AlphaFold2 on Alpine

RC Short Course

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Slides

https://bit.ly/af2alpine

CURC User Support

Background: The Protein Folding Problem

- What a protein does (it's biological function) depends on its 3D structure
- Figuring out the what shapes a protein folds into has been a grand challenge in biology for 50+ years

Can we predict a protein's 3D structure based on its 1D aa sequence?

This is computationally VERY intensive!

Background

Article | Open access | Published: 15 July 2021

Highly accurate protein st
AlphaFold

John Jumper, Richard Evans, Alexander Pritzel
Kathryn Tunyasuvunakool, Russ Bates, Augustin Ž
Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andre
Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Sti
Zielinski, ... Demis Hassabis + Show authors

Nature **596**, 583–589 (2021) | Cite this article

1.56m Accesses | **8815** Citations | **3559** Altmetric | Metrics

Access & Citations

1.57m	8815	1237
Article Accesses	Web of Science	CrossRef

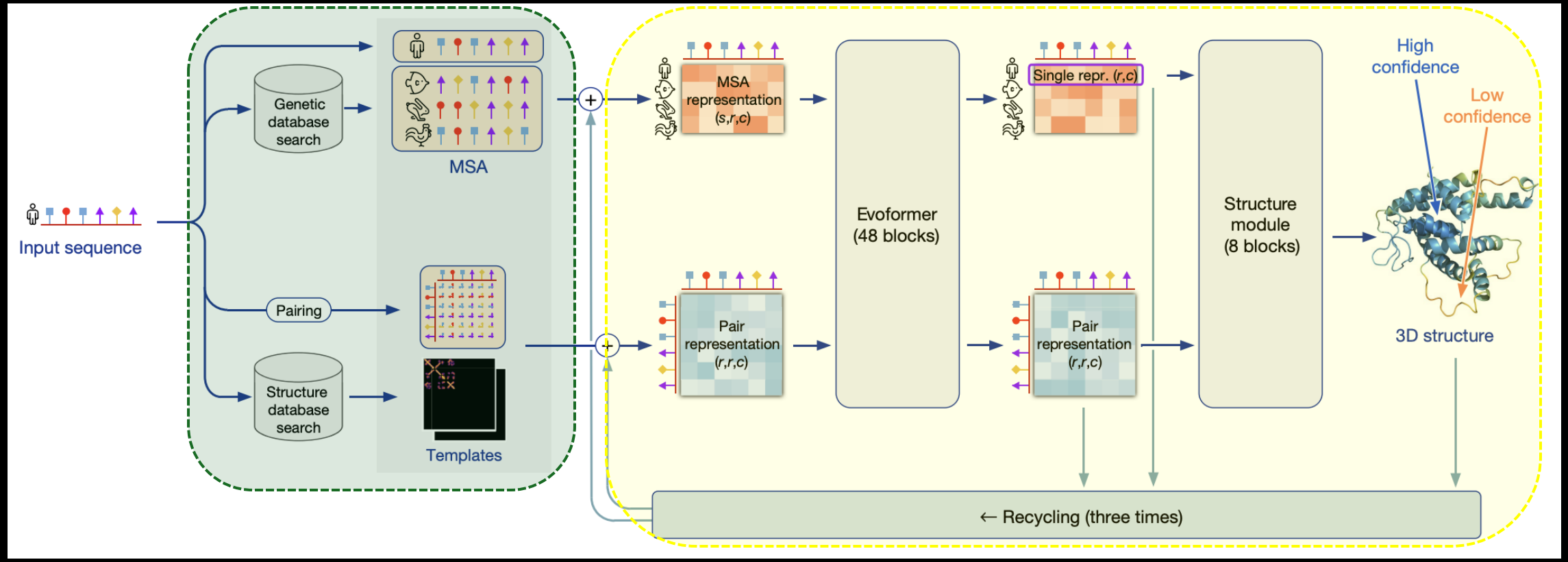
Online attention

- 5229 tweeters
- 104 blogs
- 6 Facebook pages
- 318 news outlets
- 7 Redditors
- 15 F1000
- 17 Video uploaders
- 36 Wikipedia page
- 15600 Mendeley

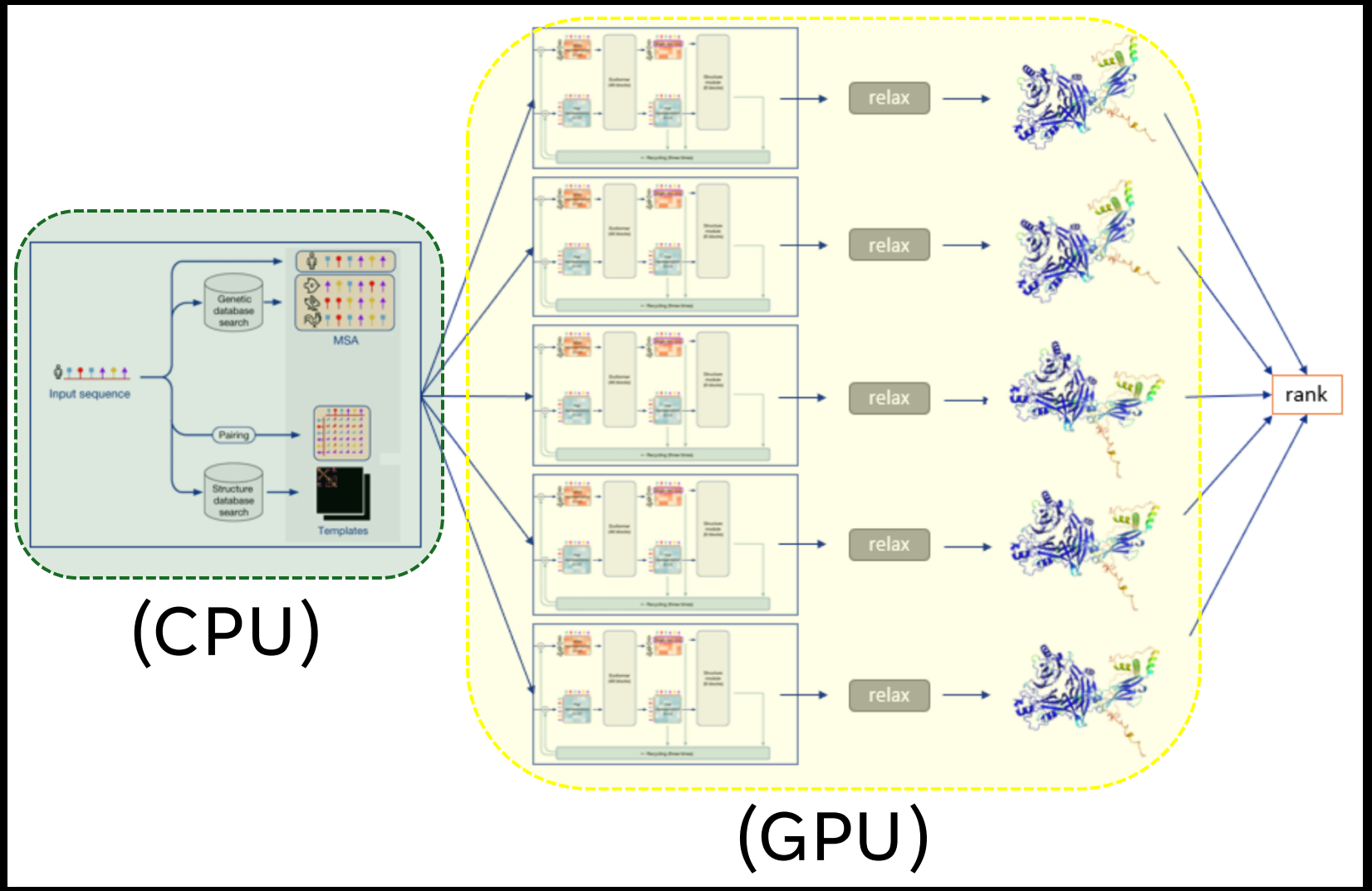
This article is in the 99th percentile (ranked 108th) of the 449,474 tracked articles of a similar age in all journals and the 98th percentile (ranked 15th) of the 927 tracked articles of a similar age in *Nature*

View more on Altmetric

The Pipeline



The Pipeline: CPU vs GPU



AlphaFold Inputs

- FASTA file (e.g., dummy.fasta, T1061.fa)

```
>T1061 T5 phage tail subcomplex, T5 phage, 949 residues|
MKKILDSAKNYLNTHDKLKTACLIALELPSSSGSAATYIYLTDYFRDVTYNGILYRSGKVKSISSHKQNRQLSIGSLSFTITGTAEDEVL
KLVQNGVSFLDRGITIHQAIINEEGNILPVDPDTDGPLLFFRGRITGGGIKDNVNTSGIGTSVITWNCSNQFYDFDRVNGRYTDDASHRG
LEVVNGTLQPSNGAKRPEYQEDYGFFHSNKSTTILAKYQVKEERYKLQSKKKLFGLSRSYSLKKYYETVTKEVDLDFNLAAKFIPVVYGV
QKIPGIPIFADTELNNPNIVYVVYAFAEGEIDGFLDFYIGDSPMICFDETDSDTRTCFGRKKIVGDTMHRLAAGTSTSQPSVHGQEYKYN
DGNGDIRIWTFHGKPDQTAAQVLVDIAKKKGFYLQNQNGNGPEYWDSRYKLLDTAYAIVRFTINENRTEIPEISAEVQGKKVKVYNSDGT
IKADKTSLNGIWQLMDYLTSDRYGADITLDQFPLQKVISEAKILDIIDESYQTSWQPYWRYVGWNDPLSENRQIVQLNTILDTSESVFKN
VQGILESFGGAINNLSGEYRITVEKYSTNPLRINFLDTYGDLDLSDTTGRNKFNSVQASLVDPALSWKTNSITFYNSKFKEQDKGLDKKL
QLSFANITNYYTARSYADRELKKSRYSRTLSFSVPYKFIGIEPNDPIAFTYERYGWKDKFFLVDEVENTRDGKINLVLQEYGEDVFINSE
QVDNSGNDIPDISNNVLPPRDFKYTPTPGGVVGAIGKNGELSWLPSLTNNVVYYSIAHSGHVNPYIVQQLENNPNERMIQEIIGEPAGLA
IFELRAVDINGRRSSPVTLSVDLNSAKNLSVVSNFRVVNTASGDVTEFVGPDVKLAWDKIPEEEIIPEIYYTLEIYDSQDRMLRSVRIED
VYTYDYLLTYNKADFALLNSGALGINRKLRFRIRAEGENGEQSVGWATI
```

A FASTA file is the only *required* AlphaFold input.

Alpine AlphaFold2 Module

Loading the AlphaFold module (ml alphafold)

- activates a conda environment containing all the necessary packages
- redirects TMPDIR to /scratch/alpine/$USER
- creates a shortcut to the AlphaFold run script
- sets convenient environment variables

  $CURC_AF_DBS

  $CURC_AF_EXAMPLES

Alpine AlphaFold2 Module

```
-- This module loads AlphaFold

-- Set local variables
local PACKAGE_PREFIX = "/curc/sw/install/bio/alphafold/2.3.1/2.3.1_env"
local USER = os.getenv("USER")

-- Load dependencies
always_load("anaconda/2022.10")

-- Set paths
prepend_path("LD_LIBRARY_PATH", pathJoin(PACKAGE_PREFIX, "lib"))

-- Set environment variables
setenv("TMPDIR", (os.getenv("SCRATCHDIR")) .. "/" .. (os.getenv("USER")))
setenv("CURC_AF_DBS", "/gpfs/alpine1/datasets/bioinformatics/alphafold")
setenv("CURC_AF_EXAMPLES", "/curc/sw/install/bio/alphafold/examples")

-- Create AlphaFold script shortcut
-- Note this will only work with bash; can add csh using $* (see https://
lmod.readthedocs.io/en/latest/015_writing_modules.html#setting-aliases-an
d-shell-functions)
set_shell_function("run_alphafold",'bash /curc/sw/install/bio/alphafold/2
.3.1/alphafold-2.3.1/run_alphafold.sh "$@"')
```

→ sets environment variables/redirects TMPDIR

→ creates a shortcut to run_alphafold.sh

Alpine AlphaFold2 Module

```
-- Activate alphafold conda environment
execute{cmd="conda activate /curc/sw/install/bio/alphafold/2.3.1/2.3.1_en
v",modeA={"load"}}

-- Software info:

help([[
Alphafold:
AlphaFold is an artificial intelligence program developed by DeepMind, a
subsidiary of Alphabet, which performs predictions of protein structure.

For detailed instructions, go to:
https://github.com/deepmind/alphafold
]])
whatis("Version: 2.3.1")
whatis("Citing AlphaFold: Jumper, J et al. Highly accurate protein struct
ure prediction with AlphaFold. Nature (2021). Varadi, M et al. AlphaFold
Protein Structure Database: massively expanding the structural coverage o
f protein-sequence space with high-accuracy models. Nucleic Acids Researc
h (2022).")
```

activates the AlphaFold conda environment

AlphaFold2 Required Parameters

```
(/curc/sw/install/bio/alphafold/2.3.1/2.3.1_env) [lafr9499@c3cpu-a2-u32-3 ~]$ run_alphafold

Please make sure all required parameters are given
Usage: /curc/sw/install/bio/alphafold/2.3.1/alphafold-2.3.1/run_alphafold.sh <OPTIONS>
Required Parameters:
-d <data_dir>          Path to directory of supporting data
-o <output_dir>        Path to a directory that will store the results.
-f <fasta_paths>       Path to FASTA files containing sequences. If a FASTA file contains multip
le sequences, then it will be folded as a multimer. To fold more sequences one after another, w
rite the files separated by a comma
-t <max_template_date> Maximum template release date to consider (ISO-8601 format - i.e. YYYY-M
M-DD). Important if folding historical test sets
```

To predict the structure of a protein in the PDB without using its experimental structure as a template, set max_template_date (-t) to before the release date of the structure.

TIP: A date like 1900-01-01 will ensure no templates are used.

AlphaFold2 Optional Parameters

```
Optional Parameters:
-g <use_gpu>           Enable NVIDIA runtime to run with GPUs (default: true)
-r <run_relax>         Whether to run the final relaxation step on the predicted models. Turning relax off might result in pred
ictions with distracting stereochemical violations but might help in case you are having issues with the relaxation stage (def
ault: true)
-e <enable_gpu_relax> Run relax on GPU if GPU is enabled (default: true)
-n <openmm_threads>    OpenMM threads (default: all available cores)
-a <gpu_devices>       Comma separated list of devices to pass to 'CUDA_VISIBLE_DEVICES' (default: 0)
-m <model_preset>      Choose preset model configuration - the monomer model, the monomer model with extra ensembling, monomer
model with pTM head, or multimer model (default: 'monomer')
-c <db_preset>         Choose preset MSA database configuration - smaller genetic database config (reduced_dbs) or full genetic
 database config (full_dbs) (default: 'full_dbs')
```

Parameter values for --model_preset (-m):
monomer
monomer_casp14
monomer_ptm
multimer

AlphaFold2 Optional Parameters Cont'd

```
-p <use_precomputed_msas> Whether to read MSAs that have been written to disk. WARNING: This will not check if the sequence, d
atabase or configuration have changed (default: 'false')
-l <num_multimer_predictions_per_model> How many predictions (each with a different random seed) will be generated per model.
E.g. if this is 2 and there are 5 models then there will be 10 predictions per input. Note: this FLAG only applies if model_pr
eset=multimer (default: 5)
-b <benchmark>        Run multiple JAX model evaluations to obtain a timing that excludes the compilation time, which should b
e more indicative of the time required for inferencing many proteins (default: 'false')
```

Using precomputed MSAs: AlphaFold looks for the MSAs in the output directory, so they must stay the same if you want to reuse them.

Folding Monomers (1 Polypeptide Chain)

- The default model preset is monomer prediction (--model_preset=monomer or -m “monomer”)
- Requires a FASTA file with a single sequence

Run Command: run_alphafold -d $CURC_AF_DBS -o . -f $CURC_AF_EXAMPLES/dummy.fasta -t 2020-05-14 -m “monomer” -g true

Folding Multimers (>1 Polypeptide Chain)

- Requires a FASTA file containing **multiple** sequences

```
>bNACHT09
MKKIFISYSWDGEEHQEWVRKLADSLEEDHAYHVVWDGYDLDPLSDKNFFMERAATNTDFILVVATEKYKEKADNRNGGVGIETYLAAARHWEMM
TGKDKKTNVITINREPDSTPNYLNGHLYVDFSKDELFNDSLVELKRLLNAEPKFKRPEKQIGVTSLKSYNLTKVSDIIGIGAKNRICLINAAEGT
DFSAGKKIKYEFWELRNPGGVITHVLALHNNIIISQTLNRAAEEIKERFQNISTLLILRPREKKKGVESMDTILEKKGYSRTVNINEYTYDEYIW
EYCIDSDFKNITPPDAIEFYTSQEVVDSKGKIYSSGVNTLSDELLKTSDYSANLIIGSGGIGKTSLCLTLVNKLIKEKSESFLTILIRSEDIRKY
IHDAGINSLYVSDIYDIYEMQAKYLNHVNVFDRNTFLLSILSGKIIIVVDGLDELSSIFGDKFDLVTFLKSLTKLHDELGETRILLTTRENNSIS
QELISDLNINVYKLLGFKLDNCIKYLNIRFKHNEYKEMITSKIRSQIEKCSLSGEERIIPFFVDVISNIYEDNLYSGSEDDEFELSQESTPYPSL
NEVNDHVIHSIFNREKVRHKYNISSLEMIELFKTLNIELGDKWDINEASVLIGVLYENQQKELTDCILKNPLLNVVSDSFTTYKYEFLHSYFNSL
SIMEFLTKNDNVENTINLLSKSSKDTPEHKDIYKFLYNKGDALQILTPSLNKAIKMSLEYEKQDKIKYERAVAAIENIIYLISMFTNGKKDEFTG
IIKKIYNSNQNDKITGLYIKGDLPPINLTGMAISHSKFKHYKRFLKSDFENSKFTYTQFINCHNENIKNTSFLAADIDRNTCDIGDLDYTFNMLT
KIQDAAEEQVSEELAKFLSSFYKGSSFVENKKIYIKFSNRLPGLKSGAFNKLLSHGLISVKAEKEVDTFYIISENYRKSVRRFLNDGYKDAKIKD
LIEFIKA
>T4_nrdC.3
MKTRKHYIDYFDSLITKHRDYQKGHREVINNILRDFLDYIGWENHICKDTQNAYSHSLGSLLEWFKRSRLLSSVIAVNNVKKFMYPSYIETNVSN
DNVVTFNIINDVKRTYLEEWFSKDSKEKFASEFSHEFNNNVNMLFKHSRRLFCHGDDRTINVNVKDWVTAKFIPSSQNGPFELLIIVCAPHEIYK
NLPYMKPCEANKHNKTIRSLTYKLRTLLSKMDVVESFDDNTNYGLSLFETKVVIKLKDPNKFKPTPKPNHGNDTMKEEREYLSTRLIEVEKQIEE
HTKVLKDLTAKANGLRNAIEVLK
```

Run Command: run_alphafold -d $CURC_AF_DBS -o . -f $CURC_AF_EXAMPLES/multimer.fa -t 2020-05-14 -m “multimer” -g true

Folding Multimers (>1 Polypeptide Chain Cont'd)

```
I0521 14:26:53.124762 140296744294208 run_alphafold.py:386] Have 25 models: ['model_1_multimer_
v3_pred_0', 'model_1_multimer_v3_pred_1', 'model_1_multimer_v3_pred_2', 'model_1_multimer_v3_pr
ed_3', 'model_1_multimer_v3_pred_4', 'model_2_multimer_v3_pred_0', 'model_2_multimer_v3_pred_1'
, 'model_2_multimer_v3_pred_2', 'model_2_multimer_v3_pred_3', 'model_2_multimer_v3_pred_4', 'mo
del_3_multimer_v3_pred_0', 'model_3_multimer_v3_pred_1', 'model_3_multimer_v3_pred_2', 'model_3
_multimer_v3_pred_3', 'model_3_multimer_v3_pred_4', 'model_4_multimer_v3_pred_0', 'model_4_mult
imer_v3_pred_1', 'model_4_multimer_v3_pred_2', 'model_4_multimer_v3_pred_3', 'model_4_multimer_
v3_pred_4', 'model_5_multimer_v3_pred_0', 'model_5_multimer_v3_pred_1', 'model_5_multimer_v3_pr
ed_2', 'model_5_multimer_v3_pred_3', 'model_5_multimer_v3_pred_4']
I0521 14:26:53.124969 140296744294208 run_alphafold.py:403] Using random seed 21484416166174026
 for the data pipeline
I0521 14:26:53.125209 140296744294208 run_alphafold.py:161] Predicting multimer
I0521 14:26:53.139846 140296744294208 pipeline_multimer.py:210] Running monomer pipeline on cha
in A: bNACHT09
I0521 14:26:53.141395 140296744294208 jackhmmer.py:133] Launching subprocess "/curc/sw/install/
bio/alphafold/2.3.1/2.3.1_env/bin/jackhmmer -o /dev/null -A /scratch/alpine/lafr9499/tmphqonmyq
d/output.sto --noali --F1 0.0005 --F2 5e-05 --F3 5e-07 --incE 0.0001 -E 0.0001 --cpu 8 -N 1 /sc
ratch/alpine/lafr9499/tmpic840x7q.fasta /gpfs/alpine1/datasets/bioinformatics/alphafold/uniref9
0/uniref90.fasta"
```

Example Job Script

- An example job script is located in /curc/sw/install/bio/alphafold/2.3.1
- Copy to any space you have write permissions and make your desired changes:

```
cd /projects/$USER
cp /curc/sw/install/bio/alphafold/2.3.1/alphafold_alphine.sh .
```

Example Job Script

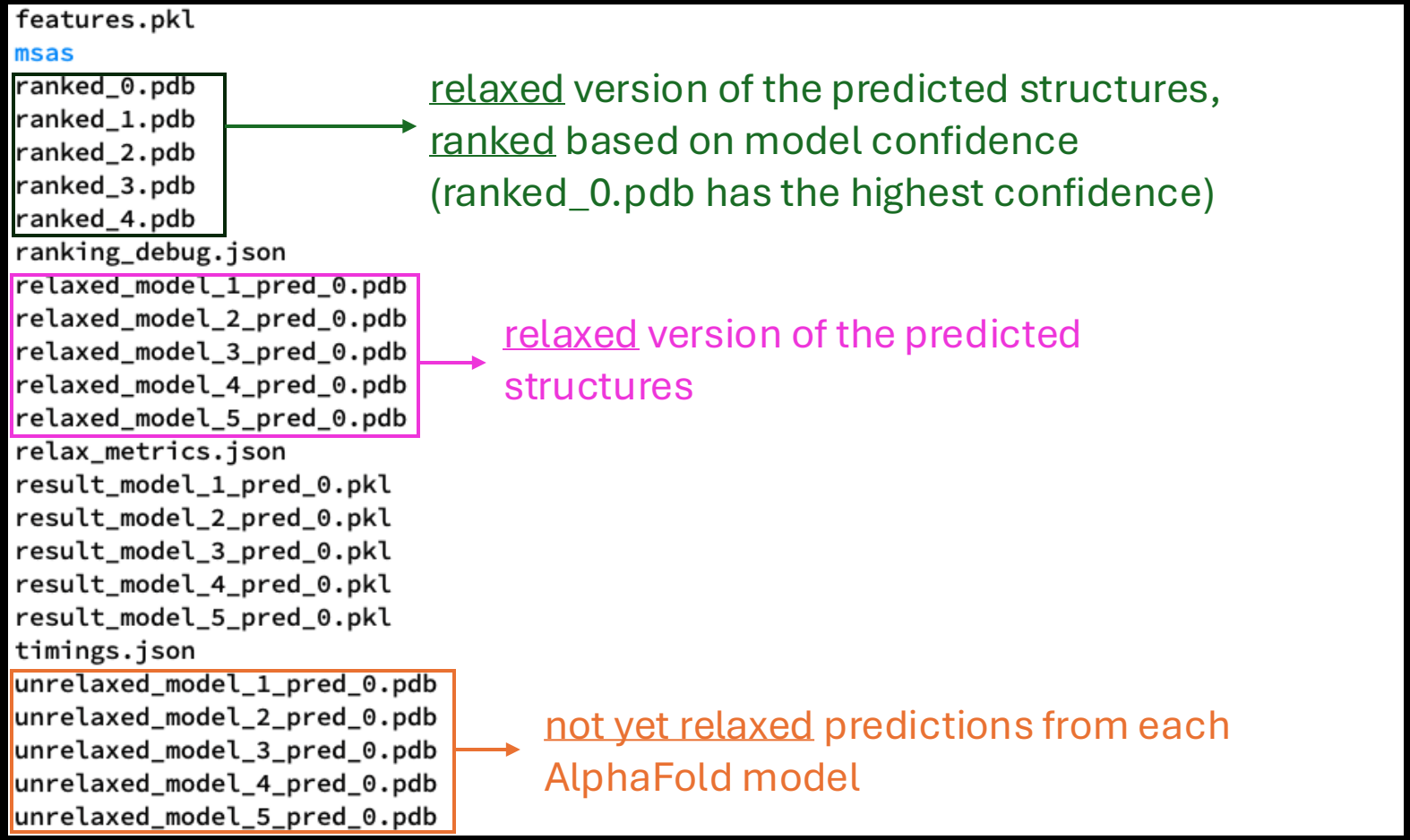
```
#!/bin/bash

#SBATCH --nodes=1
#SBATCH --time=06:00:00
#SBATCH --partition=aa100
#SBATCH --qos=normal
#SBATCH --gres=gpu:1
#SBATCH --job-name=monomer_test
#SBATCH --output=monomer_test_%j.out
#SBATCH --ntasks=8
#SBATCH --mail-type=ALL
#SBATCH --mail-user=<your email address>

module purge
module load alphafold

#change directory
cd /projects/$USER

#run AlphaFold
run_alphafold -d $CURC_AF_DBS -o . -f $CURC_AF_EXAMPLES/dummy.fasta -t 2020-05-14 -m "monomer" -g true
```

AlphaFold2 Outputs Overview

- Slurm .out file (e.g., short_course_dummy_out_example.out)
  - progress
  - warnings or errors
- a directory with the FASTA name (e.g., dummy)
  - computed MSAs
  - unrelaxed, relaxed, and ranked structures
  - raw model outputs
  - prediction metadata
  - timing information

AlphaFold2 Outputs Explained 1

.pdb protein database format (human readable)



AlphaFold2 Outputs Explained 2

.pkl

pickle data format (binary; not readable)

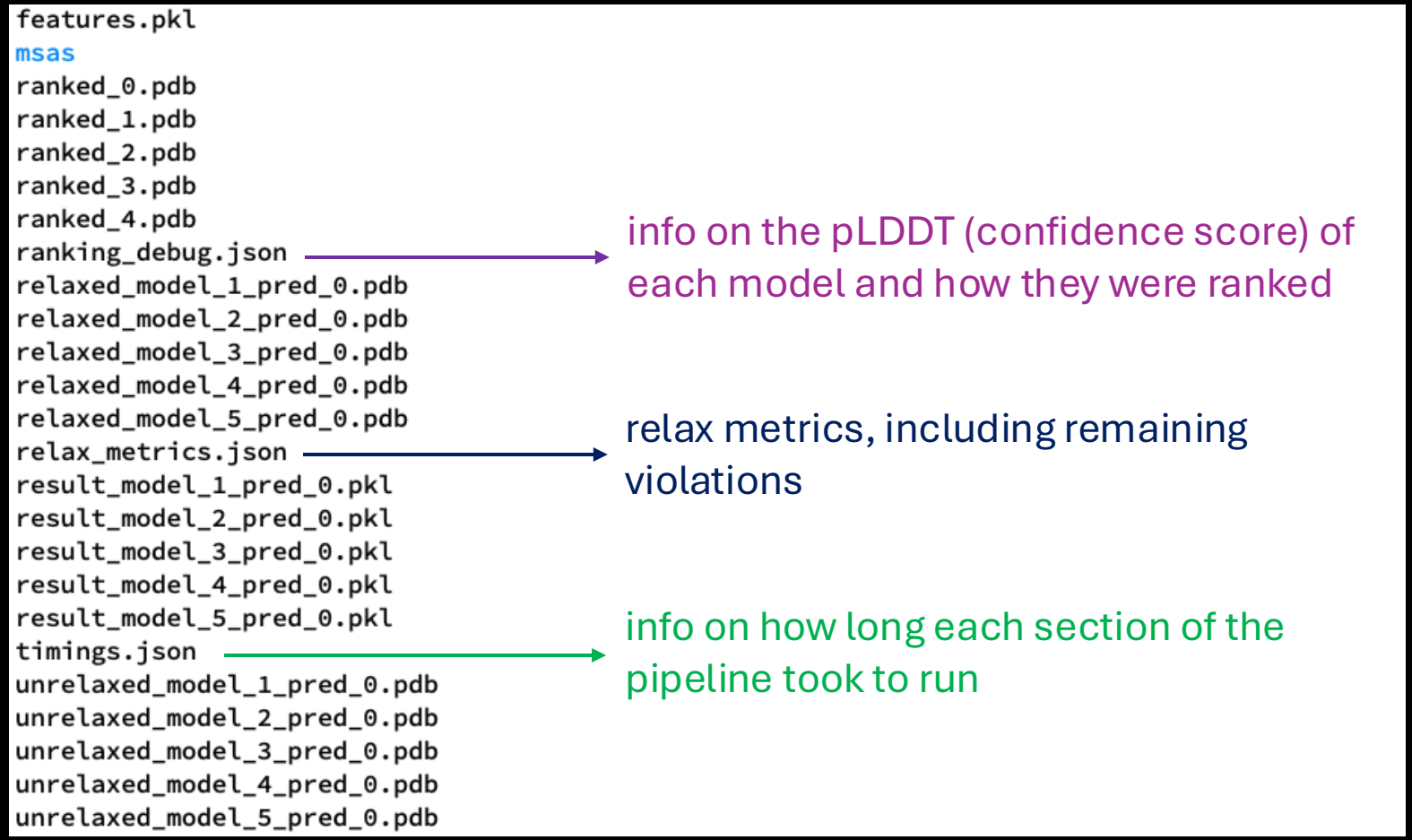
```
features.pkl
msas
ranked_0.pdb
ranked_1.pdb
ranked_2.pdb
ranked_3.pdb
ranked_4.pdb
ranking_debug.json
relaxed_model_1_pred_0.pdb
relaxed_model_2_pred_0.pdb
relaxed_model_3_pred_0.pdb
relaxed_model_4_pred_0.pdb
relaxed_model_5_pred_0.pdb
relax_metrics.json
result_model_1_pred_0.pkl
result_model_2_pred_0.pkl
result_model_3_pred_0.pkl
result_model_4_pred_0.pkl
result_model_5_pred_0.pkl
timings.json
unrelaxed_model_1_pred_0.pdb
unrelaxed_model_2_pred_0.pdb
unrelaxed_model_3_pred_0.pdb
unrelaxed_model_4_pred_0.pdb
unrelaxed_model_5_pred_0.pdb
```

processed MSA information (NumPy arrays), used as input to produce structures

outputs in .pkl format. includes pLDDT and PTM values

AlphaFold2 Outputs Explained 3

.json (readable data format)



AlphaFold2 Outputs Explained 4

Files in the msas directory are in various file formats, all human readable

```
features.pkl
msas
ranked_0.pdb
ranked_1.pdb
ranked_2.pdb
ranked_3.pdb
ranked_4.pdb
ranking_debug.json
relaxed_model_1_pred_0.pdb
relaxed_model_2_pred_0.pdb
relaxed_model_3_pred_0.pdb
relaxed_model_4_pred_0.pdb
relaxed_model_5_pred_0.pdb
relax_metrics.json
result_model_1_pred_0.pkl
result_model_2_pred_0.pkl
result_model_3_pred_0.pkl
result_model_4_pred_0.pkl
result_model_5_pred_0.pkl
timings.json
unrelaxed_model_1_pred_0.pdb
unrelaxed_model_2_pred_0.pdb
unrelaxed_model_3_pred_0.pdb
unrelaxed_model_4_pred_0.pdb
unrelaxed_model_5_pred_0.pdb
```

A directory containing the files describing the various genetic tool hits that were used to construct the input MSA

Visualizing Outputs

- To date we have left this up to the user
- Some options are
  - AlphaPickle: https://github.com/mattarnoldbio/alphapickle
  - PyMol (free for education): https://pymol.org/

- AlphaFold Protein Structure Database website provides a great resource for learning how to interpret visualizations:

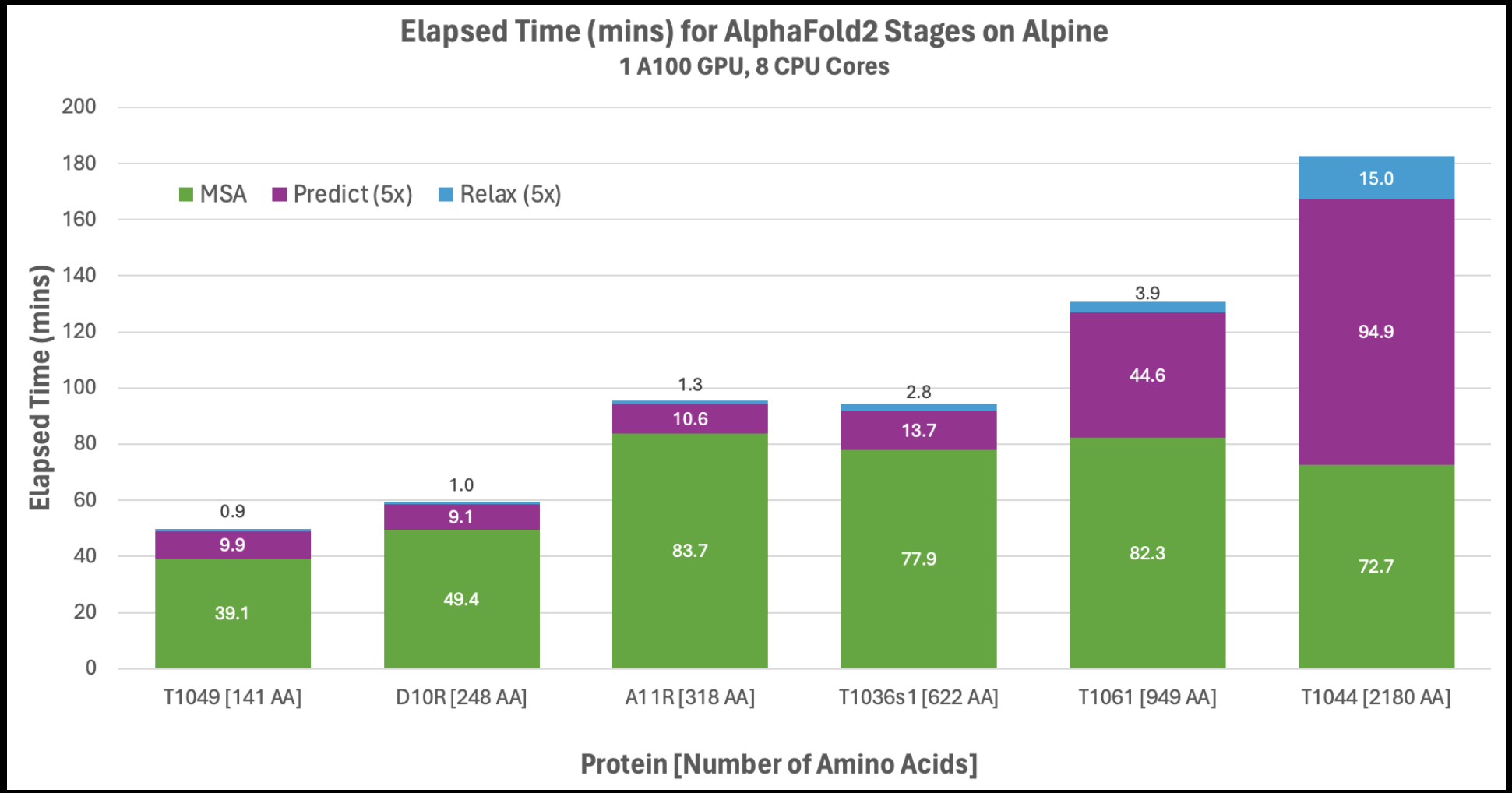
  https://alphafold.ebi.ac.uk/entry/Q9Y223#help

AlphaFold2 Performance Considerations

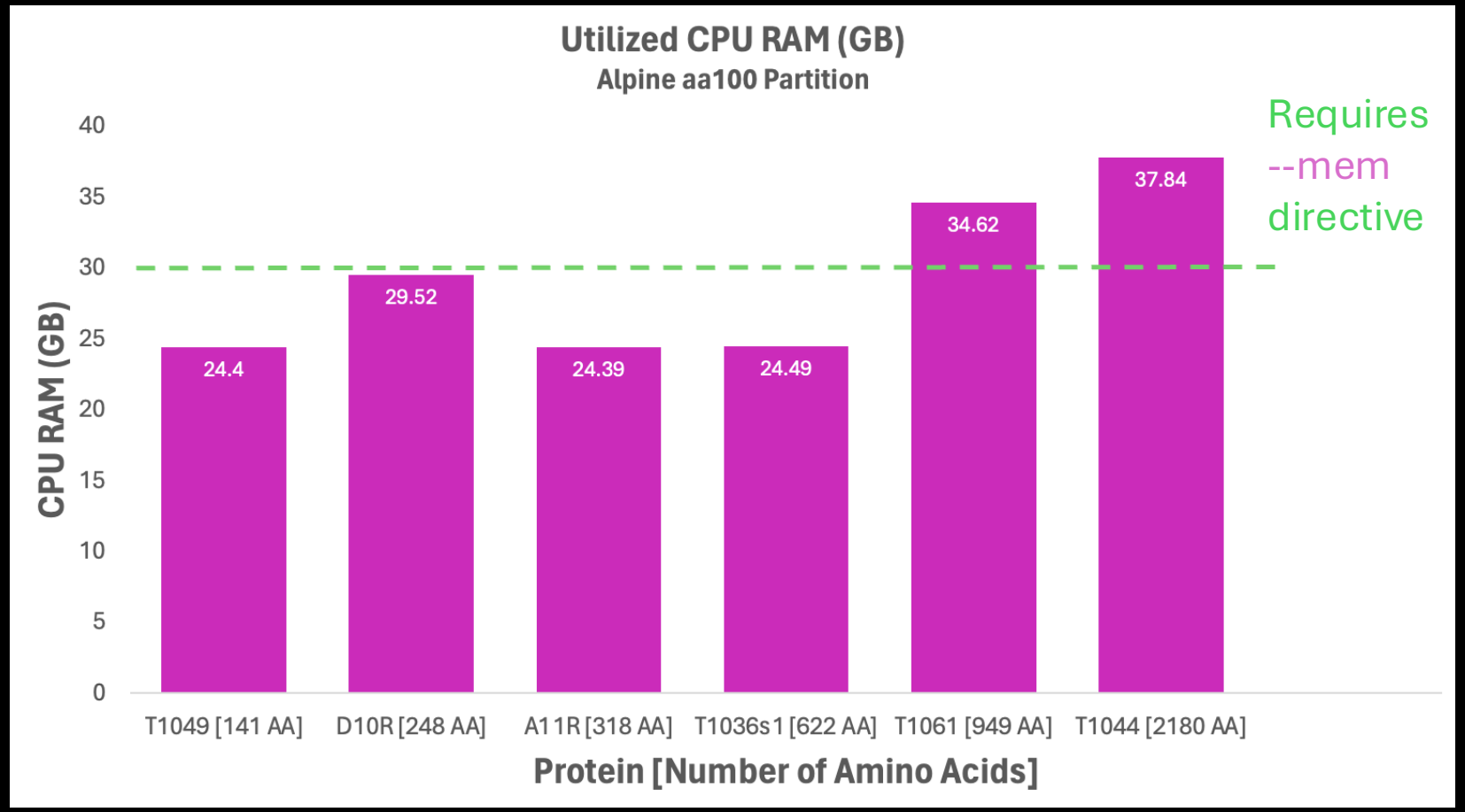
```
ice set to true (defaults to false) to enable this.
I0521 13:41:11.550761 140716613289792 run_alphafold.py:386] Have 5 models: ['model_1_pred_0', 'model_2_pred_0', 'mode
l_3_pred_0', 'model_4_pred_0', 'model_5_pred_0']
I0521 13:41:11.550974 140716613289792 run_alphafold.py:403] Using random seed 262200831417486581 for the data pipelin
e
I0521 13:41:11.551202 140716613289792 run_alphafold.py:161] Predicting dummy
I0521 13:41:11.567026 140716613289792 jackhmmer.py:133] Launching subprocess "/curc/sw/install/bio/alphafold/2.3.1/2.
3.1_env/bin/jackhmmer -o /dev/null -A /scratch/alpine/lafr9499/tmpuiv6sw6x/output.sto --noali --F1 0.0005 --F2 5e-05
--F3 5e-07 --incE 0.0001 -E 0.0001 --cpu 8 -N 1 /curc/sw/install/bio/alphafold/examples/dummy.fasta /gpfs/alpine1/dat
asets/bioinformatics/alphafold/uniref90/uniref90.fasta"
I0521 13:41:11.584823 140716613289792 utils.py:36] Started Jackhmmer (uniref90.fasta) query
```

The number of cores/threads used during MSA steps is hardcoded and so AF cannot use more than 8 CPU cores. Requesting more cores will **not** improve performance and you may end up waiting longer for your job to start.

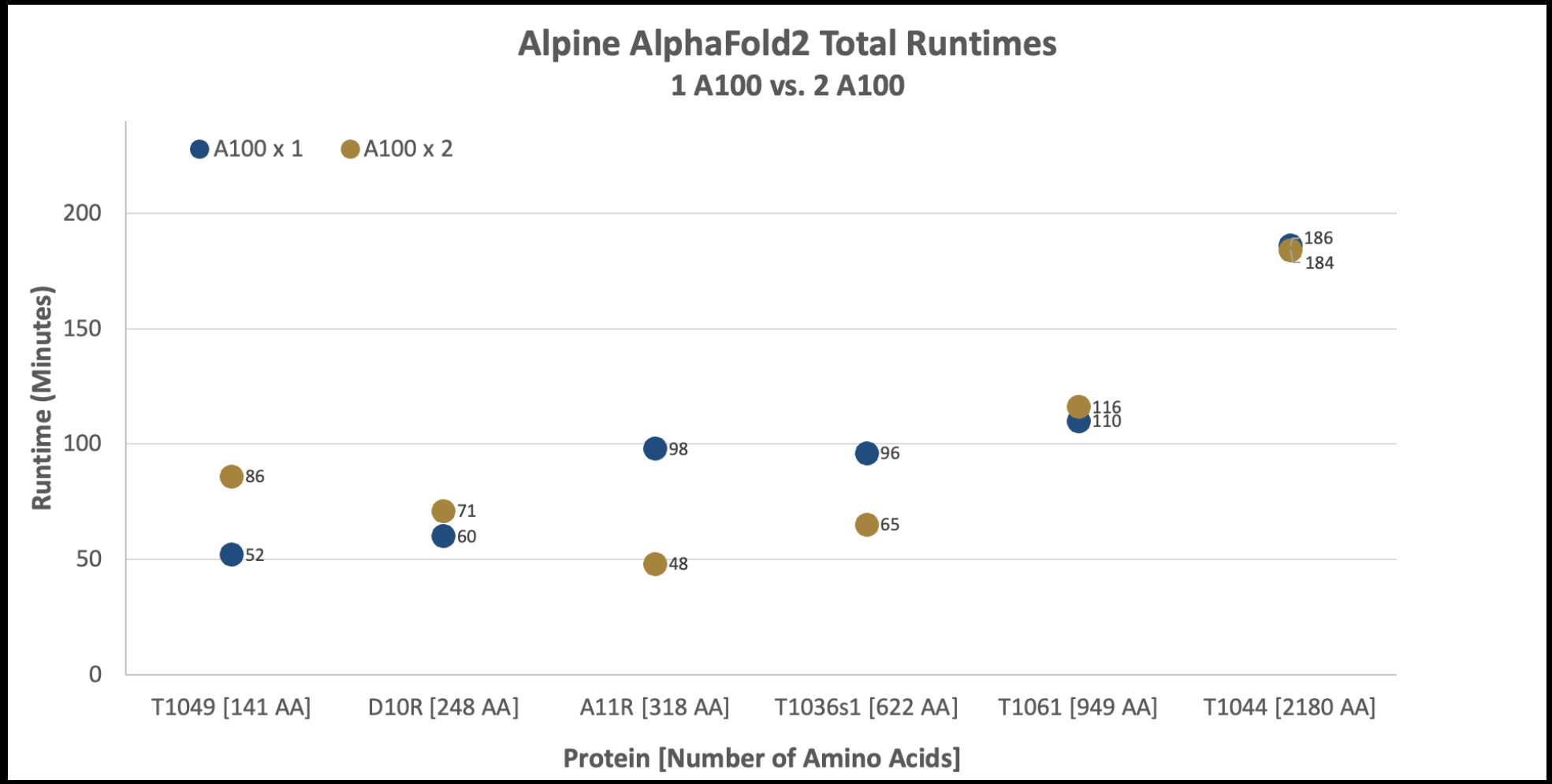
AlphaFold2 Elapsed Time



AlphaFold2 RAM Considerations



AlphaFold2 Alpine Benchmarks



AlphaFold Spin-Offs: ParaFold (ParallelFold)

- Divide CPU-intensive sections from the GPU-intensive sections
- PF isn't currently available as a module, but example scripts are available in /curc/sw/install/bio/parafold/2.0
  - parafold_cpu.sh
  - parafold_gpu.sh

AlphaFold Spin-Offs: ParaFold Job Script

```
#!/bin/bash

#SBATCH --nodes=1
#SBATCH --time=10:00:00
#SBATCH --partition=amilan
#SBATCH --qos=normal
#SBATCH --job-name=parafold_cpu
#SBATCH --output=parafold_cpu_%j.out
#SBATCH --ntasks=8
#SBATCH --ntasks-per-node=8
#SBATCH --mem=60G
#SBATCH --mail-type=ALL
#SBATCH --mail-user=<your_email_address>

module purge
module load anaconda

conda activate /curc/sw/install/bio/parafold/2.0/2.0_env

export TMPDIR=/scratch/alpine/$USER

bash /curc/sw/install/bio/parafold/2.0/ParallelFold/run_alphafold.sh -d /gpfs/alpine1/datasets/bioinformatics/alphafold
-o /projects/$USER/pf_runs -p multimer -i /curc/sw/install/bio/alphafold/examples/multimer.fa -f -g false
```

-f tells PF to stop after generating the feature.pkl file

AlphaFold Spin-Offs: ParaFold Job Script Cont'd

```
#!/bin/bash

#SBATCH --nodes=1
#SBATCH --time=24:00:00
#SBATCH --partition=aa100
#SBATCH --gres=gpu:1
#SBATCH --constraint=gpu80
#SBATCH --qos=normal
#SBATCH --job-name=parafold_gpu
#SBATCH --output=parafold_gpu_%j.out
#SBATCH --ntasks=8
#SBATCH --ntasks-per-node=8
#SBATCH --mail-type=ALL
#SBATCH --mail-user=<your_email_address>

module purge
module load cuda
module load anaconda

conda activate /curc/sw/install/bio/parafold/2.0/2.0_env

export TF_FORCE_UNIFIED_MEMORY=1
export XLA_PYTHON_CLIENT_MEM_FRACTION=4.0
export TMPDIR=/scratch/alpine/$USER

bash /curc/sw/install/bio/parafold/2.0/ParallelFold/run_alphafold.sh -d /gpfs/alpine1/datasets/bioinformatics/alphafold
-o /projects/$USER/pf_runs -p multimer -i /curc/sw/install/bio/alphafold/examples/multimer.fa -g true -m model_1_multime
r,model_2_multimer,model_3_multimer,model_4_multimer,model_5_multimer
```

Not needed for the multimer.fa example, but note that it is an option.

May need to add --mem directive for some predictions (relaxation step?)

Explore AlphaFold2 on Alpine

Slides/Self-guided material:
https://bit.ly/af2alpine

Thank you

Please give us your feedback!

http://tinyurl.com/curc-survey18

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