Research Notebook

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Bio 496
Started: 12/23/23

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AlphaFold in Microsoft Azure Virtual Machine

Objective: The purpose of this section is to configure AlphaFold/ ColabFold and Rosetta Ab Initio in the Microsoft Azure Virtual Machine, with HyperDrive enabled for concurrent modeling of both protein queries (wild type and mutant MDH). I will use a Jupyter Notebook Script derived from Dr. Colby T. Ford's GitHub repository.

Procedures:

Microsoft Azure VM setup:

1. Login to Microsoft Azure after registering for an account with the allocated VM type, and enter the Machine Learning Studio
2. Under the "Manage" tab, select "Compute" to create a new compute instance for the modeling queries
3. Create a new compute instance with CPU VM with the allocated VM type (Standard DCsv3 and DCdsv3-series used, specifically Standard_DC48ds_v3 VM with 48 physical cores, 384 GB Memory, and 2400 GiB SSD)
4. Name the compute instance, and start it. Enter it, and modify the Jupyter Notebook with the HyperDrive script.

Docker Image Creation for AlphaFold:

```
docker build -t alphafold2_aml --build-arg IMAGE_VERSION=$(git rev-parse --short HEAD) .
# docker run --name alphafold2_aml --rm -p 8787:8787 alphafold2_aml
# docker exec -it alphafold2_aml /bin/bash

az login
az account set --subscription <SUBSCRIPTION_ID>
az acr login --name <CONTAINER_REGISTRY_NAME>
docker tag mmae_aml <CONTAINER_REGISTRY_NAME>.azurecr.io/alphafold2_aml:$(git rev-parse --short
HEAD)
docker push <CONTAINER_REGISTRY_NAME>.azurecr.io/alphafold2_aml:$(git rev-parse --short HEAD)
```

Adapted Script for Rosetta in Azure VM:

Cell 1: Azure ML Compute Setup

```
from azureml.core.compute import ComputeTarget, AmlCompute
from azureml.core.compute_target import ComputeTargetException

cluster_name = "rosetta-cluster"

try:
    # Check for existing compute target
    training_cluster = ComputeTarget(workspace=ws, name=cluster_name)
    print('Found existing cluster.')
except ComputeTargetException:
    # If it doesn't already exist, create it
    try:
```

```
    compute_config = AmlCompute.provisioning_configuration(vm_size='Standard_DC48ds_v3', max_nodes=4)
    training_cluster = ComputeTarget.create(ws, cluster_name, compute_config)
    training_cluster.wait_for_completion(show_output=True)
  except Exception as ex:
    print(ex)
```

Cell 2: Environment and Script Configuration

```
from azureml.core import Experiment, ScriptRunConfig, Environment
from azureml.core.conda_dependencies import CondaDependencies

# Create a Python environment for the experiment
rosetta_env = Environment("rosetta-environment")
rosetta_env.docker.base_image = "YOUR_BASE_IMAGE"  # Replace with the appropriate base image for Rosetta
rosetta_env.python.user_managed_dependencies = True

# Create a script config
script_config = ScriptRunConfig(source_directory=".",
                        script='run_rosetta.py',
                        arguments=['--fasta_file', 'input.fasta',
                                '--frag3_file', 'frag_3mers',
                                '--frag9_file', 'frag_9mers'],
                        environment=rosetta_env,
                        compute_target=training_cluster)
```

Cell 3: Rosetta Abinitio Prediction Script

```
%%writefile run_rosetta.py
# Import necessary libraries
import argparse
from azureml.core import Run

# Set up argument parser
parser = argparse.ArgumentParser()
parser.add_argument("--fasta_file", type=str, help="Path to FASTA file")
parser.add_argument("--frag3_file", type=str, help="Path to 3-mer fragment file")
parser.add_argument("--frag9_file", type=str, help="Path to 9-mer fragment file")
args = parser.parse_args()
run = Run.get_context()

# TODO: Add code to run Rosetta AbInitio with the provided files
run.complete()
```

Docker Image Creation for Rosetta AbInitio:

```
git clone https://github.com/Metaphorme/Rosetta2Go.git
cd Rosetta2Go
git checkout 3.13
chmod +x build4docker.sh
./build4docker.sh
# update notebook environment configuration:
```

rosetta_env = Environment("rosetta-environment")
rosetta_env.docker.base_image = "rosetta2go:3.13"
rosetta_env.python.user_managed_dependencies = True

Compile Rosetta:

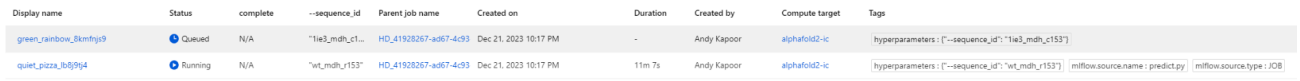
tar -xvjf rosetta.binary.linux.release-362.tar.bz2 **or** lbzip2 -dc rosetta.binary.linux.release-362.tar.bz2 | tar xvf -

Data:

Contents of AlphaFold sequences.fasta:

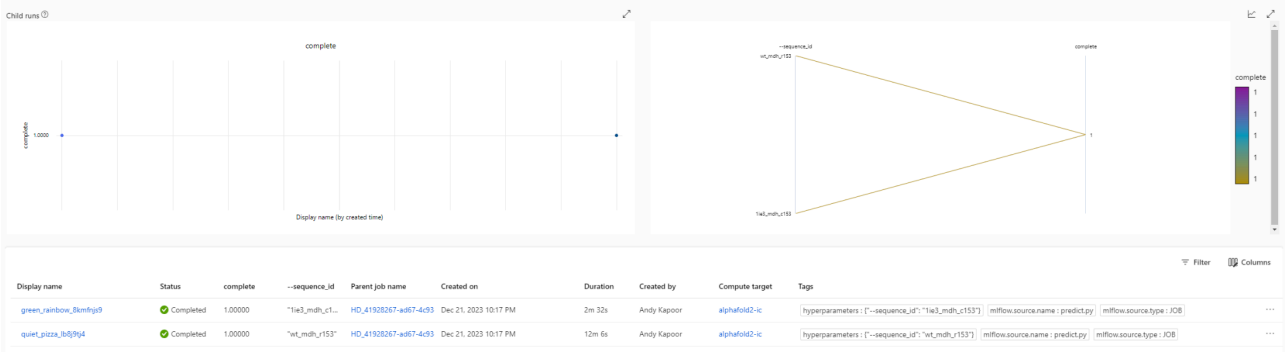
>wt_mdh_r153
MKVAVLGAAGGIGQALALLLKTQLPSGSELSLYDIAPVTPGVAVDLSHIPTAVKIKGFSGEDATPALEGADVV
LISAGVARKPGMDRSDLFNVNAGIVKNLVQQVAKTCPKACIGIITNPVNTTVAIAAEVLKKAGVYDKNKLF
GVTTLDIIRSNTFVAELKGKQPGEVEVPVIGGHSGVTILPLLSQVPGVSFTEQEVADLTKRIQNAGTEVVEAK
AGGGSATLSMGQAAARFGLSLVRALQGEQGVVECAYVEGDGQYARFFSQPLLLGKNGVEERKSIGTLSAFE
QNALEGMLDTLKKDIALGQEFVNK
>1ie3_mdh_c153
MKVAVLGAAGGIGQALALLLKTQLPSGSELSLYDIAPVTPGVAVDLSHIPTAVKIKGFSGEDATPALEGADVV
LISAGVARKPGMDRSDLFNVNAGIVKNLVQQVAKTCPKACIGIITNPVNTTVAIAAEVLKKAGVYDKNKLF
GVTTLDIICSNTFVAELKGKQPGEVEVPVIGGHSGVTILPLLSQVPGVSFTEQEVADLTKRIQNAGTEVVEAK
AGGGSATLSMGQAAARFGLSLVRALQGEQGVVECAYVEGDGQYARFFSQPLLLGKNGVEERKSIGTLSAFE
QNALEGMLDTLKKDIALGQEFVNK

AlphaFold Queued Runs:

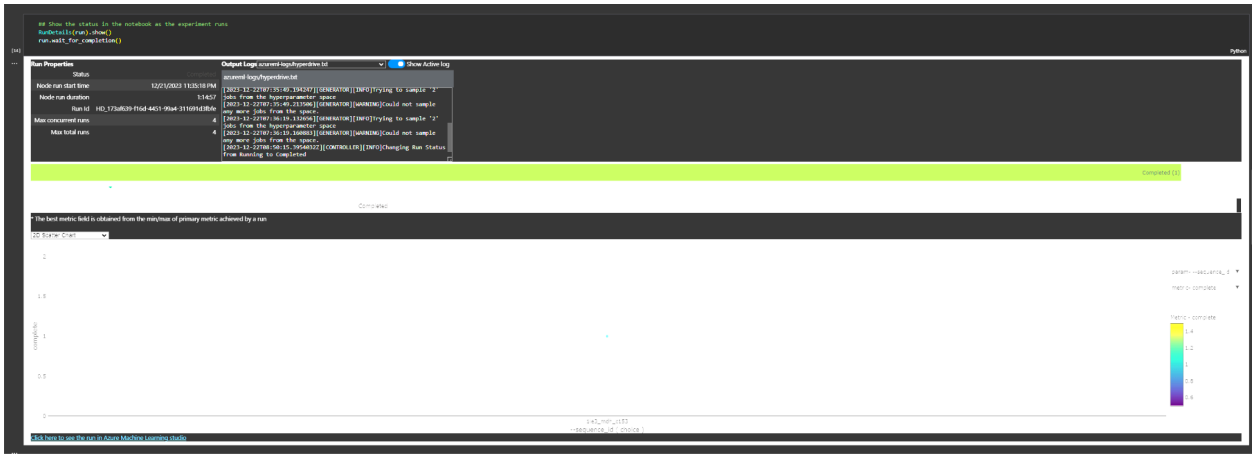


Display name	Status	complete	--sequence_id	Parent job name	Created on	Duration	Created by	Compute target	Tags
green_rainbow_8kmfnjs9	Queued	N/A	"1ie3_mdh_c1...	HD_41928267-ad67-4c93	Dec 21, 2023 10:17 PM	-	Andy Kapoor	alphafold2-ic	hyperparameters : {"--sequence_id": "1ie3_mdh_c153"}
quiet_pizza_lb8j9tj4	Running	N/A	"wt_mdh_r153"	HD_41928267-ad67-4c93	Dec 21, 2023 10:17 PM	11m 7s	Andy Kapoor	alphafold2-ic	hyperparameters : {"--sequence_id": "wt_mdh_r153"} mlflow.source.name : predict.py mlflow.source.type : JOB

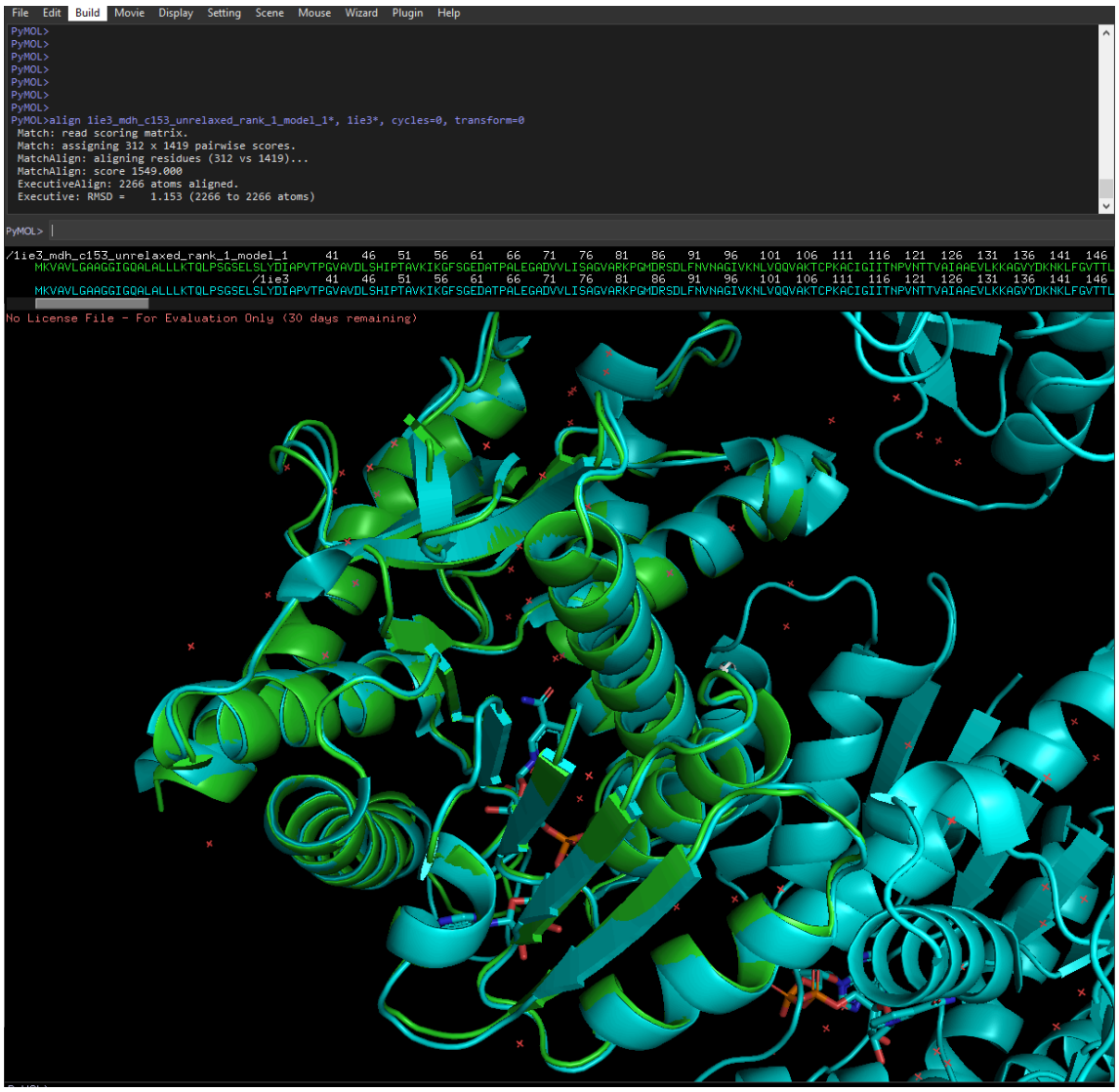
AlphaFold Completed Runs:



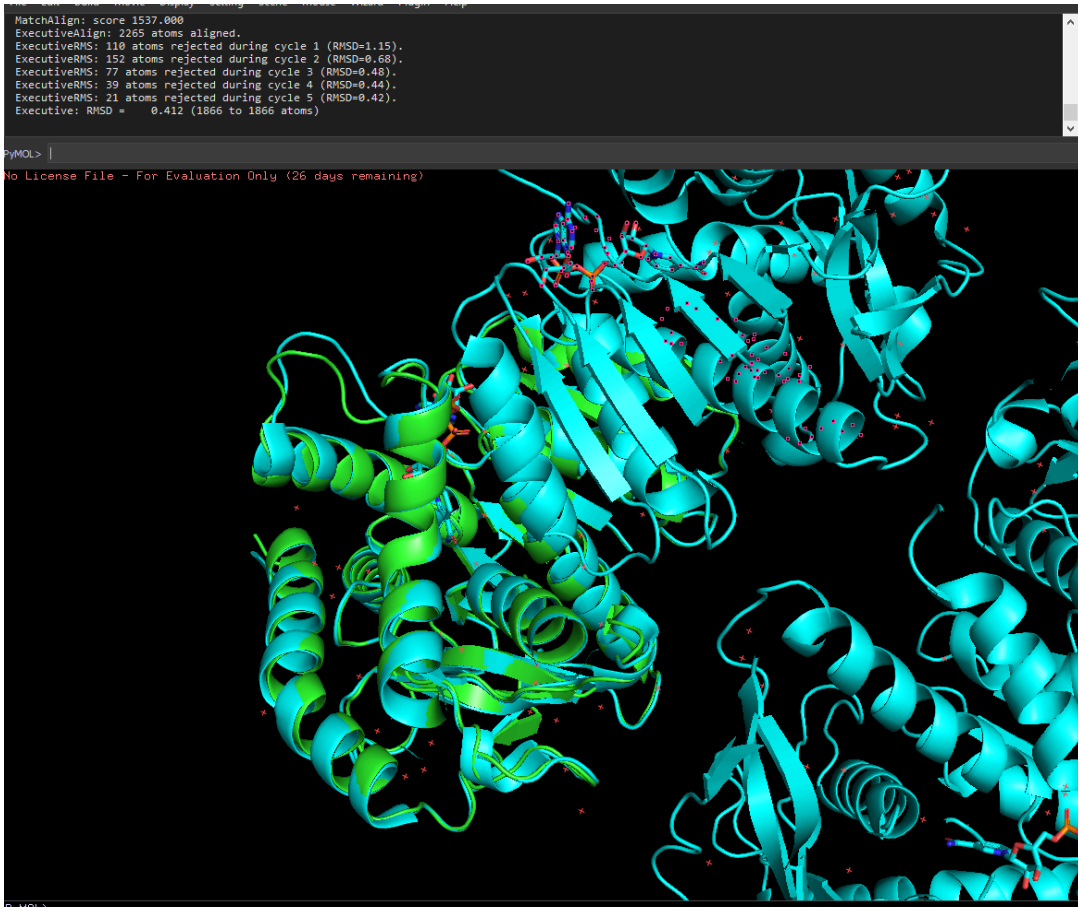
Display name	Status	complete	--sequence_id	Parent job name	Created on	Duration	Created by	Compute target	Tags
green_rainbow_8kmfnjs9	Completed	1.00000	"1ie3_mdh_c1...	HD_41928267-ad67-4c93	Dec 21, 2023 10:17 PM	2m 32s	Andy Kapoor	alphafold2-ic	hyperparameters : {"--sequence_id": "1ie3_mdh_c153"} mlflow.source.name : predict.py mlflow.source.type : JOB
quiet_pizza_lb8j9tj4	Completed	1.00000	"wt_mdh_r153"	HD_41928267-ad67-4c93	Dec 21, 2023 10:17 PM	12m 6s	Andy Kapoor	alphafold2-ic	hyperparameters : {"--sequence_id": "wt_mdh_r153"} mlflow.source.name : predict.py mlflow.source.type : JOB



AlphaFold Mutant Protein Overlayed With Incorrect PDB Reference Structure:

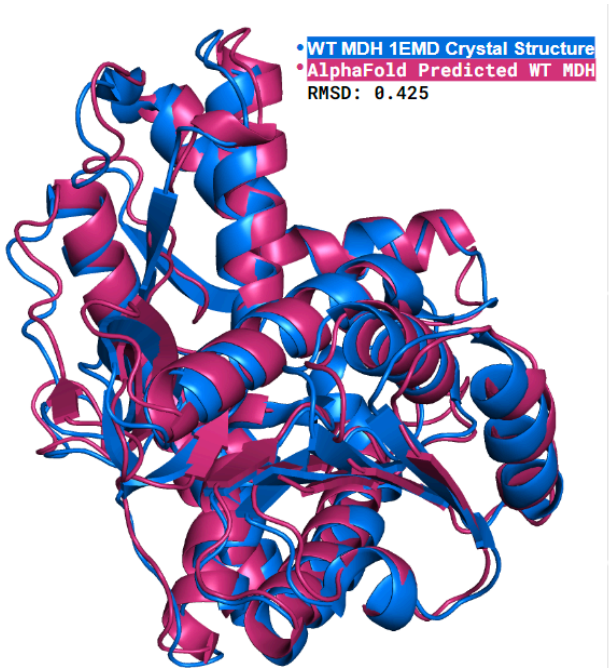


AlphaFold Wild Type Protein Overlayed With Incorrect PDB Reference Structure:



AlphaFold Wild Type Protein Overlayed With Correct PDB Reference Structure (1EMD):

- wt_mdh_r153_unrelaxed_rank_1_model_1.pdb from AlphaFold had an RMSD of 0.425 (1794 to 1794 atoms) in pyMOL Molecular Visualization using “orient” and “align 1emd, wt_mdh_r153_unrelaxed_rank_1_model_1” commands. Mutant (R153C) MDH from AlphaFold with filename mdh_c153_unrelaxed_rank_1_model_1.pdb had RMSD 0.443 (1810 to 1810 atoms) using “orient” and “align 1emd, mdh_c153_unrelaxed_rank_1_model_1” commands.



Conclusion: AlphaFold was successfully setup in the Microsoft Azure Virtual Machine with HyperDrive able to concurrently feed the FASTA sequences into the program through the Jupyter Notebook. Products of AlphaFold were overlaid in PyMol with the crystal reference structure 1EMD from the Protein Data Base. The Root Mean Squared Deviation scores indicate high accuracy of the program when the product's similarity is compared to the reference. Rosetta AbInitio was unable to be compiled in the Azure VM, prompting searching for an alternative to preserve the 'concurrent' modeling aspect to maintain high efficiency and throughput of the programs. Inability possible due to corrupted archive, filesystem limitations, permission issues, or version incompatibility. Integrity of the files and commands were confirmed with root user, but Rosetta could not be compiled in the Azure environment.

12/29/23

Rosetta AbInitio in Oracle VirtualBox Ubuntu Virtual Machine

Objective: The purpose of this section is to outline the alternative to setting up Rosetta in the Azure VM. Due to incompatibility between the program and the environment, Rosetta will be set up in a personal Ubuntu environment.

Procedures:

Downloading and Installing Ubuntu VM:

1. New system with sufficient computing power was built for Ubuntu Virtual Machine
    a. Ryzen 9 5900X
    b. Asus RTX 3060
    c. 32 GB DDR4 RAM
    d. 1 TB SSD Storage
    e. 1200 W Gold Rated PSU
2. Oracle VirtualBox downloaded
3. New Virtual Machine setup initiated
4. Destination folder automatically set
5. Ubuntu 24.04.4 Desktop ISO Image downloaded from Canonical Ubuntu
6. ISO selected in Oracle VirtualBox setup
7. CPU and Memory allocated

Compiling Rosetta AbInitio:

1. Register for account at University of Washington COMOTION
2. Download Rosetta Academic License and upload to the virtual machine
3. Compile:

```
sudo apt install zlibig-dev scons build-essential -y
tar -xvzf rosetta_src_<version>_bundle.tgz
cd {ROSETTA}/main/source
./scons.py -j<number_of_processors_to_use> mode=release bin
```

Running Rosetta AbInitio:

1. Parameters:
    a. Download Reference PDB Structure
    b. Retreive FASTA Sequence
    c. Register with old.robetta fragment server and submit FASTA queries to Fragment Libraries to receive fragment files
        i. Download 09_05.200_v1_3, 03_05.200_v1_3, and .psipred_ss2 files.
    d. Create Flag Files:
        i. Delineate locations of files, and inherent parameters of Rosetta

WT Flag File	Mutant Flag File
-database /home/modelingvm/Rosetta/main/database	-database /home/modelingvm/Rosetta/main/database
-in:file:native ./wt_1emd.pdb	-in:file:native ./mut_1emd.pdb
-in:file:fasta ./wt_rcsb_pdb_1EMD.fasta	-in:file:fasta ./mut_rcsb_pdb_1EMD.fasta
-in:file:frag3 ./wt_aat000_03_05.200_v1_3	-in:file:frag3 ./mut_aat000_03_05.200_v1_3
-in:file:frag9 ./wt_aat000_09_05.200_v1_3	-in:file:frag9 ./mut_aat000_09_05.200_v1_3
-psipred_ss2 ./wt_t000_.psipred_ss2	-psipred_ss2 ./mut_t000_.psipred_ss2
-nstruct 50	-nstruct 50
-abinitio:relax	-abinitio:relax
-use_filters true	-use_filters true
-abinitio::increase_cycles 10	-abinitio::increase_cycles 10
-abinitio::rg_reweight 0.5	-abinitio::rg_reweight 0.5
-abinitio::rsd_wt_helix 0.5	-abinitio::rsd_wt_helix 0.5
-abinitio::rsd_wt_loop 0.5	-abinitio::rsd_wt_loop 0.5
-relax::fast	-relax::fast
-out:file:silent ./50x_wt_fold_silent.out	-out:file:silent ./50x_mut_fold_silent.out
Formatting of flag files to produce 50 structures each of wt and mutant MDH. All data reported in silent file.	

2. Download and install GNU screens to separate modeling queries:

   sudo apt-get update
   sudo apt-get installs screen
   screen -S session_name
   screen -ls
   screen -r session_name

3. Run Rosetta AbInitio:

   modelingvm@modelingvm:~$ Rosetta/main/source/bin/AbinitioRelax.default.linuxgccrelease @mut_flags.txt
   modelingvm@modelingvm:~$ Rosetta/main/source/bin/AbinitioRelax.default.linuxgccrelease @wt_flags.txt

4. Extracting the pdbs (wt)

   grep SCORE 50x_wt_fold_silent.out | sort -nk 2 | awk {'print $32'} | more
   grep SCORE 50x_wt_fold_silent.out | sort -nk 2 | awk {'print $32'} > wt_temp
   cat wt_temp | awk '{print}' ORS=" " > ./wt_liststring
   xargs Rosetta/main/source/bin/extract_pdbs.default.linuxgccrelease -in::file::silent ./50x_wt_fold_silent.out -out:pdb
   -in:file:tags < wt_liststring
   grep SCORE 50x_wt_fold_silent.out | awk '{print $2 "\t" $32}' | sort -nk 1 > ./wt_score_against_pdbs.txt

5. Extracting the pdbs (mut)

```
grep SCORE 50x_mut_fold_silent.out | sort -nk 2 | awk {'print $32'} | more
grep SCORE 50x_mut_fold_silent.out | sort -nk 2 | awk {'print $32'} > mut_temp
cat mut_temp | awk '{print}' ORS=" " > ./mut_liststring
xargs Rosetta/main/source/bin/extract_pdbs.default.linuxgccrelease -in::file::silent ./50x_mut_fold_silent.out -out:pdb
-in:file:tags < mut_liststring
grep SCORE 50x_mut_fold_silent.out | awk '{print $2 "\t" $32}' | sort -nk 1 > ./mut_score_against_pdbs.txt
```

6. Make VALUES_LIST files for 50x mut and wt:

```
grep SCORE 50x_mut_fold_silent.out | awk '{print $2 "\t" $27}' | sort -nk 2 > 50x_mut_VALUELIST.txt
grep SCORE 50x_wt_fold_silent.out | awk '{print $2 "\t" $27}' | sort -nk 2 > 50x_wt_VALUELIST.txt
```

7. Overlay best Rosetta Outputs in PyMol

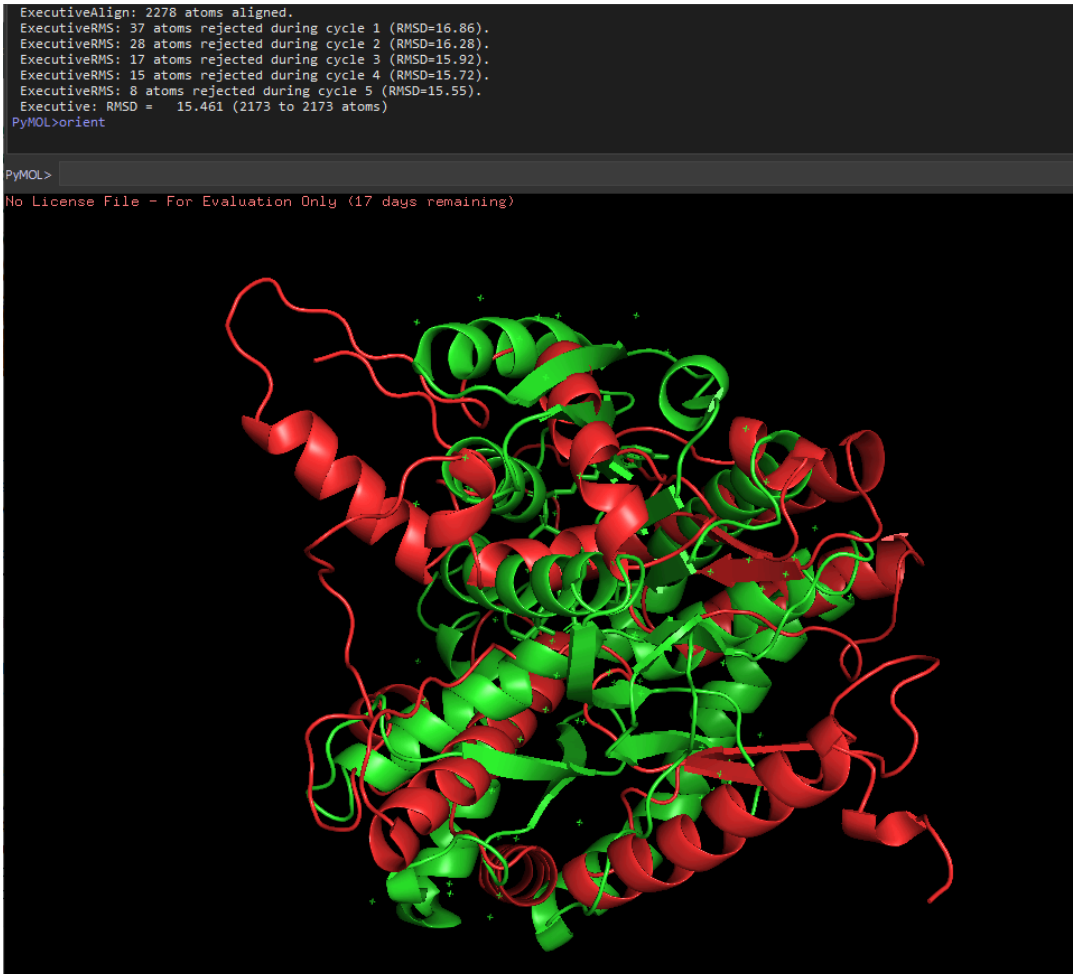
<u>Analysis of RMSD from 50x Rosetta Output</u>:

1. Given 50x_wt_VALUELIST.txt and 50x_mut_VALUELIST.txt, create stripchart in R to show distribution of the RMSD values for all 100 runs (50 each):

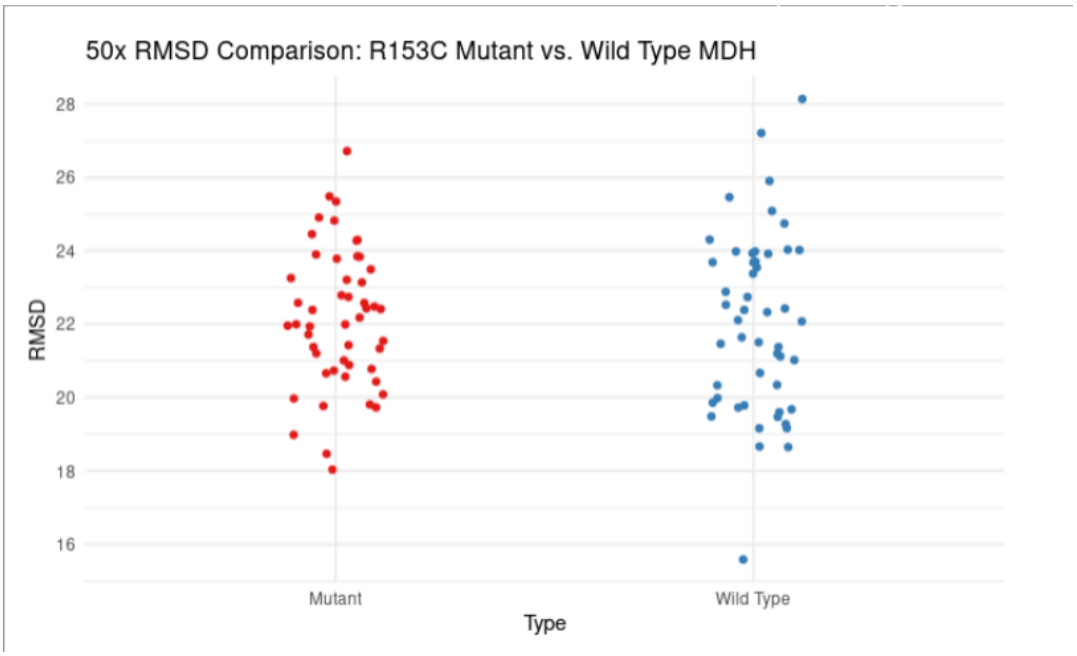
```r
MutValues$Type <- 'Mutant'
WTValues$Type <- 'Wild Type'
combinedData <- rbind(MutValues, WTValues)

ggplot(combinedData, aes(x = Type, y = rms, color = Type)) +
  geom_jitter(width = 0.12, height = 0.1) +
  labs(title = "50x RMSD Comparison: R153C Mutant vs. Wild Type MDH",
       x = "Type",
       y = "RMSD") +
  scale_color_brewer(palette = "Set1") +
  theme_minimal() +
  theme(legend.position = "none") +
  scale_y_continuous(breaks = seq(floor(min(combinedData$rms)-5), ceiling(max(combinedData$rms)+2),
by = 2))
```

Data:

Rosetta AbInitio 7x Trial Best Output Overlayed with Reference Structure (RMSD:16):



Rosetta AbInitio Strip Chart of 50x runs for wt and mutant MDH:



Conclusions: To keep the vital aspect of the project and increase the efficiency of computational modeling by concurrently modeling the proteins, Rosetta was set up in an Ubuntu VM with GNU screens installed. GNU screens allow allocation of one query to one core of the CPU, overcoming the limitation of Rosetta's inherent lack of multicore processing. The products of Rosetta were unusable in AutoDock analysis, yielding RMSD values well above the required threshold (0-2 for significant similarity).

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AutoDock Vina Enzyme-Substrate Binding

Objective: The goal of the AutoDock Binding simulations was to assess the binding of MDH with oxaloacetate before and after the simple missense R153C mutation. The two proteins from AlphaFold would be used as the protein PDB model paired with a downloaded oxaloacetate file from PubChem. Docking would occur with both models from AlphaFold since it produced the only viable models within the RMSD range necessary (RMSD 0-2).

Procedures:

Preparing the PDB Outputs from AlphaFold:

1. Import protein.pdb and Edit → delete water (ensures accurate affinity)
2. Edit → add hydrogens (all hydrogens, noBondOrder (fine since auto dock uses atom type, not bond order) renumber atoms: No
3. Edit → charges → Kollman charges (known was 2.0)
4. Grid → macromolecules → choose protein
5. Save it as the new file format

Preparing the ligand from PubChem:

1. Open ligand sdf in pyMOL
2. Export as pdb

Setup in AutoDock Tools:

1. Download and open AutoDock Tools/ MGLTools in Ubuntu VM or windows machine to use graphical user interface
2. Ligand → choose → ligand → message reports accurate setup of oxaloacetate
3. Ligand → output → as pdbqt file
4. Setting grid box:
    a. Right-click protein under All Molecules → show sequences → click residues of interest one after another (81,87,153) (now they're highlighted in yellow)
5. Adjust offset and dimensions to put the residues in the grid space (leaving extra space for rotations)
6. Import all pdb structures and export as .pdbqt format for docking analysis

Setup Config File:

1. Center and size will be x,y, and z centers from the grid box Grid Options Window. The size parameters are the number of points for x,y, and z from the Grid Options Window. Format:

```
receptor = protein.pdbqt
ligand = ligand.pdbqt

center_x =  2
center_y =  6
```

center_z = -7

size_x = 25
size_y = 25
size_z = 25

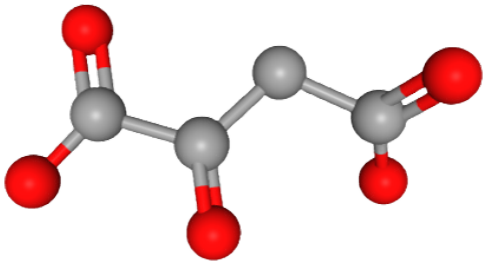
energy_range = 4

Running AutoDock In CommandLine:

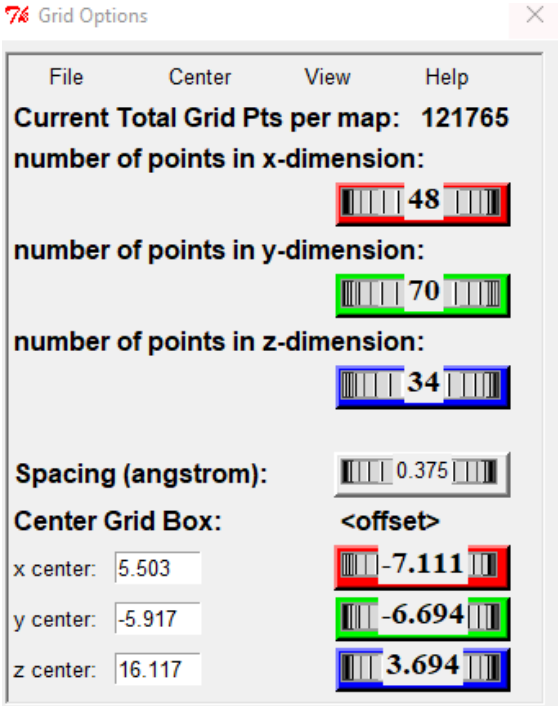
1. Locate files
    a. C:\Users\Andy\Downloads>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand ligand.pdbqt --config config.txt --log Vina_WT_Log.txt --out Vina_WT_Output.pdbqt

Data:

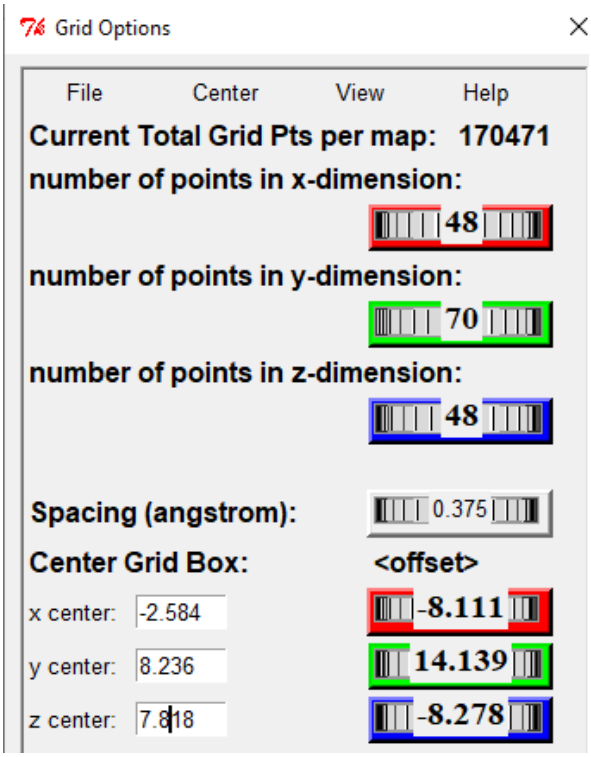
Oxaloacetate structure downloaded from PubChem in sdf format:



Wild Type grid box grid options window parameters:



Mutant grid box grid options window parameters:



Commandline results of AutoDock binding:

```
C:\Users\Andy\Downloads>"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand ligand.pdbqt
--config config.txt --log Vina_WT_Log.txt --out Vina_WT_Output.pdbqt
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 16 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1236987120
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -4.4      0.000      0.000
   2         -4.1      0.538      2.733
   3         -4.1      1.524      2.398
   4         -4.1      1.243      2.986
   5         -4.0     26.827     27.144
   6         -3.8     30.842     31.557
   7         -3.8     12.359     13.196
   8         -3.8      1.264      2.760
   9         -3.8     12.774     13.865
Writing output ... done.

C:\Users\Andy\Downloads>
```

Reformatted results of AutoDock binding for wt and mutant MDH:

Wild Type Docking Results				**Mutant Docking Results**			
Mode	Affinity	RMSD Lower Bound	RMSD Upper Bound	Mode	Affinity	RMSD Lower Bound	RMSD Upper Bound
1	-4-4	0.000	0.000	**1**	-5.8	0.000	0.000
2	-4.1	0.538	2.733	**2**	-5.1	24.884	25.615
3	-4.1	1.524	2.398	**3**	-5.0	35.368	36.027
4	-4.1	1.243	2.986	**4**	-5.0	24.334	24.923
5	-4.0	26.827	27.144	**5**	-4.9	20.821	21.230
6	-3.8	30.842	31.557	**6**	-4.9	30.581	31.115
7	-3.8	12.359	13.196	**7**	-4.8	30.528	31.200
8	-3.8	1.264	2.760	**8**	-4.8	24.854	25.587
9	-3.8	12.774	13.865	**9**	-4.8	20.865	21.455

Conclusions: The use of MGLTools and PyMol to reformat and input the objects into AutoDock Vina for binding analysis was largely successful, only encountering few errors with file naming of the mutant instance. Grid box parameters were set around the Arginine residues involved in substrate coordination, as they were the residues of interest in the study, not the full active site. Enough space was provided around the residues to let AutoDock attempt binding in many conformations, as would be expected in the protein. The deviation in RMSD of the mutant results indicate a large amount of energetically unfavorable conformations between MDH and the substrate, supported by laboratory data reporting more flexible binding and incorrect binding between MDH and many substrates. Mutant affinity values show oxaloacetate binding tighter to MDH, unexpected, but explainable as MDH may potentially be binding so tight it inhibits proper enzymatic activity/ enzymatic chemistry.

References

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