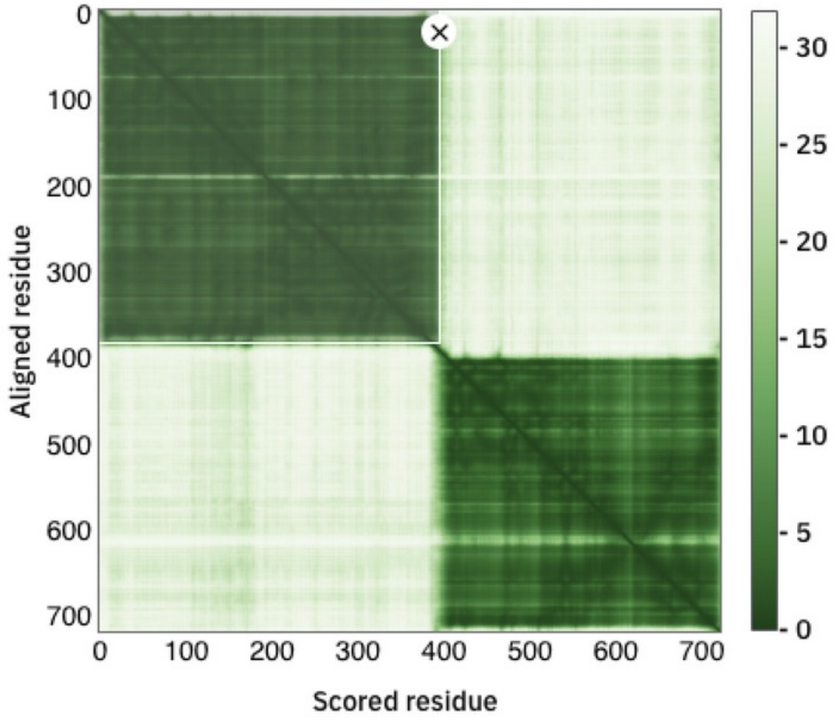






This is the “hardest” plot to interpret and takes practice

Bad PAE Example



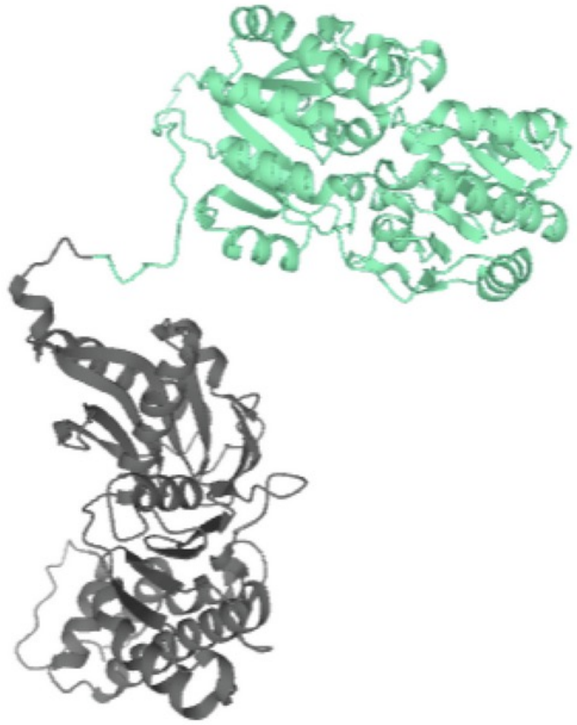


Photo: AlphaFold2 Database, PAE Tutorial

Good PAE Example

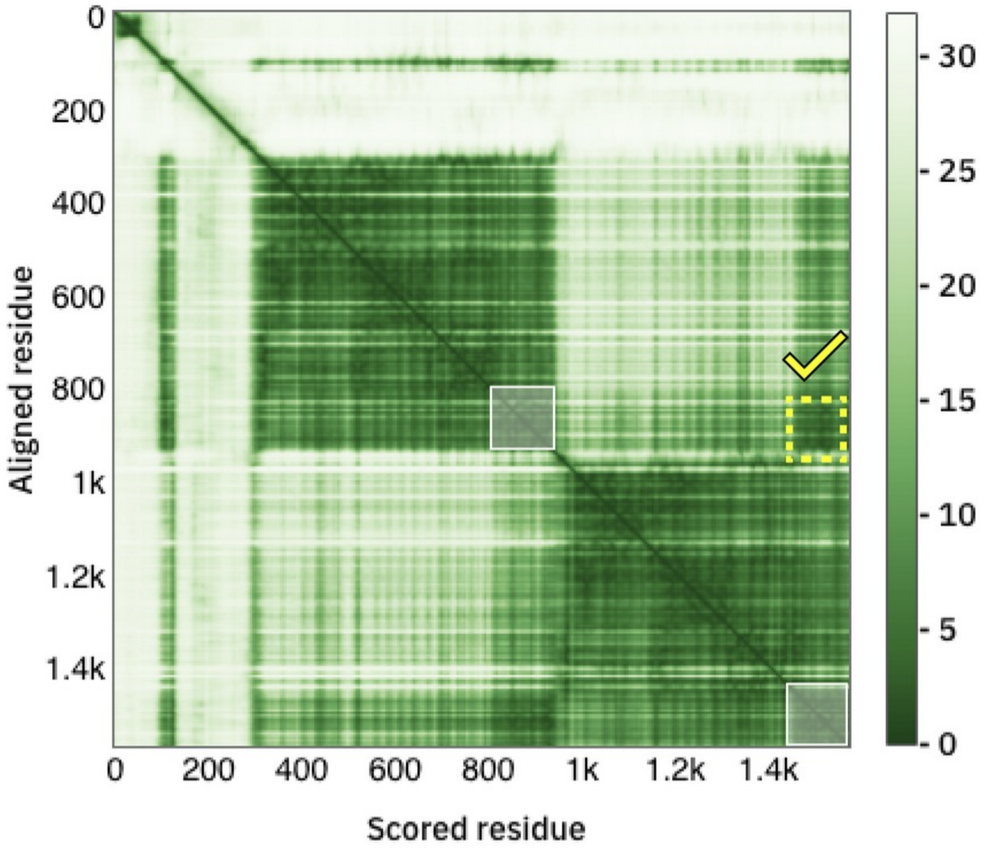
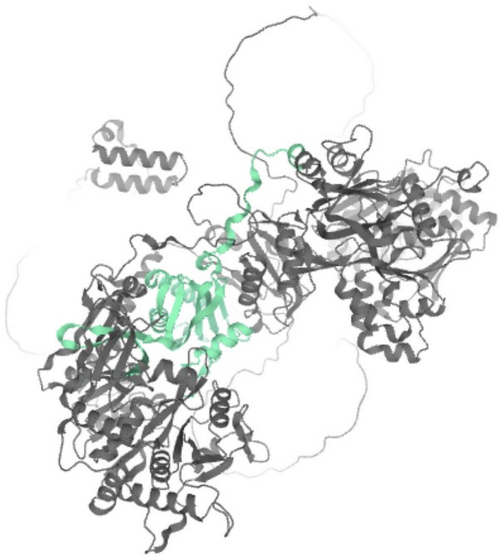
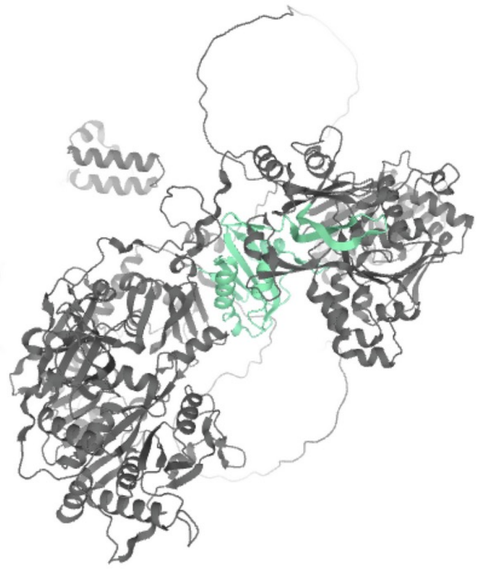


Photo: AlphaFold2 Database, PAE Tutorial

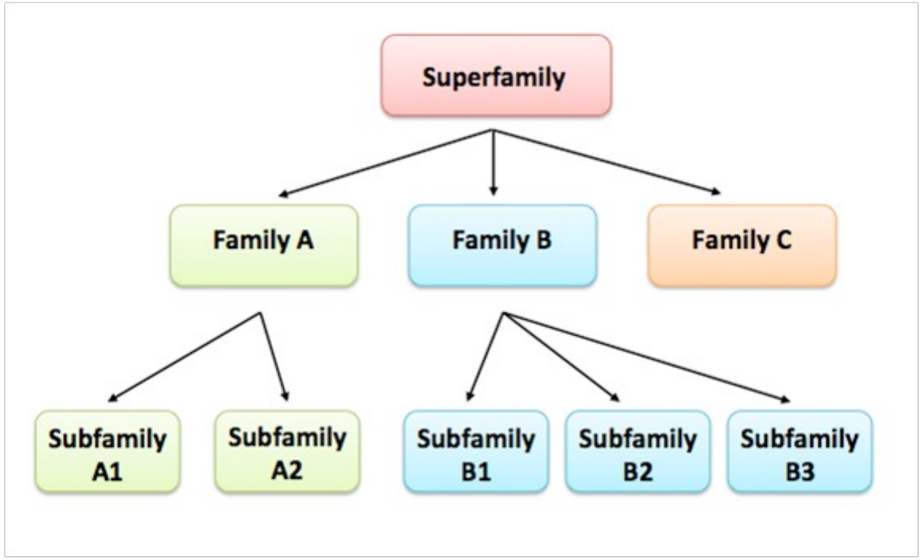
1.

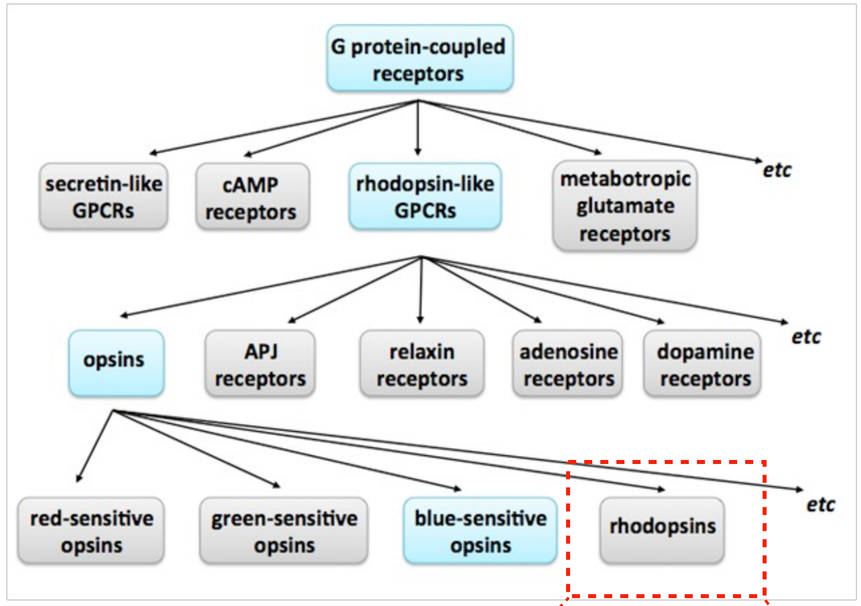


2.

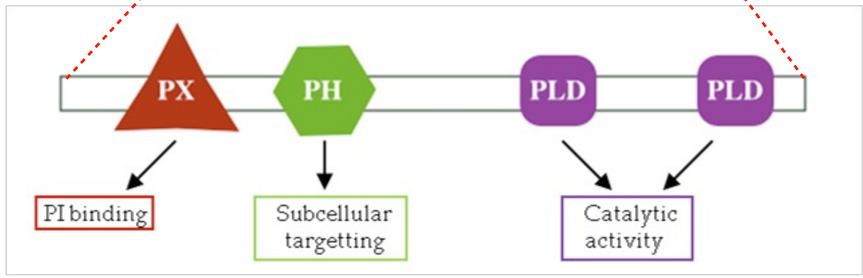


Using Protein Conserved Domains to Probe Function





We can leverage this classification system to probe potential function of our protein sequences.



Photos: EMBL-EBI Rescources, Protein Classification

Combining Predicted Structures & Conserved Domain For Sequence Annotation

- So far you have….
  - predicted 3D structure
  - analyzed sequence for conserved domains
- Combine all of this information
  - structure confidence
  - predicted protein function
- Can you think of a way we could test our structure for cofactor/substrate binding?
- Conclusions:
  - What metrics do we have for scoring AF2 model confidence?
    - pLDDT (3D structure depedent, R chain placement)
    - pAE (3D structure indepedent, inter-domain placement)
  - What situtations is AF2 evaluated for?
    - Single monomeric, naturally occuring protein chains
  - What is the largest limitation in AF2?
    - Homologous sequence coverage