Introduction to Protein Structure Prediction With AlphaFold 2

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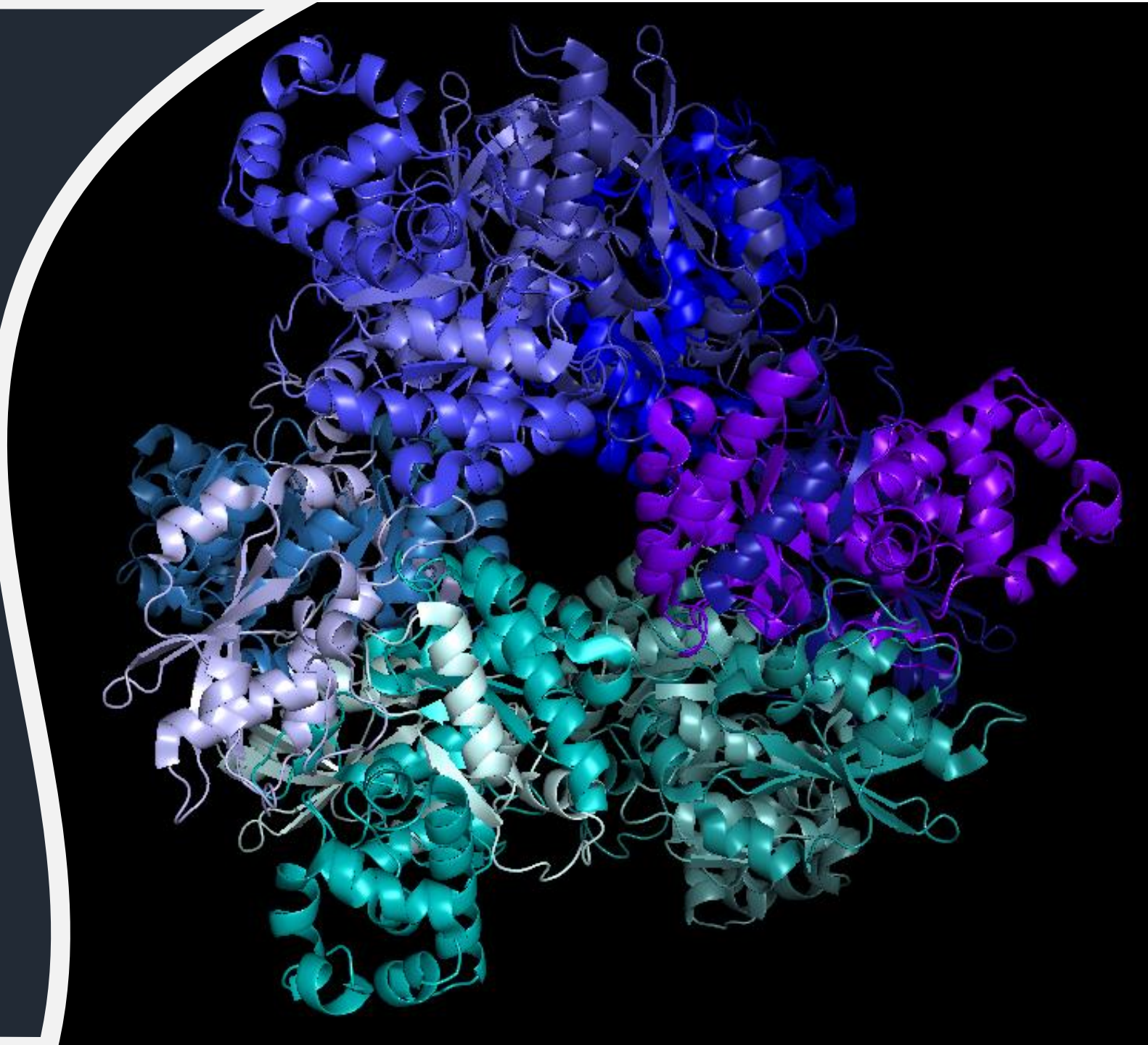

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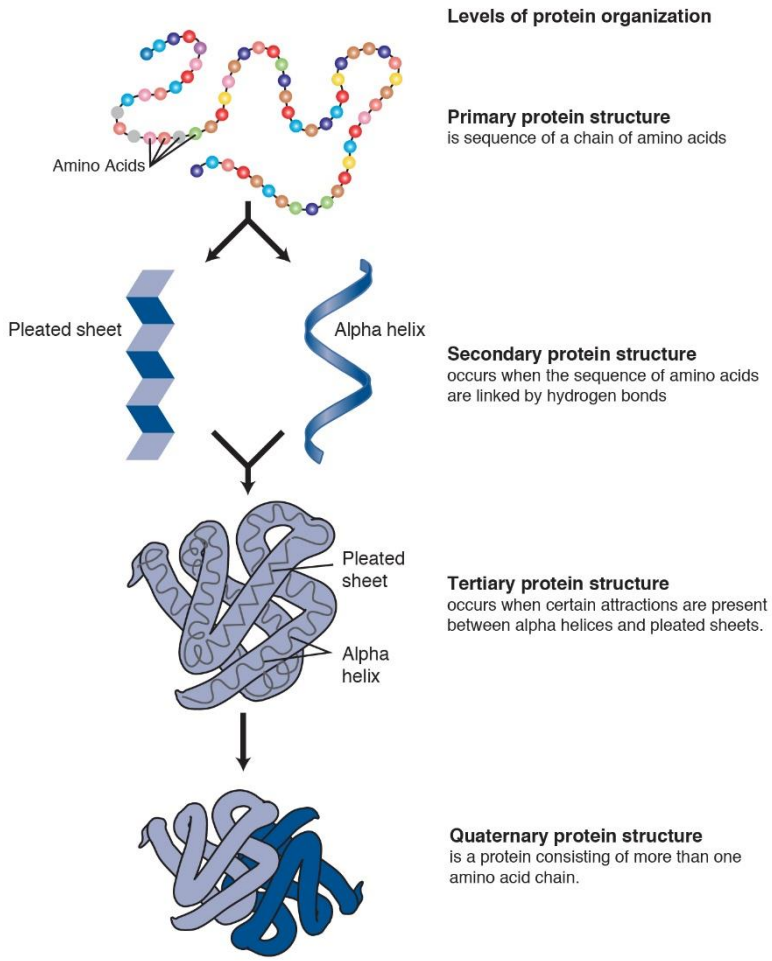
- ✓ Consultation on Projects and Grants
- ✓ High Performance Compute Cluster
- ✓ Workshops

https://it.tufts.edu/research-technology

Protein Organization

- Primary Structure: amino acid sequence
- Secondary Structure: amino acid sequences linked by hydrogen bonds
- Tertiary Structure: organization of secondary structures
- Quaternary Structure: organization of multiple amino acid chains

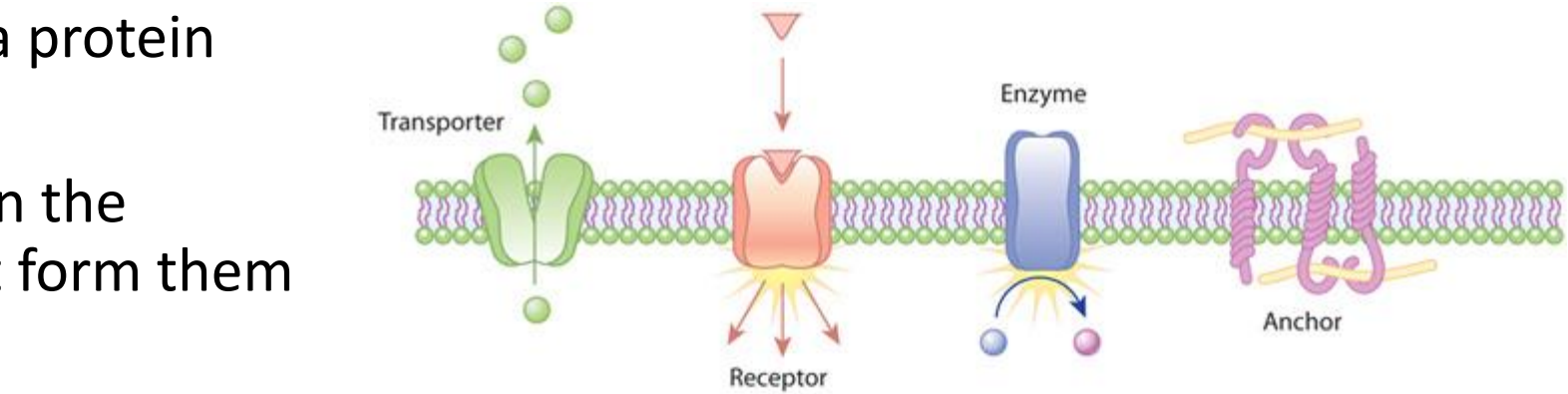


https://www.genome.gov/genetics-glossary/Protein

The Importance of Protein Structure

- Can help determine what a protein does
- Often more conserved than the amino acid sequences that form them

Examples of Different Proteins



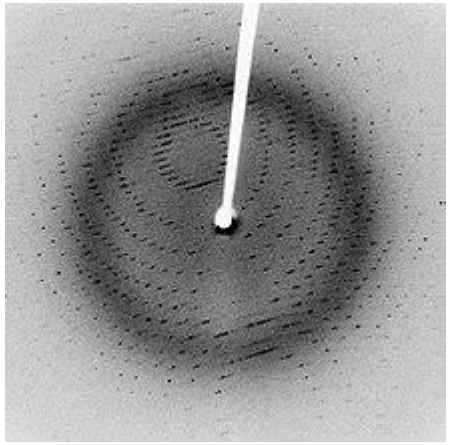
https://www.nature.com/scitable/topicpage/protein-function-14123348/

https://www.ncbi.nlm.nih.gov/books/NBK26820/

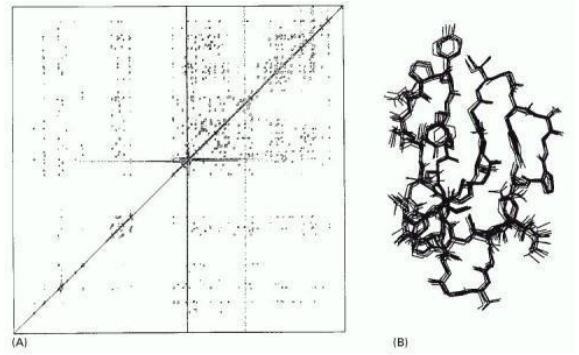
Laboratory Means To Determine Protein Structure

- X-ray Crystallography
- Nuclear Magnetic Resonance (NMR) Spectroscopy
- 3D Electron Microscopy

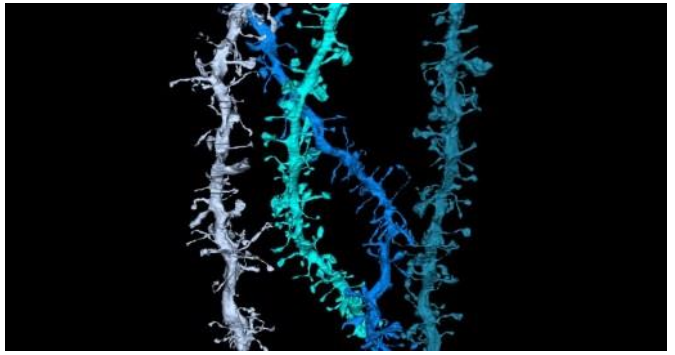
X-ray Crystallography



NMR Spectroscopy



3D Electron Microscopy



https://directorsblog.nih.gov/tag/serial-scanning-3d-electron-microscopy/

https://www.ncbi.nlm.nih.gov/books/NBK26820/

https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/methods-for-determining-structure

https://simple.wikipedia.org/wiki/X-ray_crystallography

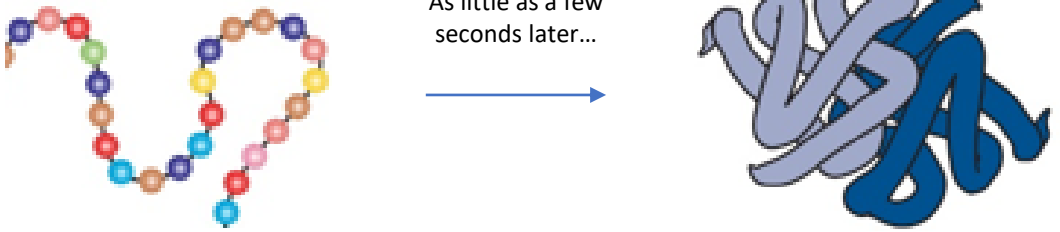
The Protein Structure Problem

- 100,000,000 known distinct proteins
- Each has a unique structure that determines function
- Determining protein structure is time consuming
- Only a small fraction of exact 3D structures are known

https://deepmind.com/research/case-studies/alphafold

Levinthal's Paradox

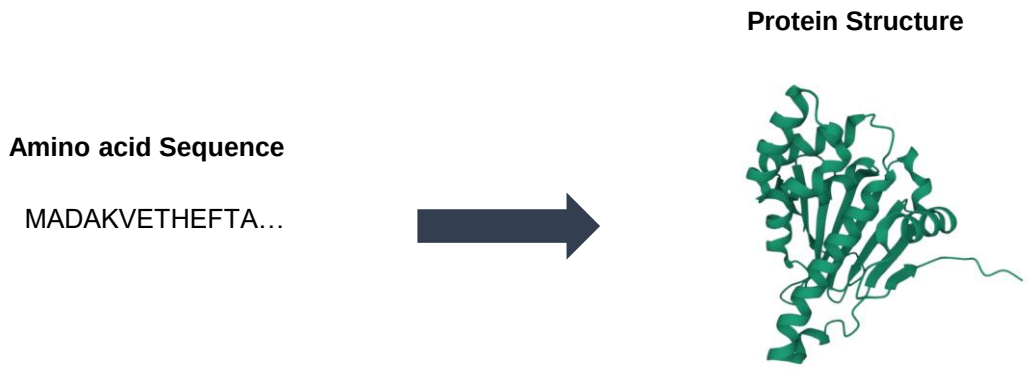
- Finding the native folded state of a protein by random searching of all possible configurations would take an enormous amount of time
- However, proteins can often fold within seconds
- Meaning some process must be guiding this folding



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC48166/

Using Sequence To Predict Structure

- Instead of laboratory experimentation, there have been massive efforts to use a protein's sequence to determine structure
- 1994, the Critical Assessment of Structure Protein (CASP) was established as a biennial assessment of methods to predict structure from sequence

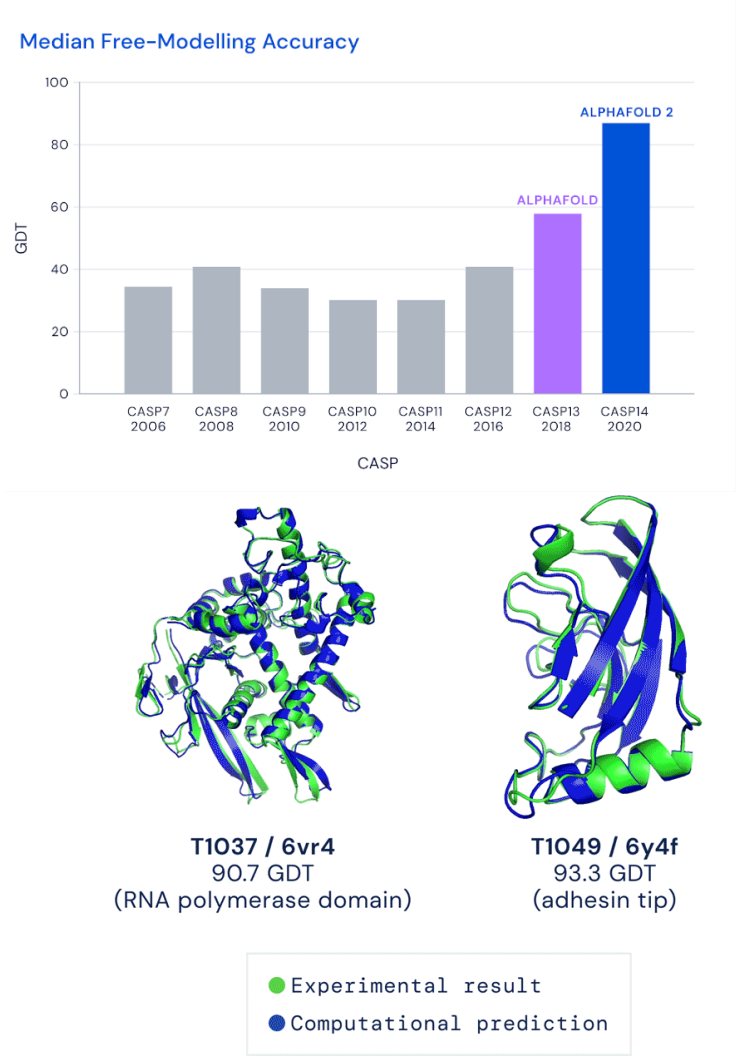


https://deepmind.com/blog/article/alphafold-a-solution-to-a-50-year-old-grand-challenge-in-biology

https://predictioncenter.org/

Enter AlphaFold 2

- Google's DeepMind team Entered AlphaFold 2 in CASP14
- Achieved a median Global Distance Test Score of 92.4
- AlphaFold 2 works by finding similar sequences to the query, extracts the information using a neural network, then passes that information to another neural network that construct a theoretical structure



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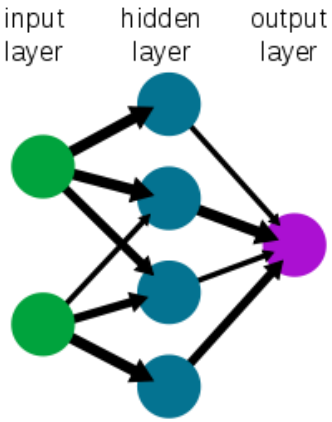
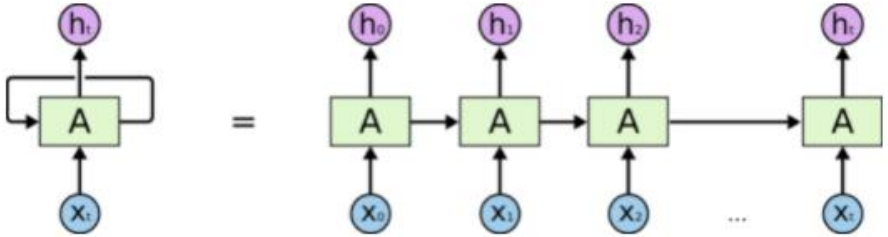
Simple v. Recurrent Neural Network

- A neural network is a machine learning algorithm commonly used in predictive modelling
  - Composed of an input layer, hidden layer, and an output layer
  - Traditionally learn from training
- A Recurrent Neural Network learns from training and from previous inputs
- However, the memory is poor when pulling from old connections

Simple Neural Network



Recurrent Neural Network



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

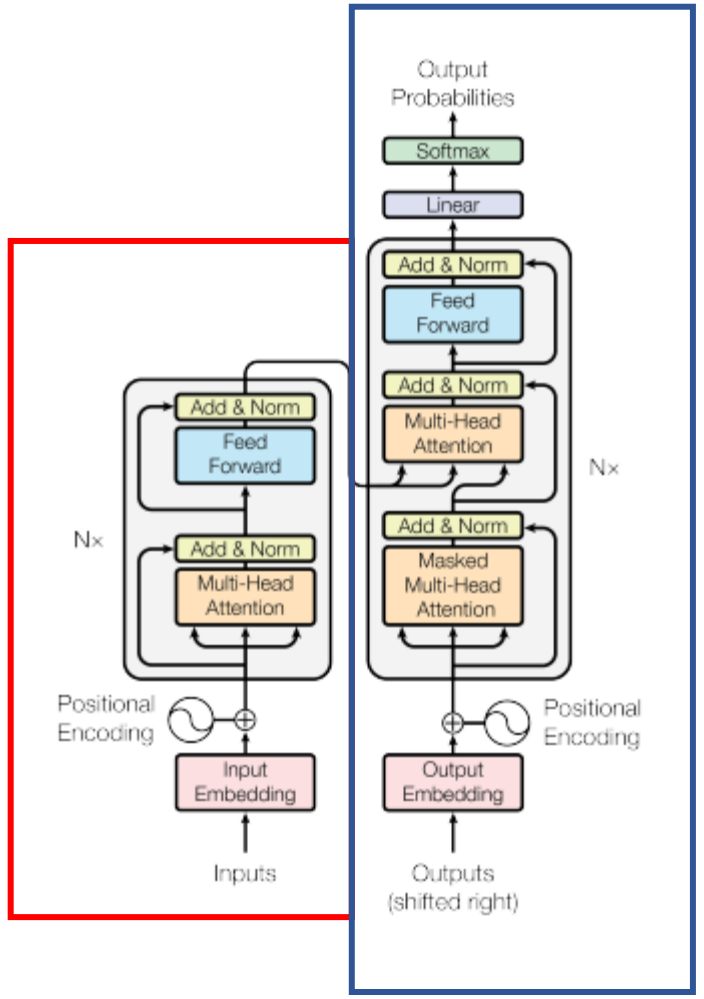
https://colah.github.io/posts/2015-08-Understanding-LSTMs/

The Transformer

- AlphaFold 2 uses an evolution of the Recurrent Neural Network called a Transformer
- The Transformers can be broken up into two blocks: the Encoder Block and the Decoder Block
- **Encoder Block:** turn sequences into vectors w/ positional information, the attention is limited by each character's interaction w/ the rest of the sequence
- **Decoder Block:** information from the previous block is converted to probability distributions

Encoder Block

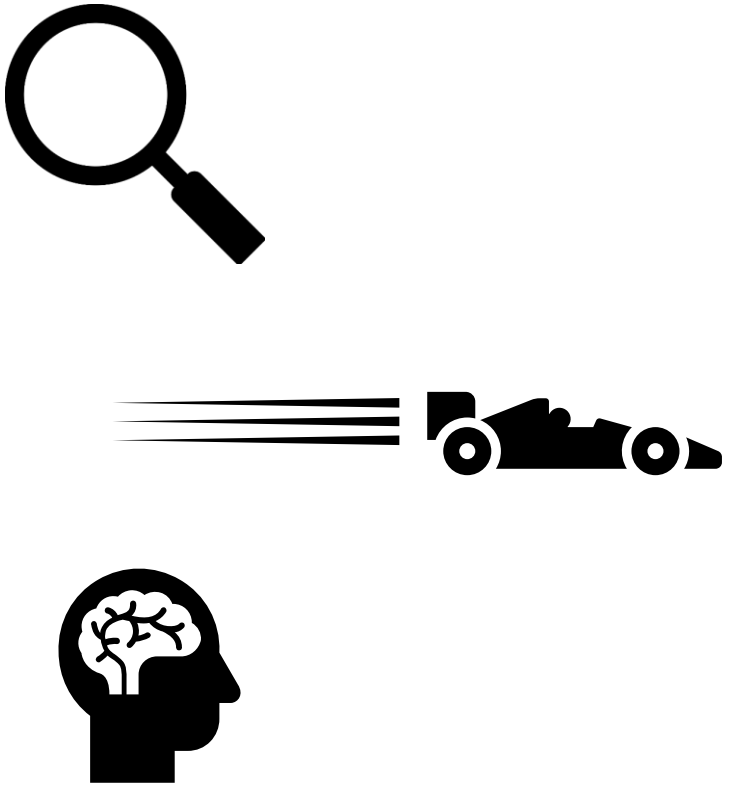
Decoder Block



https://proceedings.neurips.cc/paper/2017/file/3f5ee243547dee91fbd053c1c4a845aa-Paper.pdf

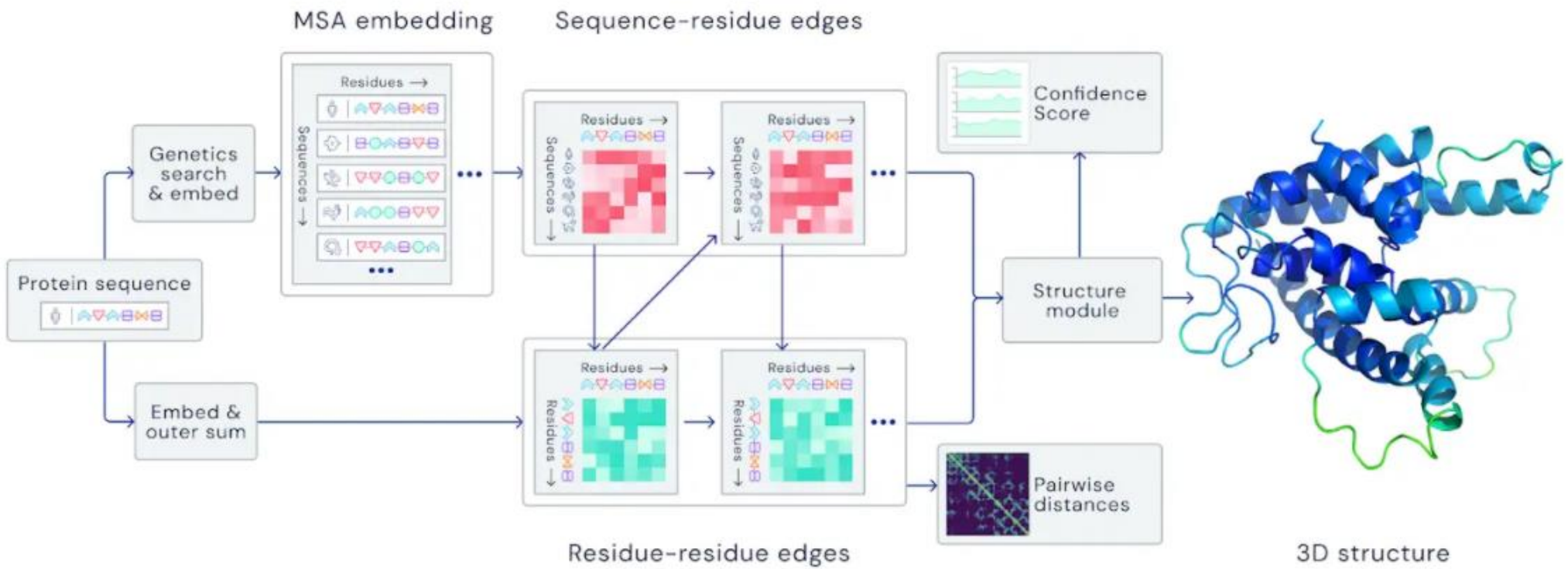
Transformer Benefits

- Limited attention means better memory utilization
- Faster model training times
- Overcome the issue of the memory being poor when pulling from old connections



https://towardsdatascience.com/transformer-neural-network-step-by-step-breakdown-of-the-beast-b3e096dc857f

The AlphaFold 2 Workflow



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Protein Sequence Information

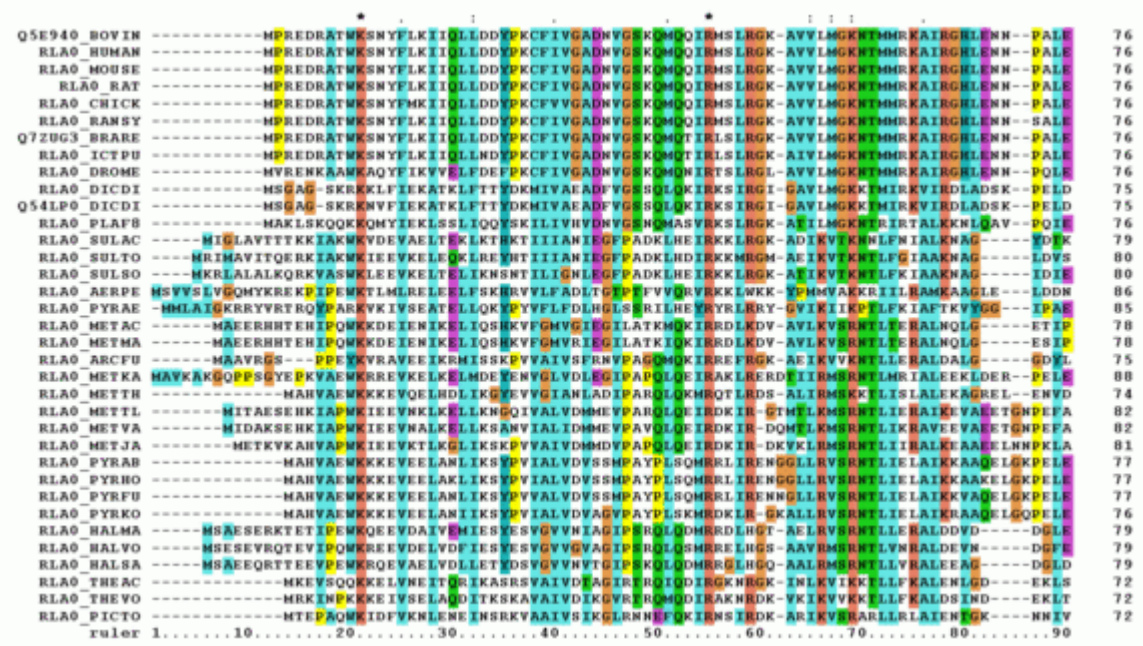
- Protein sequence information stored as a fasta file. Consists of a:
  - Header
  - sequence

```
>sp|P46598|HSP90_CANAL Heat shock protein 90 homolog OS=Candida albicans
(strain SC5314 / ATCC MYA-2876) OX=237561 GN=HSP90 PE=1 SV=1
MADAKVETHEFTAEISQLMSLIINTVYSNKEIFLRELISNASDALDKIRYQALSDPSQLE
SEPELFIRIIPQKDQKVLEIRDSGIGMTKADLVNNLGTIAKSGTKSFMEALSAGADVSMI
GQFGVGFYSLFLVADHVQVISKHNDDEQYVWESNAGGKFTVTLDETNERLGRGTMLRLFL
KEDQLEYLEEKRIKEVVKKHSEFVAYPIQLVVTKEVEKEVPETEEEDKAAEEDDKKPKLE
EVKDEEDEKKEKKTKTVKEEVTETEELNKTKPLWTRNPSDITQDEYNAFYKSISNDWEDP
LAVKHFSVEGQLEFRAILFVPKRAPFDAFESKKKKNNIKLYVRRVFITDDAEELIPEWLS
FIKGVVDSEDLPLNLSREMLQQNKILKVIRKNIVKKMIETFNEISEDQEQFNQFYTAFSK
NIKLGIHEDAQNRQSLAKLLRFYSTKSSEEMTSLSDYVTRMPEHQKNIYYITGESIKAVE
KSPFLDALKAKNFEVLFMVDPIDEYAMTQLKEFEDKKLVDITKDFELEESDEEKAAREKE
IKEYEPLTKALKDILGDQVEKVVVSYKLVDAPAAIRTGQFGWSANMERIMKAQALRDTTM
SSYMSSKKTFEISPSSPIIKELKKKVETDGAEDKTVKDLTTLLFDTALLTSGFTLDEPSN
FAHRINRLIALGLNIDDDSEETAVEPEATTTASTDEPAGESAMEEVD
```

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

Building a Multiple Sequence Alignment (MSA)

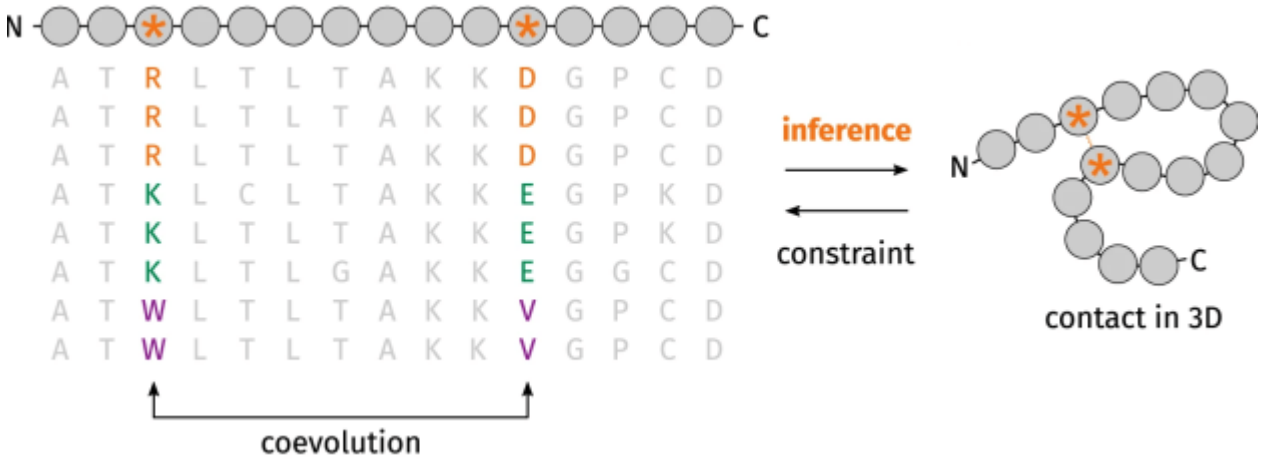
- The sequence is checked against a reference database of sequences - UniRef90 database
- Sequences with sections that align well to our query are then used as input



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

Coevolution of Residues

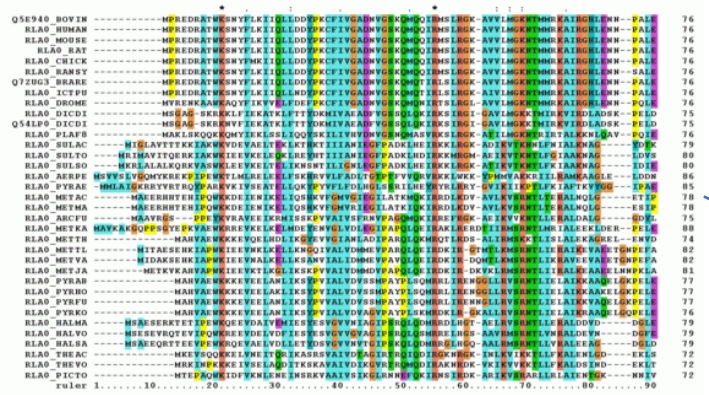
- So how does one go from an alignment to a structure?
- The theory is that residues that coevolve are generally close to each other in the protein's folded state



https://www.pnas.org/content/114/34/9122

Template Information

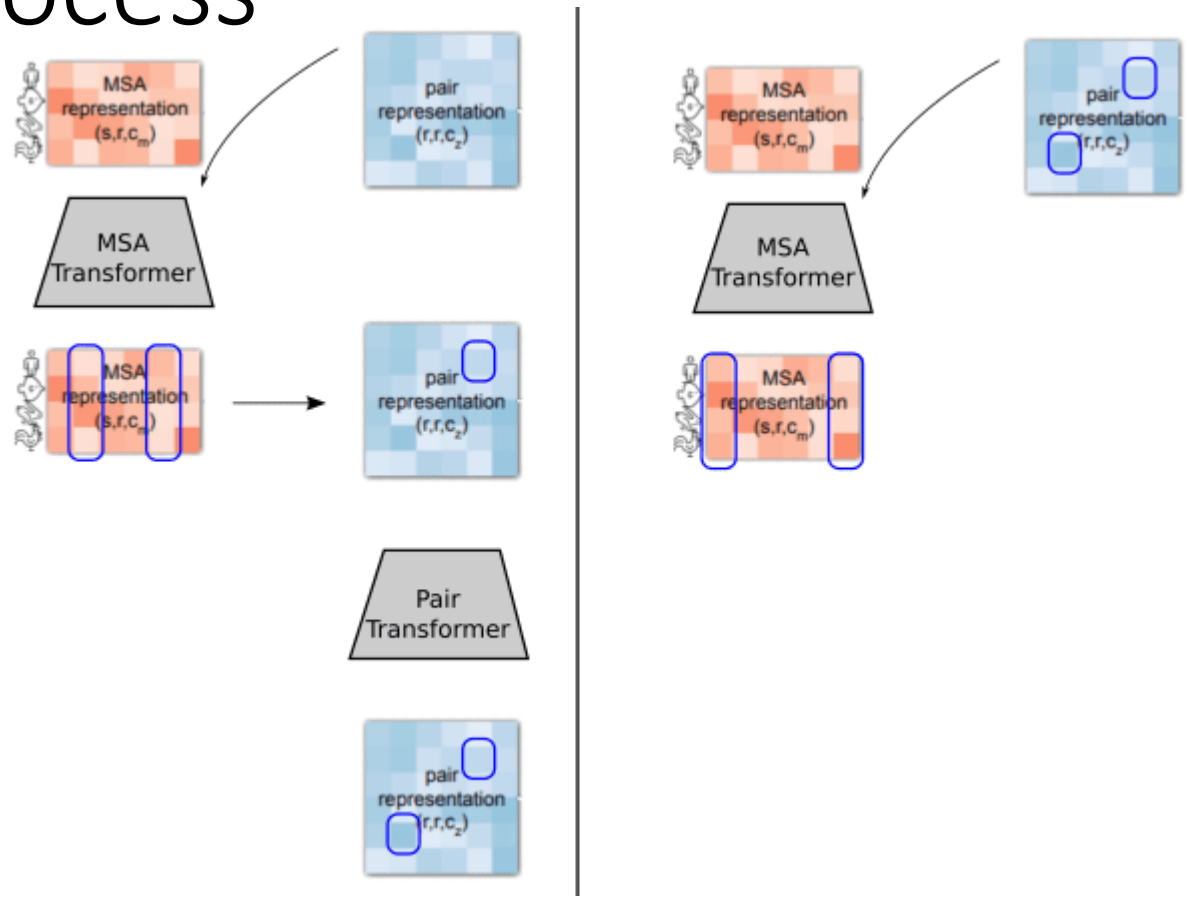
- The similar sequences pulled from also have structural information
- These templates can be converted into distance matrices to determine the distance between residues



https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

The Evoformer Process

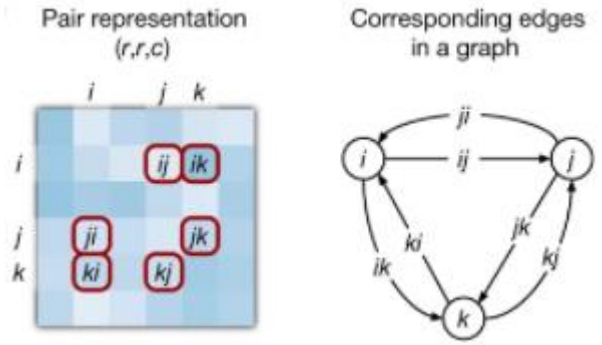
- Takes the MSA representation and the pair representation
- Uses the pair representation to limit the attention of the MSA transformer
- Model then determines two residues are close
- Given this information, the Pair Transformer notes that another two residues could be close
- Process is iterated until a possible structure is resolved

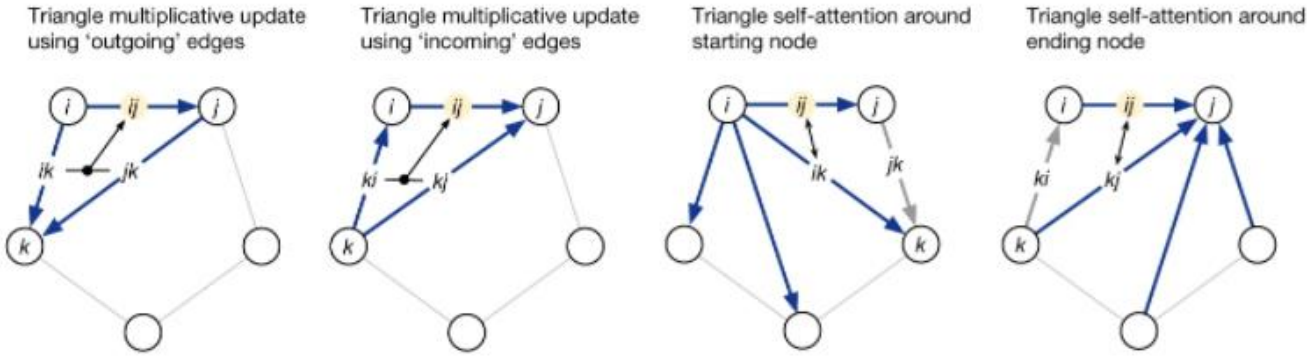


https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

A Closer Look at the Pair Transformer

- The pair transformer works on the principle of Triangle inequality, where the sum of two sides must be greater than or equal to the third side.
- Using this theorem, we can determine the likely distance residues have from one another because the distance between three points can never break that theorem

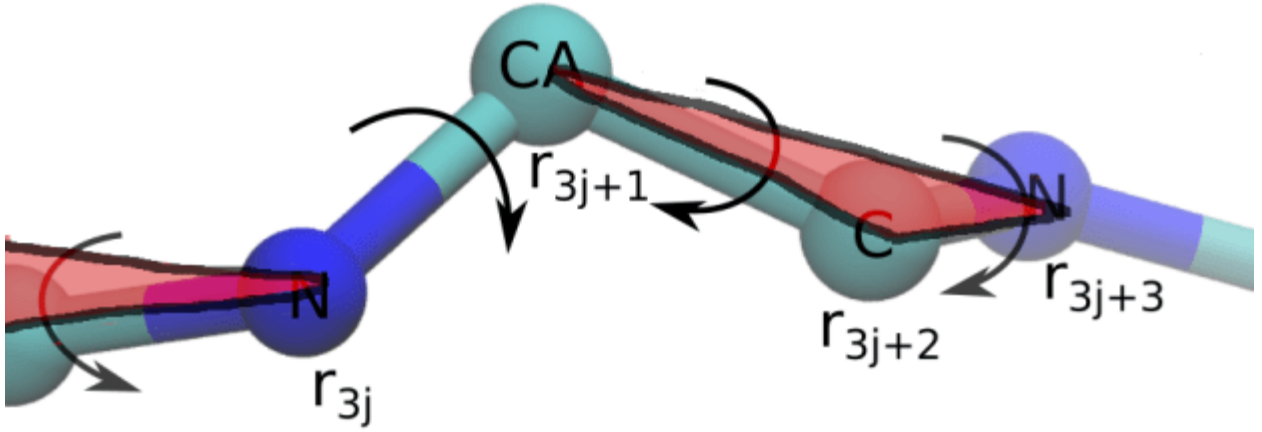




https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

The Structure Module

- Begins with each amino acid as a residue gas, or triangle with points at Nitrogen, R group Carbon and the Alpha Carbon
- These “gases” start at an origin point and are moved by the model using the pair distances and the information from the pairwise distance matrix and the MSA



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AlphaFold File Output

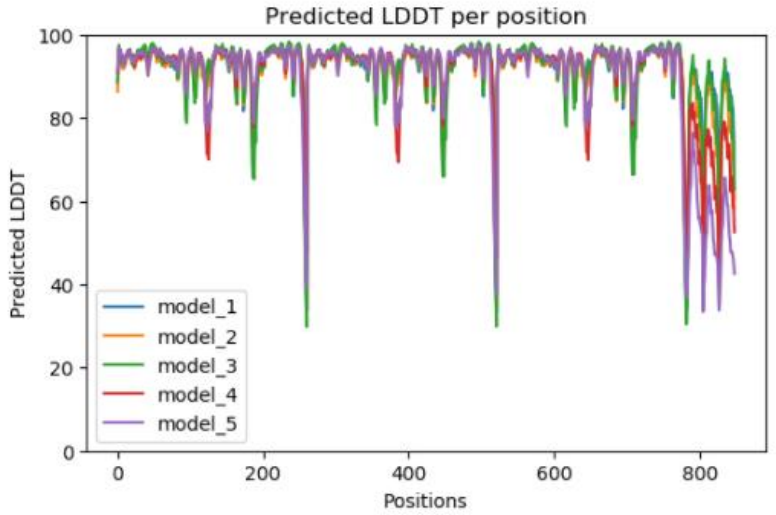
features.pkl	unrelaxed_model_*.pdb	relaxed_model_*.pdb	ranked_*.pdb	ranking_debug.json	timings.json	msas/	result_model_*.pkl
A pickle file w/ input feature NumPy arrays	A PDB file w/ predicted structure, exactly as outputted by the model	A PDB file w/ predicted structure, after performing an Amber relaxation procedure on the unrelaxed structure prediction	A PDB file w/ relaxed predicted structures, after reordering by model confidence (using predicted LDDT (pLDDT) scores). ranked_0.pdb = highest confidence ranked_4.pdb = lowest confidence	A JSON file w/ pLDDT values used to perform the model ranking, and a mapping back to the original model names.	A JSON file w/ times taken to run each section of the AlphaFold pipeline.	A directory containing the files describing the various genetic tool hits that were used to construct the input MSA.	– A pickle file w/ a nested dictionary of the various NumPy arrays directly produced by the model: • Structure Module Output • Distograms • Per-residue pLDDT scores • predicted TM-score • predicted pairwise aligned errors

https://github.com/deepmind/alphafold

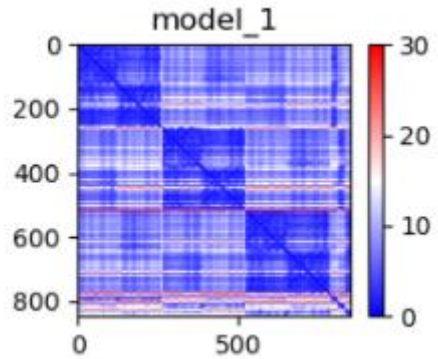
Assessing AlphaFold Accuracy

- We can assess the accuracy of the AlphaFold prediction using:
  - Predicted Local Distance Difference Test (pLDDT)
  - Predicted Alignment Error

Predicted Local Distance Difference Test (pLDDT)



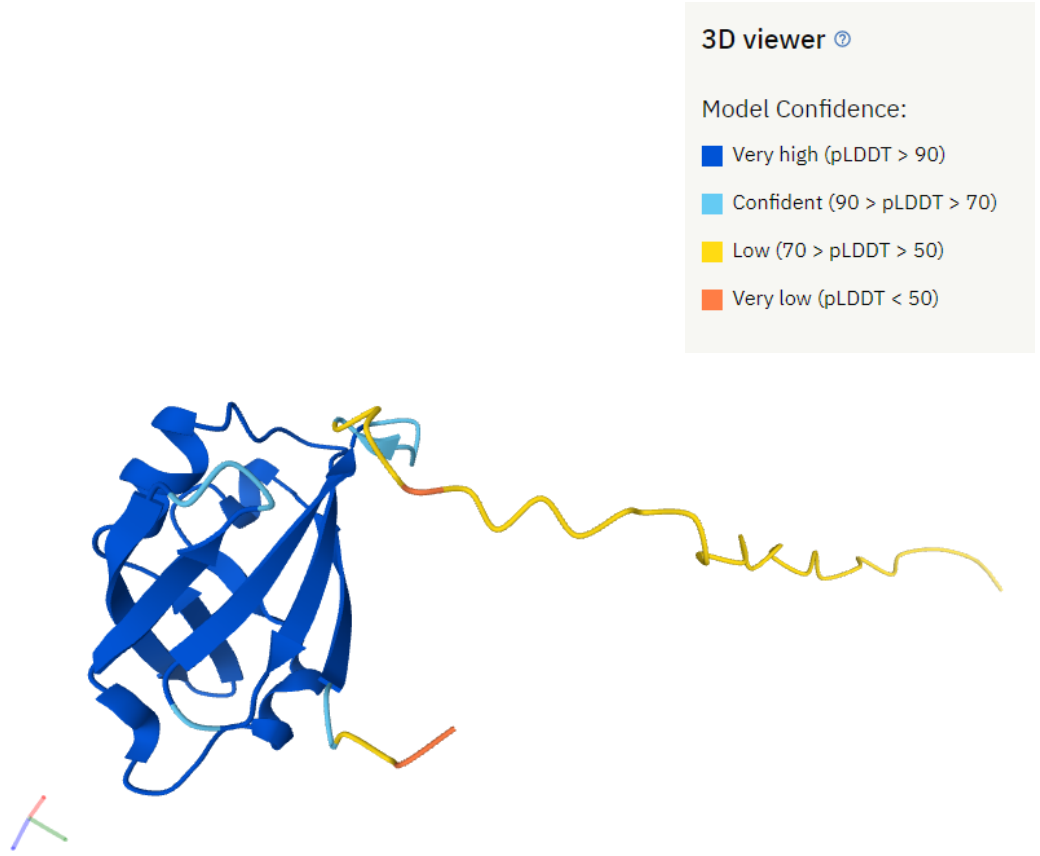
Predicted Alignment Error (PAE)



https://github.com/deepmind/alphafold

Predicted Local Distance Difference Test (pLDDT)

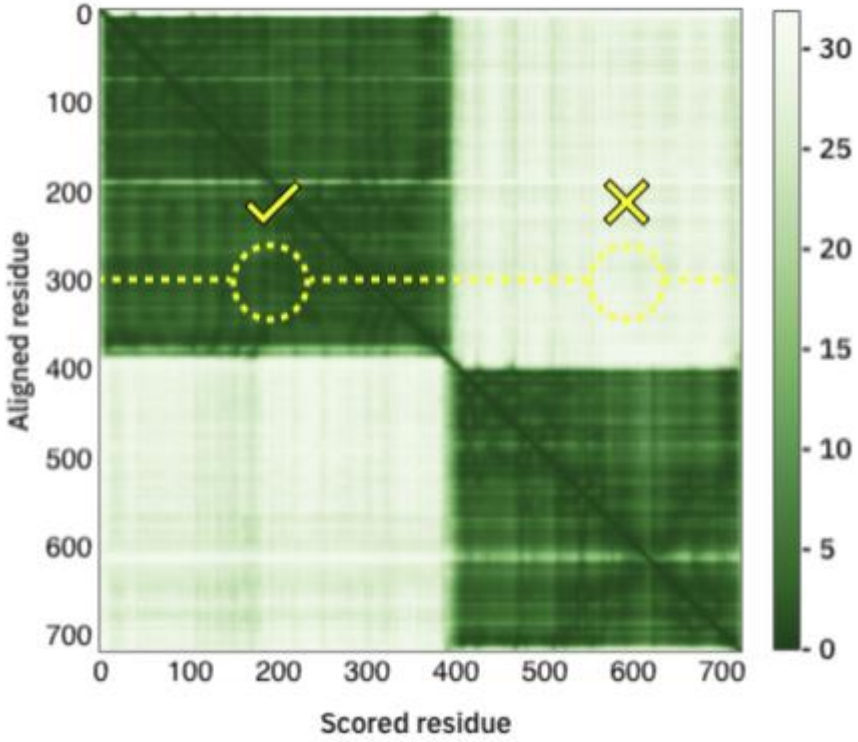
- per-residue confidence metric ranging from 0-100 (100 being the highest confidence)
- Regions below 50 could indicate disordered regions
- This information can be found in each model's result_model_*.pkl file where * is the model number



https://alphafold.com/entry/Q9FX77

Predicted Alignment Error (PAE)

- The color at (x, y) corresponds to the expected distance error in residue x's position, when the prediction and true structure are aligned on residue y.
- So, in the example to the right:
  - The darker color indicates a lower error
  - When we are aligning on residue 300, we are more confident in the position of residue 200 and less confident in the position of residue 600



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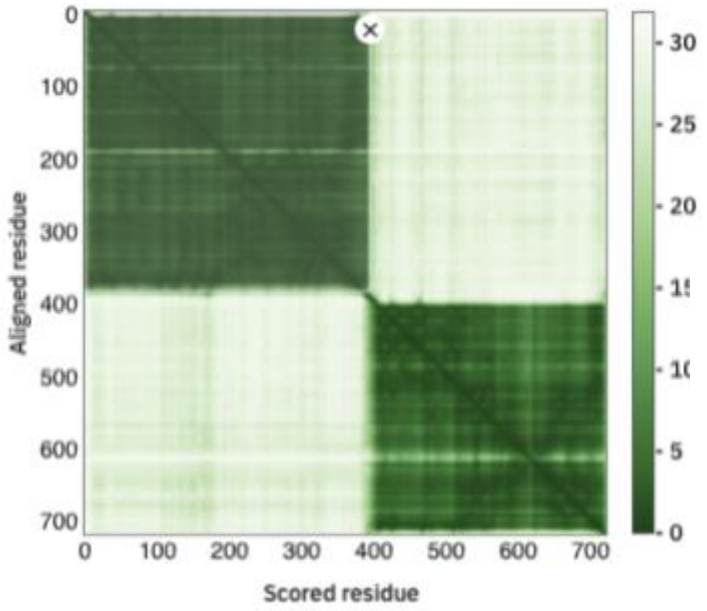
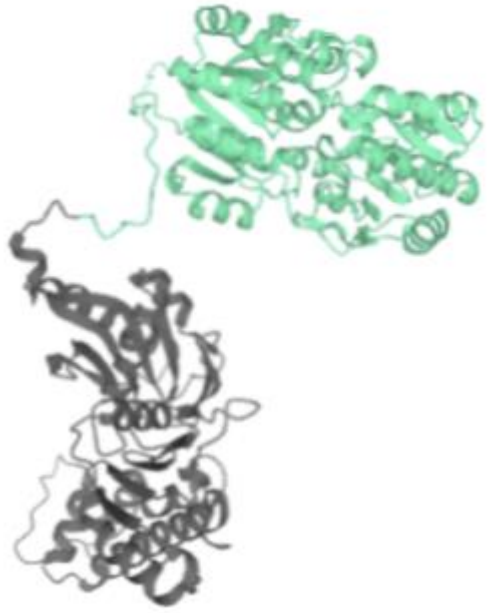
Predicted Alignment Error (PAE) cont.

- The example in the previous slide came from a multimer prediction
- Here we see that the error is higher when assessing the position between the two chains





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Acknowledgement

Much of this tutorial has been adapted from the Oxford Protein Informatics Group's explanation on AlphaFold 2

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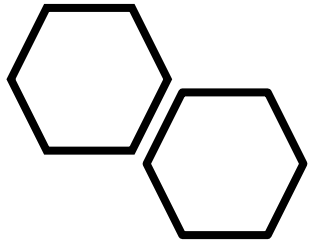

Next: Setup