# AlphaFold and Protein Structure Prediction

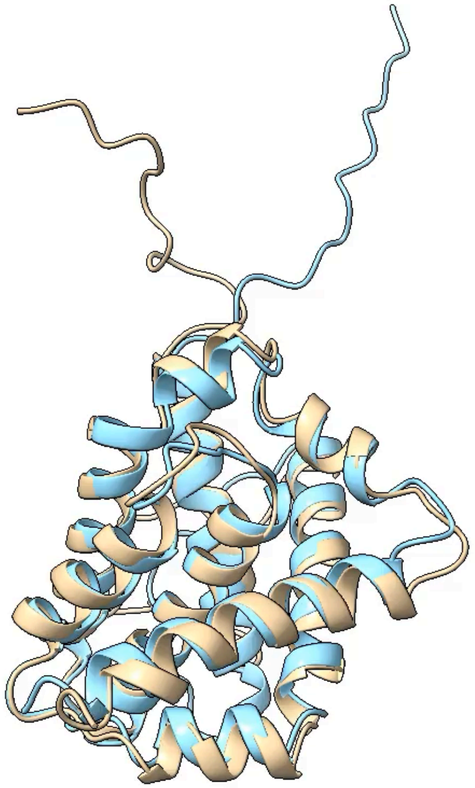
## CCATS Group

# Alphafold Tutorial

- **Goals:**
  A. Predict the structure of Bax
  B. Predict the complex of PaParDE (Bourne Lab)

- The working directory I prepared is organized as follow:

```
V /home/van/MacroMol_Modelling_Tutorial/02_alphafold/
  > Results/
  V Tutorial/
    V 00_monomer_pred/
      > monomer.slurm
    V 01_complex_pred/
      > complex.slurm
```



NMR Structure (PDB 1F16)

Alphafold Prediction

# Alphafold Workflow

1. Input
   - Amino acid Sequence (FASTA format)
2. Alphafold Program
   - Conda environment (contains dependencies; ~ 2.2 Gb)
   - Alphafold Software (~14 Mb)
3. Alphafold Databases (~2.2 Tb)
   - BDF, MGnify, PDB70, PDB MMCIF, PDB SEQRES, Uniclust30, Uniprot, Uniref90
4. GPU (not required but strongly encouraged)
5. Set a max template date

**If all goes well, you get 5 to 25 predicted structures!**

# FASTA Files

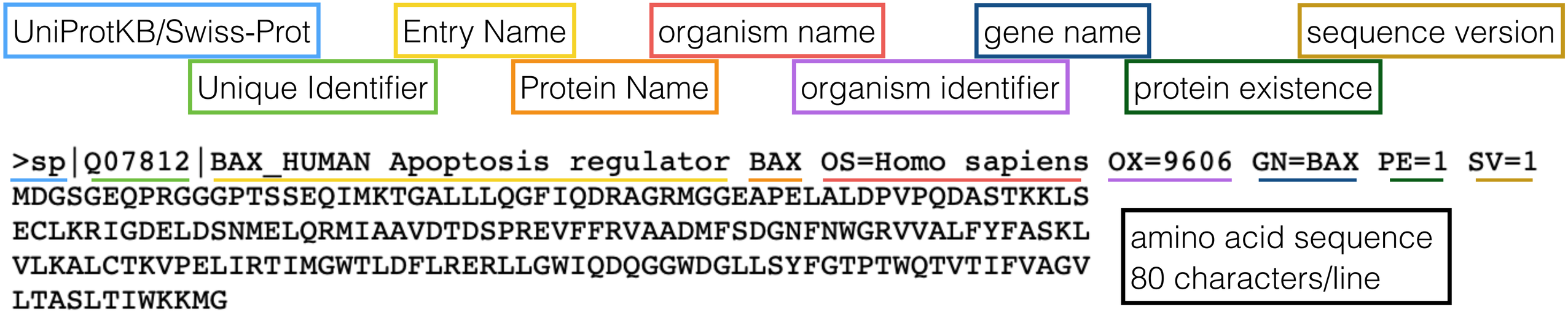
- FASTA means “FAST All,” which is an extension from the previous “FAST-N” or “FAST-P”
  - Format originated from FASTA Program for sequence alignments (1985)
  - Now the universal standard for bioinformatic tools (BLAST, CLUSTAL, etc.)

1. First line contains “>” which denotes a comment
   - Formally, this line also contain descriptions such as:
     - UNIPROT entry, Gene Identity, Organism, Accession Numbers, etc.
2. Next line contains one letter code of nucleotide or amino acid sequence
   - **AA sequence for Alphafold!**
   - Usually 80 characters/line
3. Followed by empty line for multiple sequences.

# FASTA Files

## Example Bax UNIPROT Data:

- https://www.uniprot.org/uniprotkb/Q07812
- https://rest.uniprot.org/uniprotkb/Q07812.fasta



# Alphafold Program on OSCER

- https://github.com/deepmind/alphafold
- Installation: 1) NVIDIA Container Toolkit, and 2) Docker file (*cannot do this as normal user*)
- My workaround:

  1. Conda environment (Contains NVIDA Toolkit, Tensorflow, OpenMM, HHsuite, etc.)
     - Anaconda/Miniconda for package management (**conda activate af2.2.0**)
  2. Download my own copy of Alphafold (**/home/van/Programs/alphafold**)

- Database for Alphafold is stored on OSCER
  - Half of the database is stored in: **/opt/oscer/software/alphafold/2.0.0**
  - Other half requires periodic updates which is automated in: **/data/alphafold** (done at the beginning of every month)

# Alphafold Databases

- BDF
  - Clustered protein sequences (MSA)
- MGnify
  - Genetic microbiome data (MSA)
- PDB70
  - Sequence for HHsuite (MSA)
- PDB MMCIF
  - MMCIF files from RCSB
- PDB SEQRES
  - RCSB data for complex prediction
- Uniclust30
  - Clustered sequences based on 30% pairwise identity
- Uniprot
  - Gene data for complex prediction
- Uniref90
  - Clustered sequences from Uniref100 database (sequences shorter than 11 have 1 representative member here)

# Differences for Monomer v. Complex Prediction

## 1. Input File

(Amino Acid Sequence(s) in FASTA format)

| **$ vi monomer.fasta** |
| --- |
| 1 > name_1 |
| 2 [amino acid sequence 1] |

| **$ vi complex.fasta** |
| --- |
| 1 > name_1 |
| 2 [amino acid sequence 1] |
| 3 > name_2 |
| 4 [amino acid sequence 2] |
| .. … |

## Example:

### Monomer FASTA

```
1 >bax
2 MDGSGEQPRGGGPTSSEQIMKTGALLLQGFIQDRAGRMGGEAPELA
  LDPVPQDASTKKLSECLKRIGDELDSNMELQRMIAAVDTDSPREVF
  FRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMG
  WTLDFLRERLLGWIQDQGGWDGLLSYFGTPTWQTVTIFVAGVLTAS
  LTIWKKMG
```

### Complex FASTA (PaParDE - Bourne Lab)

```
1 > ParD
2 GSHMMSLKWTRKAAADLDAIYDHYVVLIGPEKALKAVQDIVEQVKP
  LQQVANQGAGRPSEVPGVRTLTLERWPFSAPFRVKGKEIQILRIDR
  VEITP
3 >ParE
4 GSHMMSTVVSFRADDALVAALDELARATHRDRPYHLRQALAQYLER
  QQWQVAAIDEGLADANAGRLLEHIEIEKRWGLQ
```

# Differences for Monomer v. Complex Prediction

## 2. Alphafold Databases

Monomer Prediction Database

A. BFD
B. MGnify
C. PDB MMCIF
**D. PDB70**
E. Uniclust30
F. Uniref90
**G. —**
**H. —**

Complex Prediction Database

A. BFD
B. MGnify
C. PDB MMCIF
**D. —**
E. Uniclust30
F. Uniref90
**G. PDB SEQRES**
**H. UNIPROT**

# Tutorial/00_monomer_pred/monomer.slurm (First 35 Lines)

```
#!/bin/bash
#SBATCH --partition=gpu
#SBATCH --exclusive
#SBATCH --ntasks=20
#SBATCH --ntasks-per-node=20
#SBATCH --mem=0
#SBATCH --output=%j.out
#SBATCH --error=%j.err
#SBATCH --time=48:00:00
#SBATCH --job-name=INPUT

# THIS SCRIPT IS FOR MONOMER PREDICTION

#Loading module for GPU
module load CUDA/11.3.1

# Activates Richard's Conda environment prepared for Alphafold (Tensorflow, CuDNN, etc.)
eval "$(/ourdisk/hpc/ccats/dont_archive/van/.Programs/miniconda3/bin/conda shell.bash hook)"
conda activate af2.2.0

# Use Richard's Alphafold program
AF="/home/van/Programs/alphafold/run_alphafold.py"

# Sets output directory to the current folder
OUT_DIR=`pwd`

# Databases for Alphafold
#       DATA_DIR contains the sequence database for MSAs
#       DATA_DIR2 contains mmCIF and other files that require periodic updating
DATA_DIR="/opt/oscer/software/alphafold/2.0.0/data"
DATA_DIR2="/data/alphafold"

# Input name of sequence file. CHANGE THIS BETWEEN DIFFERENT RUNS!
input="INPUT"

```

SLURM Allocation requests

- Rename INPUT to job name

Load CUDA library (for GPU)

Loads my conda environment for Alphafold

Variable “AF” points to alphafold program

Variable “OUT_DIR” points to current working directory

Variable “DATA_DIR” points to AF databases

Variable “DATA_DIR2” points to AF databases that update the beginning of every month

Variable “input” is the name of your pasta file (rename INPUT)

# Tutorial/00_monomer_pred/monomer.slurm (Lines 35 to EOF)

Max template year for PDBS

```
36 # Set year for the maximum template year (oldest PDB you want included to the prediction).
37 year="2000"
38
39 python ${AF} \
40  --fasta_paths=${input}.fasta \
41  --output_dir=${OUT_DIR} \
42  --max_template_date=${year}-01-01 \
43  --use_gpu_relax=True \
44  --jackhmmer_binary_path=/opt/oscer/software/HMMER/3.2.1-GCC-8.2.0-2.31.1/bin/jackhmmer \
45  --data_dir=${DATA_DIR2} \
46  --bfd_database_path=${DATA_DIR}/bfd/bfd_metaclust_clu_complete_id30_c90_final_seq.sorted_opt \
47  --uniref90_database_path=${DATA_DIR}/uniref90/uniref90.fasta \
48  --mgnify_database_path=${DATA_DIR}/mgnify/mgy_clusters.fa \
49  --pdb70_database_path=${DATA_DIR}/pdb70/pdb70 \
50  --uniclust30_database_path=${DATA_DIR}/uniclust30/uniclust30_2018_08/uniclust30_2018_08 \
51  --template_mmcif_dir=${DATA_DIR2}/pdb_mmcif/mmcif_files \
52  --obsolete_pdbs_path=${DATA_DIR2}/pdb_mmcif/obsolete.dat
53
```

* The symbol “\” means continue reading next line (ignores line breaks)

- *fasta_paths*

  Input sequence (e.g. bax.fasta)
- *output_dir*

  Predictions are saved to output directory in this directory
- *max_template_date*

  For PDB search
- *use_gpu_relax*

  Use GPU acceleratedminimization
- *jackhmmer_binary_path*

  Points to jackhammer program (probably not needed anymore)
- *The rest are flags pointing to the appropriate databases.*

# Monomer Tutorial (~2.75 hours, node: c301)

1. Make input file:

   $ vi bax.fasta

   >bax

   MDGSGEQPRGGGPTSSEQIMKTGALLLQGFIQDRAGRMGGEAPELALDPVPQDASTKKLSECLKRIGDEL
   DSNMELQRMIAAVDTDSPREVFFRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLD
   FLRERLLGWIQDQGGWDGLLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG

2. Change INPUT to bax in monomer.slurm (lines 10 & 34)

   sed -i ‘s/INPUT/bax/’ monomer.slurm

3. Submit the SLURM script

   sbatch monomer.slurm

# Tutorial/01_complex_pred/complex.slurm (First 35 Lines)

```
 1 #!/bin/bash
 2 #SBATCH --partition=gpu
 3 #SBATCH --exclusive
 4 #SBATCH --ntasks=20
 5 #SBATCH --ntasks-per-node=20
 6 #SBATCH --mem=0
 7 #SBATCH --output=%j.out
 8 #SBATCH --error=%j.err
 9 #SBATCH --time=48:00:00
10 #SBATCH --job-name=INPUT
11
12 # THIS SCRIPT IS ONLY FOR MULTIMER PREDICTION!!!!!!!!
13
14 # Loading module for GPU
15 module load CUDA/11.3.1
16
17 # Activates Richard's Conda environment prepared for Alphafold (Tensorflow, CuDNN, etc.)
18 eval "$(/ourdisk/hpc/ccats/dont_archive/van/.Programs/miniconda3/bin/conda shell.bash hook)"
19 conda activate af2.2.0
20
21 # Use Richard's Alphafold program
22 AF="/home/van/Programs/alphafold/run_alphafold.py"
23
24 # Sets output directory to the current folder
25 OUT_DIR=`pwd`
26
27 # Databases for Alphafold
28 #       DATA_DIR contains the sequence database for MSAs
29 #       DATA_DIR2 contains mmCIF and other files that require periodic updating
30 DATA_DIR="/opt/oscer/software/alphafold/2.0.0/data"
31 DATA_DIR2="/data/alphafold"
32
33 # Input name of sequence file. CHANGE THIS BETWEEN DIFFERENT RUNS!
34 input="INPUT"
35
```

SLURM Allocation requests

- Rename INPUT to job name

**Same as monomer.sh so far..**

**(Except for comment on line 12)**

Load CUDA library (for GPU)

Loads my conda environment for Alphafold

Variable “AF” points to alphafold program

Variable “OUT_DIR” points to current working directory

Variable “DATA_DIR” points to AF databases

Variable “DATA_DIR2” points to AF databases that update the beginning of every month

Variable “input” is the name of your pasta file (rename INPUT)

# Tutorial/01_complex_pred/complex.slurm (Lines 35 to EOF)

Max template year for PDBS

```
36 # Set year for the maximum template year (oldest PDB you want included to the prediction).
37 year="2000"
38
39 python ${AF} \
40         --fasta_paths=${input}.fasta \
41         --output_dir=${OUT_DIR} \
42         --max_template_date=${year}-01-01 \
43         --use_gpu_relax=True \
44         --model_preset=multimer \
45         --jackhmmer_binary_path=/opt/oscer/software/HMMER/3.2.1-GCC-8.2.0-2.31.1/bin/jackhmmer \
46         --data_dir=${DATA_DIR2} \
47         --bfd_database_path=${DATA_DIR}/bfd/bfd_metaclust_clu_complete_id30_c90_final_seq.sorted_opt \
48         --uniref90_database_path=${DATA_DIR}/uniref90/uniref90.fasta \
49         --mgnify_database_path=${DATA_DIR}/mgnify/mgy_clusters.fa \
50         --uniclust30_database_path=${DATA_DIR}/uniclust30/uniclust30_2018_08/uniclust30_2018_08 \
51         --template_mmcif_dir=${DATA_DIR2}/pdb_mmcif/mmcif_files \
52         --obsolete_pdbs_path=${DATA_DIR2}/pdb_mmcif/obsolete.dat \
53         --pdb_seqres_database_path=${DATA_DIR2}/pdb_seqres/pdb_seqres.txt \
54         --uniprot_database_path=${DATA_DIR2}/uniprot/uniprot.fasta
```

- All flags are the same as monomer.slurm
- Differences are in lines 53, 54, and removed the PDB70 database

* The symbol “\” means continue reading next line (ignores line breaks)

# Complex Tutorial

1. Make input file:

   $ vi paparde.fasta

   > ParD

   GSHMMSLKWTRKAAADLDAIYDHYVVLIGPEKALKAVQDIVEQVKPLQQVANQGAGRPSEVPGVRTLTLERWPFSAPF
   RVKGKEIQILRIDRVEITP

   >ParE

   GSHMMSTVVSFRADDALVAALDELARATHRDRPYHLRQALAQYLERQQWQVAAIDEGLADANAGRLLEHIEIEKRWGL
   Q

2. Change INPUT to paparde to complex.slurm (lines 10 & 34)

   sed -i ‘s/INPUT/paparde/’ complex.slurm

3. Submit the SLURM script

   sbatch complex.slurm

# SLURM Commands

- sbatch *[file.slurm] ;* Submits SLURM script to scheduler
- squeue ; Check the SLURM queue
  - squeue -u *[username]* ; Check your SLURM queue
  - squeue -p *[partition]* ; Check the queue for a specific partition
- scancel *[job number]* ; Cancel a submitted job

# SLURM Commands

- module load *[name]* ; Loads the environment for specified program
- module purge ; Cleans loaded modules
- module list ; List all currently loaded modules
- module avail ; List all available modules
- module spider *[name]* ; Searches for program

- Alternatively, you can look for software in: **/opt/oscer/software**