Introduction to AlphaFold: Bioinformatics for 3D Protein Structure Prediction

Devon J. Boland
Norman Borlaug Endowed Research Scholar

The Field of Bioinformatics

"Omics"-Based
- Genome
- Transcriptome
- Proteome

Molecular Dynamic Simulations

Annotation

Gene/Protein Expression

De Maio, C., et al. (2018). Text Mining Basics in Bioinformatics.

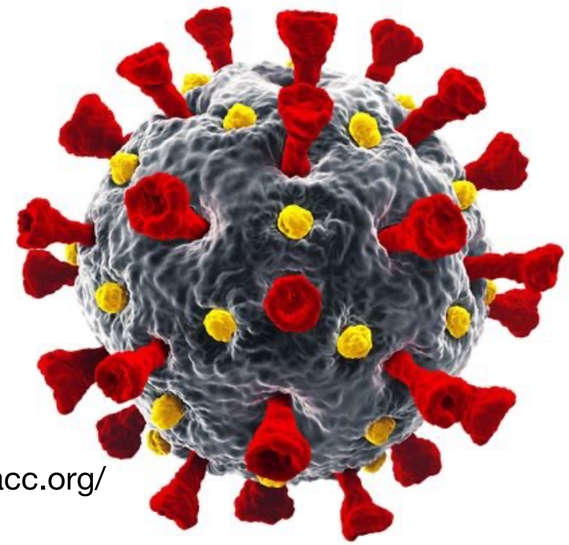
Image/Microscopy Analysis

Structural Proteomics

What Does Protein Structure Tell Us?

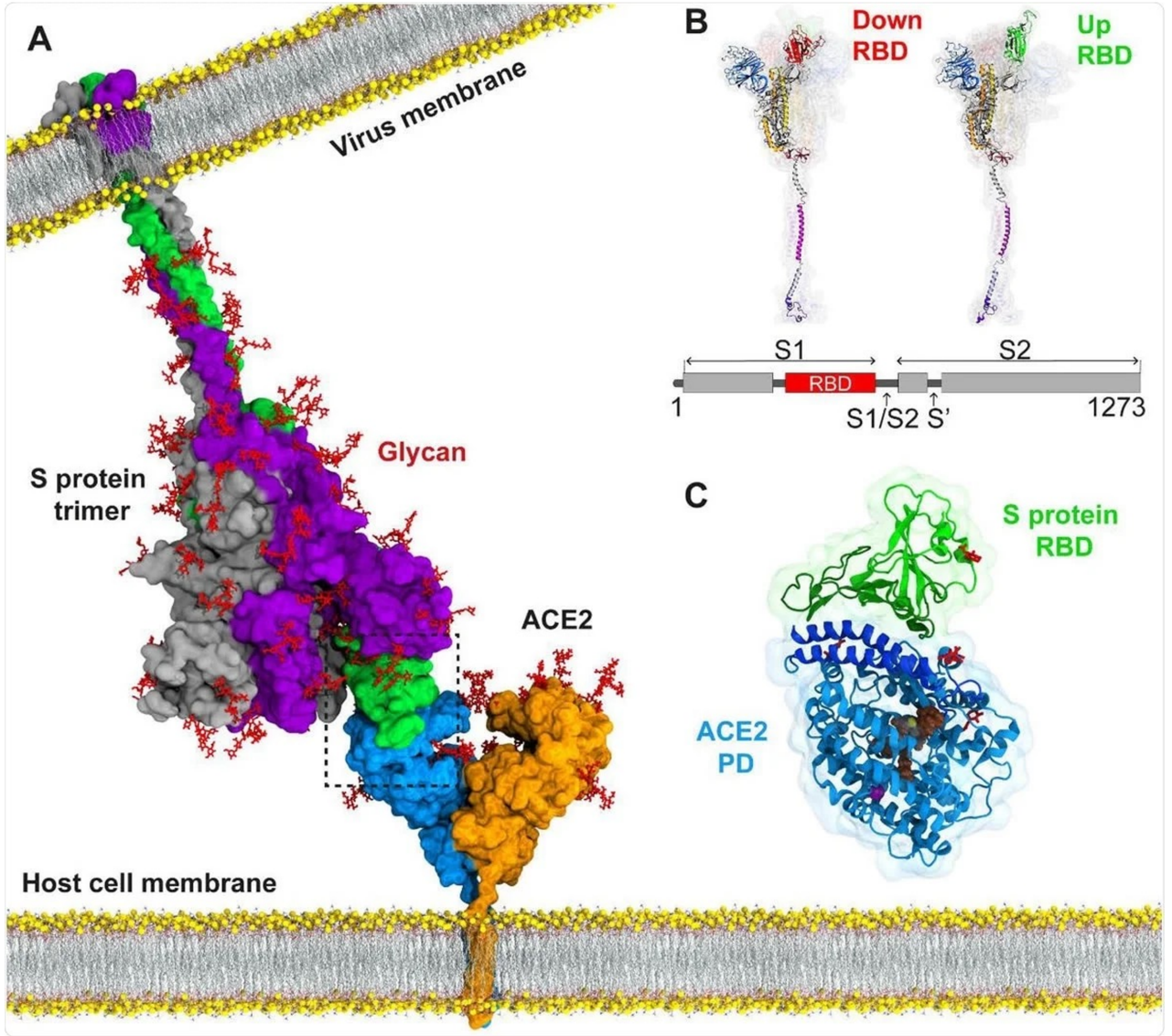
Structure = Function → Function = Structure

- visualizing binding interface
- location of allosteric inhibition
- conformational changes
- molecular scaffold for molecular docking
- mutational design
- MANY OTHER FACETS…



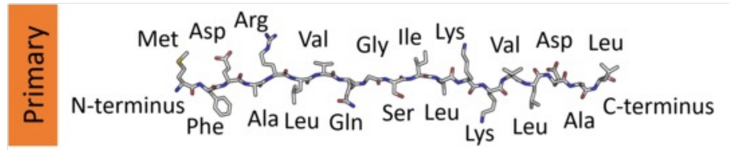
https://www.acc.org/

SARS-COV-2



Taka, E., et al. (2021). The Journal of Physical Chemistry B 125(21): 5537-5548.

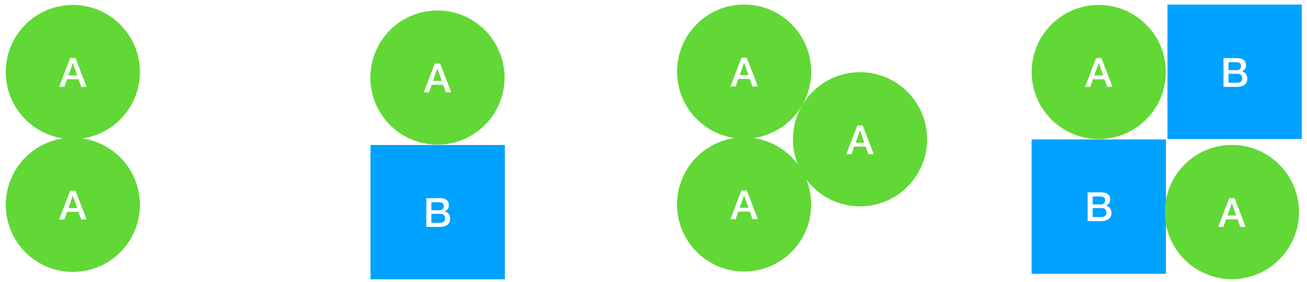
Basics of 3D Protein Structure



Four Tiers of Protein Structure

- 1° - Sequence of AA's (polypeptides)
- 2° - Interactions of the carbon backbone of 1°
- 3° - Folding of 2° onto itself
- 4° - Multiple 3° units (Monomers) assembling together

Different Types of Quaternary Structure



Dimer (homo) | **Dimer (hetero)** | **Trimer (homo)** | **Tetramer (hetero)**

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

Methods for Elucidating Protein Structure

X-Ray Crystallography

https://www.businesswire.com/

pros:
- widely used
- high-throughput
- ~2.5-1Å resolution

cons:
- crystallization process
- high variation

NMR Spectroscopy

pros:
- native state
- non-destructive
- real-time

cons:
- only works on “small” proteins
  - <100kDa
- high [protein]
- $$$ maintenance

Cryo-EM

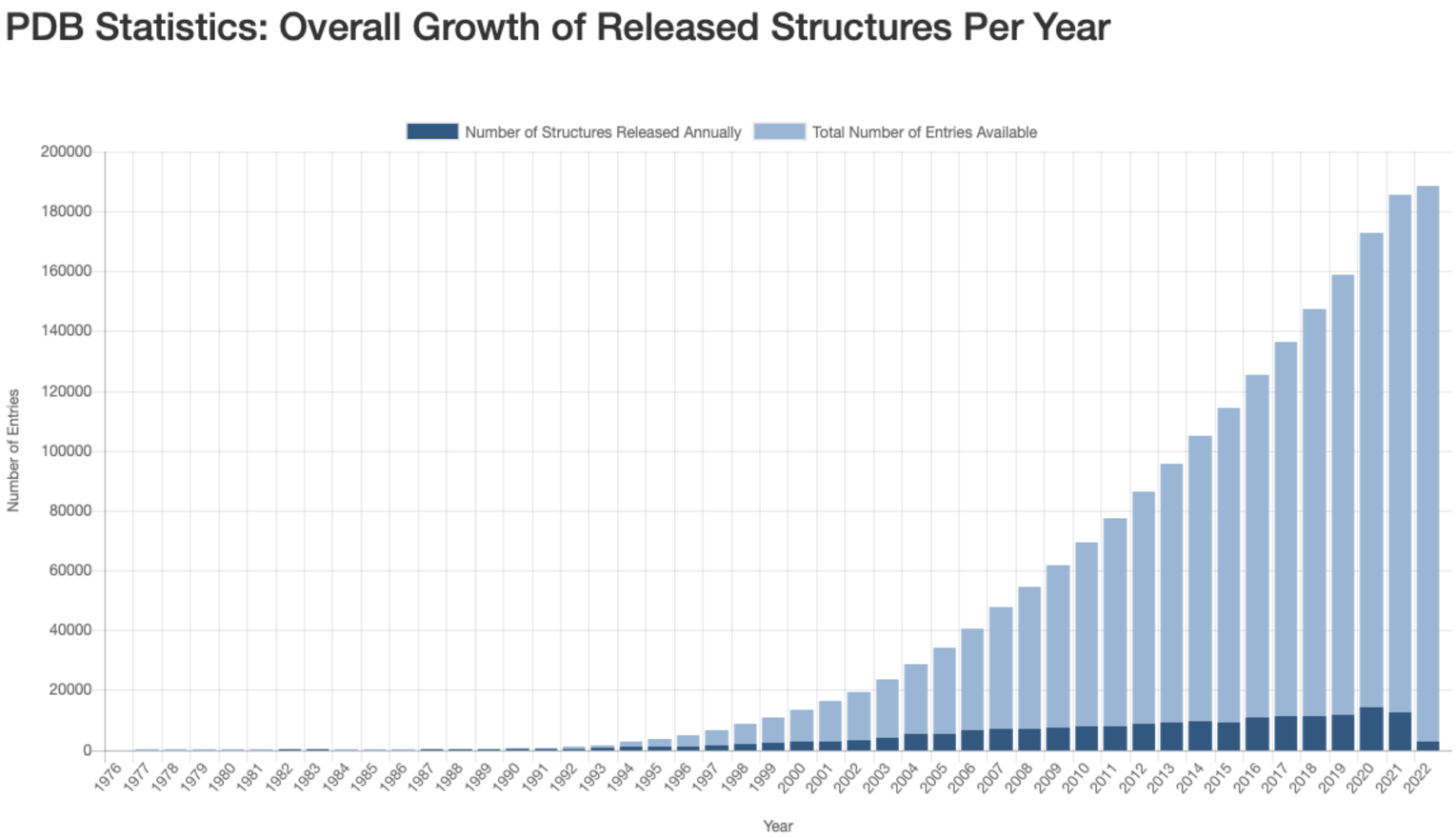
https://www.thermoscientific.com/

pros:
- large proteins/complexes
- near-native state

cons:
- only works on “large” proteins
  - >200kDa
- freezing samples
- computationally intensive
- $$$ maintenance

Protein Data Bank (PDB)

After we elucidate a structure where does it go?



Most publishing journals require a structure be deposited to the PDB prior to publication of a study!

Enter Computers! (Not The One In Your Pocket)

Parameters that MUST be considered:

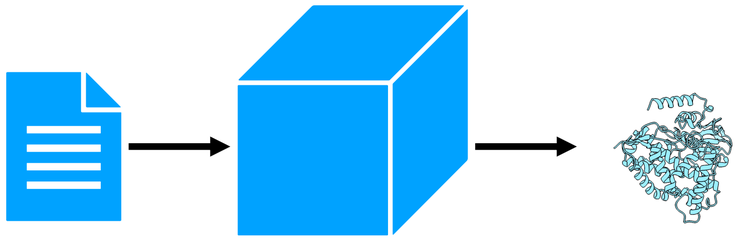
1. **Primary Structure (bond angles)**
2. **Secondary Structure (α-helix, β-sheet, loops)**
3. **Tertiary Structure (folding of secondary structure)**
4. **Quaternary Structure (Optional)**

3°/4° Structure Have Additional Parameters:

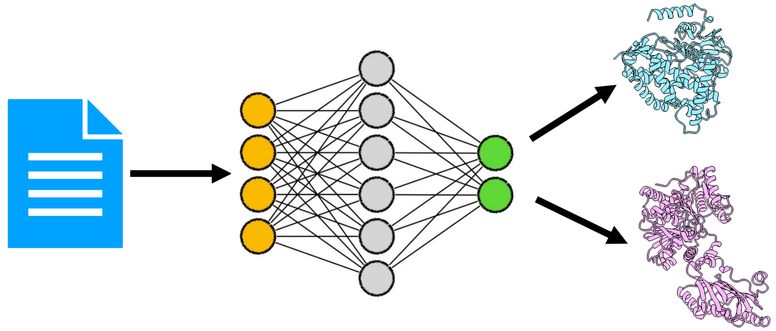
1. **Conserved protein domains**
2. **Protein families/superfamilies/clans**

Ideally, we would like to predict a protein's 3-D structure given only its AA sequence (1° stucture)

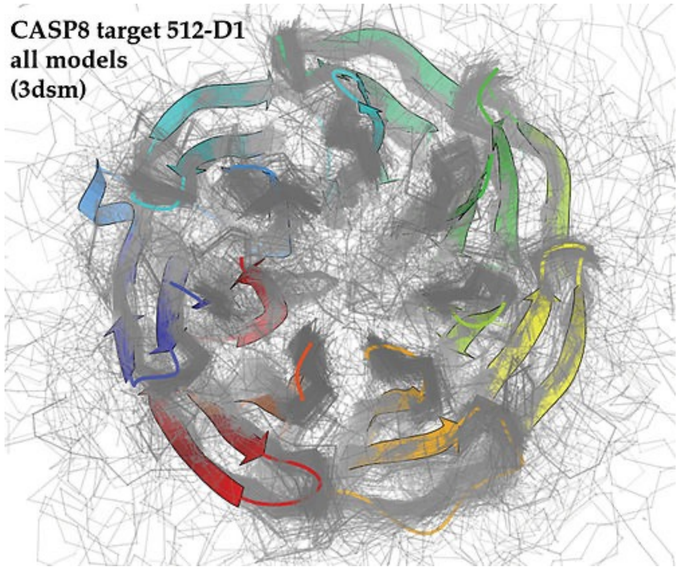
Algorithm-Based



Machine Learning (AI)



Assessing Methods for Protein Structure Prediction



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

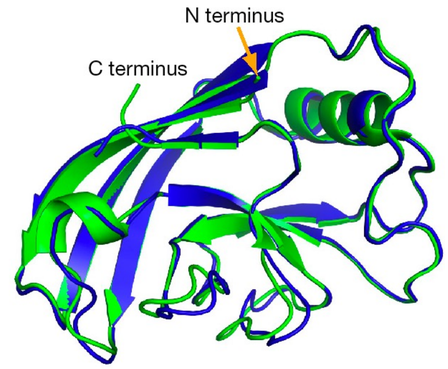
CASP evaluates current methods of predicting protein structure since 1994.

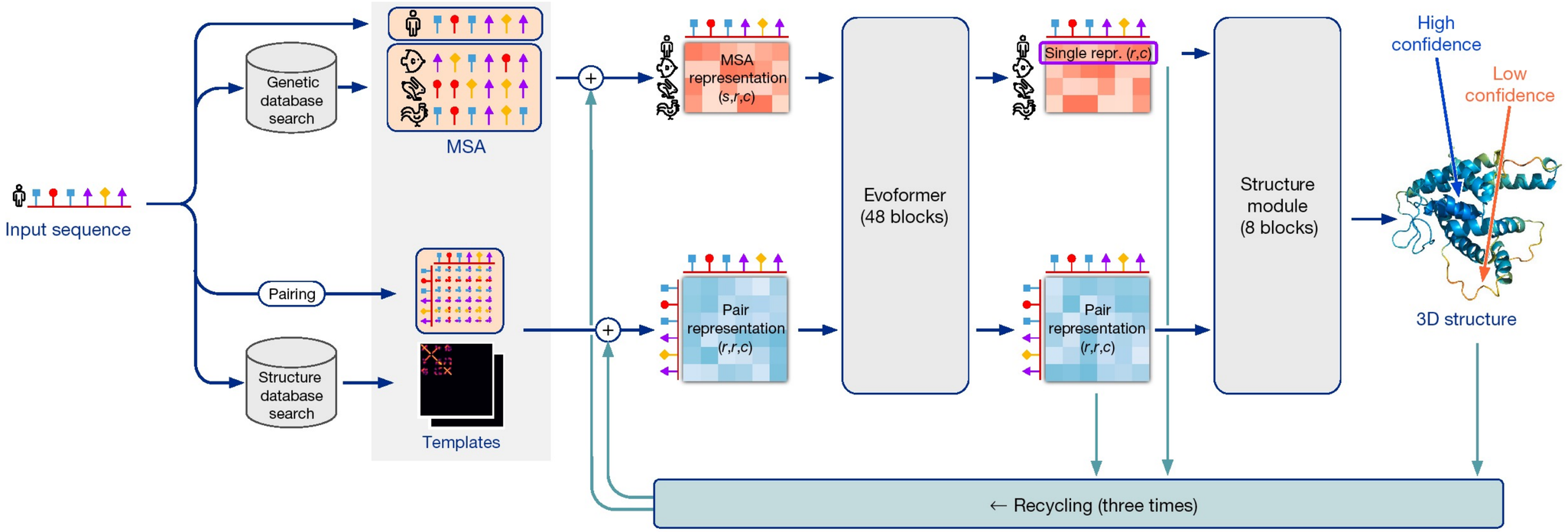
"These techniques are expensive and slow: it can take hundreds of thousands of dollars and years of trial and error for each protein. AlphaFold can find a protein's shape in a few days." - MIT Technology Review

- **2020 was the first year a program met a true success rate (>90%)**
  - **Google DeepMind's AlphaFold2**
  - **Worked on both homologous and novel proteins**
  - **Only needed to provide the AA sequence**
  - **AlphaFold2 was released under the Apache Common Use license in 2021**

AlphaFold2: A Neural Network Trained To Predict Protein 3-D Structure

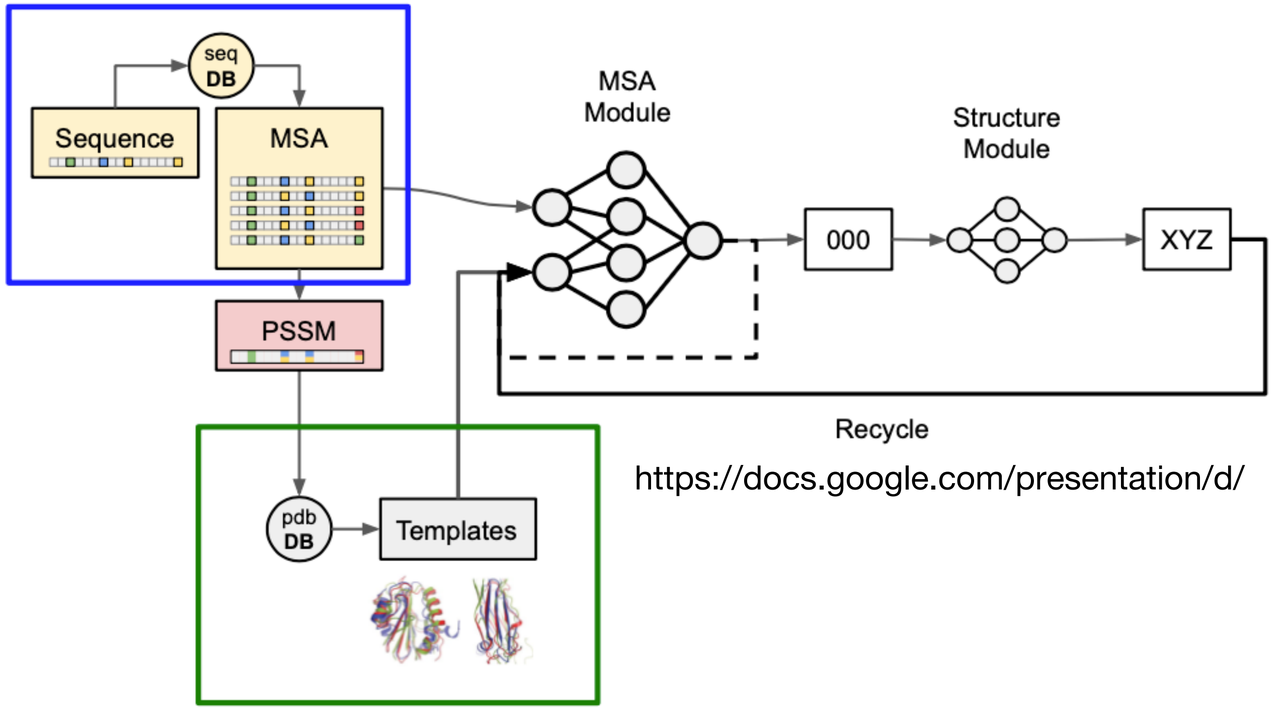
- **First program to ever meet the success rate at CASP**
- **Assessed on alignment of predicted structure to experimental**
- **AF2 custom value to "quantify" model confidence**



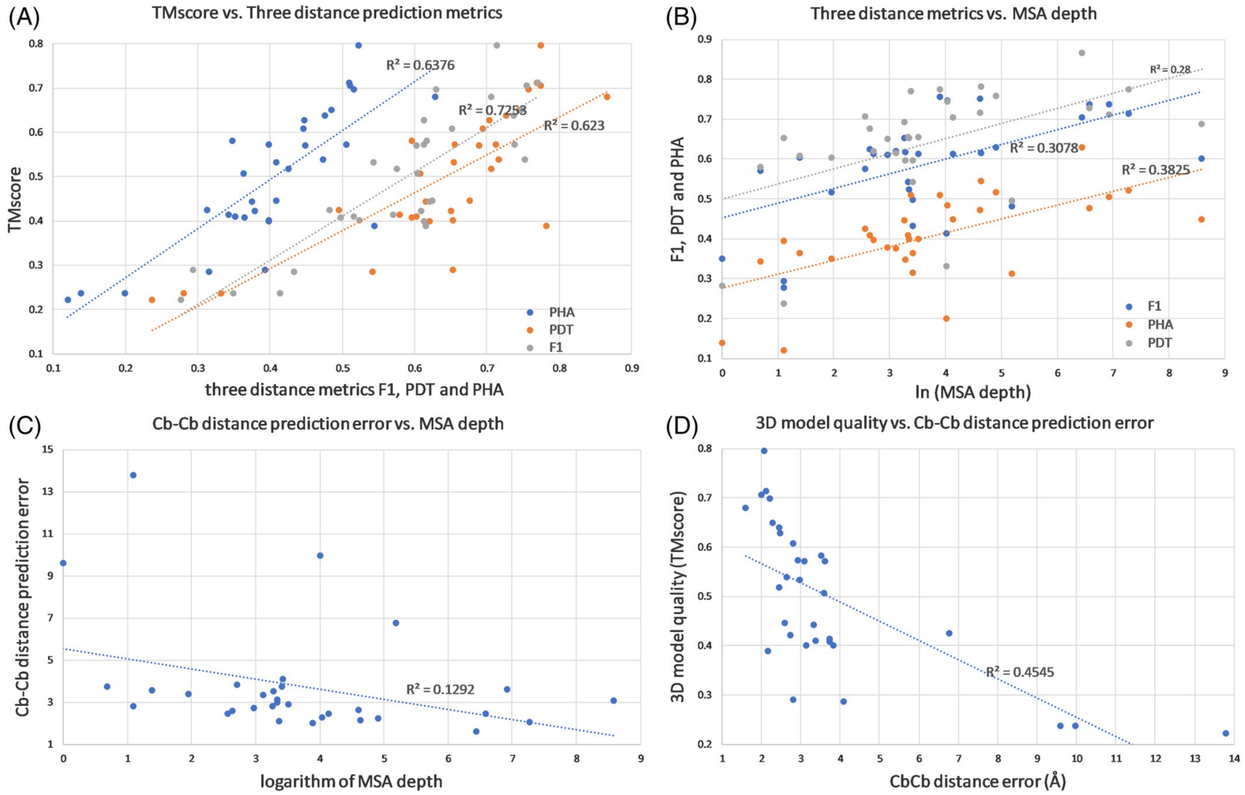


MSA Depedency of AF2

Structure Prediction is only as good as the input MSA



https://docs.google.com/presentation/d/



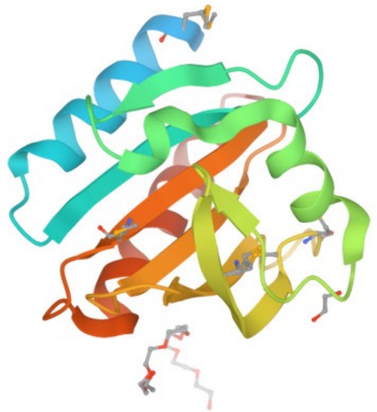
Xu, J.*et. al*. *Proteins* 2019, *87* (12), 1069-1081.

Typically MSA depth of >100 sequences/residue produce a highly accurate and confident model

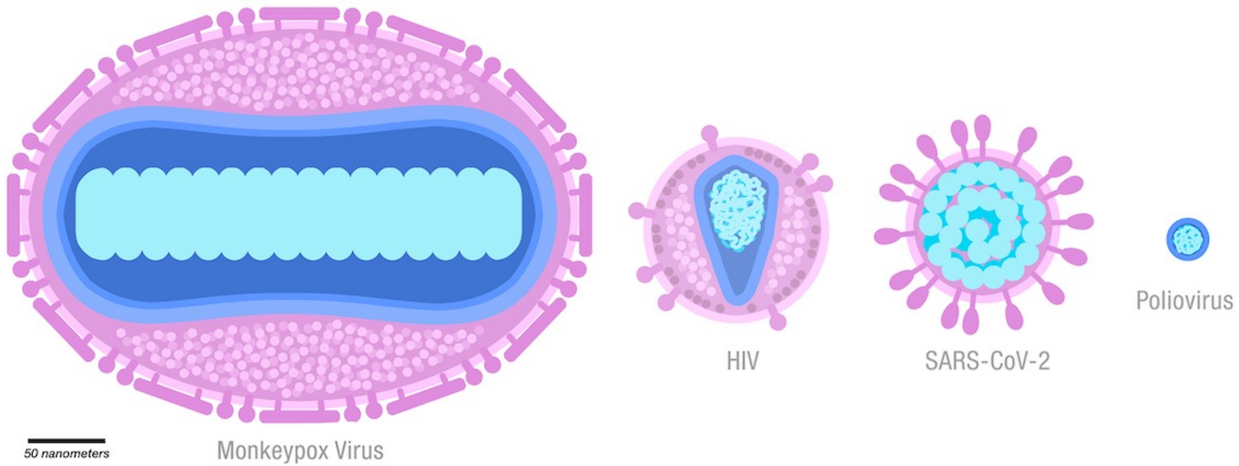
MPOX (Monkey Pox)

- **MPOX** first isolated 1958
  - Copenhagen, Denmark
  - Started in children from a playgroup
- Easily diagnosed by lesions that develop in 2-27 days
- Can spread to genitalia
- Can cause:
  - pneumonia, sepsis, and stillbirth (pregnancy)
- Genome ~197kb
  - Contains 190 protein-encoding genes
  - We will predict structures for 60 of these proteins
- To date only two proteins have been elucidated
  - MPXV polymerase holoenzyme in replicating state
  - Profilin-like Protein

PDB: 8HG1

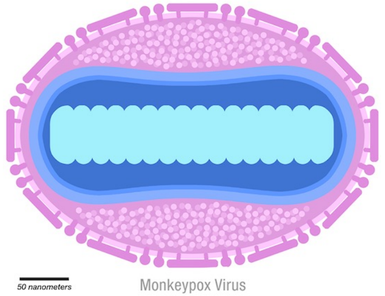


PDB: 4QWO



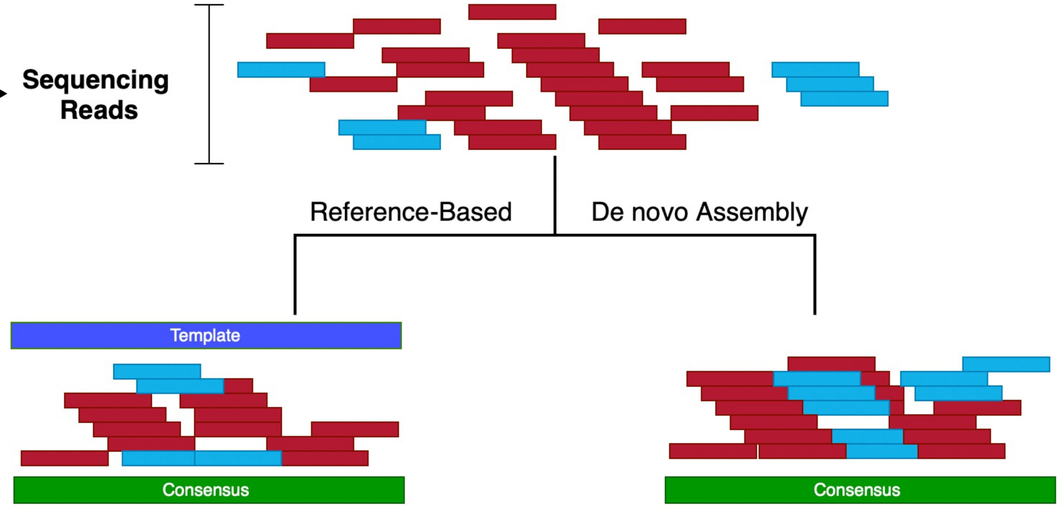
https://en.wikipedia.org/wiki/Mpox

Assembly of the MPOX-22 Global Outbreak Genome



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

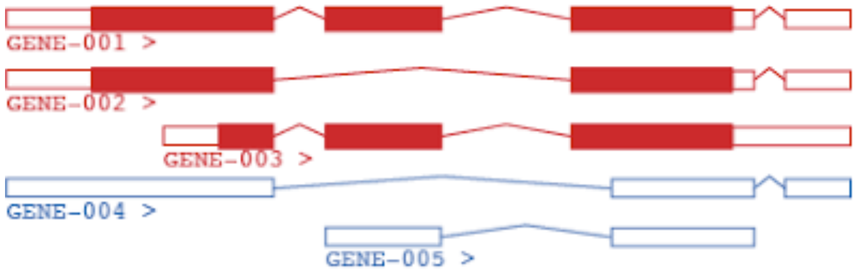
https://illumina.com/



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

Genome Sequencing & Assembly

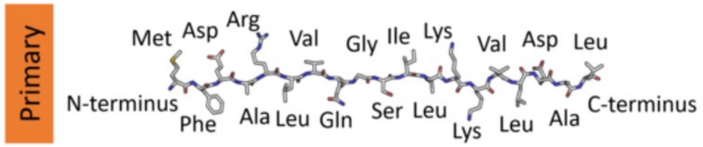
Gene ORF Prediction & Annotation



https://grch37.ensembl.org/info/genome/genebuild/index.html

Pearson Fasta Format

***In vivo* "Biological" Representation**



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

***In silico* "Computer" Representation**

```
>MCHU - Calmodulin - Human, rabbit, bovine, rat, and chicken
MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTID
FPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREA
DIDGDGQVNYEEFVQMMTAK*
```

- They are separated by the '>' sign
  - Followed by a descriptor, known as a 'header'

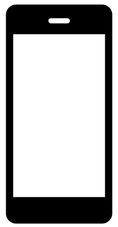
```
>lcl|ON563414.3_prot_URK20440.1_1
MKQYIVLACMCLVAAAMPTSLQQSSSSCTEEENKHHMGIDVIIKVTKQDQTPTNDKICQSVTEVTETEDD
```

- Each sequence is represented as two lines
  - The next line contains the amino acid sequence

How would you represent a genome on a computer? What about a transcriptome? or proteome?

Running AlphaFold2 In A Timely Manner

>50 years



4-8 CPU core

CUSTOM Graphics Chip

8-16Gb RAM

Months - Years

8-10 CPU core

NVIDIA/AMD Entry GPU

16-32Gb RAM

Hours-Days

https://hprc.tamu.edu/resources/

925 nodes (40cpu per node)

NIVIDIA A100/AMD Ryzen GPUs

>3TB RAM

Architecture of HPC Clusters

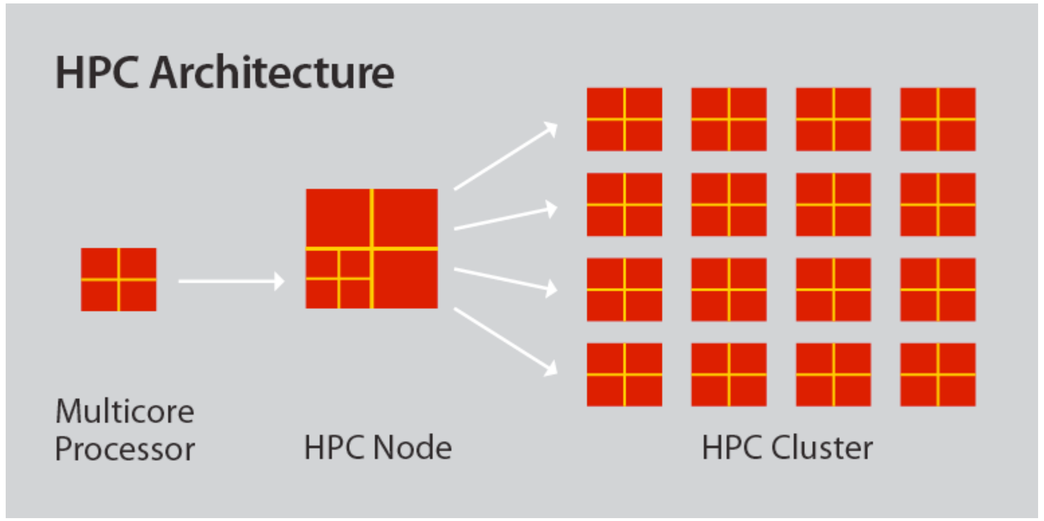
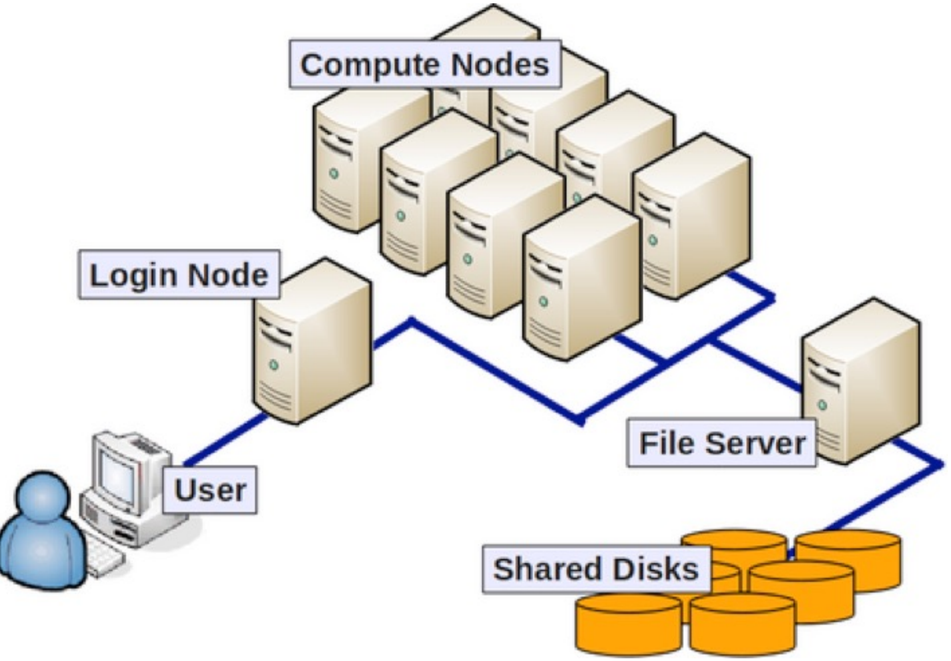
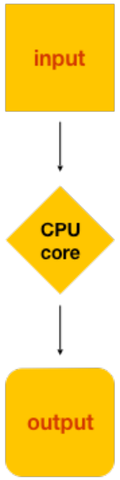
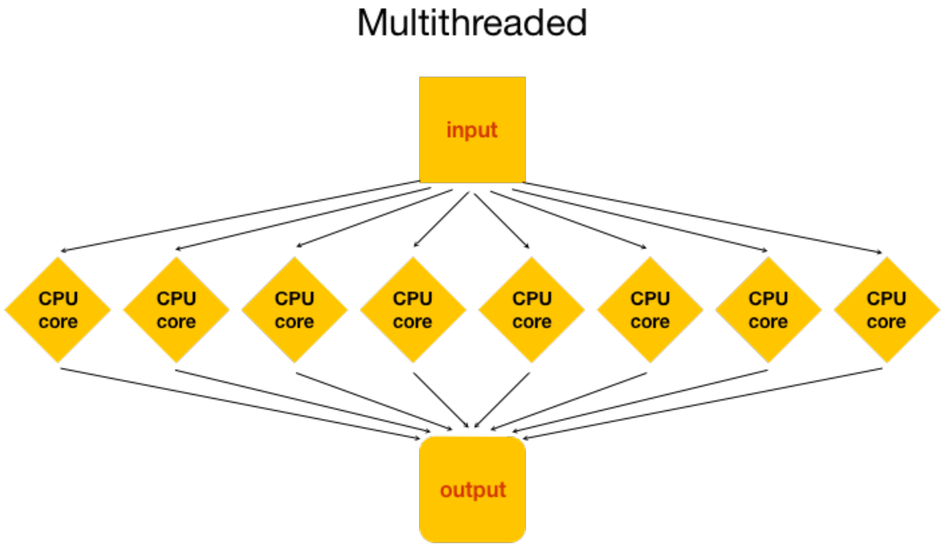


Photo: Illinois State University (ISU.EDU)



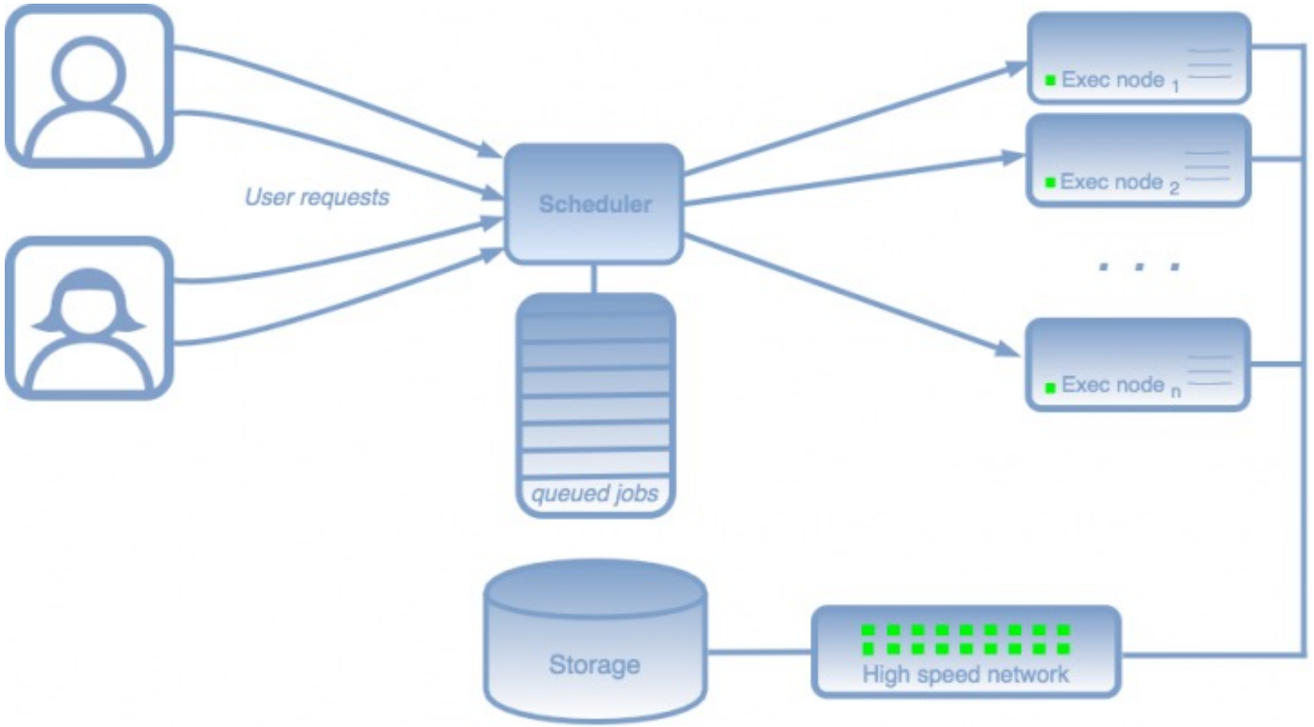




Photos: GitHub HPCTraining

Process of Running A Job On Grace

- Unlike to your personal computer, Grace will not do things automatically
- We will build a list of commands which will include running AlphaFold2
  - This is called “scripting/building” a job file
- Our job file will contain “directives” and “commands”
  - Directives - tell Grace what resources to use (CPU/GPU/Time/RAM)
  - Commands - will execute programs or lines of code



https://rdlab.cs.upc.edu/wp-content/uploads/documentation/manuals/html/manual_rdlab_hpc/index.html

The AlphaFold2 Job Script

```
#!/bin/bash
#SBATCH --job-name=LASTNAME_FIRSTNAME_PROTEIN_HEADER    # Edit this line with your name and protein fasta header
#SBATCH --time=1-00:00:00           # Leave this and all other lines starting with #SBATCH alone
#SBATCH --ntasks-per-node=1
#SBATCH --cpus-per-task=24
#SBATCH --mem=180G
#SBATCH --output=stdout.%j.txt
#SBATCH --error=stderr.%j.txt
```

Directives - tells the computer what hardware to use

Modules - tells the computer what software to use

```
######## Load Program Modules ############
# This will load the modules (programs) needed to run AF2 and analyze the output
module purge
module load GCC/10.2.0  CUDA/11.1.1  OpenMPI/4.0.5  AlphaPickle/1.4.1
```

Variables - our input for the program

```
################################## VARIABLES ##################################

########## INPUTS ##########
# Edit this variable to point towards your fasta file
protein_fasta='/Path/to/your/fasta_file.fasta'

```

Parameters - “arguments” the program uses, typically specific to your data

```
######## PARAMETERS ########
# Leave these variables alone
DOWNLOAD_DIR='/scratch/data/bio/alphafold/2.3.0'
max_template_date='2023-1-1'
model_preset='monomer'            # monomer, monomer_casp14, monomer_ptm, multimer
db_preset='full_dbs'              # full_dbs, reduced_dbs
```

Variables - specific to the program you are using

```
########## OUTPUTS #########
protein_basename=$(basename ${protein_fasta%.*})
output_dir="out_${protein_basename}_${model_preset}"
pickle_out_dir=$protein_basename
```

Commands - specific to the program, and tell the computer what to do

```
################################## COMMANDS ###################################
singularity exec --nv /sw/hprc/sw/bio/containers/alphafold/alphafold_2.3.0.sif python /app/alphafold/run_alphafold.py \
 --data_dir=$DOWNLOAD_DIR \
 --uniref90_database_path=$DOWNLOAD_DIR/uniref90/uniref90.fasta \
 --mgnify_database_path=$DOWNLOAD_DIR/mgnify/mgy_clusters_2022_05.fa \
 --bfd_database_path=$DOWNLOAD_DIR/bfd/bfd_metaclust_clu_complete_id30_c90_final_seq.sorted_opt \
 --uniref30_database_path=$DOWNLOAD_DIR/uniref30/UniRef30_2021_03 \
 --pdb_seqres_database_path=$DOWNLOAD_DIR/pdb_seqres/pdb_seqres.txt \
 --template_mmcif_dir=$DOWNLOAD_DIR/pdb_mmcif/mmcif_files \
 --obsolete_pdbs_path=$DOWNLOAD_DIR/pdb_mmcif/obsolete.dat \
 --uniprot_database_path=$DOWNLOAD_DIR/uniprot/uniprot.fasta \
 --model_preset=$model_preset \
 --max_template_date=$max_template_date \
 --db_preset=$db_preset \
 --output_dir=$output_dir \
 --fasta_paths=$protein_fasta
# graph pLDDT and PAE .pkl files
# This line command will run a python script to plot our pLDDT and pAE plots
# if you would like more information on how this works please email Devon
run_AlphaPickle.py -od $output_dir/$pickle_out_dir
```

Summary

- AlphaFold2 was the first program to be considered a success for protein structure prediction
- It works on proteins with structural homologs, and those without
- Its major limitation is still requiring homologous sequences for accurate structure prediction
- Its dependent on two parts:
  - Finding homologous sequences
  - Finding homologous structures
- The confidence of the model is expressed as pLDDT and pAE
- When in doubt, run AF2 on your protein, worst that happens is you waste your time

Next Time
We will analyze the predicted structures