Introduction to Protein Structure Prediction With AlphaFold 2

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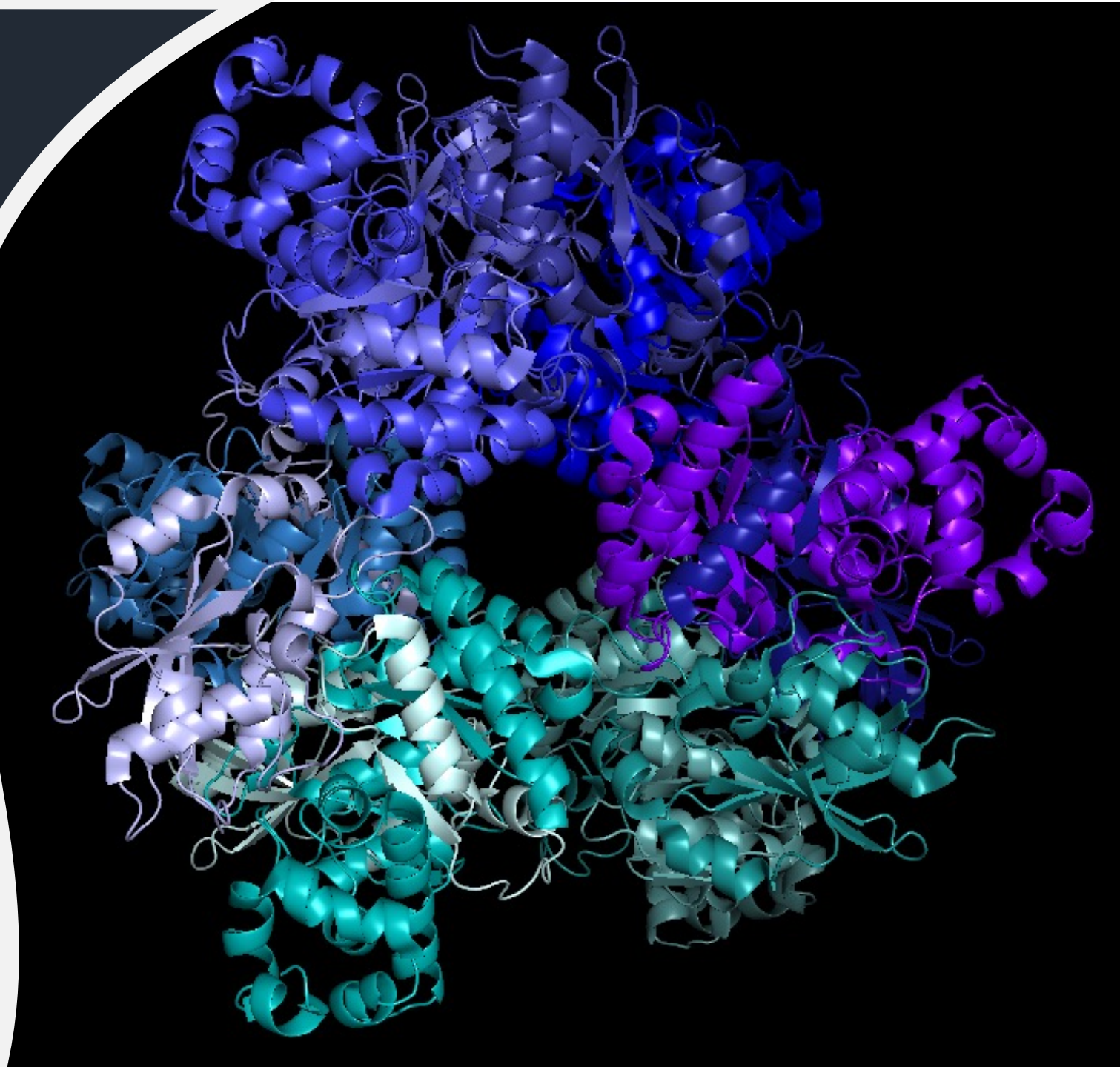
Bioinformatics Scientist



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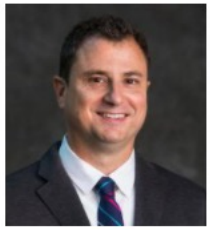

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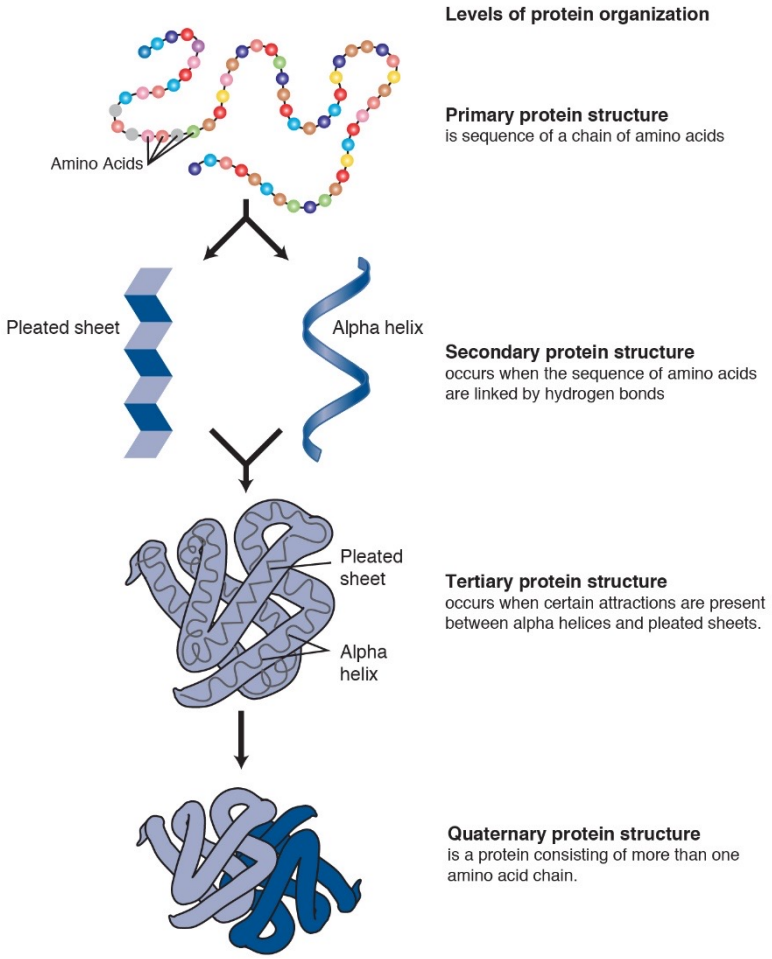
- ✓ Consultation on Projects and Grants
- ✓ High Performance Compute Cluster
- ✓ Workshops

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

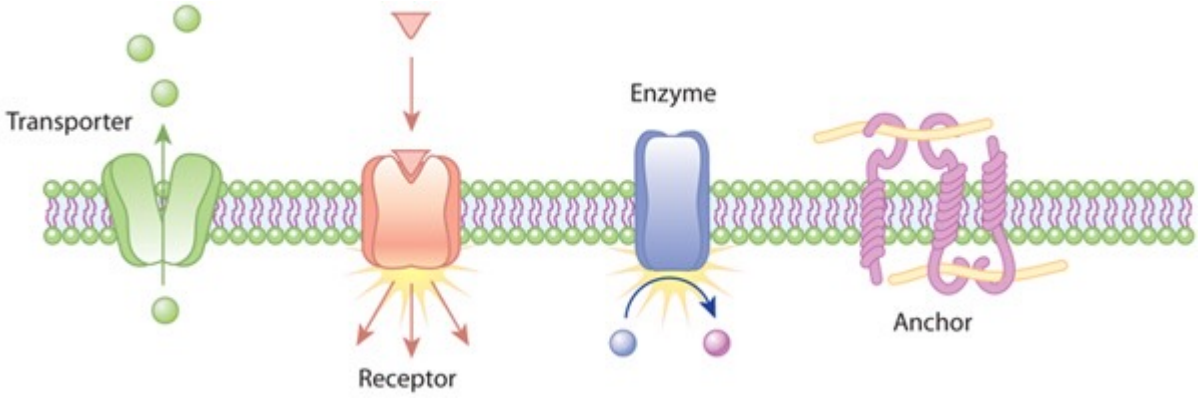


https://www.genome.gov/genetics-glossary/Protein

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



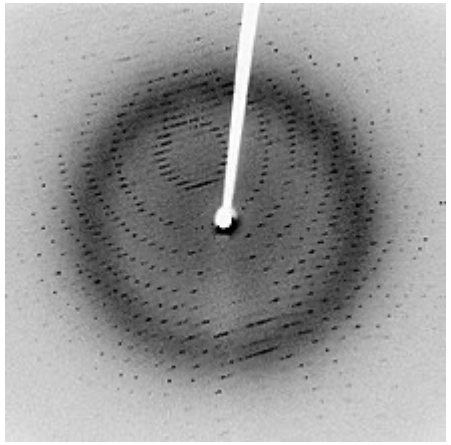
https://www.nature.com/scitable/topicpage/protein-function-14123348/

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

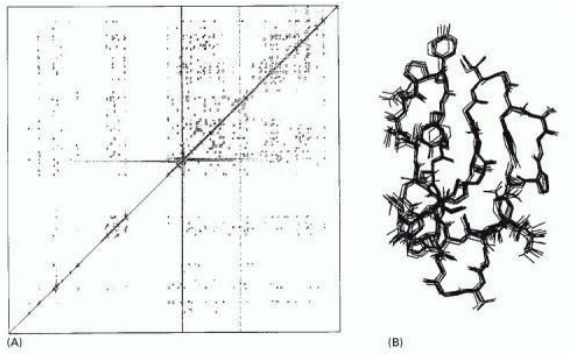
Laboratory Means To Determine Protein Structure

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

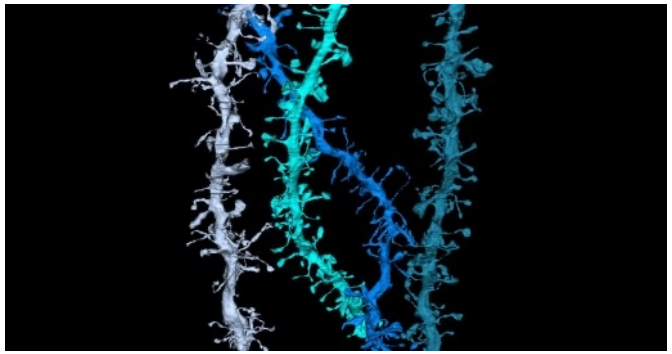
X-ray Crystallography



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

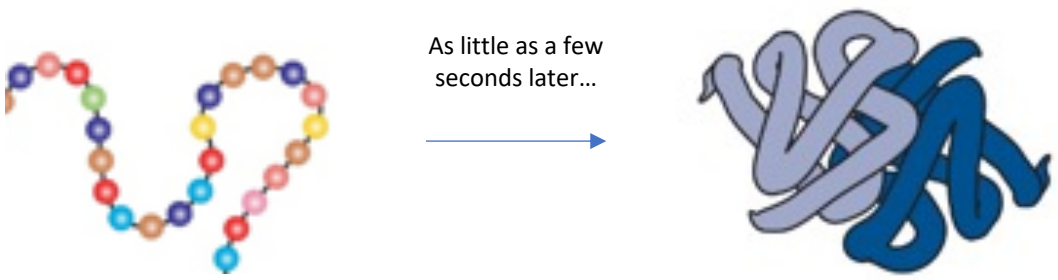
The Protein Structure Problem

- 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

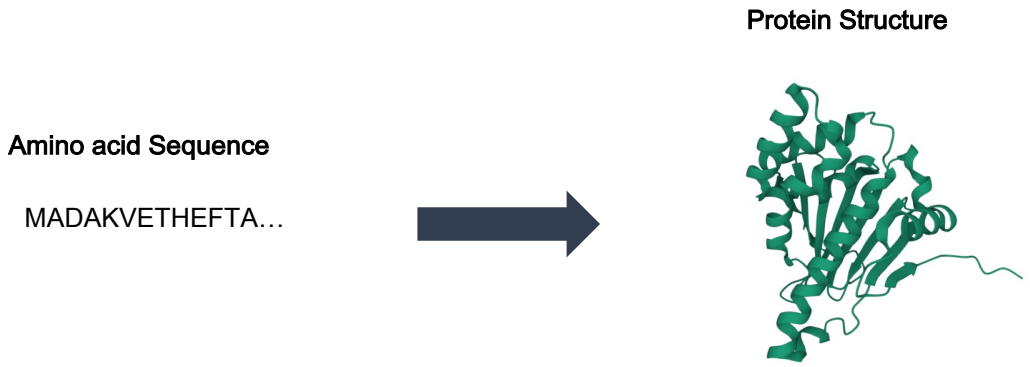
- Finding the native folded state of a protein by random searching of all possible configurations would take an enormous amount of time
- However, proteins can often fold within seconds
- Meaning some process must be guiding this folding



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

Using Sequence To Predict Structure

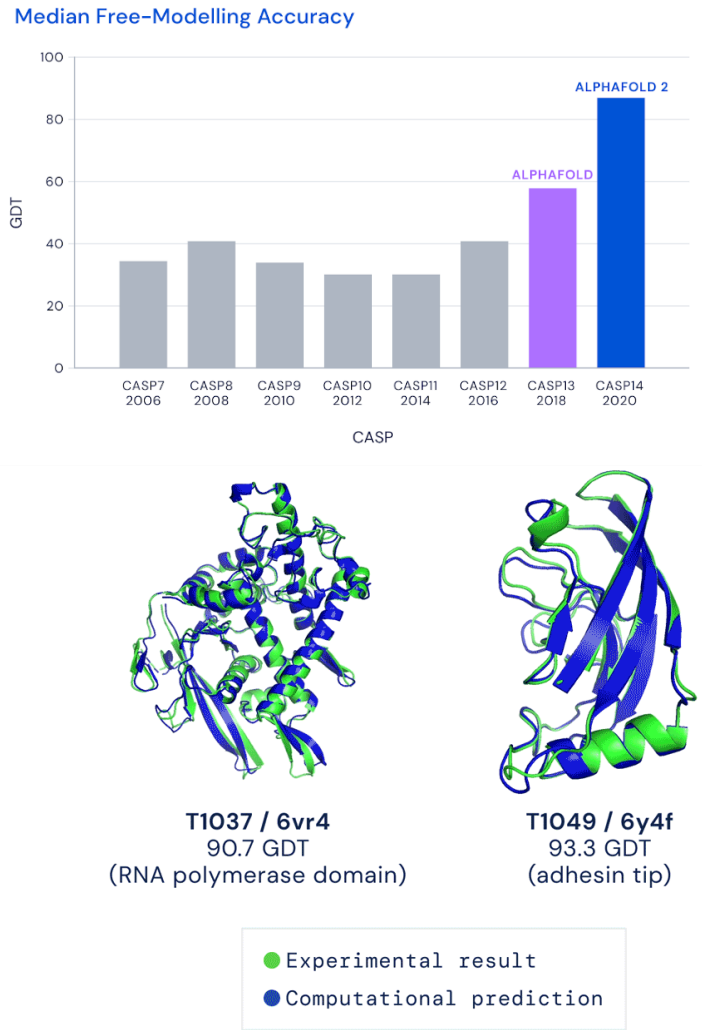
- Instead of laboratory experimentation, there have been massive efforts to use a protein's sequence to determine structure
- In 1994, the Critical Assessment of Structure Protein (CASP) was established as a biennial assessment of methods to predict structure from sequence



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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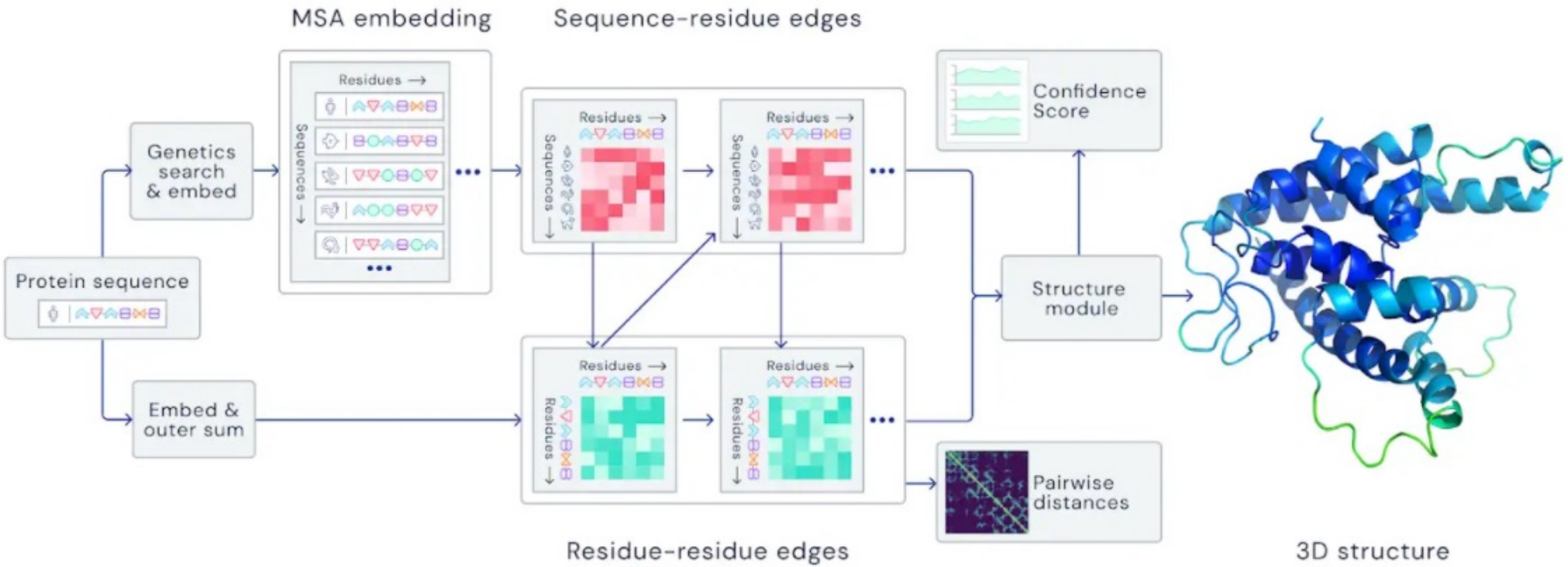
The AlphaFold 2 Workflow



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

Protein Sequence Information

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

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
KEDQLEYLEEKRIKEVVKKHSEFVAYPIQLVVTKEVEKEVPETEE
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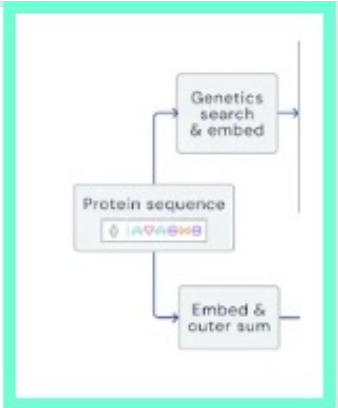
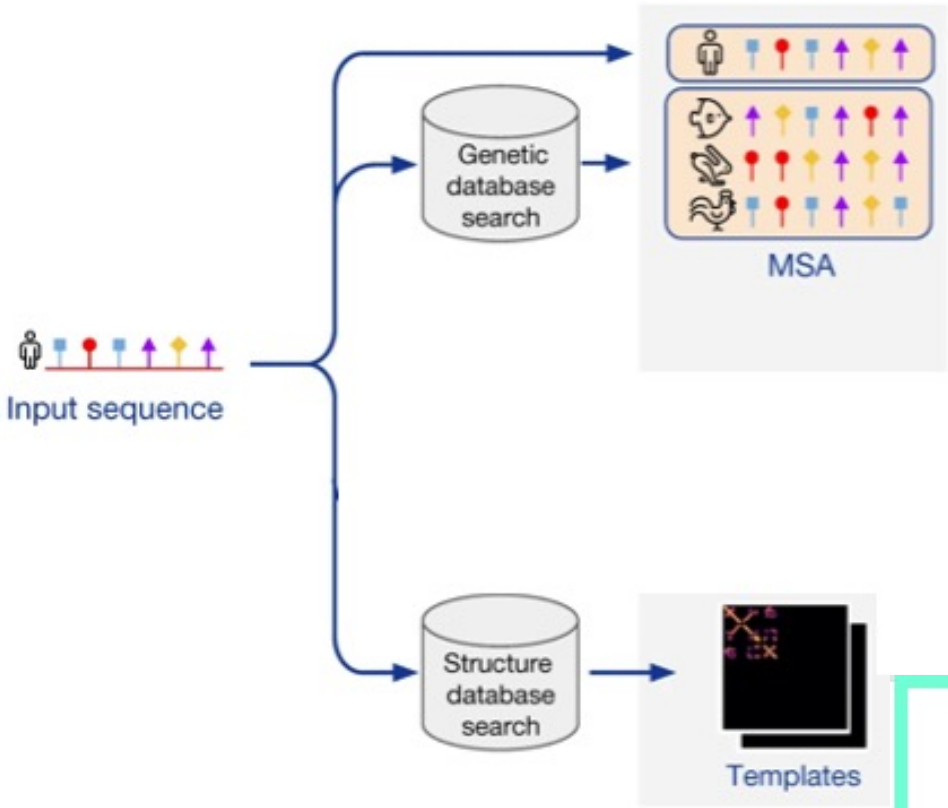
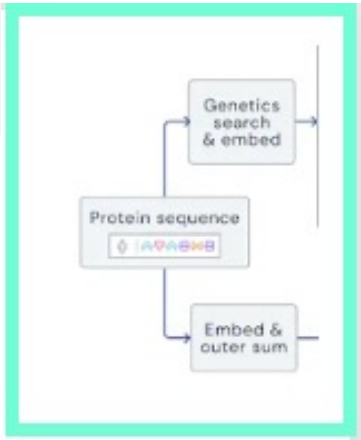


https://en.wikipedia.org/wiki/FASTA_format

Searching for Similar Sequences

- This query sequence is compared to:
  - **UniRef90 database:** to find similar sequences
  - **PDB70** to find similar structures
- Sequences that are too similar to our query are filtered out so that we don't just build a replicate based on that sequence
- These sequences are arranged as an MSA





https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/
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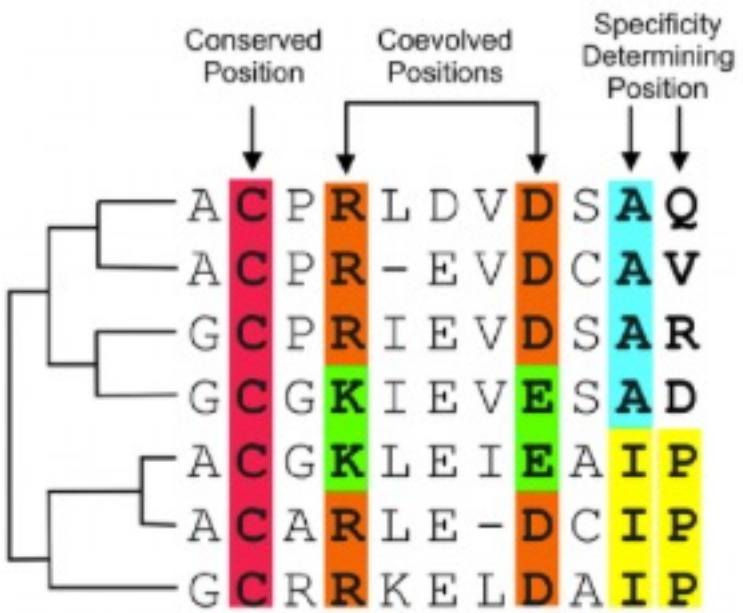
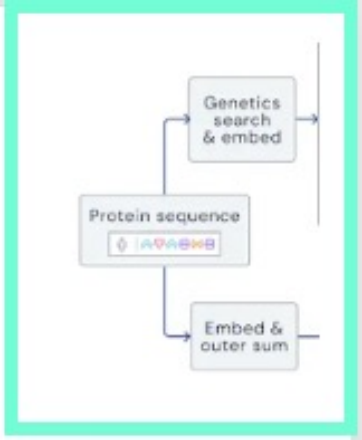
Multiple Sequence Alignment (MSA)

- An MSA is an array of sequences
- These sequences are ***aligned*** with one another as to best match similar regions
- These sequences don't always line up perfectly and as such we see:
  - **Conserved positions:** where the letter does not change
  - **Coevolved positions:** where the letter will change with another letter
  - **Specificity Determining positions:** where the letter is consistently different



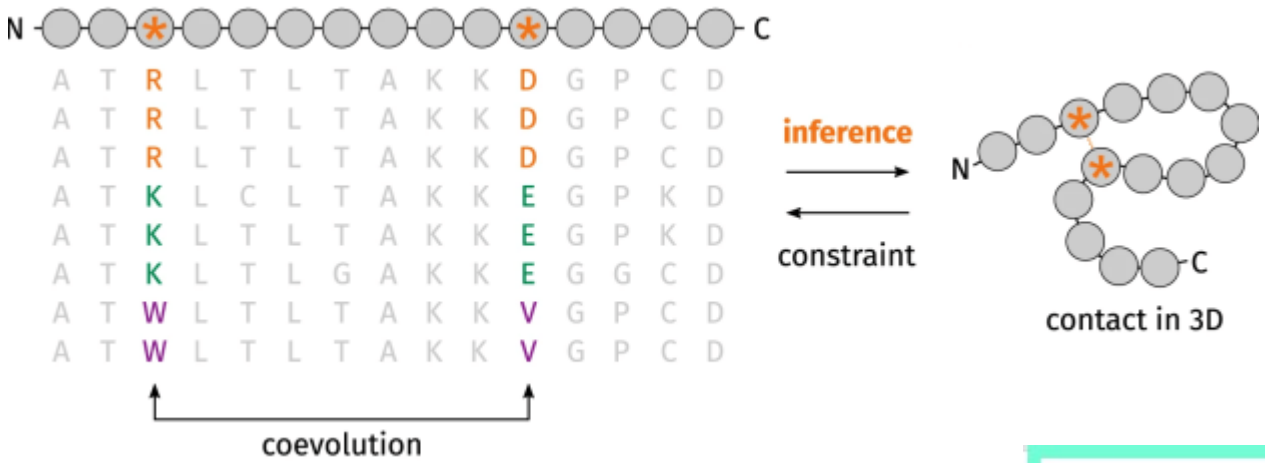


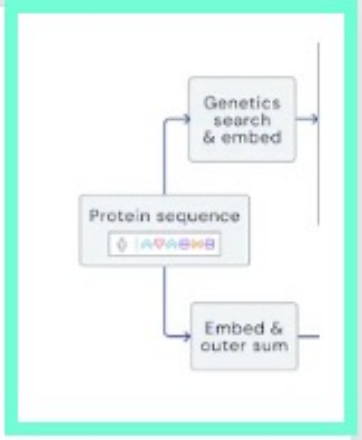
https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-13-235

Why is an MSA Useful In Structure Prediction?

- The theory is that residues that coevolve are generally close to each other in the protein's folded state
- So, by assessing what residues change together we get an idea of where they might be spatially!

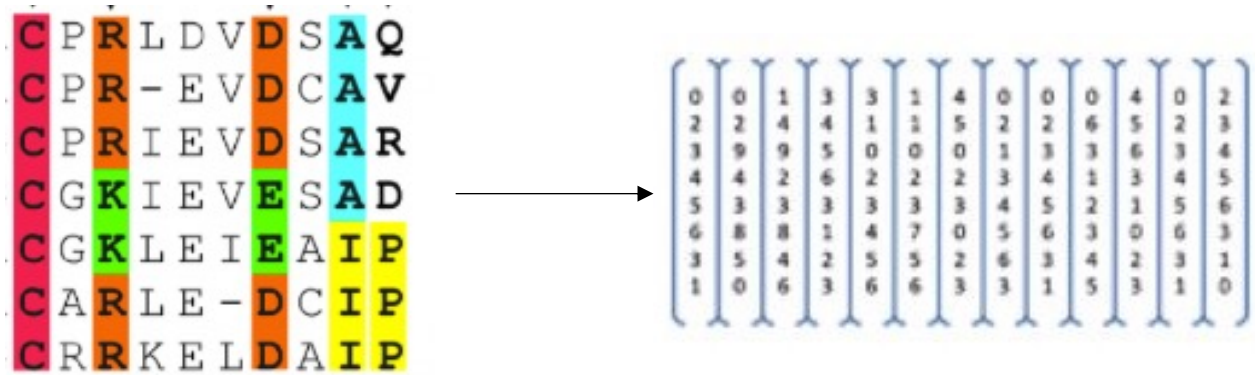


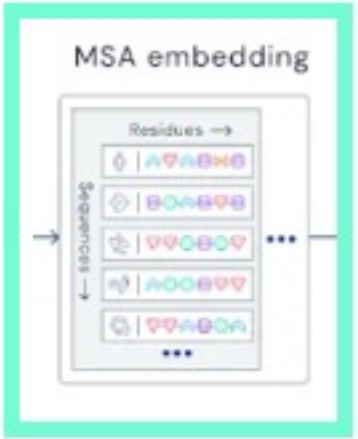


https://www.pnas.org/content/114/34/9122

MSA Embedding

- An MSA is still essentially an array of letters
- To be more computer friendly these letters are ***embedded*** as numbers using their positional information
- AlphaFold embeds these letter values as numeric ones and terms this the MSA representation





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

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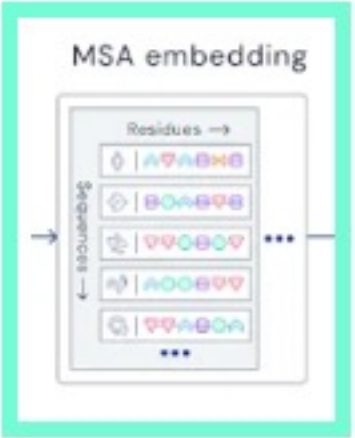
Embedding Example

- Take for example the sentence "**I ate an apple and played the piano**"
- This string is embedded by positional information.
- **e.g. ate** was the second word so there is a 1 in the second column at row **"ate"**

	1	2	3	4	5	6	7	8
I	1	0	0	0	0	0	0	0
ate	0	1	0	0	0	0	0	0
an	0	0	1	0	0	0	0	0
apple	0	0	0	1	0	0	0	0
and	0	0	0	0	1	0	0	0
played	0	0	0	0	0	1	0	0
the	0	0	0	0	0	0	1	0
piano	0	0	0	0	0	0	0	1



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

- Similar Structures were also queried for using our protein sequence.
- These structure files (A.K.A Crystallographic Information Files (CIF)) contain 3D coordinates for a protein's atoms in space
- These coordinates are used to initialize a pairwise distance matrix between residues that AlphaFold calls the pair representation

Residue	x	y	z
C	-3.8	-1.12	0.57
N	0.96	-1.07	-0.89
H	0.05	0.95	0.39

	C	N	H
C	0	-1.12	.94
N	0.54	0	3.1
H	0.05	1.32	0

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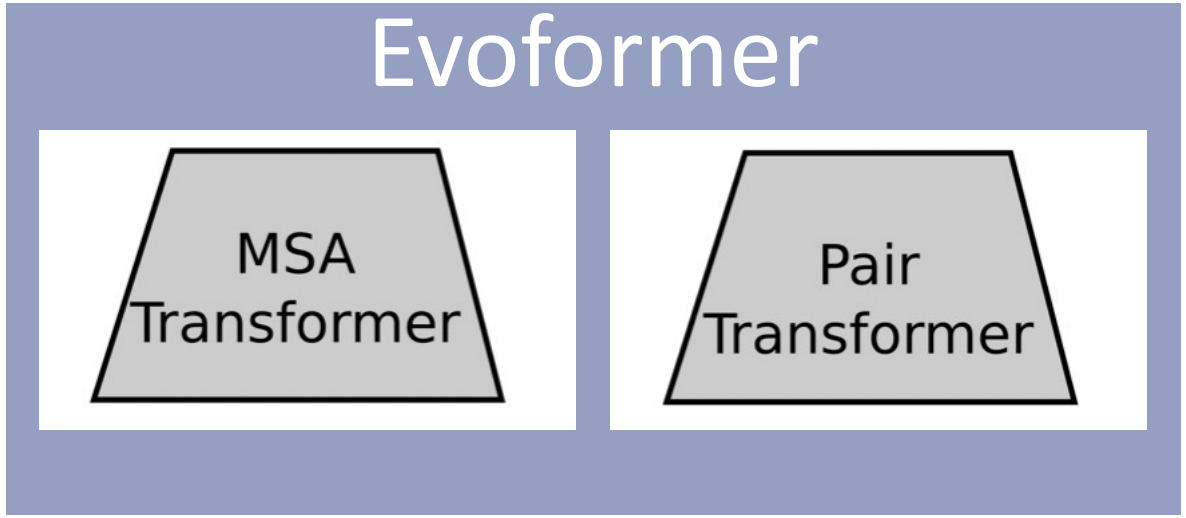
The Evoformer

- The MSA representation and the pair representation are fed into in special type of neural network that AlphaFold terms the Evoformer
- The Evoformer is a combination of two special types of neural networks called Transformers



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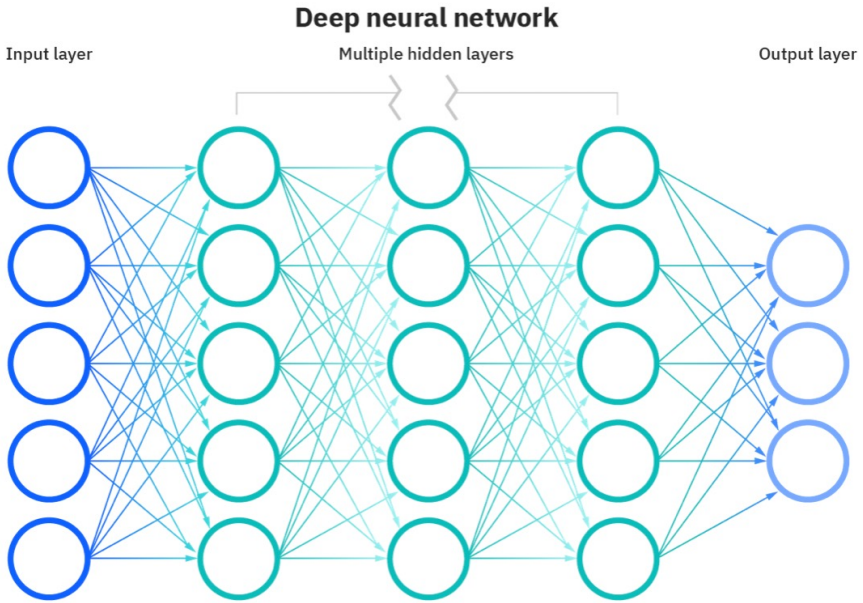
What Are Neural Networks?

- Neural Networks are machine learning algorithms that mimic the way neurons communicate
- They usually consist an input, hidden and output layer
- Each node has a threshold and if the output of the node isn't above that threshold it doesn't communicate with the next node



https://www.ibm.com/cloud/learn/neural-networks

What Is In A Node?

- Each node can be thought of as a linear regression model with input data, weights, a bias term and an output
- The weights are assigned as to weight importance – the larger the weight the more important the variable

A Single Node

$$= \sum_{i=1}^{m} w_i x_i + bias = w_1 x_1 + w_2 x_2 + w_3 x_3 + bias$$

https://www.ibm.com/cloud/learn/neural-networks

To Communicate Or Not Communicate?

- Each node will have an output based on this regression function
- That output is then fed into something called an activation function
- The output of this activation function is compared to some threshold
- If the threshold is met it ”fires” and communicates with the next layer

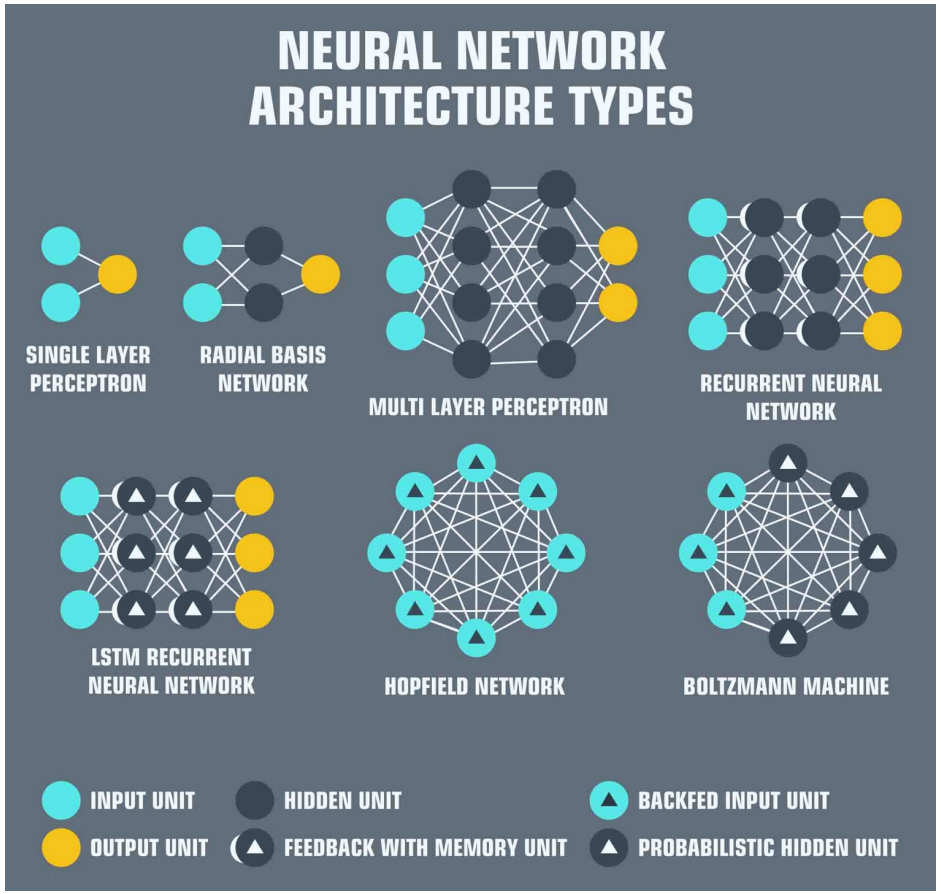
$$\sum_{i=1}^{m} w_i x_i + bias = w_1 x_1 + w_2 x_2 + w_3 x_3 + bias$$

$$\text{output} = \underbrace{f(x)}_{\text{Activation Function}} = \begin{cases} 1 \text{ if } \sum w_1 x_1 + b \geq 0 \\ 0 \text{ if } \sum w_1 x_1 + b < 0 \end{cases}$$

https://www.ibm.com/cloud/learn/neural-networks

Neural Network Customization

- There are different types of neural networks depending on what functions you use and how you organize node communication
- AlphaFold uses a Recurrent Neural Network

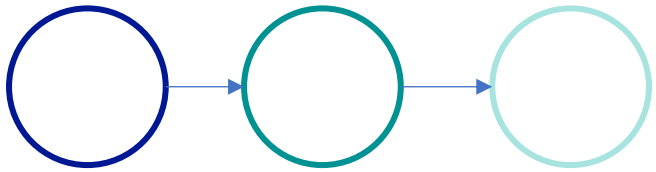


https://www.allerin.com/blog/3-types-of-neural-networks-that-ai-uses

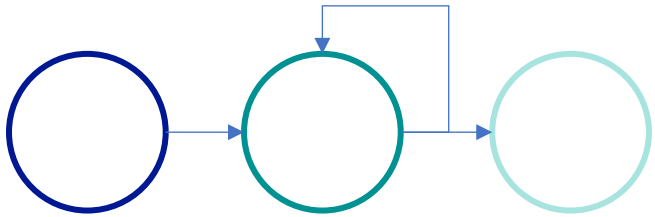
Recurrent Neural Network

- In a feed forward neural network you have input that is processed through a node and if that node is activated it communicates with the next node
- In a recurrent neural network, the output of a node can be used to inform and change the output of the node
- Naturally this comes at a memory cost when it tries to pull from old connections

Feed-Forward Neural Network



Recurrent Neural Network



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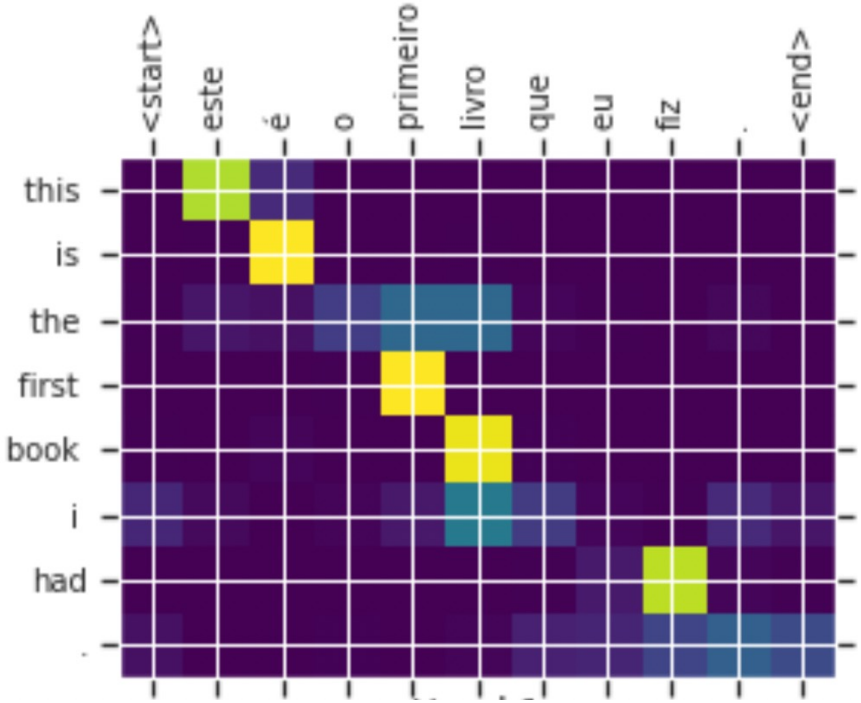
Transformer And Attention

- To save on computational cost, Recurrent Neural Networks can have their attention limited
- Basically, values are scaled down to reveal which data points are worth paying ***attention*** to
- This focused recurrent neural network is called a Transformer

An Example Using Language Translation



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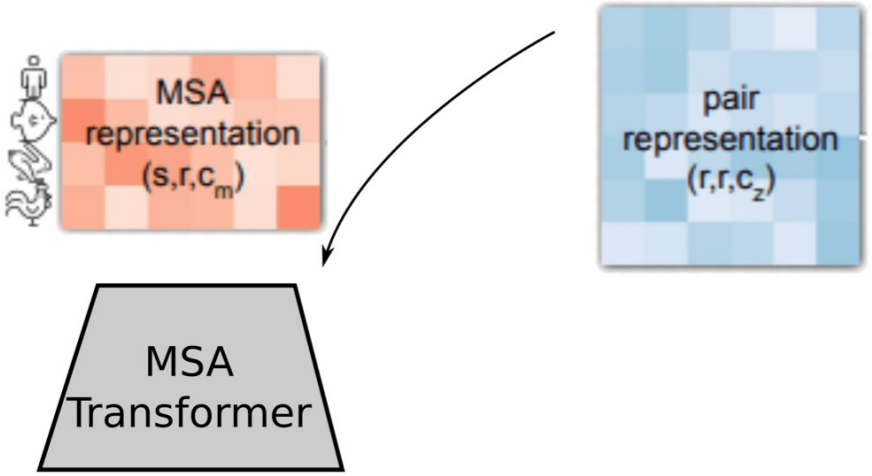
MSA Transformer

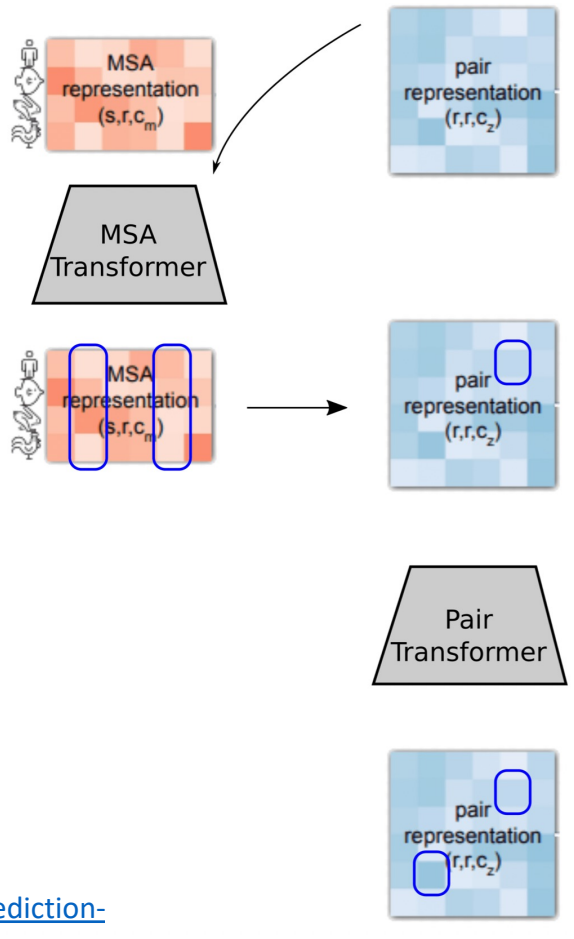
- The MSA Transformer limits its attention two ways:
  - Row-wise: to determine which residues are most related
  - Column-wise: to determine which sequences are most important
- The limited MSA along with the Pair Representation are then fed into the first head of the Evoformer

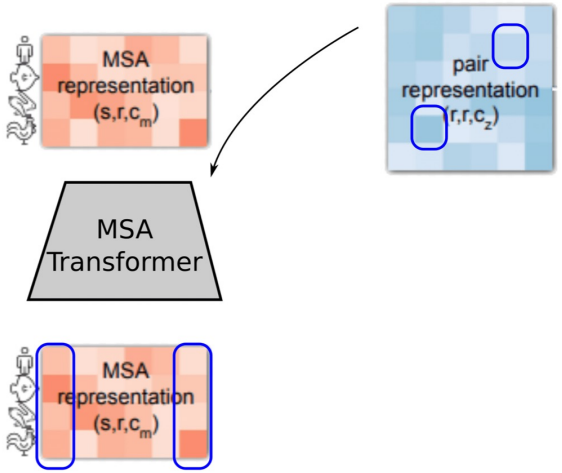


https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

Evoformer Part 1

- The first block of the Evoformer works to determine how close residues are
- start with correlations between two sets of residues, say A and B
- Highly correlation indicates these residues are close
- Now process is iterated - residue C is correlated with B
- So, B and C are close
- This process is repeated for all residues

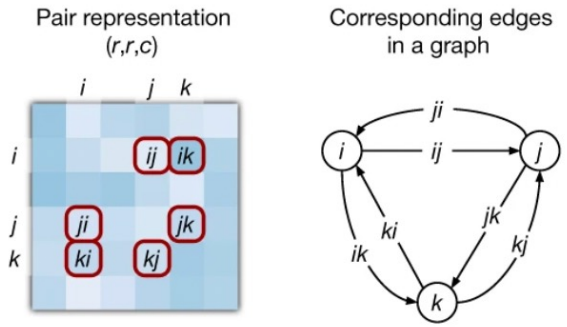


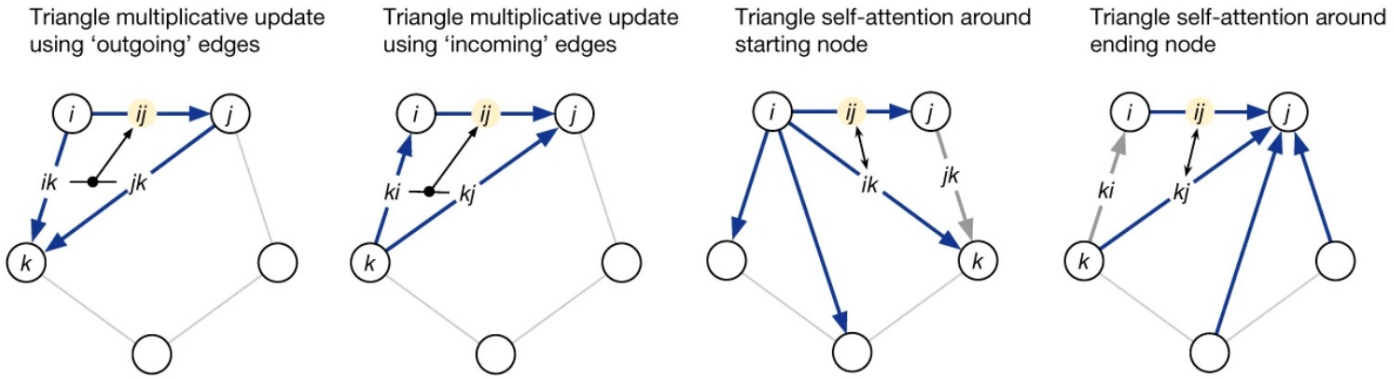


https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/

Evoformer Part 2

- The second block of the Evoformer works through pair wise distances between residues
- Here 3 residues are compared, and triangle inequality is enforced
- So, one side of the triangle must be less than or equal to the other two sides





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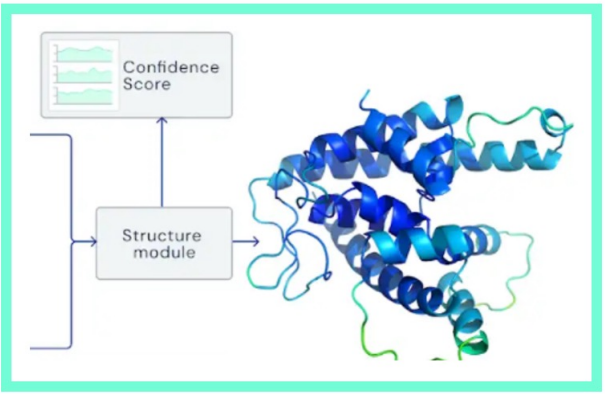
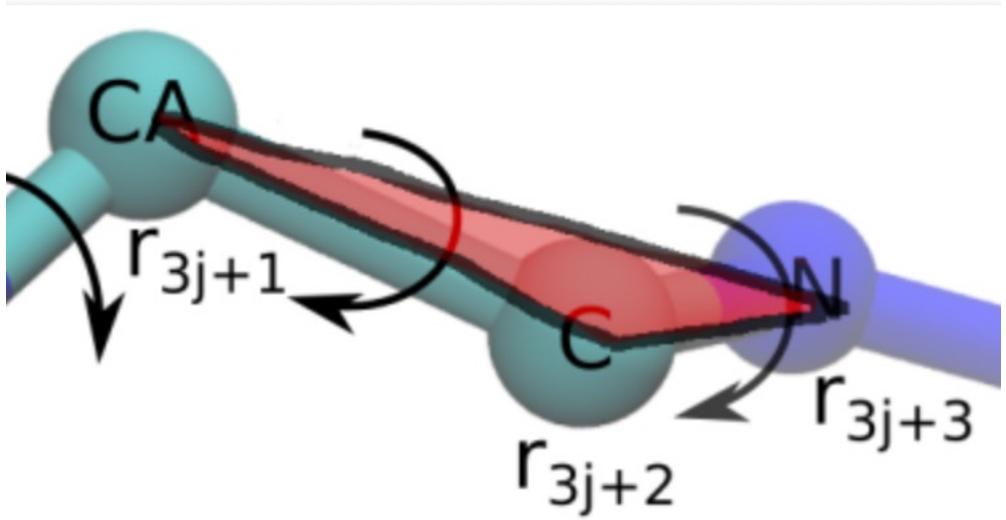
Structure Module



- The Evoformer outputs distances between residues, but residues are themselves three dimensional objects
- How are they oriented?
- Each residue starts as a “residue gas” or triangle between the Alpha Carbon, R-group Carbon, and the Nitrogen



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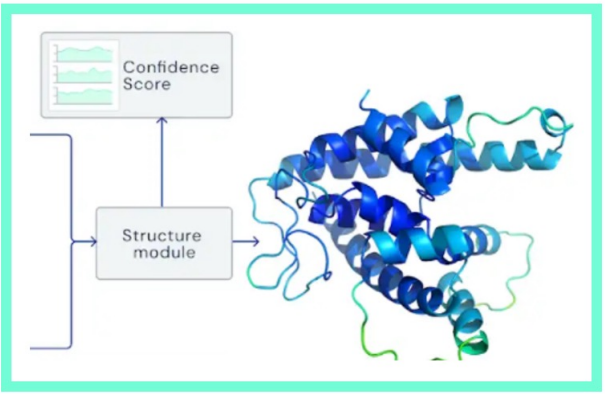


- All residue gases start at the origin of the coordinate system
- Each position is defined as an affine matrix, or **xyz** coordinates for the three points of the triangle, which is multiplied by a displacement vector to "move" the residue gas to its final location

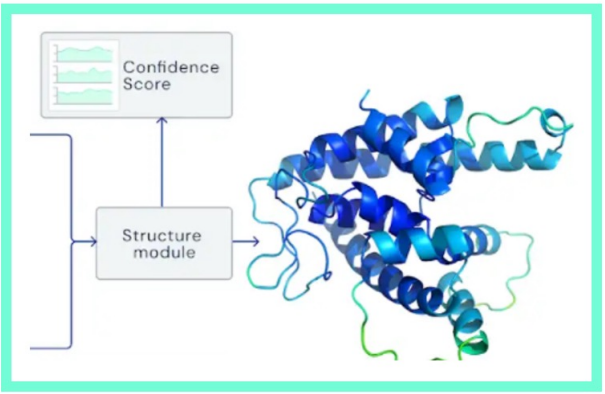
Displacement Vector

$$\mathbf{M} = \begin{pmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

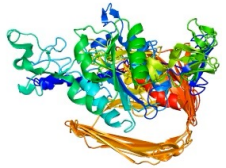
Affine Matrix

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

Structure Module – Invariant Point Attention



- The Structure Module also uses an attention mechanism called Invariant Point Attention
- This limits the data the model needs because points in 3D space are ***invariant*** to translation/rotation
- Basically, this means that no matter how you rotate/translate the final structure you still produce the same answer



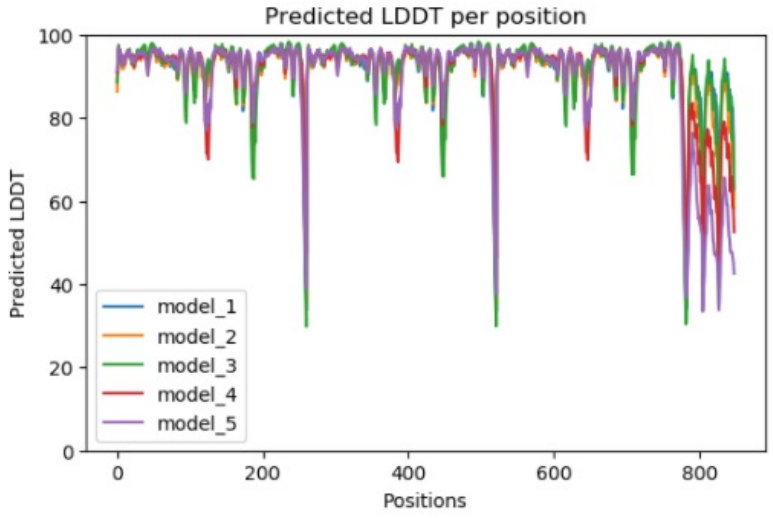
Recycling iteration 0, block 01
Secondary structure assigned from the final prediction

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

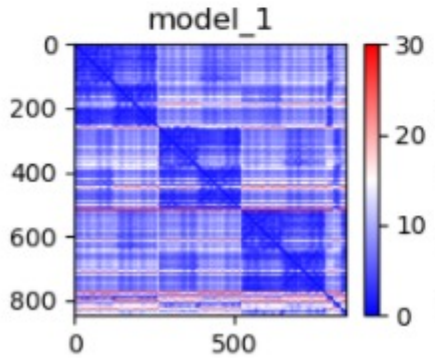
Assessing AlphaFold Accuracy

- 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)



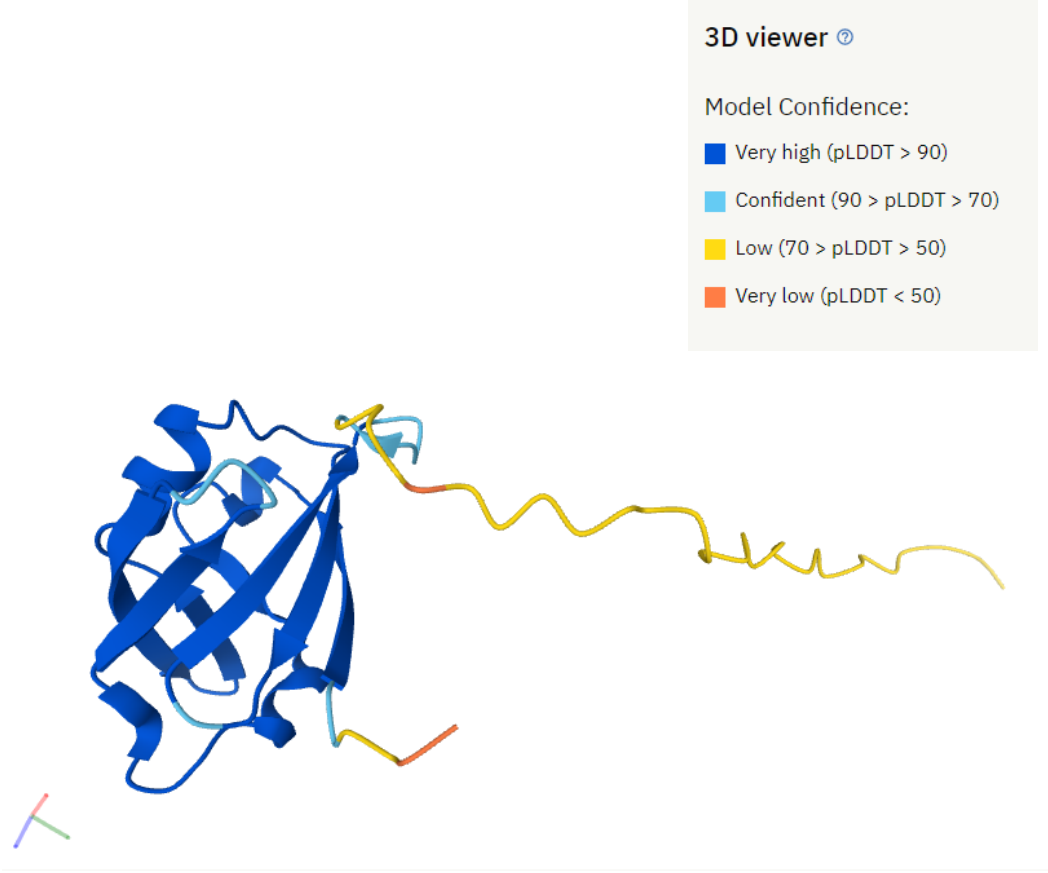
Predicted Alignment Error (PAE)



https://github.com/deepmind/alphafold

Predicted Local Distance Difference Test (pLDDT)

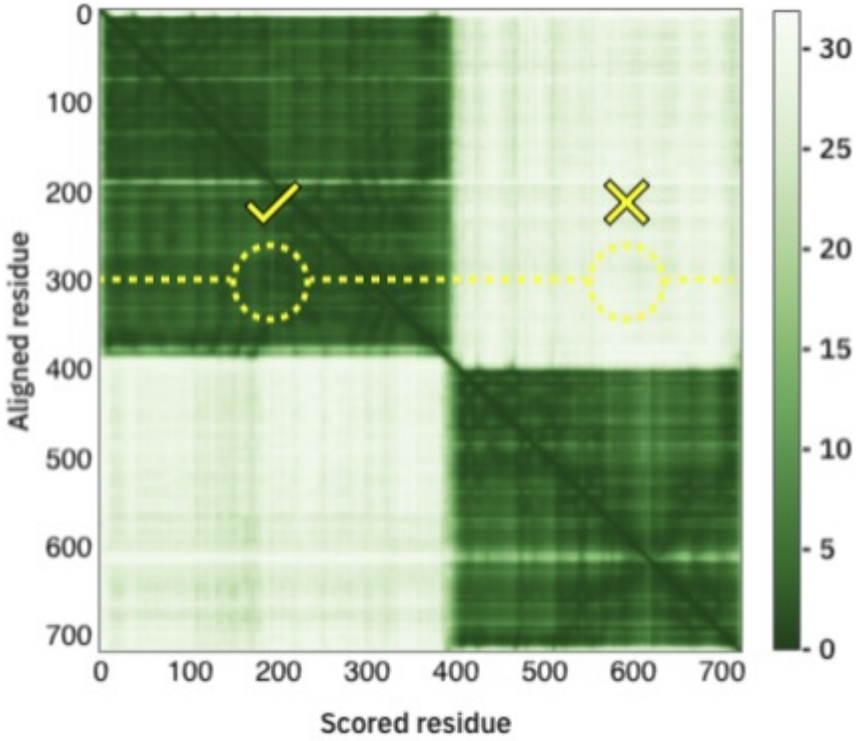
- per-residue confidence metric ranging from 0-100 (100 being the highest confidence)
- Regions below 50 could indicate disordered regions



https://alphafold.com/entry/Q9FX77

Predicted Alignment Error (PAE)

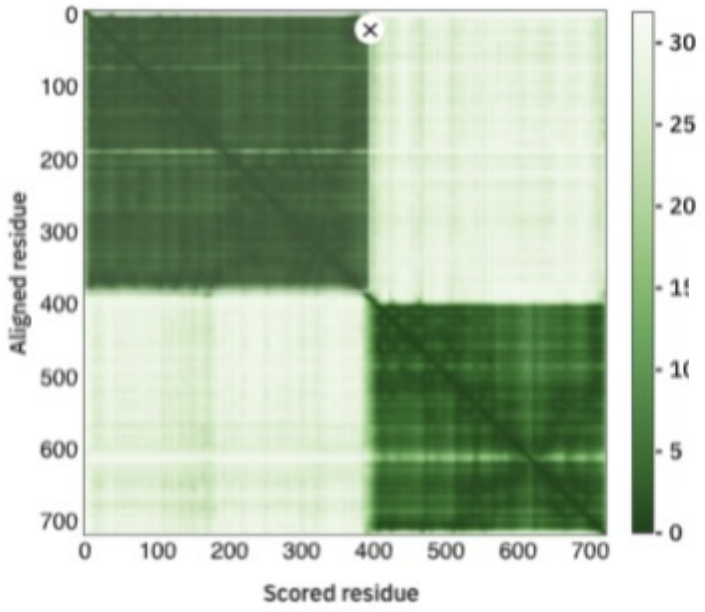
- The color at (x, y) corresponds to the expected distance error in residue x's position, when the prediction and true structure are aligned on residue y.
- So, in the example to the right:
  - The darker color indicates a lower error
  - When we are aligning on residue 300, we are more confident in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

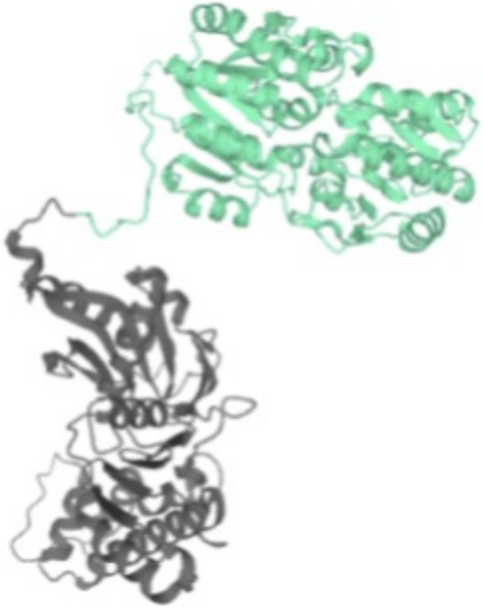


https://alphafold.com/entry/Q9FX77

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





https://alphafold.com/entry/Q9FX77

Acknowledgement

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

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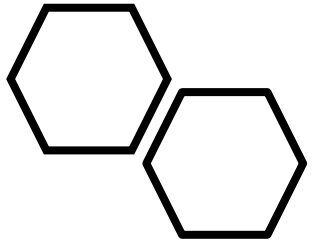

Next: Setup