An Introduction to Protein Structure Prediction with AlphaFold

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QLS
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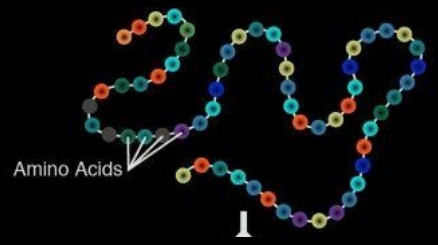
Seminar Overview

1. Summary of **protein structure** and modeling
2. Overview of **AlphaFold functionality**
3. **How to use** AlphaFold and why you may want to
4. Processing outputs and **next steps**

Protein Structure Modeling – A brief history

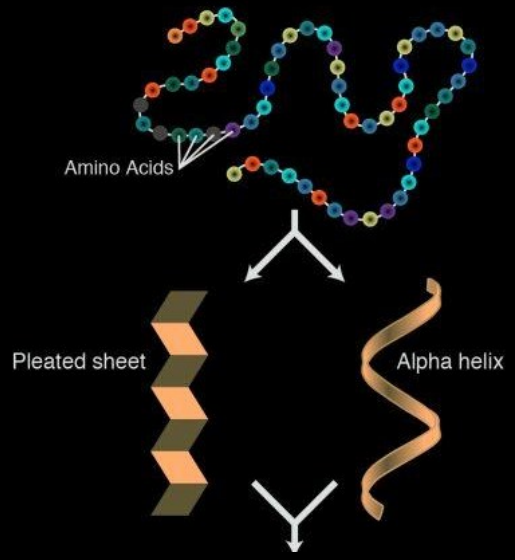


Primary Structure → Amino acid sequence of a protein.

Christian B. Anfinsen

Anfinsen's Dogma: The 3-dimensional structure, under standard physiological conditions, is governed by the amino acid sequence of a protein

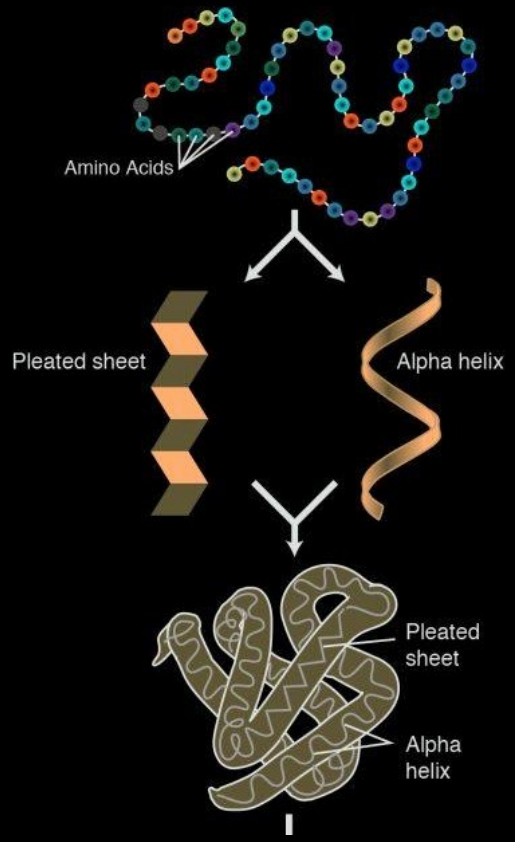
Protein Structure Modeling – A brief history



Primary Structure → Amino acid sequence of a protein.

Secondary Structure → Hydrogen bonds cause local folding into sheets and helices

Protein Structure Modeling – A brief history

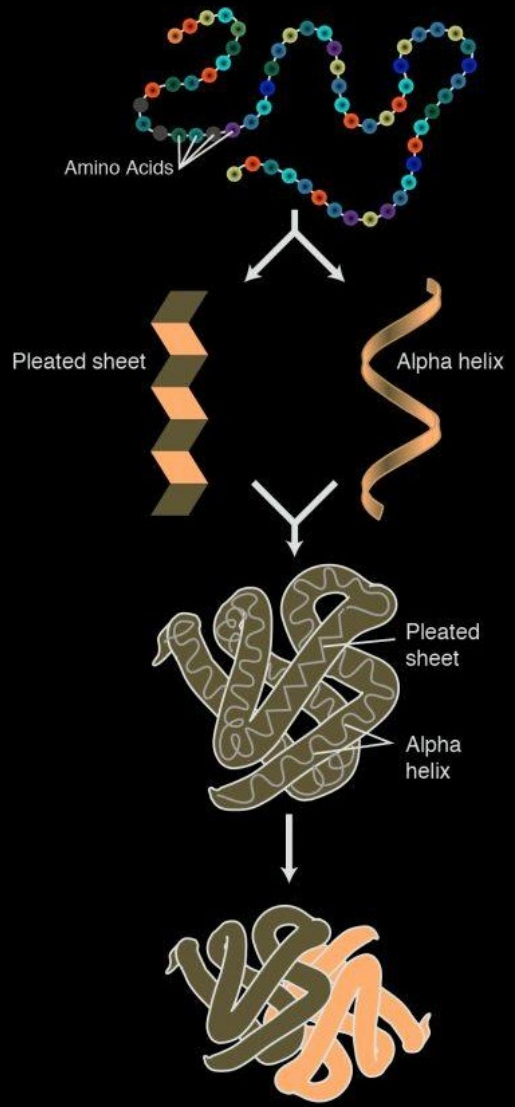


Primary Structure → Amino acid sequence of a protein.

Secondary Structure → Hydrogen bonds cause local folding into sheets and helices

Tertiary Structure → Functional protein stabilized by side-chain interactions

Protein Structure Modeling – A brief history



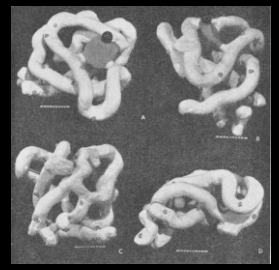
Primary Structure → Amino acid sequence of a protein.

Secondary Structure → Hydrogen bonds cause local folding into sheets and helices

Tertiary Structure → Functional protein stabilized by side-chain interactions

Quaternary Structure → Multi-chain protein structure (channels, dimers, etc.)

Protein Structure Modeling – A brief history

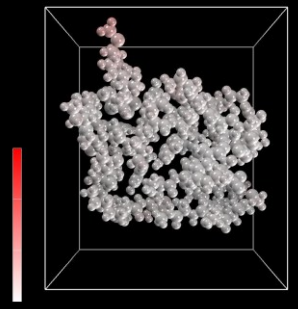


Myoglobin structure solved

1958

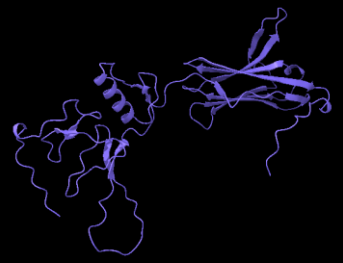
Protein structure linked to sequence

1973



Energy minimization tools AMBER and CHARMM

1980s



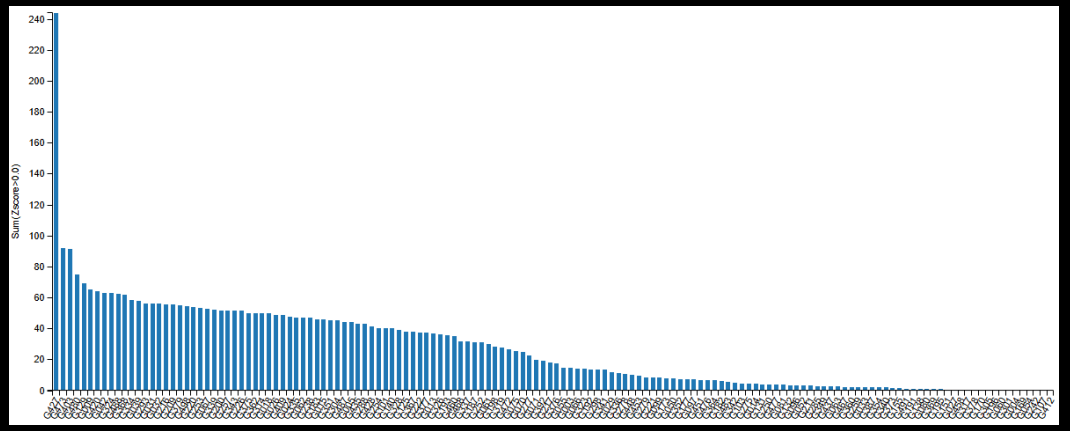
Swiss-Model, an early homology modeling server

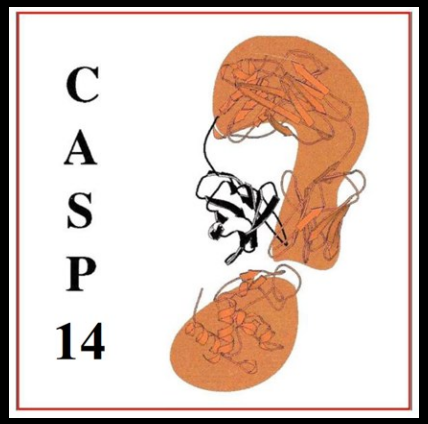
1993

HMMs, deep learning, and evolutionary analyses

2000-2010s

2018

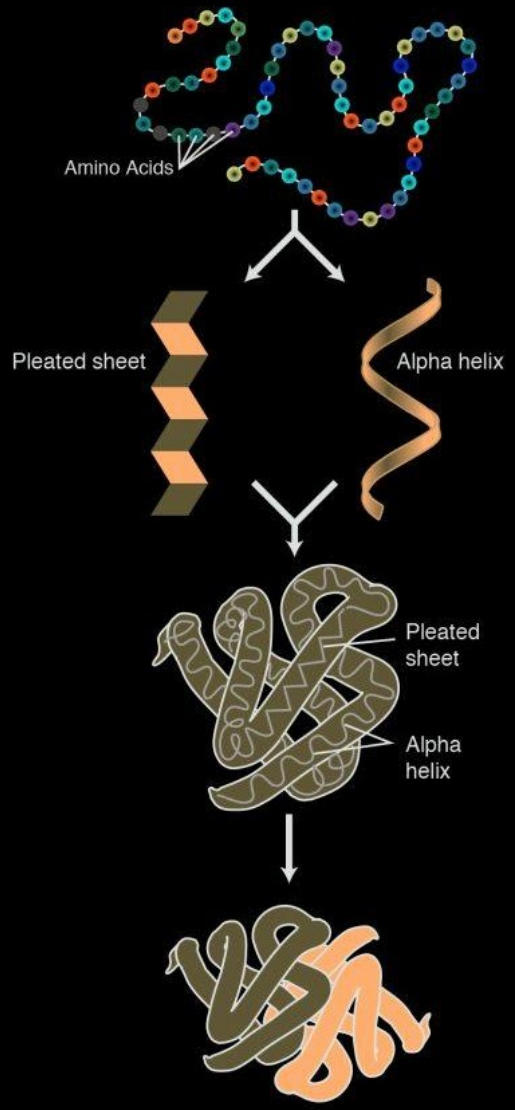




Google DeepMind

To date, Alphafold has solved the structures of over 200 million different proteins.

AlphaFold is not a Large Language Model



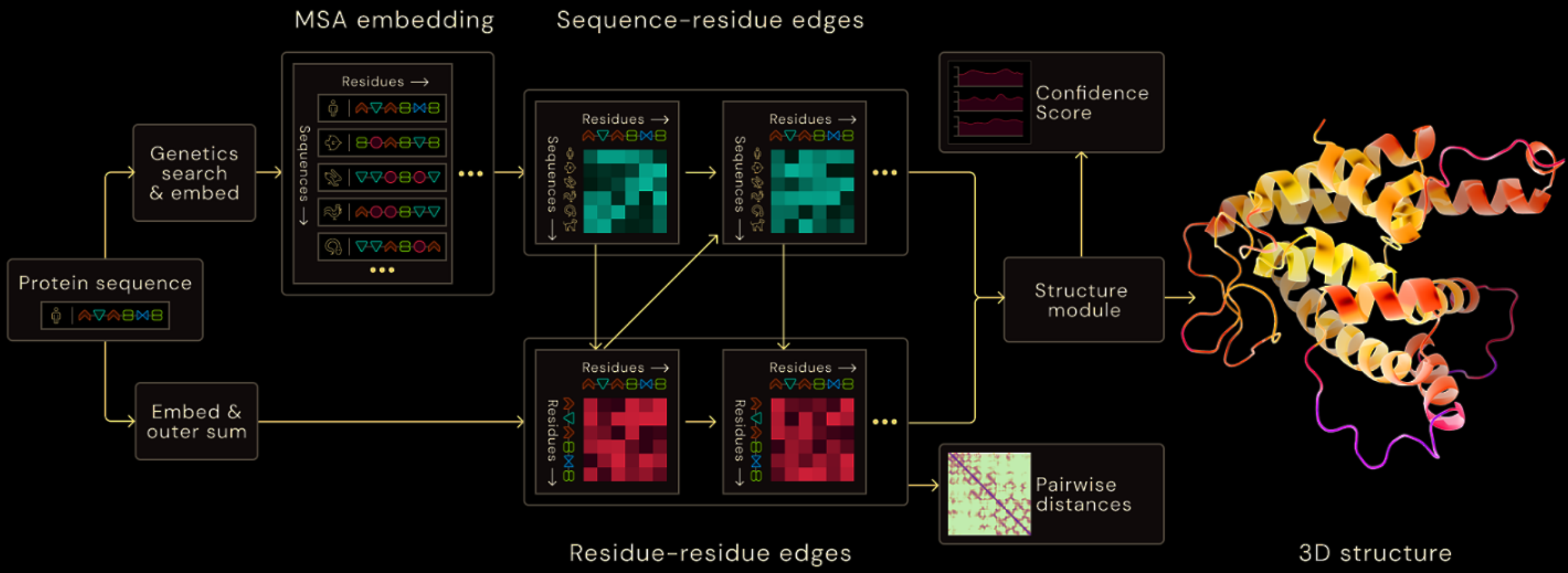
Primary Structure → Alphabets; Letters

Secondary Structure → Individual Words

Tertiary Structure → Complete Sentences

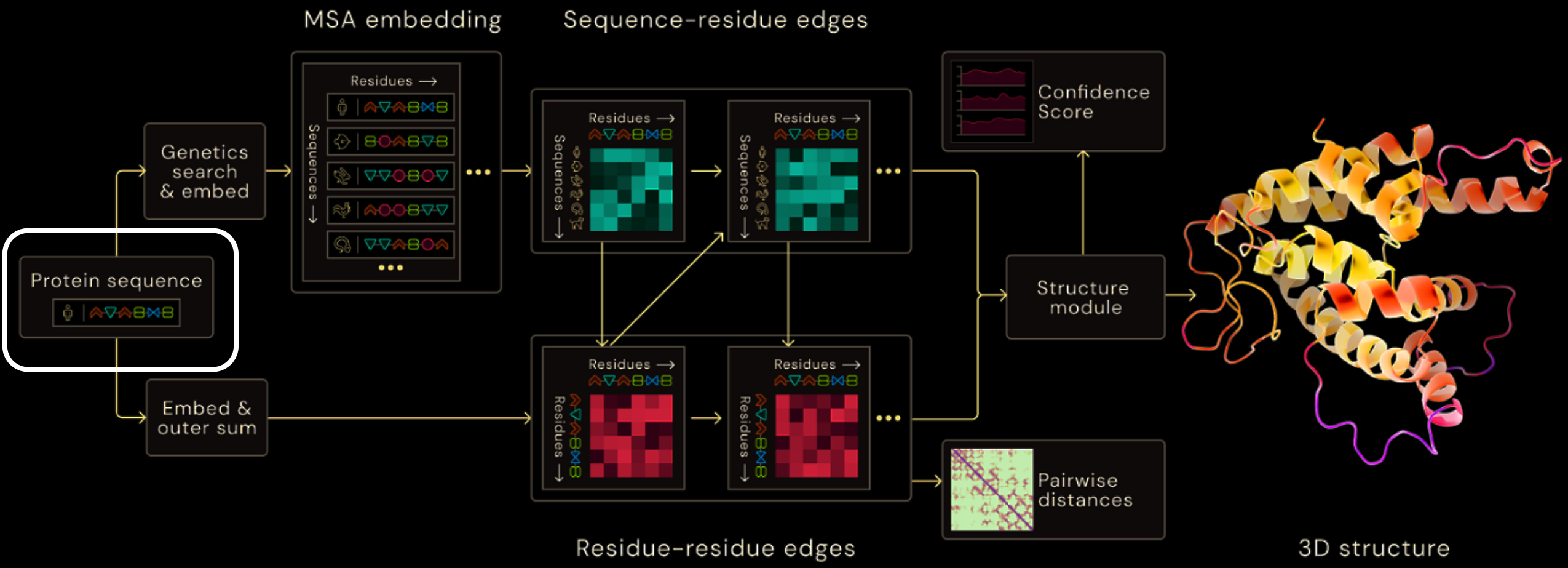
Quaternary Structure → Full Paragraphs; Passages

What is AlphaFold?



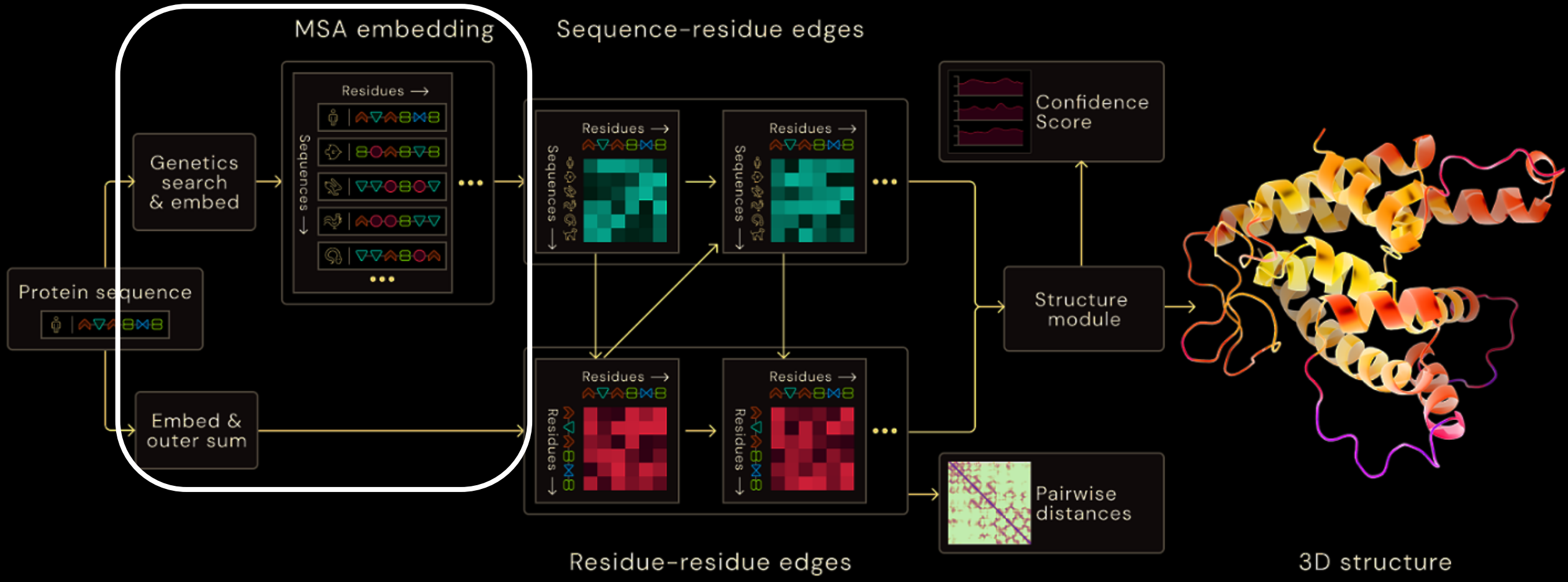
AlphaFold is a neural network-based AI model

How does AlphaFold work? - Inputs



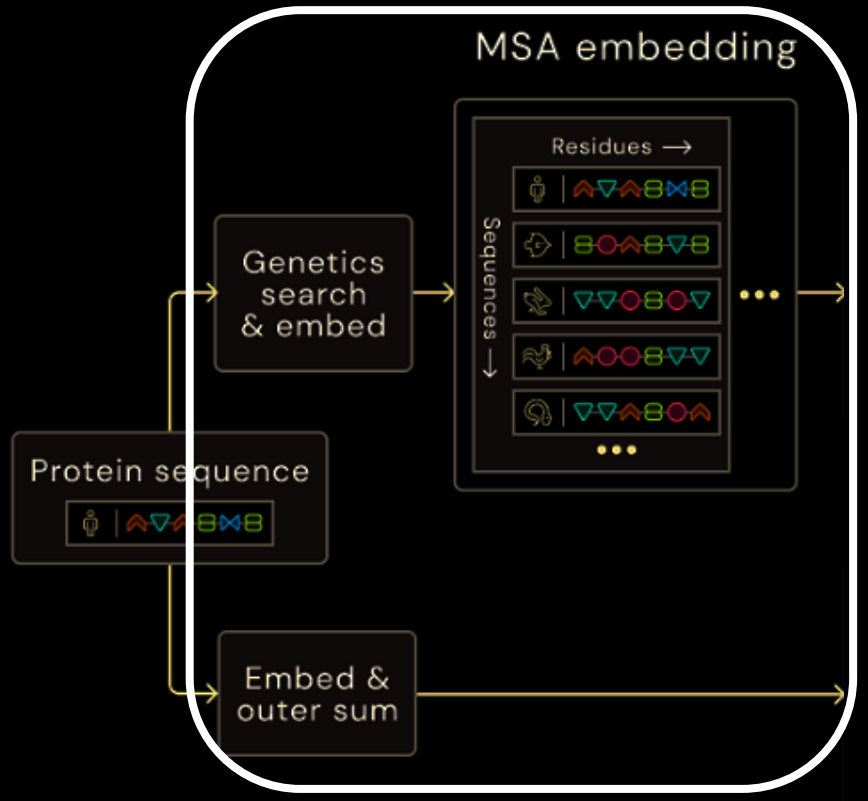
- Input protein sequence and desired cofactors (AF3: PTMs, DNA, Ions, etc)

How does AlphaFold work? - Embedding



- Generation of sequence alignments and templates. AF is building a list of reference sequences/structures that are most similar or evolutionarily relevant to the input.

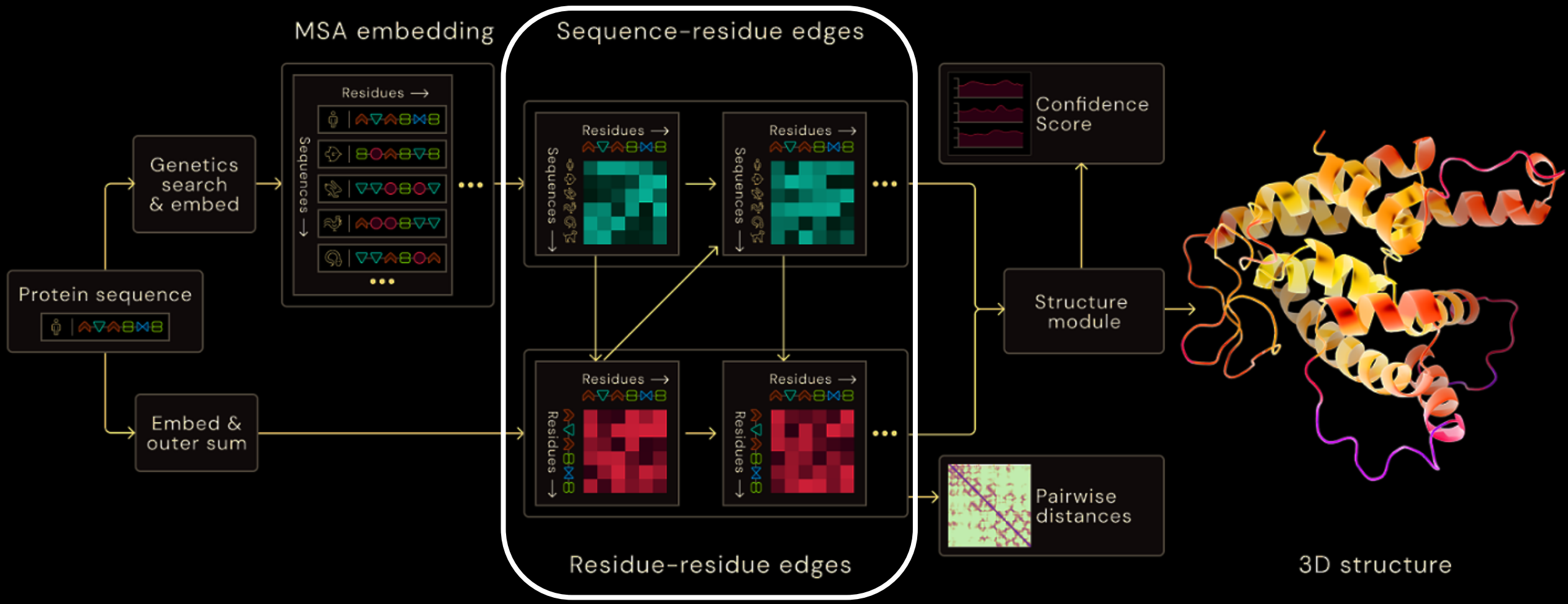
Database search and sequence alignment



This is where your question-dependent customizations will happen.

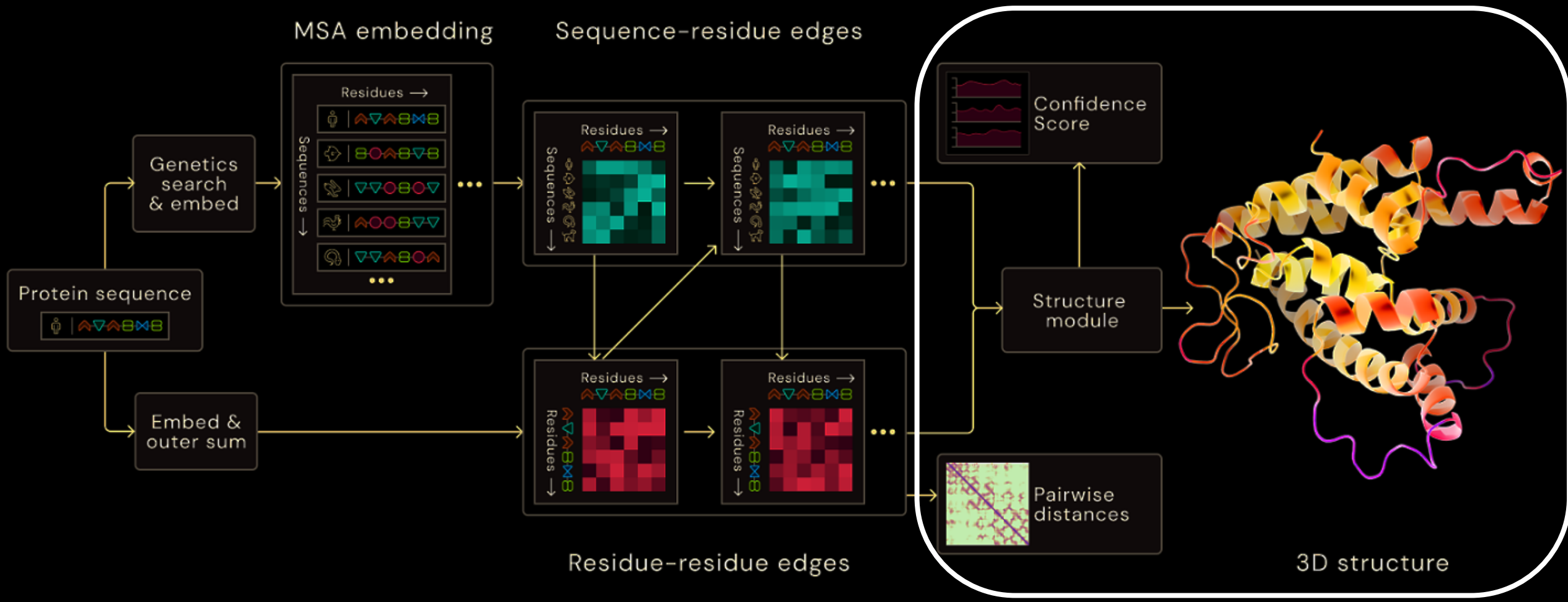
- Custom MSAs/templates can help increase accuracy of predictions in certain cases
- Mainly applicable to evolutionary queries or to proteins with very little similarity to UniREF and AlphaFold databases.
- Can be used to probe specific conformational states more accurately
- Typically, default MSAs will be best

How does AlphaFold work? - Evoformer



- AF is generating and refining pairwise representations of residues within the protein structure and using that information to refine MSAs and repeat.

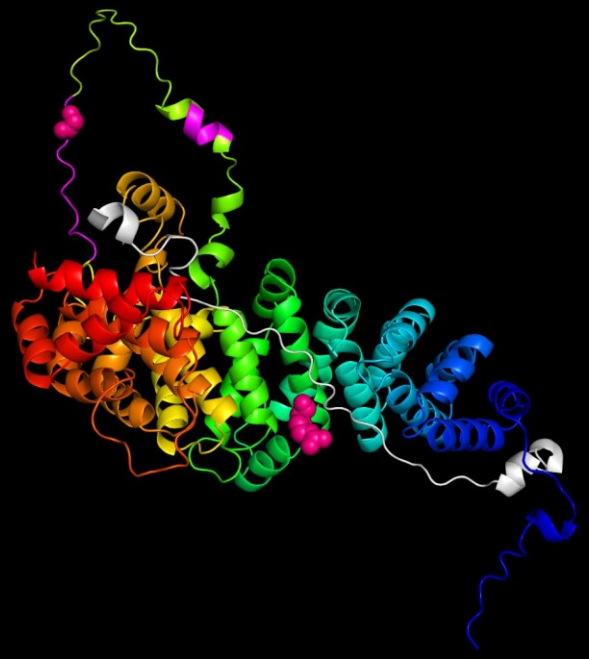
How does AlphaFold work? – Structure Module

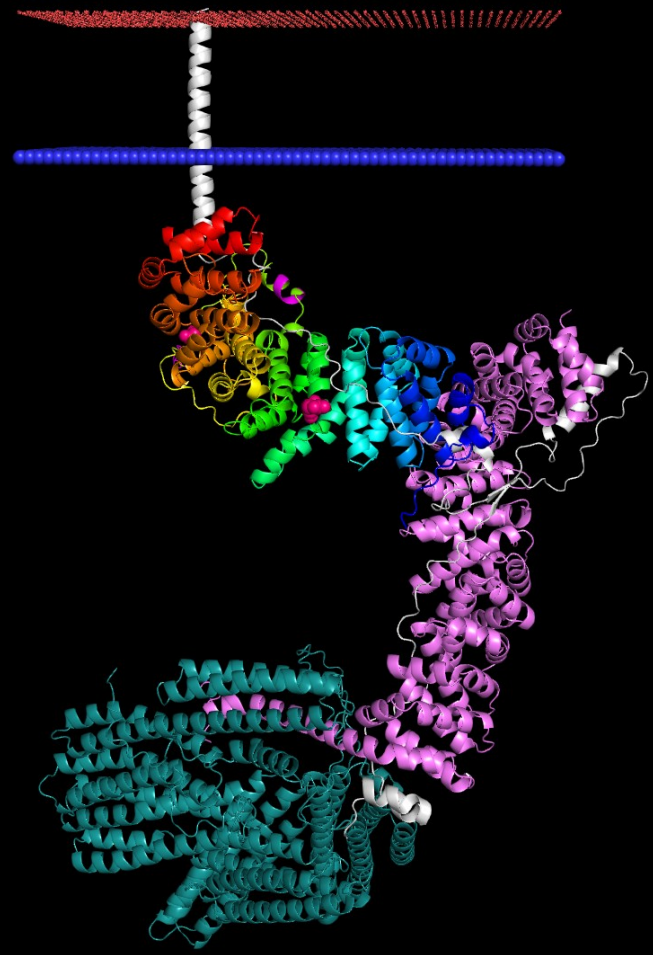


- Alphafold combines the MSA and pairwise data from the Evoformer to represent amino acids in 3D space and to guide structure assembly.

Why should you care about protein modeling?

- Structures can add biological context to experimental observations.





Why should you care about protein modeling?

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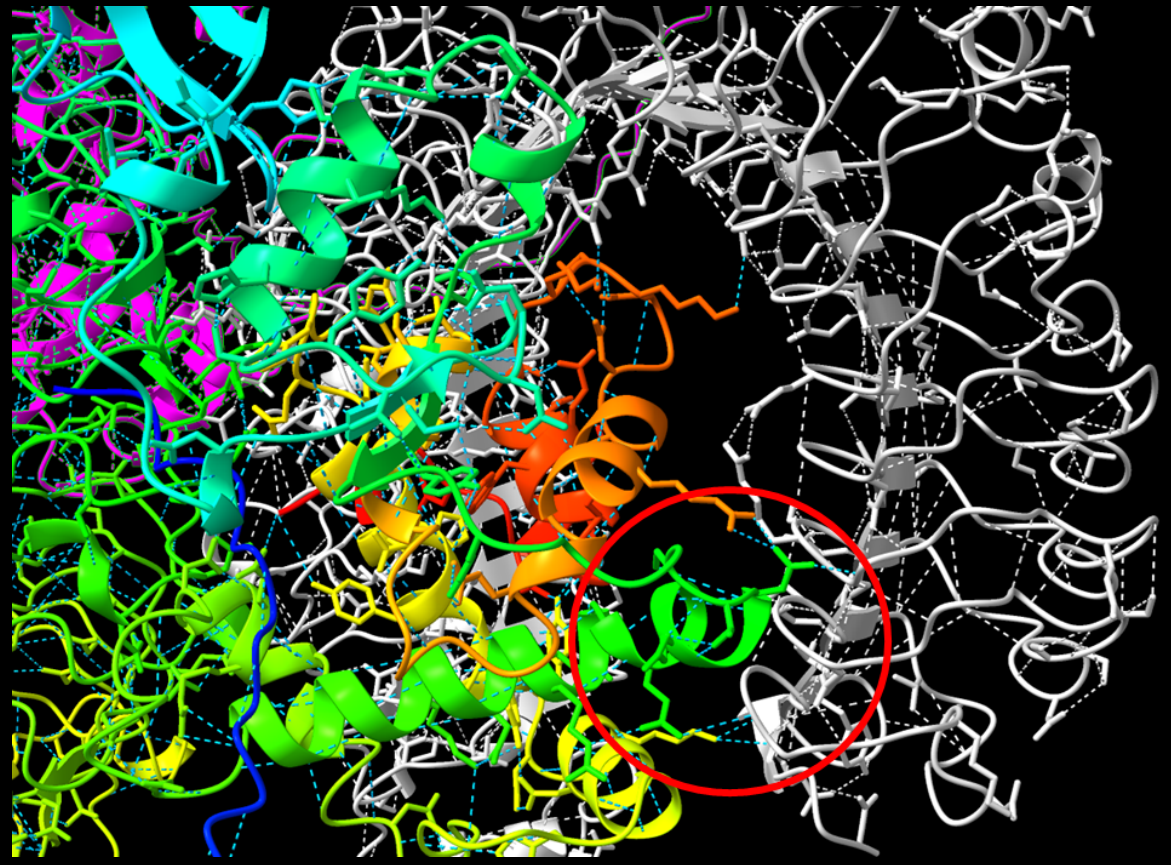
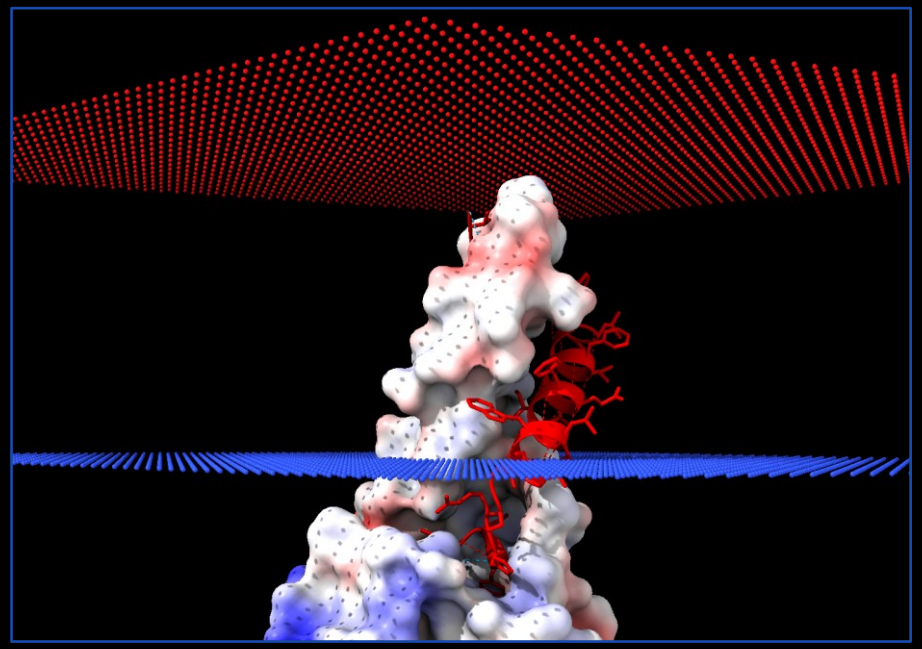
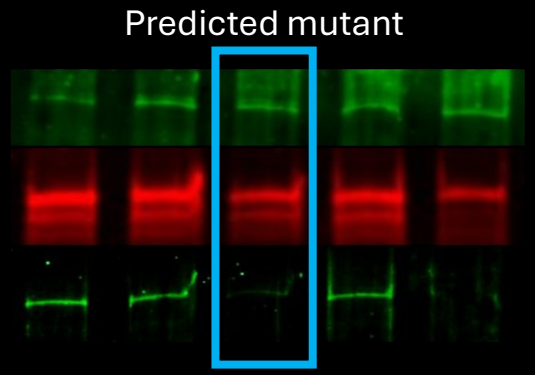
- Structures can be used to guide experiment design.



Why should you care about protein modeling?

- Structures can add biological context to experimental observations.
- Structures can be used to guide experiment design.
- Can even be used experimentally to facilitate wet lab assays.





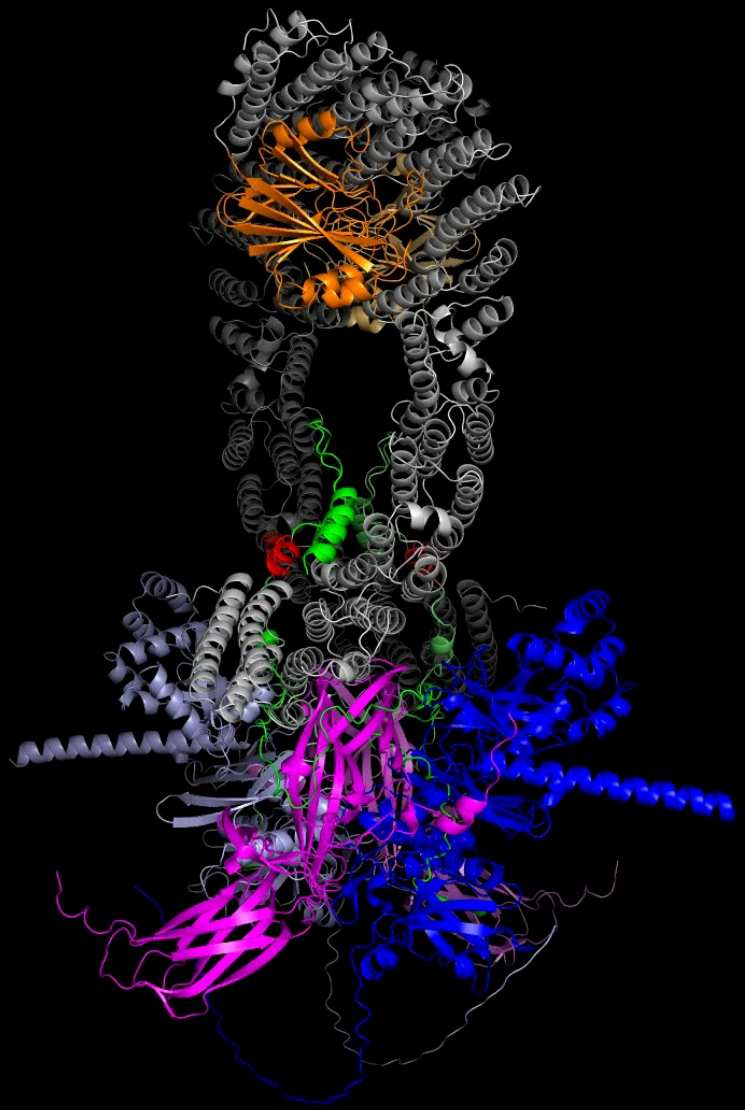
Why should you care about protein modeling?

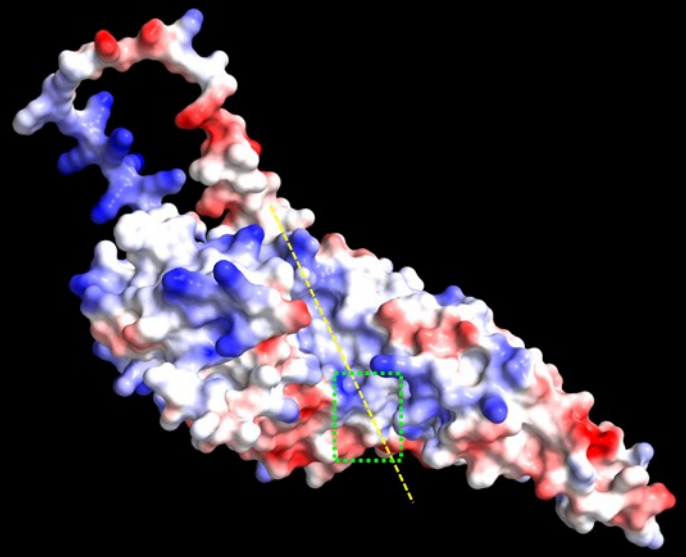
- Structures can add biological context to experimental observations.
- Structures can be used to guide experiment design.
- Can even be used experimentally to facilitate wet lab assays.

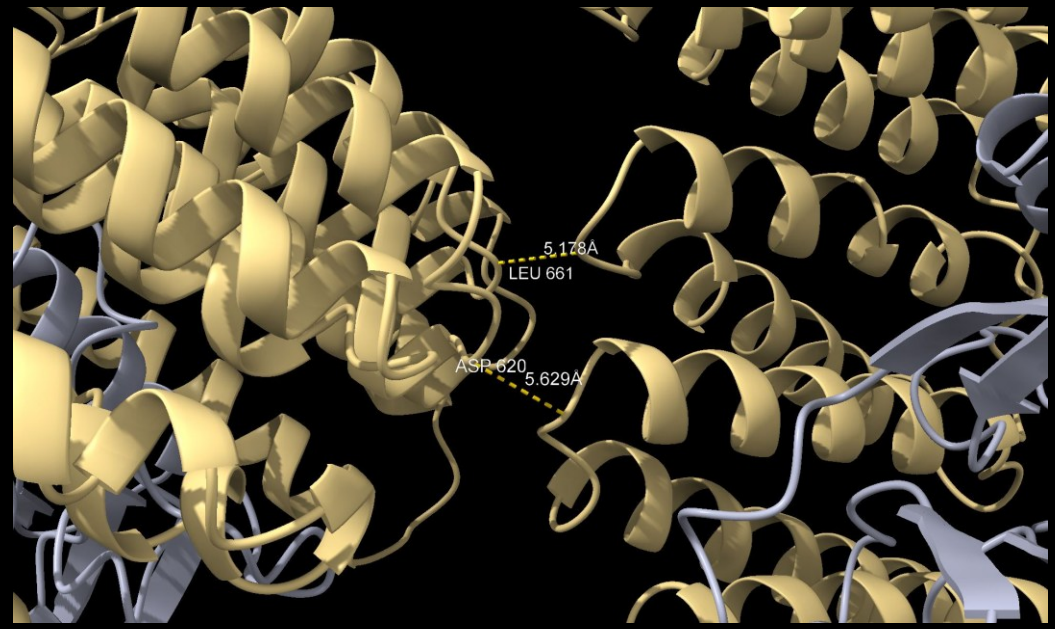
<u>But most of all:</u>

- **It is easily accessible!**

Biological context: Assess impacts of disease-linked mutations

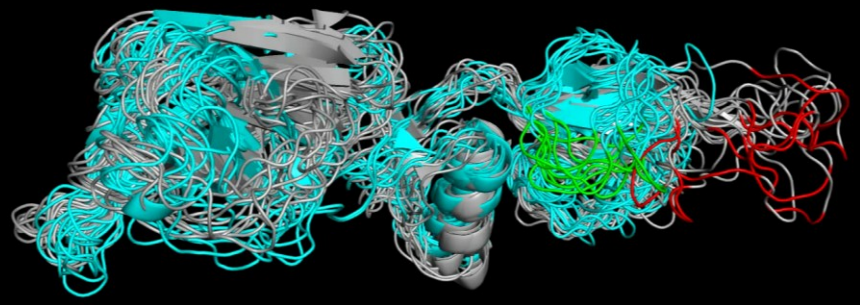




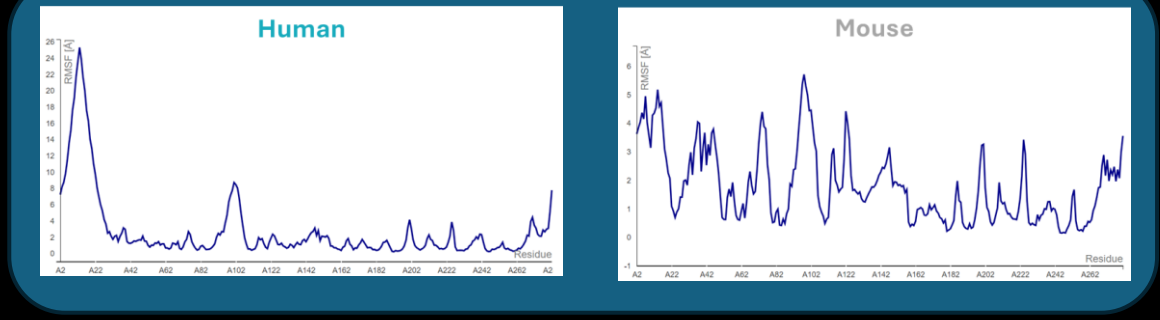


Biological context: Identify clues of functional differences between species

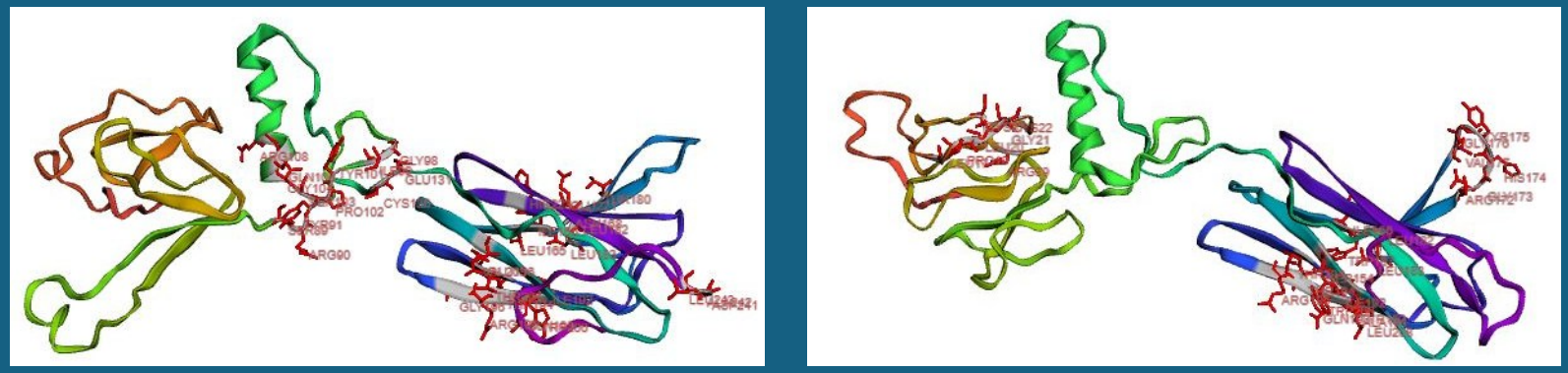
Predicted structures



Flexibility analyses

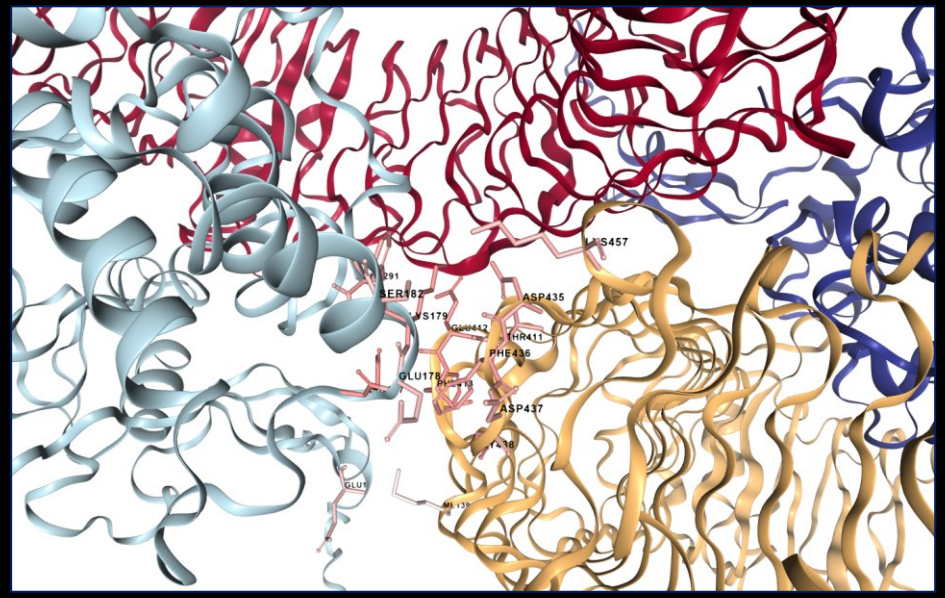


Exposed residue analyses



Experiment Design: Identify interaction-disrupting point mutations and modes of competition

- Downstream pipelines can generate a tremendous amount of information from AlphaFold structure predictions!



Using Alphafold

Three Approaches:

Local Installation

- Most involved process (requires moderate coding knowledge)
- Most customizable option
- Most accurate (in some cases)

- Unnecessary for most casual applications
- Cost prohibitive

Colab Notebook

- Very approachable (requires minimal coding knowledge)
- Highly customizable and still benefits from dedicated GPU
- More accurate than server

- Was most common until AF3 server
- Requires subscription

AlphaFold3 Server

- Very approachable (requires zero coding knowledge)
- Allows for PTM and non-protein modeling
- Not customizable at all

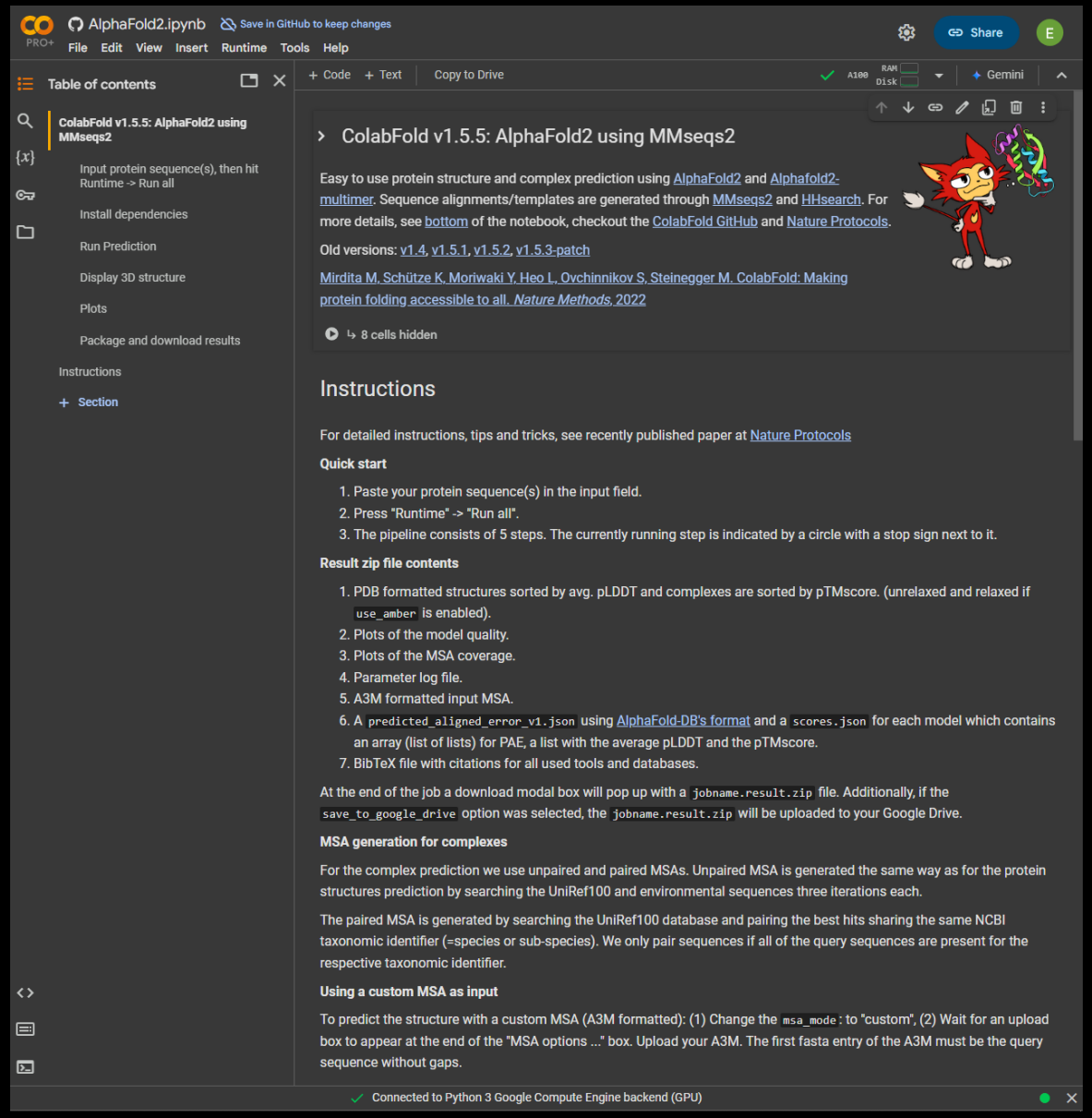
- Only way to use AF3 until recently (November 2024)
- Free

Using Alphafold3 Locally

- Requires installation on Linux system
- Requires extensive knowledge of python language
- Requires a dedicated system
  - A100 GPU is ~ $8,000
- I do not use AlphaFold locally.

Using Alphafold2 ColabFold

- AlphaFold2-Multimer
  - Predecessor to AF3
  - AF3 adds non-protein inputs
- Specialized for predicting multi-chain (multi-protein) structures
- Exists as a prebuilt notebook
  - AF3 Colab requires installation



Using Alphafold2 ColabFold - Input

- Input protein sequence, file name, relaxation, and template mode

Input protein sequence(s), then hit `Runtime` -> `Run all`

```
[ ] #@title Input protein sequence(s), then hit `Runtime` -> `Run all`
    from google.colab import files
    import os
    import re
    import hashlib
    import random

    from sys import version_info
    python_version = f"{version_info.major}.{version_info.minor}"

    def add_hash(x,y):
      return x+"_"+hashlib.sha1(y.encode()).hexdigest()[:5]

    query_sequence = 'MPRLSLLLPLLLLLLLPLLPPLSPSLGIRDVGGRRPKCGPCRPEGCPAPAPCP
    #@markdown  - Use `:` to specify inter-protein chainbreaks for **modeli
    jobname = 'test' #@param {type:"string"}
    # number of models to use
    print("jobname",jobname)
    print("sequence",query_sequence)
    print("length",len(query_sequence.replace(":","")))
```

Choose Files | No file chosen | Cancel upload

query_sequence: "MPRLSLLLPLLLLLLLPLLPPLSPSLGIRDVGGRRPI"

- Use `:` to specify inter-protein chainbreaks for **modeling complexes** (supports homo- and hetro-oligomers). For example **PI...SK:PI...SK** for a homodimer

jobname: "test"

num_relax: 0

- specify how many of the top ranked structures to relax using amber

template_mode: none

- `none` = no template information is used. `pdb100` = detect templates in pdb100 (see notes). `custom` - upload and search own templates (PDB or mmCIF format, see notes)

Using Alphafold2 ColabFold – Customizations

- Increasing recycles can lead to vastly increased structure quality

```
#@markdown ### Advanced settings
model_type = "auto" #@param ["auto", "alphafold2_ptm", "alphafold2_multimer_v1", "alphafold2_multimer_v2", "alphafold2_multimer_v3", "deepfold_v1", "alpha
#@markdown - if `auto` selected, will use `alphafold2_ptm` for monomer prediction and `alphafold2_multimer_v3` for complex prediction.
#@markdown Any of the mode_types can be used (regardless if input is monomer or complex).
num_recycles = "3" #@param ["auto", "0", "1", "3", "6", "12", "24", "48"]
#@markdown - if `auto` selected, will use `num_recycles=20` if `model_type=alphafold2_multimer_v3`, else `num_recycles=3` .
recycle_early_stop_tolerance = "auto" #@param ["auto", "0.0", "0.5", "1.0"]
#@markdown - if `auto` selected, will use `tol=0.5` if `model_type=alphafold2_multimer_v3` else `tol=0.0`.
relax_max_iterations = 200 #@param [0, 200, 2000] {type:"raw"}
#@markdown - max amber relax iterations, `0` = unlimited (AlphaFold2 default, can take very long)
pairing_strategy = "greedy" #@param ["greedy", "complete"] {type:"string"}
#@markdown - `greedy` = pair any taxonomically matching subsets, `complete` = all sequences have to match in one line.
calc_extra_ptm = False #@param {type:"boolean"}
#@markdown - return pairwise chain iptm/actifptm

#@markdown #### Sample settings
#@markdown -  enable dropouts and increase number of seeds to sample predictions from uncertainty of the model.
#@markdown -  decrease `max_msa` to increase uncertainity
max_msa = "auto" #@param ["auto", "512:1024", "256:512", "64:128", "32:64", "16:32"]
num_seeds = 1 #@param [1,2,4,8,16] {type:"raw"}
use_dropout = False #@param {type:"boolean"}

num_recycles = None if num_recycles == "auto" else int(num_recycles)
recycle_early_stop_tolerance = None if recycle_early_stop_tolerance == "auto" else float(recycle_early_stop_tolerance)
if max_msa == "auto": max_msa = None

#@markdown #### Save settings
save_all = False #@param {type:"boolean"}
save_recycles = False #@param {type:"boolean"}
save_to_google_drive = False #@param {type:"boolean"}
#@markdown -  if the save_to_google_drive option was selected, the result zip will be uploaded to your Google Drive
dpi = 200 #@param {type:"integer"}
#@markdown - set dpi for image resolution

if save_to_google_drive:
  from pydrive2.drive import GoogleDrive
  from pydrive2.auth import GoogleAuth
  from google.colab import auth
  from oauth2client.client import GoogleCredentials
  auth.authenticate_user()
  gauth = GoogleAuth()
  gauth.credentials = GoogleCredentials.get_application_default()
  drive = GoogleDrive(gauth)
  print("You are logged into Google Drive and are good to go!")

#@markdown Don't forget to hit `Runtime` -> `Run all` after updating the form.
```

Advanced settings

model_type: auto

- if `auto` selected, will use `alphafold2_ptm` for monomer prediction and `alphafold2_multimer_v3` for complex prediction. Any of the mode_types can be used (regardless if input is monomer or complex).

num_recycles: 3

- if `auto` selected, will use `num_recycles=20` if `model_type=alphafold2_multimer_v3`, else `num_recycles=3` .

recycle_early_stop_tolerance: auto

- if `auto` selected, will use `tol=0.5` if `model_type=alphafold2_multimer_v3` else `tol=0.0`.

relax_max_iterations: 200

- max amber relax iterations, `0` = unlimited (AlphaFold2 default, can take very long)

pairing_strategy: greedy

- `greedy` = pair any taxonomically matching subsets, `complete` = all sequences have to match in one line.

calc_extra_ptm: ☐

- return pairwise chain iptm/actifptm

Sample settings

- enable dropouts and increase number of seeds to sample predictions from uncertainty of the model.
- decrease `max_msa` to increase uncertainity

max_msa: auto

num_seeds: 1

use_dropout: ☐

Save settings

save_all: ☐

save_recycles: ☐

save_to_google_drive: ☐

- if the save_to_google_drive option was selected, the result zip will be uploaded to your Google Drive

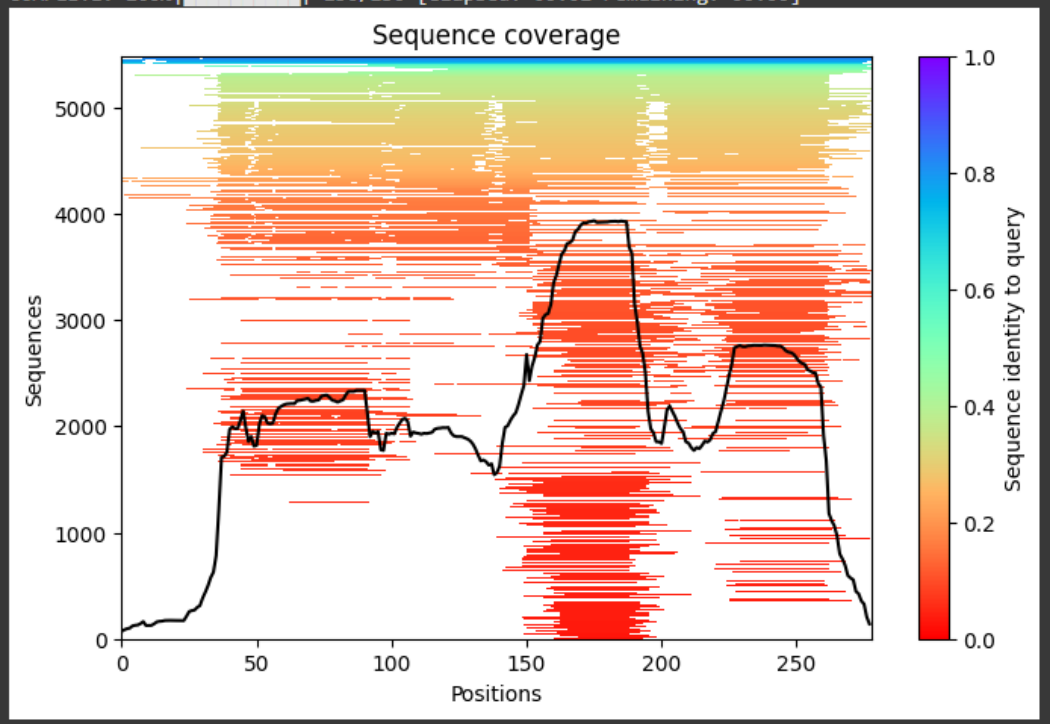
dpi: 200

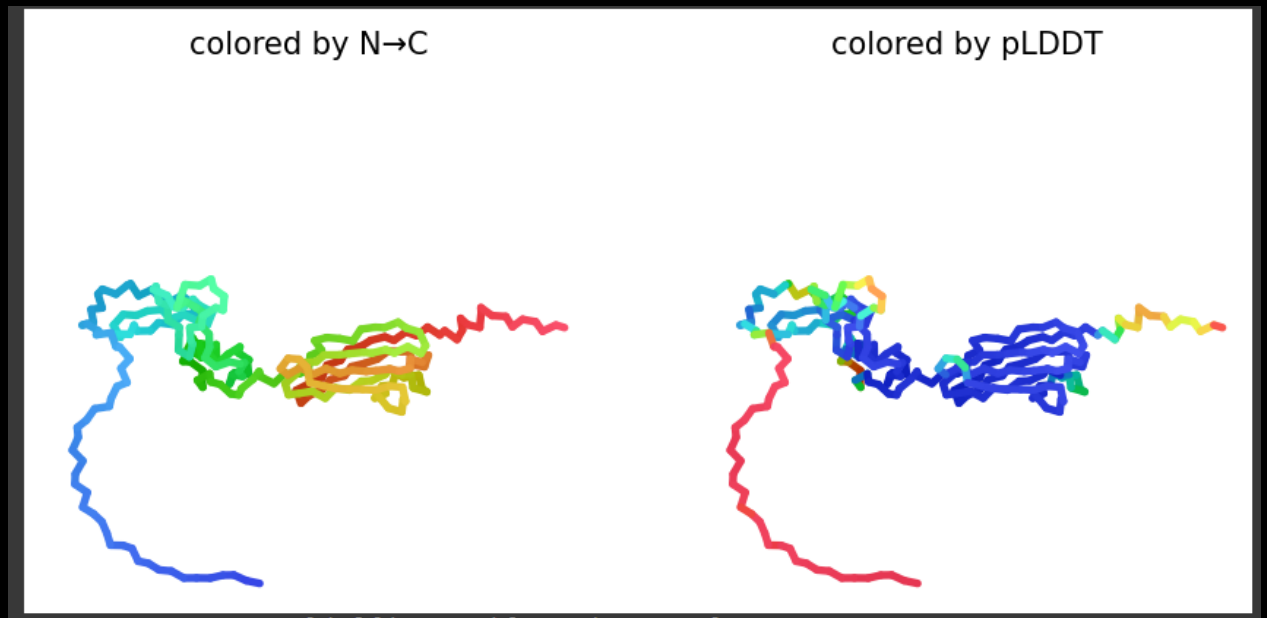
- set dpi for image resolution

Using Alphafold2 ColabFold – Output

- MSA Sequence coverage chart and a series of structures with recycle data and confidence scores. **Structures output as PDB files.**

```
Downloading alphafold2_ptm weights to .: 100%|██████████| 3.47G/3.47G [00:13<00:00, 268MB/s]
2025-02-11 21:18:06,221 Running on GPU
2025-02-11 21:18:06,690 Found 5 citations for tools or databases
2025-02-11 21:18:06,690 Query 1/1: test_27faa (length 278)
COMPLETE: 100%|██████████| 150/150 [elapsed: 00:01 remaining: 00:00]
```



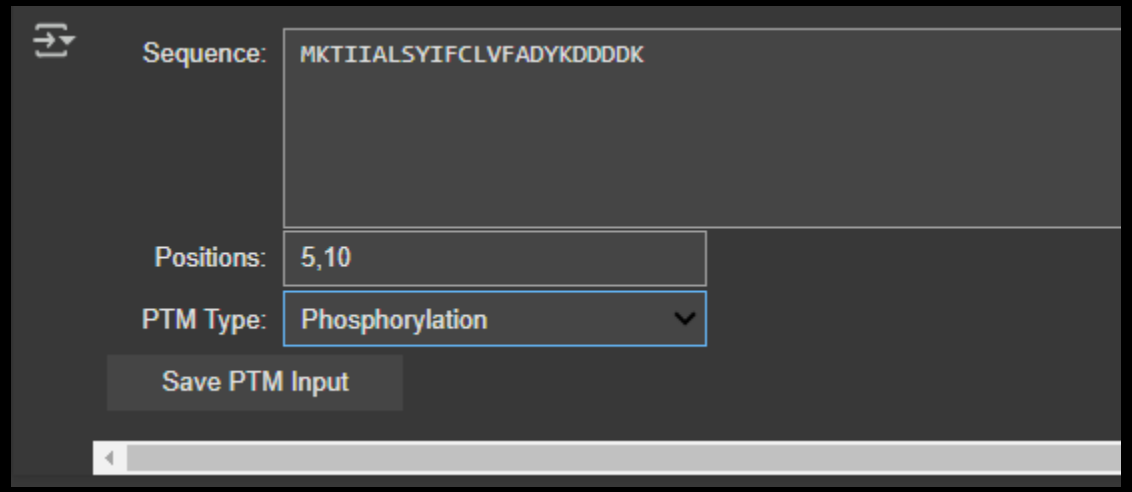



```
2025-02-11 21:19:37,475 alphafold2_ptm_model_3_seed_000 recycle=0 pLDDT=81.9 pTM=0.654
2025-02-11 21:19:40,721 alphafold2_ptm_model_3_seed_000 recycle=1 pLDDT=83.5 pTM=0.682 tol=1.91
2025-02-11 21:19:43,947 alphafold2_ptm_model_3_seed_000 recycle=2 pLDDT=83.6 pTM=0.687 tol=0.78
2025-02-11 21:19:47,175 alphafold2_ptm_model_3_seed_000 recycle=3 pLDDT=83.6 pTM=0.689 tol=0.655
2025-02-11 21:19:47,177 alphafold2_ptm_model_3_seed_000 took 12.9s (3 recycles)
```

Confidence scores are reported as pLDDT and pTM

Using Alphafold3 in a Colab Notebook

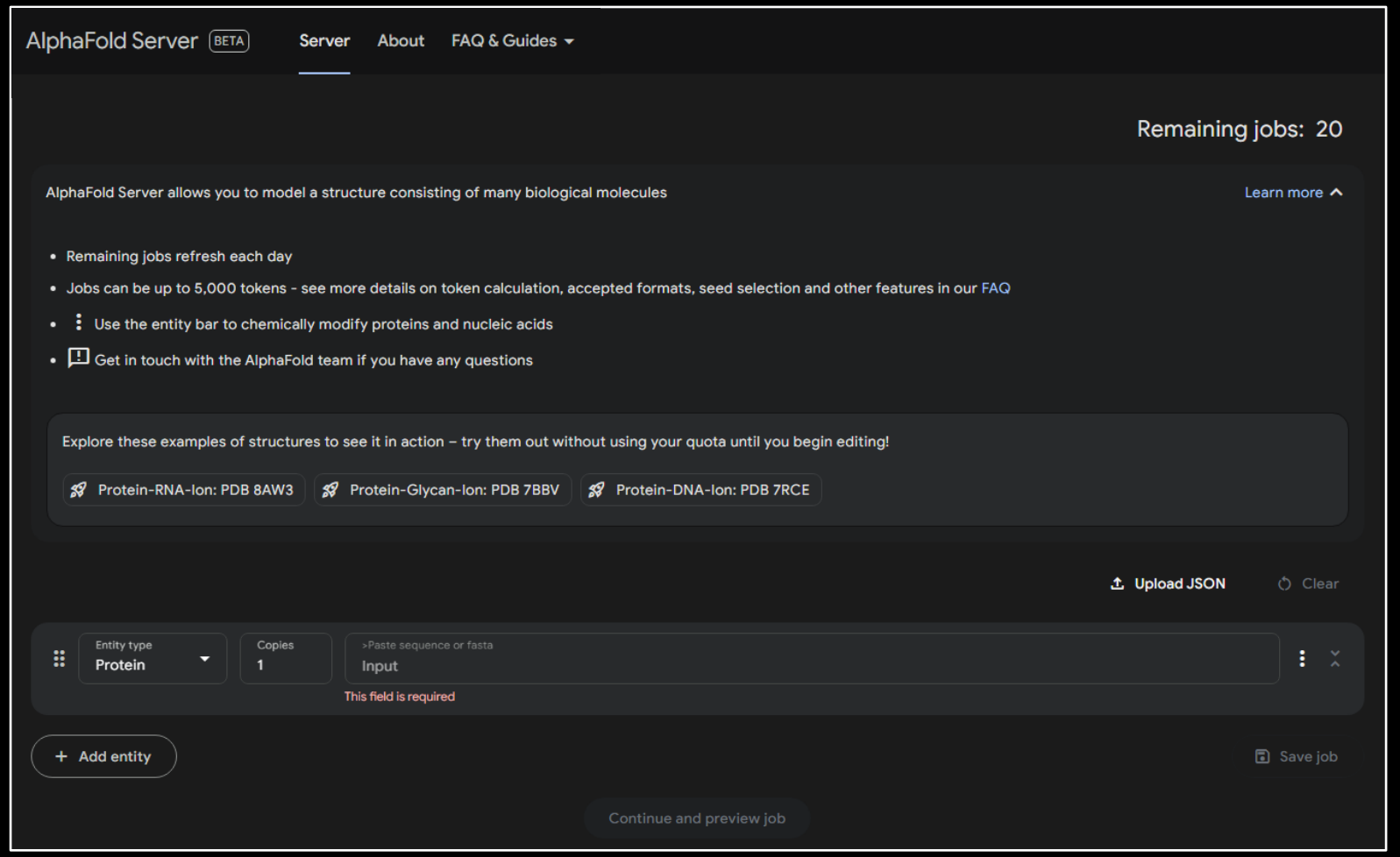
- Requires installation
- Requires some knowledge of python language
- Similar to using locally, but Colab handles most of the common causes for headache.




```
# ============================
# ⚙️ Step 2: Install AlphaFold 3
# ============================
!git clone --quiet https://github.com/google-deepmind/alphafold3.git
!pip install -q --no-warn-conflicts -r alphafold3/requirements.txt

# Set model directory
os.environ['ALPHAFOLD3_MODEL_DIR'] = "/content/alphafold3/params"

print("✅ AlphaFold 3 installed successfully!")

# ================================
# 📥 Step 3: Download Required Data
# ================================
!alphafold3/fetch_databases.sh /content/alphafold3/data

print("✅ Databases downloaded successfully!")

# ================================
# 🚀 Step 4: Run AlphaFold 3 Model
# ================================
OUTPUT_DIR = "output"
DATA_DIR = "/content/alphafold3/data"

cmd = f"""
python alphafold3/run_alphafold.py \
  --input_json=input.json \
  --output_dir={OUTPUT_DIR} \
  --model_preset=multimer \
  --data_dir={DATA_DIR} \
  --use_gpu_relax=True
"""
```

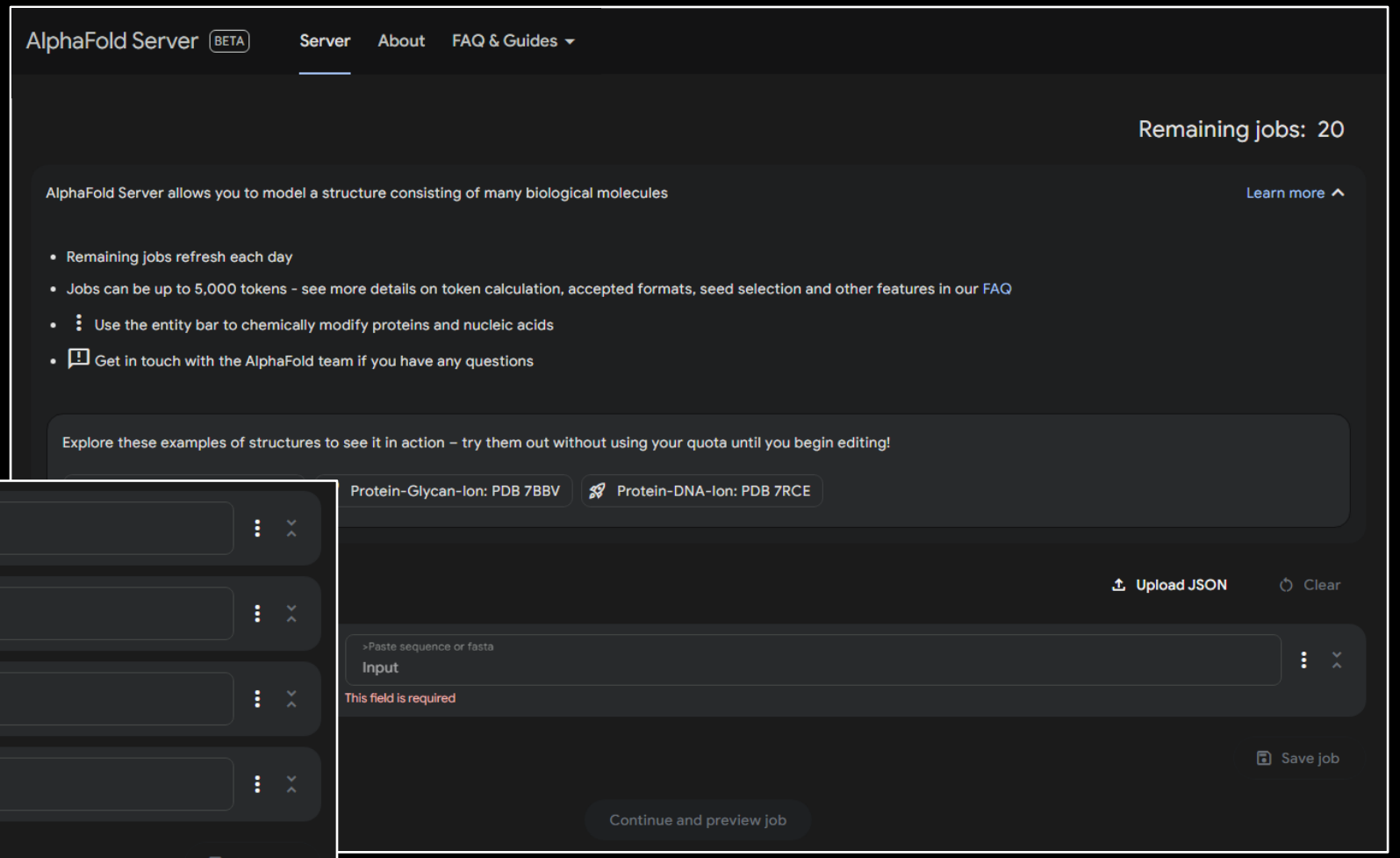
Using AlphaFold3 Server

- Very simple – Log in and input sequence
- Requires Google account, but is free to use
  - 20 uses per day
  - Jobs limited to 5,000 tokens (1 token ~ 1 residue)
- Lacks customization of recycles and MSA properties



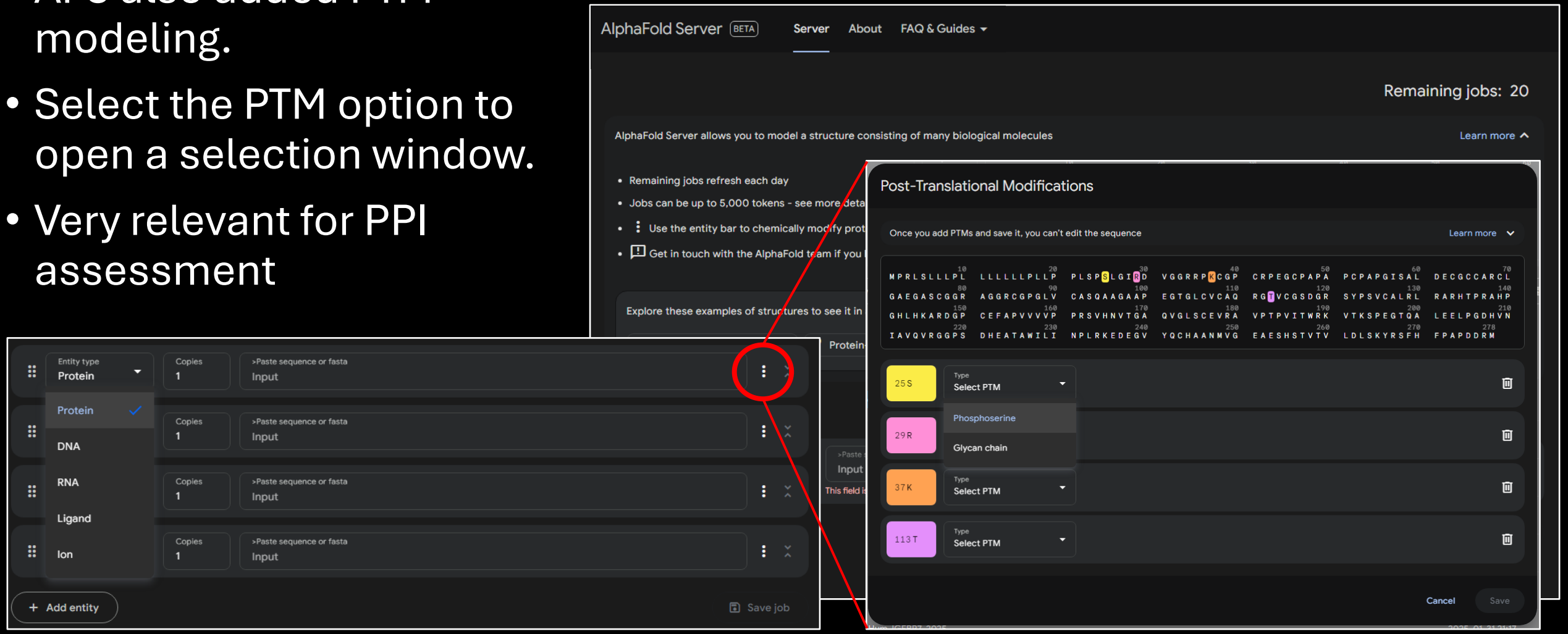
Using AlphaFold3 Server

- AF3 added the possibility to model non-protein entities
- Specify entity type in drop down menu.
- Specify number of copies



Using AlphaFold3 Server

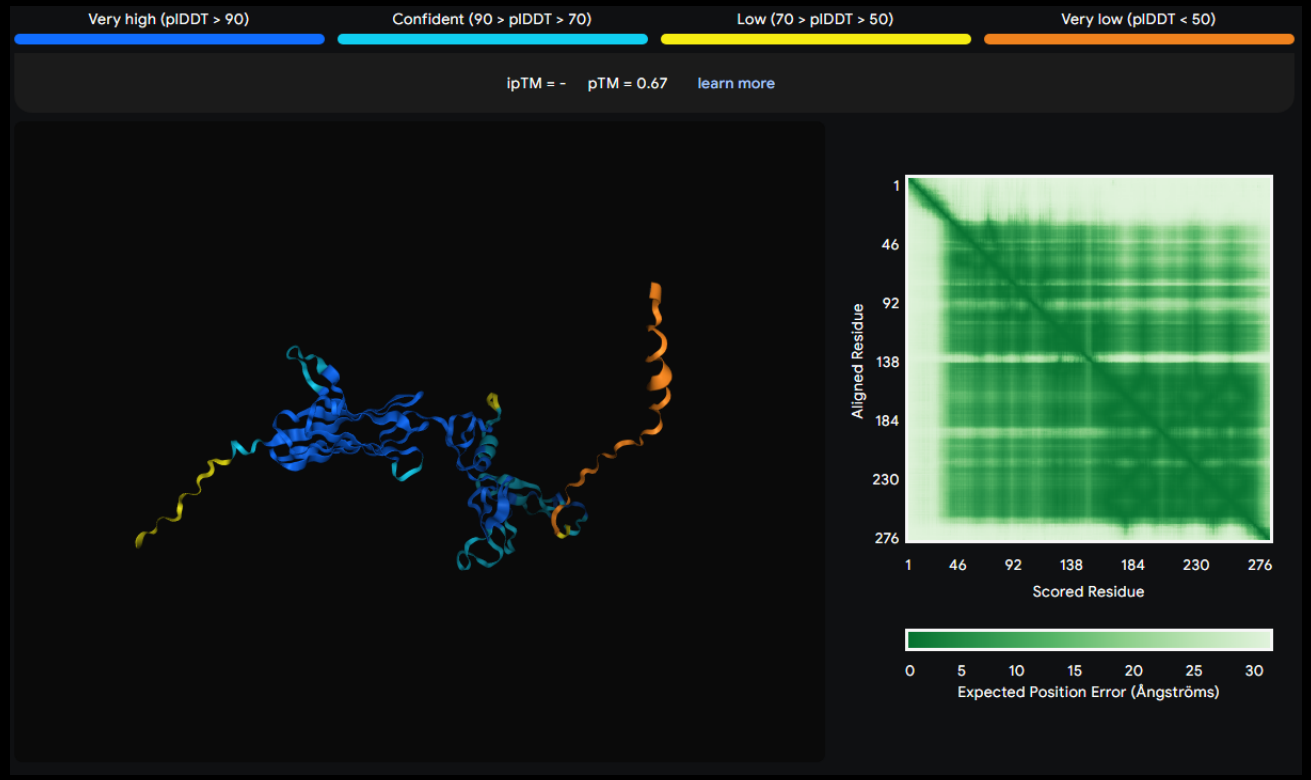
- AF3 also added PTM modeling.
- Select the PTM option to open a selection window.
- Very relevant for PPI assessment



Using AlphaFold3 Server - Output

- Similar to ColabFold
  - (Confidence scores and PDB files)
- Need special software to open PDB


```
"fraction_disordered": 0.16,
"has_clash": 0.0,
"iptm": 0.92,
"num_recycles": 10.0,
"ptm": 0.78,
"ranking_score": 0.97
```

cat-cadherin.pdb

File Edit View


```
ATOM      1  N   ILE A   1     -65.952  21.848  82.645  1.00 46.67      A    N
ATOM      2  CA  ILE A   1     -66.828  21.079  81.738  1.00 53.02      A    C
ATOM      3  C   ILE A   1     -66.435  21.303  80.280  1.00 55.51      A    C
ATOM      4  O   ILE A   1     -66.245  20.328  79.562  1.00 51.42      A    O
ATOM      5  CB  ILE A   1     -68.322  21.376  81.992  1.00 50.67      A    C
ATOM      6  CG1 ILE A   1     -68.706  20.981  83.438  1.00 46.78      A    C
ATOM      7  CG2 ILE A   1     -69.202  20.610  80.975  1.00 46.21      A    C
ATOM      8  CD1 ILE A   1     -70.138  21.375  83.842  1.00 41.25      A    C
ATOM      9  N   ALA A   2     -66.269  22.560  79.866  1.00 47.12      A    N
ATOM     10  CA  ALA A   2     -65.848  22.876  78.495  1.00 53.42      A    C
ATOM     11  C   ALA A   2     -64.479  22.278  78.143  1.00 54.07      A    C
ATOM     12  O   ALA A   2     -64.316  21.708  77.067  1.00 53.80      A    O
ATOM     13  CB  ALA A   2     -65.849  24.400  78.319  1.00 52.70      A    C
ATOM     14  N   ILE A   3     -63.526  22.330  79.084  1.00 53.57      A    N
ATOM     15  CA  ILE A   3     -62.201  21.716  78.898  1.00 57.37      A    C
ATOM     16  C   ILE A   3     -62.327  20.193  78.800  1.00 58.40      A    C
ATOM     17  O   ILE A   3     -61.719  19.590  77.919  1.00 58.22      A    O
ATOM     18  CB  ILE A   3     -61.233  22.141  80.018  1.00 55.84      A    C
ATOM     19  CG1 ILE A   3     -61.009  23.672  79.983  1.00 52.70      A    C
ATOM     20  CG2 ILE A   3     -59.889  21.405  79.892  1.00 51.46      A    C
ATOM     21  CD1 ILE A   3     -60.210  24.222  81.175  1.00 47.43      A    C
ATOM     22  N   LEU A   4     -63.161  19.570  79.652  1.00 52.54      A    N
ATOM     23  CA  LEU A   4     -63.443  18.133  79.579  1.00 55.85      A    C
ATOM     24  C   LEU A   4     -64.131  17.756  78.266  1.00 56.08      A    C
ATOM     25  O   LEU A   4     -63.759  16.756  77.664  1.00 58.52      A    O
ATOM     26  CB  LEU A   4     -64.290  17.704  80.788  1.00 54.75      A    C
ATOM     27  CG  LEU A   4     -63.441  17.080  81.906  1.00 52.17      A    C
ATOM     28  CD1 LEU A   4     -64.108  17.263  83.261  1.00 46.73      A    C
ATOM     29  CD2 LEU A   4     -63.233  15.592  81.663  1.00 51.22      A    C
ATOM     30  N   LEU A   5     -65.097  18.582  77.803  1.00 55.32      A    N
ATOM     31  CA  LEU A   5     -65.719  18.397  76.488  1.00 58.56      A    C
ATOM     32  C   LEU A   5     -64.704  18.546  75.357  1.00 59.04      A    C
ATOM     33  O   LEU A   5     -64.708  17.720  74.452  1.00 59.95      A    O
ATOM     34  CB  LEU A   5     -66.887  19.381  76.307  1.00 57.00      A    C
ATOM     35  CG  LEU A   5     -68.231  18.783  76.751  1.00 52.99      A    C
ATOM     36  CD1 LEU A   5     -69.238  19.887  77.060  1.00 48.19      A    C
ATOM     37  CD2 LEU A   5     -68.813  17.888  75.666  1.00 51.41      A    C
ATOM     38  N   CYS A   6     -63.824  19.543  75.442  1.00 61.27      A    N
ATOM     39  CA  CYS A   6     -62.741  19.674  74.465  1.00 63.73      A    C
ATOM     40  C   CYS A   6     -61.791  18.478  74.505  1.00 63.19      A    C
ATOM     41  O   CYS A   6     -61.422  17.979  73.449  1.00 64.83      A    O
ATOM     42  CB  CYS A   6     -61.968  20.976  74.685  1.00 62.96      A    C
ATOM     43  SG  CYS A   6     -62.890  22.371  73.985  1.00 55.84      A    S
ATOM     44  N   ILE A   7     -61.427  17.988  75.703  1.00 61.19      A    N
ATOM     45  CA  ILE A   7     -60.582  16.791  75.838  1.00 62.94      A    C
ATOM     46  C   ILE A   7     -61.316  15.553  75.318  1.00 62.47      A    C
ATOM     47  O   ILE A   7     -60.706  14.746  74.622  1.00 64.37      A    O
```


Ln 25, Col 81 | 1,402,466 characters | 100% | Unix (LF) | UTF-8

Output – Confidence Scores

pLDDT – predicted local distance difference test

- Local, residue-level confidence score
- Can provide indications of disordered or unstructured regions

Ranges from 0 to 100

- >90: Very high confidence

pTM – predicted temptale modeling

- Global, structure-level confidence score

piTM – predicted interface template modeling

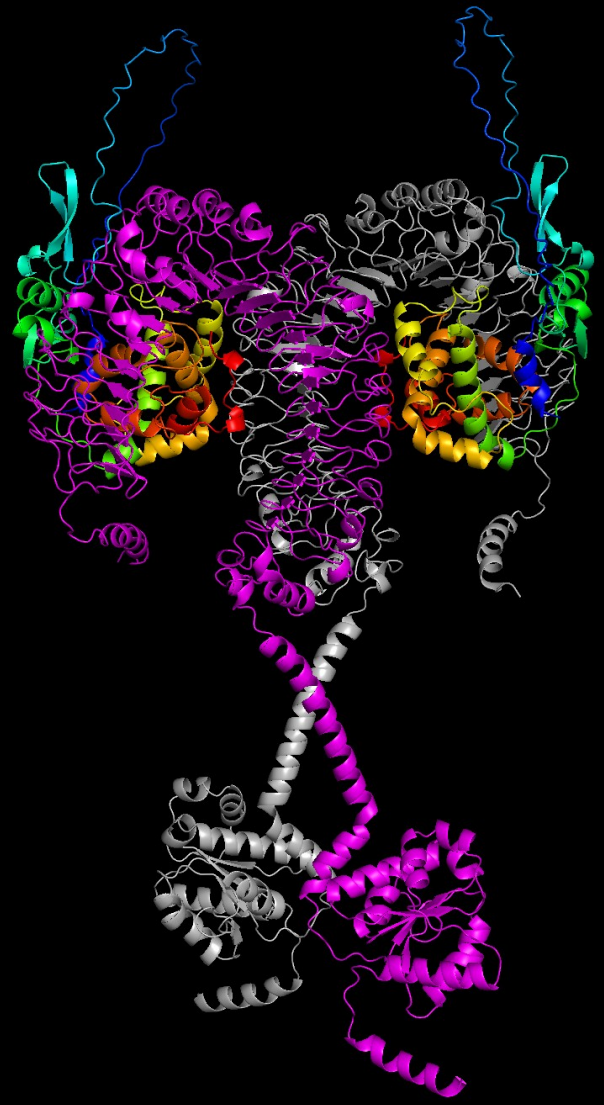
- Evaluation of how well chains interact at protein interfaces

pTM and piTM range from 0 to 1

- >0.6: Likely a real structure

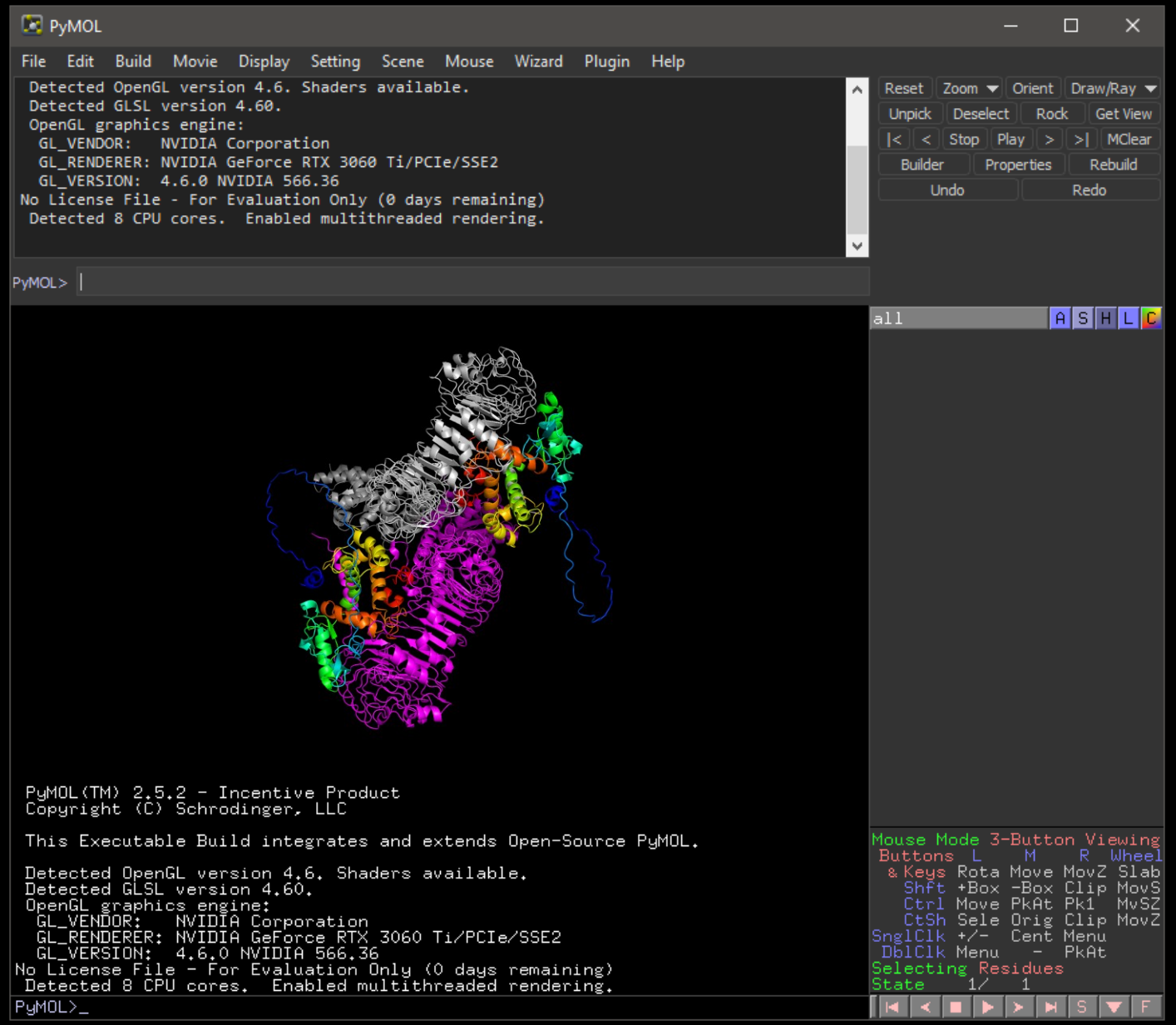
Output – PDB Files: Visualizing your structures

- Need molecular visualization software to open files
- PyMOL and ChimeraX are the most common choices
- Both are free to use and work with Windows/MacOS
- Best to have both on hand



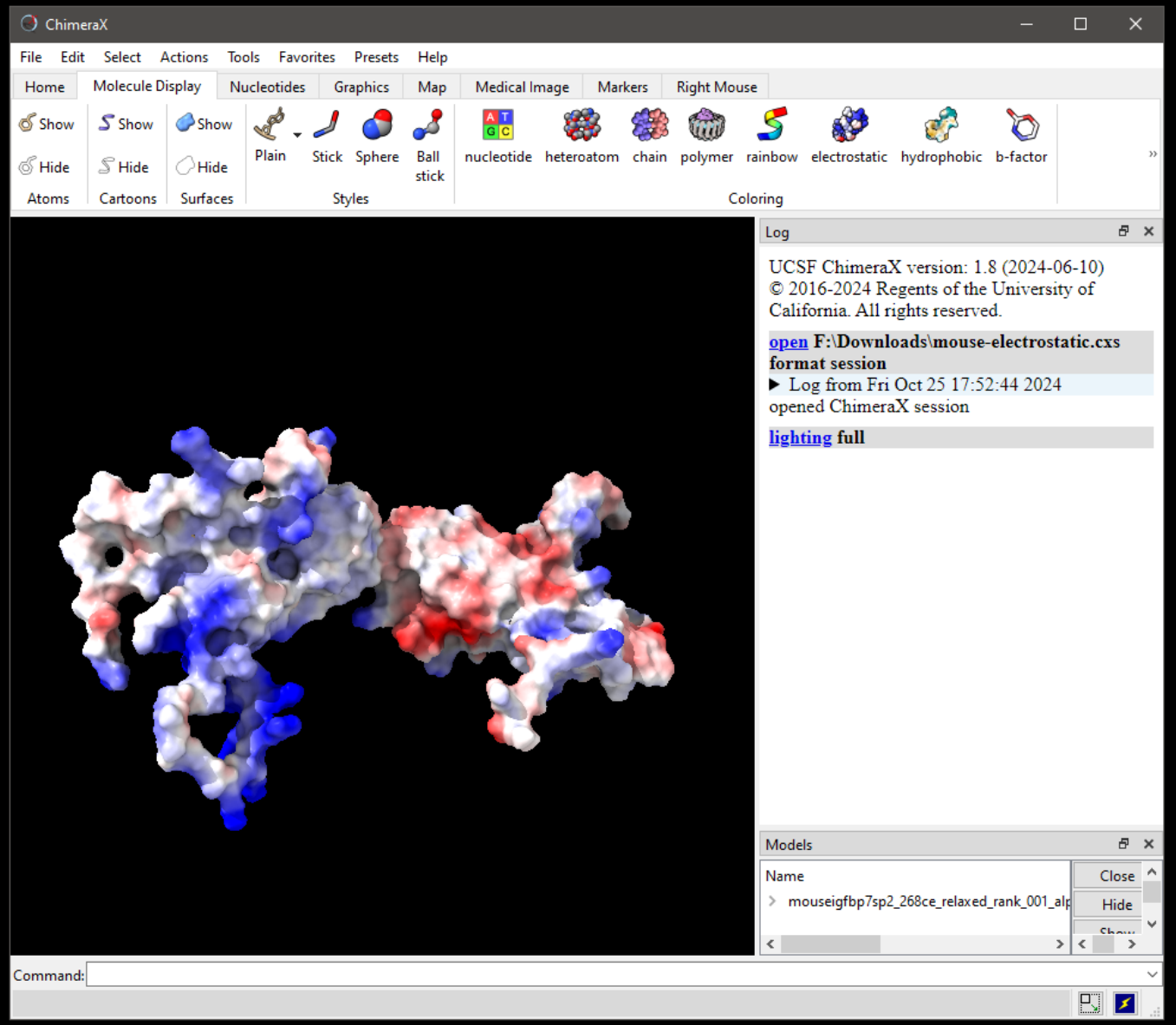
PyMOL

- Open-source, Python-based molecular visualization tool
- Utilizes a Python-based command line for most operations
- Most functions/commands are covered in the PyMOL wiki
- Not as flashy as ChimeraX, but more granular



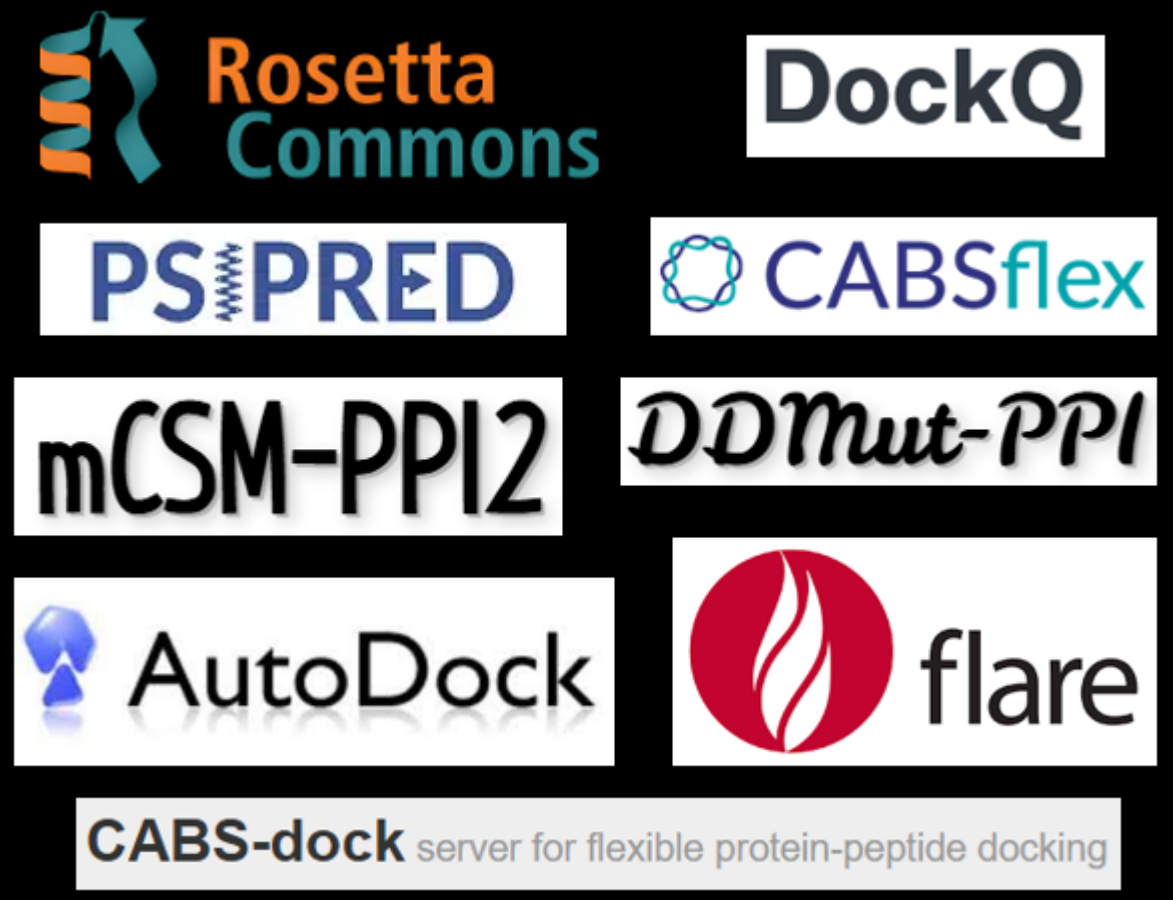
UCSF-ChimeraX

- Open-access molecular visualization software developed by UCSF
- Utilizes a GUI for most features
  - Proprietary command list and .py scripts are also used in some cases
- ChimeraX wiki is comprehensive and covers commands in detail
- Excels at figure design



Next Steps – Analyze your structure

- Further analyses can be conducted on your structure
- Servers and software packages are typically purpose-built and rather specific
- Some analyses are user-friendly GUIs and others are command-line based
- **Google is your friend!**



Summary

- Protein structures can reveal a lot about their functions
- AlphaFold is a highly-accessible tool for predicting protein and protein complex structures
- PyMOL and ChimeraX can be used to freely visualize and analyze structures
- Assay-specific downstream software is readily available

February 26th, 2025

- Downstream software examples
- PPI prediction and manipulation
- DockQ analysis