Rosetta Basics:
IO

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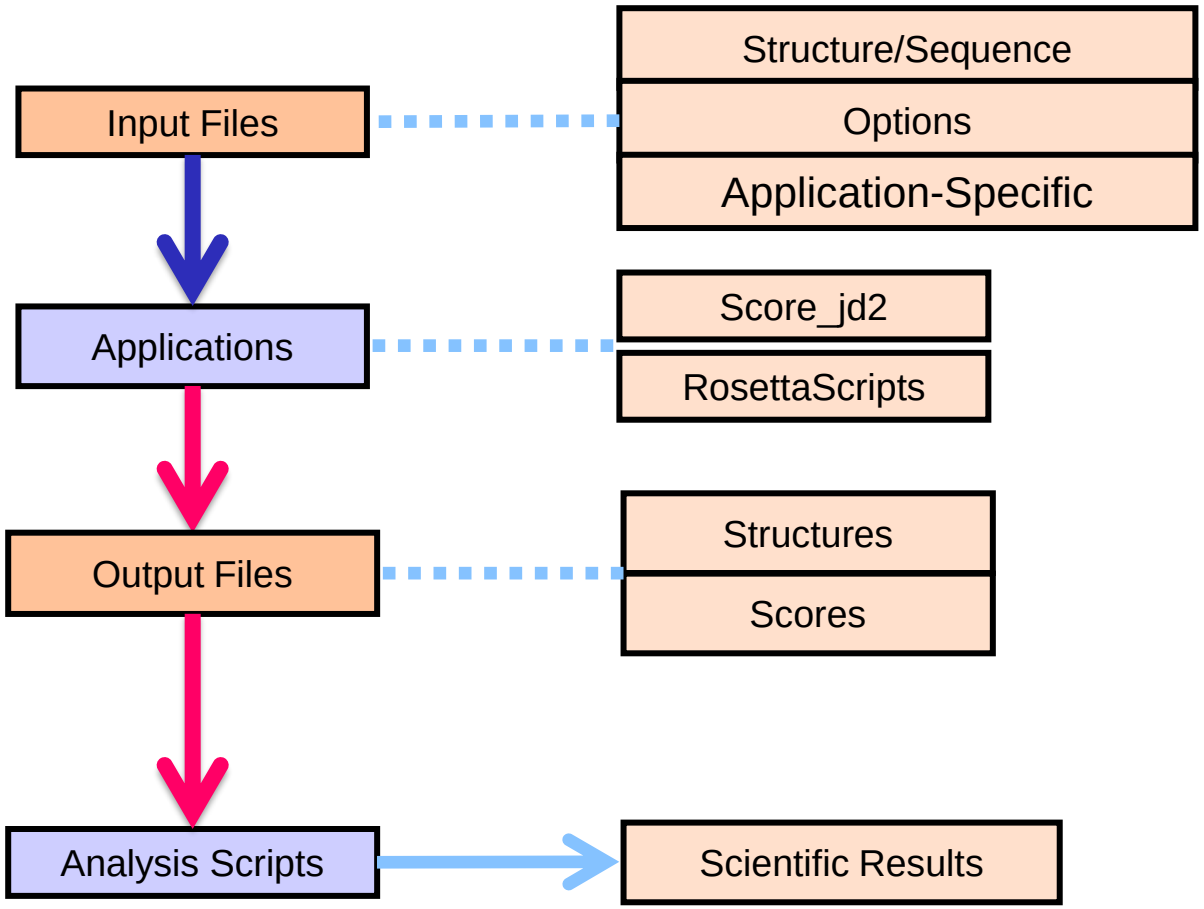
Goals for this Talk

1. General Rosetta Concepts:
   - How do I run basic Rosetta applications?
   - Input/Output: file types, options, etc.

2. Learn where things are in Rosetta
   - Your working directory is independent of these Rosetta directories (AKA your data is stored outside of Rosetta)

QUESTIONS ARE ENCOURAGED!

General Workflow



How do I run a Rosetta command?

Every command has the same basic layout:

Path to the Rosetta application

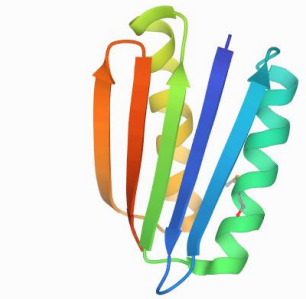
Arguments/flags/options

```
<path_to_rosetta>/main/source/bin/<app_name>.default.linuxgccrelease  –arg1 –arg2
```

Arguments consist of multiple things:

1. Point to input files
2. Point to where you want output files to go
3. Other arguments are protocol-dependent

Your first Rosetta command:



- Scoring 1qys (https://www.rcsb.org/structure/1QYS)
- Crystal structure of Top7: A computationally designed protein with a novel fold

<path_to_rosetta>/main/source/bin/score_jd2.default.linuxgccrelease –in:file:s 1qys.pdb -out:pdb > 1qys_score.log

- Inputs:
  - Running score_jd2 application, which simply scores in the input protein
  - -in:file:s 1qys.pdb : tells Rosetta we're inputting the 1qys.pdb PDB file
  - -out:pdb argument tells Rosetta that we want to save the output PDB file of the scored protein
  - > 1qys_score.log : print terminal output to file called 1qys_score.log
- Outputs:
  - 1qys_0001.pdb : output PDB
  - score.sc : default name for output scorefile
  - 1qys_score.log: tracer of run AKA what is output to the terminal screen when running command

Kuhlman B, Dantas G, Ireton GC, Varani G, Stoddard BL, Baker D. Design of a novel globular protein fold with atomic-level accuracy. *Science*. 2003;302(5649):1364-1368. doi:10.1126/science.1089427

Reading structures into Rosetta

PDB files

- International standard
- Readable by PyMol, MOE, Chimera, etc
- One line per atom
- Useful for small number of structures

www.wwpdb.org/documentation/file-format

Silent files

- Specific to Rosetta
- Compact
- One line per residue
- Useful for archiving many structures
- Binary silent files: more compact, but not human-readable

https://www.rosettacommons.org/docs/latest/rosetta_basics/file_types/silent-file

Common command line arguments

- Common input options:
  - -in:file:s example.pdb ## input a PDB structure file
  - -parser:protocol example.xml ## RosettaScripts XML file
  - -in:file:fasta example.fasta ## input a FASTA sequence file
  - -in:file:silent example.silent ## input a Rosetta silent file
  - -nstruct 42 ## produce 42 outputs
- Common output options:
  - -out:file:silent example_out.silent ## output structures to silent file
  - -out:file:scorefile example_out.sc ## output scorefile for run

Examples of output: the scorefile

score.sc

- One output per line—name of output is in the very last column
- Each column defines a specific score term for the respective output structure
- Second column is the “total_score”
- The following columns are individual scoreterms (described in detail in later talk)
- Excerpt of example scorefile here, but recommend you look at your own score.sc output file

```
SEQUENCE:
SCORE:     score     fa_atr     fa_rep     fa_sol    fa_intra_rep    fa_elec    ...     omega     fa_dun    p_aa_pp        ref                    description
SCORE:  -1217.209  -2778.696    266.309   1545.149           5.900   -301.320    ...    63.032    684.989   -109.110    -32.534      3gbm_HA_3gbn_Ab_full_0011
SCORE:  -1217.028  -2792.422    263.906   1549.738           5.867   -295.799    ...    66.036    682.694   -108.402    -32.534      3gbm_HA_3gbn_Ab_full_0012
SCORE:  -1204.280  -2760.354    259.175   1534.072           5.913   -293.050    ...    65.391    674.840   -108.393    -32.534      3gbm_HA_3gbn_Ab_full_0013
SCORE:  -1207.127  -2768.191    260.443   1541.857           5.881   -301.847    ...    67.951    686.381   -110.919    -32.534      3gbm_HA_3gbn_Ab_full_0014
SCORE:  -1208.390  -2769.872    262.398   1539.668           5.879   -297.571    ...    64.073    681.731   -109.633    -32.534      3gbm_HA_3gbn_Ab_full_0015
```

Examples of output: the output PDB

1qys_0001.pdb

- One atom/line just like normal PDBs

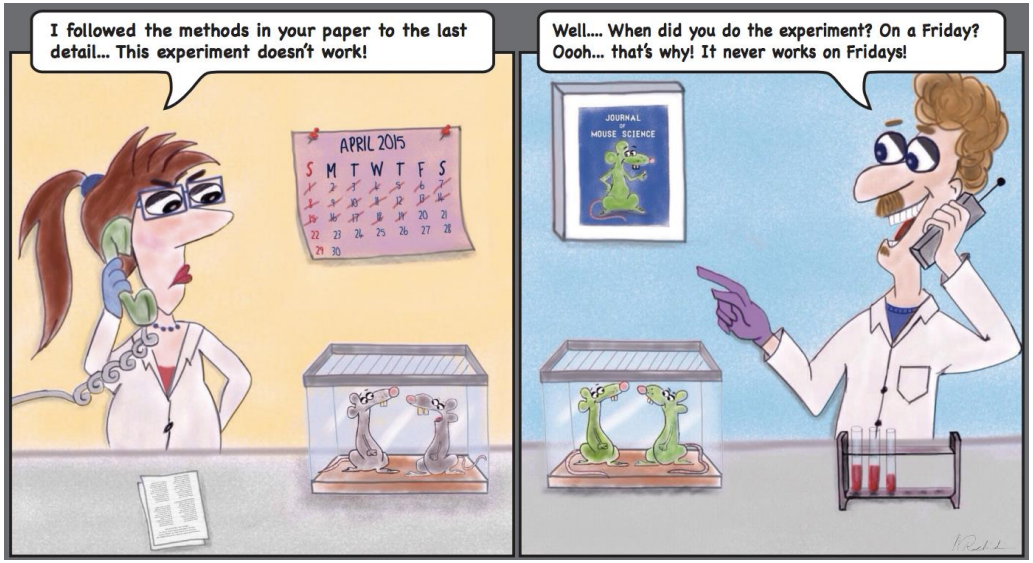
- Scroll to the bottom and there is per residue score information

```
ATOM   3378  HB  THR L 227     -36.166  22.580  28.848  1.00  0.00           H
ATOM   3379  HG1 THR L 227     -34.994  19.987  29.136  1.00  0.00           H
ATOM   3380 1HG2 THR L 227     -34.138  22.579  30.246  1.00  0.00           H
ATOM   3381 2HG2 THR L 227     -35.593  22.831  31.238  1.00  0.00           H
ATOM   3382 3HG2 THR L 227     -34.799  21.240  31.213  1.00  0.00           H
TER
# All scores below are weighted scores, not raw scores.
#BEGIN_POSE_ENERGIES_TABLE 3gbn_Ab_0005.pdb
label fa_atr fa_rep fa_sol fa_intra_rep fa_elec pro_close hbond_sr_bb hbond_lr_bb hbond_bb_sc hbond_sc dslf_fa13 rama omega fa_dun p_aa_pp yhh_planarity ref total
weights 1 0.55 0.9375 0.005 0.875 1.25 1.17 1.17 1.17 1.1 1.25 0.25 0.625 0.7 0.4 0.625 1 NA
pose -994.338 137.719 561.027 2.15688 -112.612 19.7634 -12.2069 -79.5391 -24.1449 -23.4441 -1.15166 -7.47192 71.8572 276.633 -29.8673 0.09431 13.9828 -201.541
GLU:NtermProteinFull_1 -1.58225 0.53996 1.45283 0.00353 0.06909 0 0 0 0 0 0 0 0.01109 6.53174 0 0 -1.96094 5.06505
VAL_2 -3.34255 0.45648 1.46378 0.01322 -0.08167 0 0 0 0 0 0 -0.16095 0.87346 0.30715 0.39992 0 0.97964 0.90848
GLN_3 -2.79445 0.10936 1.74929 0.00451 -0.52743 0 0 0 -0.35772 0 0 -0.09682 0.35321 2.59775 0.02034 0 -1.51717 -0.45911
LEU_4 -5.13483 0.73792 1.6574 0.00685 -0.16379 0 0 0 0 0 0 0.06265 0.2281 2.29891 -0.1217 0 0.76113 0.33264
VAL_5 -2.72905 0.12167 1.72074 0.00789 -0.45069 0 0 0 0 0 0 -0.27382 0.01969 0.02557 -0.49649 0 0.97964 -1.07485
```

Examples of output: Tracer output (log files)

1qys_score.log

- Shows exactly the command line you are running at the beginning
- Which databases are being used, calling protocols, warnings, errors, etc.
- Very useful for debugging to figure out where problems are coming from
- Makes your protocol reproducible!!



Examples of output: Tracer output (log files)

1qys_score.log

```
core.init: Rosetta version exported  from http://www.rosettacommons.org
core.init: command: /dors/meilerlab/apps/rosetta/rosetta_2016.08.58479/main/source/bin/rosetta_scripts.default.linuxgccrelease @docking.options -parser:protocol docking
core.init: 'RNG device' seed mode, using '/dev/urandom', seed=1059677151 seed_offset=0 real_seed=1059677151
core.init.random: RandomGenerator:init: Normal mode, seed=1059677151 RG_type=mt19937
core.init: Resolved executable path: /dors/meilerlab/apps/rosetta/rosetta_2016.08.58479/main/source/build/src/release/linux/2.6/64/x86/gcc/5.2/default/rosetta_scripts.d
core.init: Looking for database based on location of executable: /dors/meilerlab/apps/rosetta/rosetta_2016.08.58479/main/database/
protocols.jd2.PDBJobInputter: Instantiate PDBJobInputter
protocols.jd2.PDBJobInputter: PDBJobInputter::fill_jobs
protocols.jd2.PDBJobInputter: pushed 3gbm_HA_3gbn_Ab.pdb nstruct indices 1 - 50
protocols.evaluation.ChiWellRmsdEvaluatorCreator: Evaluation Creator active ...
protocols.jd2.JobDistributor: Parser is present.  Input mover will be overwritten with whatever the parser creates.
protocols.jd2.PDBJobInputter: PDBJobInputter::pose_from_job
protocols.jd2.PDBJobInputter: filling pose from PDB 3gbm_HA_3gbn_Ab.pdb
core.chemical.ResidueTypeSet: Finished initializing fa_standard residue type set.  Created 384 residue types
core.chemical.ResidueTypeSet: Total time to initialize 0.43 seconds.
core.conformation.Conformation: Found disulfide between residues 7 461
...
protocols.rosetta_scripts.ParsedProtocol.REPORT: ============End report for ==================
protocols.rosetta_scripts.ParsedProtocol.REPORT: ============Begin report for ==================
protocols.rosetta_scripts.ParsedProtocol.REPORT: ============End report for ==================
protocols.jd2.JobDistributor: 3gbm_HA_3gbn_Ab_full_0050 reported success in 381 seconds
protocols.jd2.JobDistributor: no more batches to process...
protocols.jd2.JobDistributor: 50 jobs considered, 50 jobs attempted in 16297 seconds
~
~
~
```

Options to control tracer output *these files can get very long!*:

- Silence certain tracers:

  -mute core.chemical.ResidueTypeSet

- Change verbosity level (Error/Warning/Info/Debug/Trace)

  -out:levels all:Warning core.init:Info

Other files: application-specific

- Span File: Defines which residues are in the membrane
- Loops File: Identifies the loop residues for loop closure
- Params File: Custom parameters for small molecules or non-canonical amino acids
- Constraint File: Experimentally derived restraints
- Fragment File: Short protein segments used for comparative modeling and de novo folding
- Res Files: Indicates which residue positions should be designed

Protocols can get complicated…

Toward high-resolution prediction and design of transmembrane helical protein structures

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```
$ROSETTA/main/source/bin/AbinitioRelax.linuxgccrelease -database
../../rosetta_database -in:file:fasta ./input_files/1elwA.fasta -
in:file:native ./input_files/1elw.pdb -in:file:frag3
./input_files/aa1elwA03_05.200_v1_3 -in:file:frag9
./input_files/aa1elwA09_05.200_v1_3 -abinitio:relax -relax:fast -
abinitio::increase_cycles 10 -abinitio::rg_reweight 0.5 -
abinitio::rsd_wt_helix 0.5 -abinitio::rsd_wt_loop 0.5 -use_filters
true -psipred_ss2 ./input_files/1elwA.psipred_ss2 -kill_hairpins -
out:file:silent 1elwA_silent.out
-nstruct 10
```

OR

$ROSETTA/main/source/bin/AbinitioRelax.linuxgccrelease @options.txt

Use an options file for your runs

Why?

- Easier to read/organize
- Reproducibility!

$ROSETTA/main/source/bin/AbinitioRelax.linuxgccrelease @options.txt

```
-in:file
      -fasta ./input_files/1e1wA.fasta
      -native ./input_files/1e1w.pdb
      -frag3 ./input_files/aa1elwA03_05.200_v1_3
      -frag9 ./input_files/aa1elwA09_05.200_v1_3
-psipred_ss2 ./input_files/1elwA.psipred_ss2
-abinitio:relax
-relax:fast
-abinitio::increase_cycles 10
-abinitio::rg_reweight 0.5
…
-out:file:silent ./output_files/1elwA_silent.out
-nstruct 10
```

Rosetta Resources for Users:

https://www.rosettacommons.org

- Documentation
- User guides
- Forum
- Software Download
- Tutorials (meilerlab.org)

Any Questions?