De novo / Ab Initio Protein Folding in Rosetta

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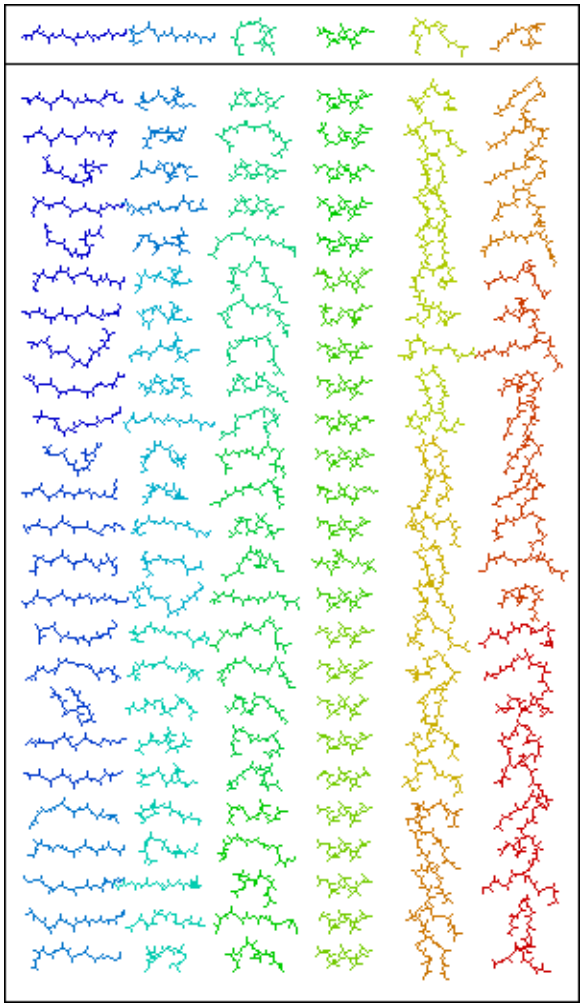
What is *de novo* protein folding?

- A technique to determine tertiary structure *from a primary sequence* using secondary structure prediction and peptide fragments selected from the PDB
- Differs from homology modeling, which starts with a template structure

What are fragments?



- 3 and 9 amino acid peptides generated from the PDB
- Fragments change the geometry of the protein
- Scoring functions identify and maintain good fragments

Figure courtesy of Jens Meiler

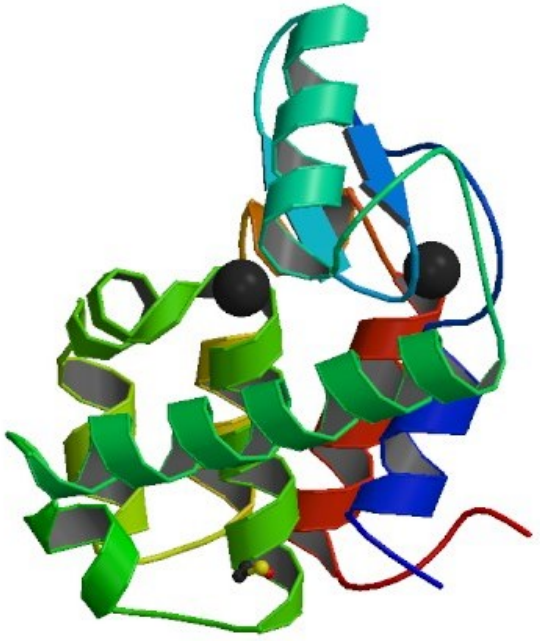
What is *de novo* protein folding?

MNIFEMLRIDEGLRLKI
YKDTEGYYTIGIGHLLT
KSPSLNASKSELDKAIG
RNTNGVITKDEAEKLFN
QDVDAAVRGILRNAKLK
PVYDSLDAVRRAALINM
VFQMGETGVAGFTNSLR
MLQQKRWDEAAVNLAKS
RWYNQTPNRAKRVITTF
RTGTWDAYKNL

Primary Sequence

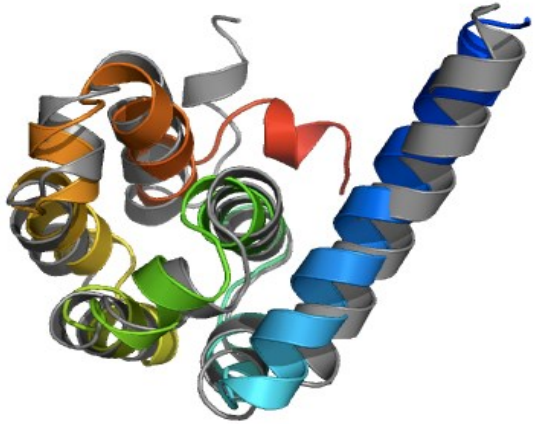
Secondary Structure Prediction, PDB Fragments, Rosetta Scoring Functions

***de novo* folding**



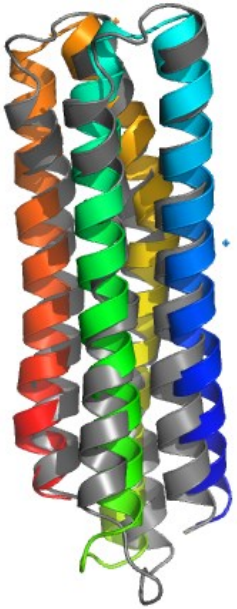
Tertiary Structure

What can Rosetta actually fold?



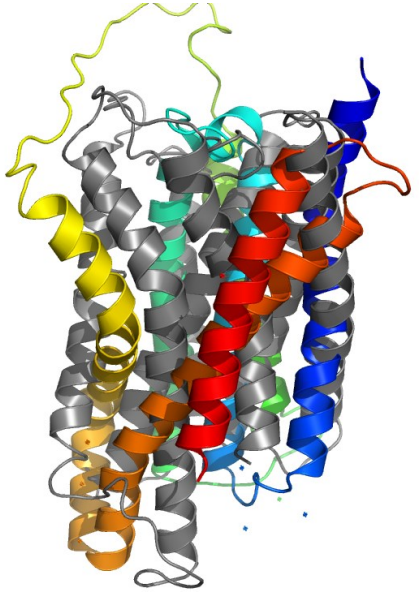
Small, globular, soluble proteins

T4-lysozyme C-terminal domain



Small, simple membrane proteins

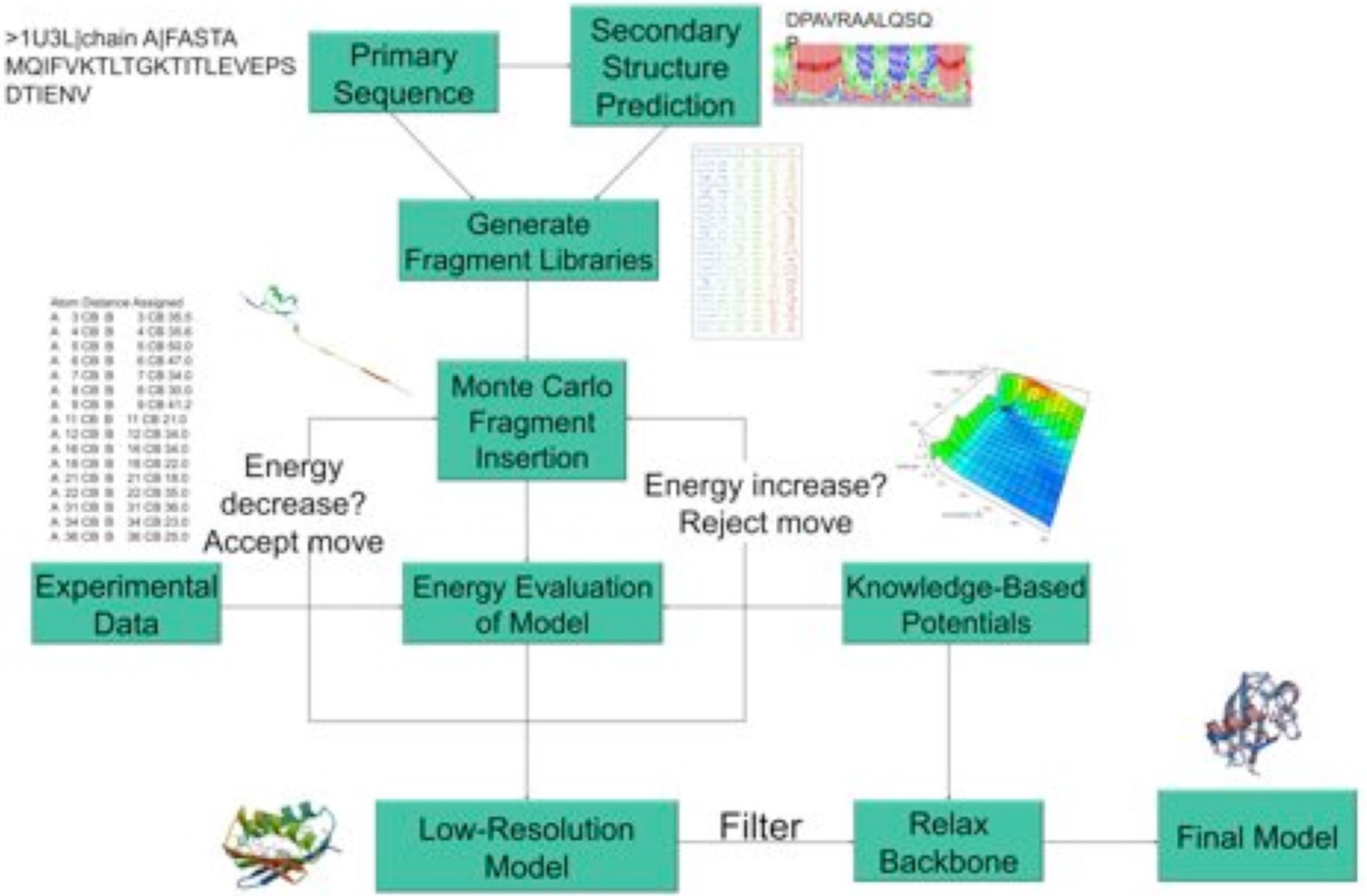
V-type Na^+ ATP synthase subunit



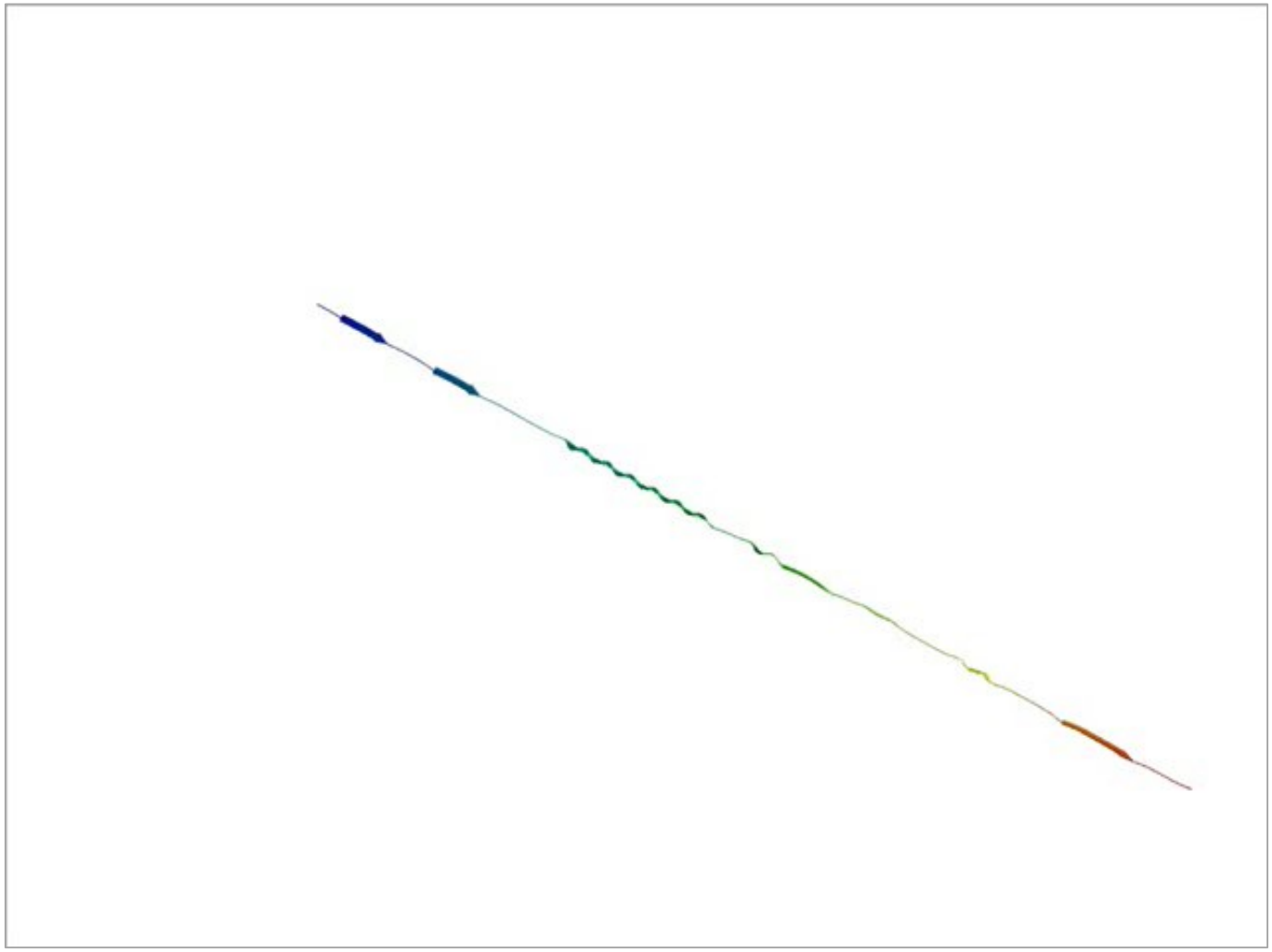
…but not large, complex proteins

Rhodopsin

Rosetta *de novo* folding protocol



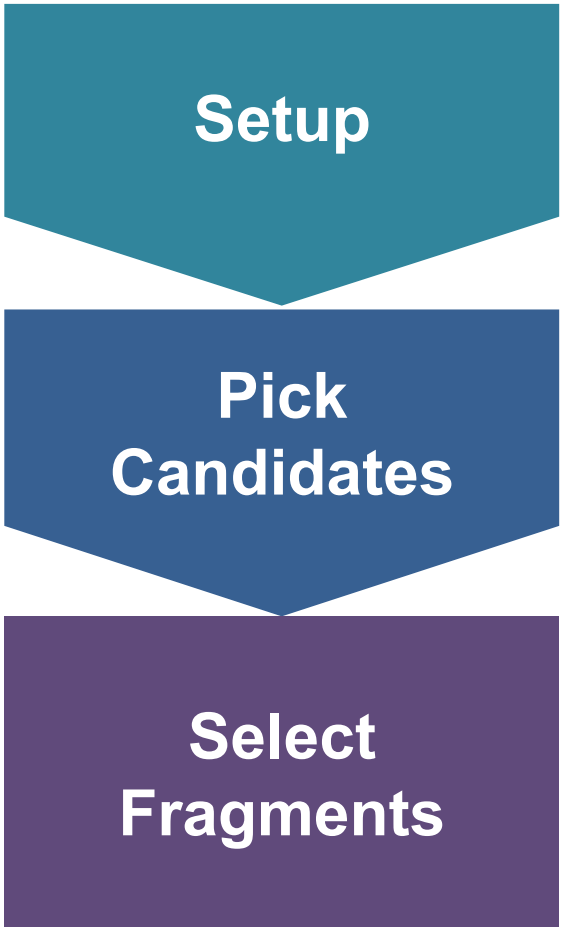
Running a folding job: ubiquitin



Necessary input files for the *de novo* folding protocol

- FASTA file of your protein sequence
- Secondary structure prediction files
- **Fragment library files**
- **Options file**
- Topology broker setup file
- *(Optional) PDB file of native structure*
- ***(Optional) Constraints file***

Fragment file generation



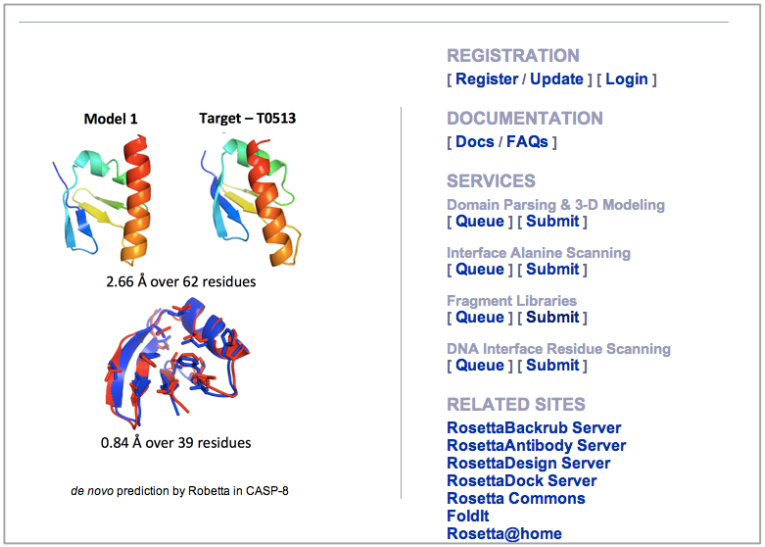
- Vall database
- Primary sequence
- Secondary structure prediction
- NMR data (if applicable)

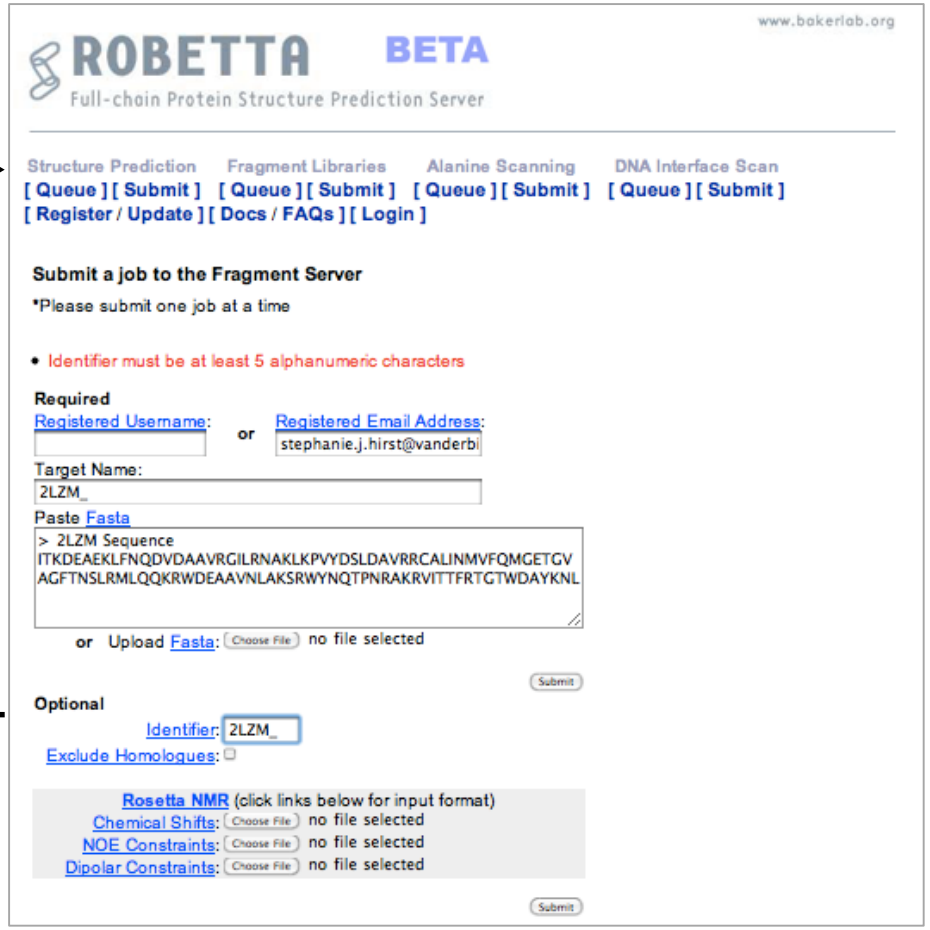
- Gather all possible fragments
- Score candidates based on input

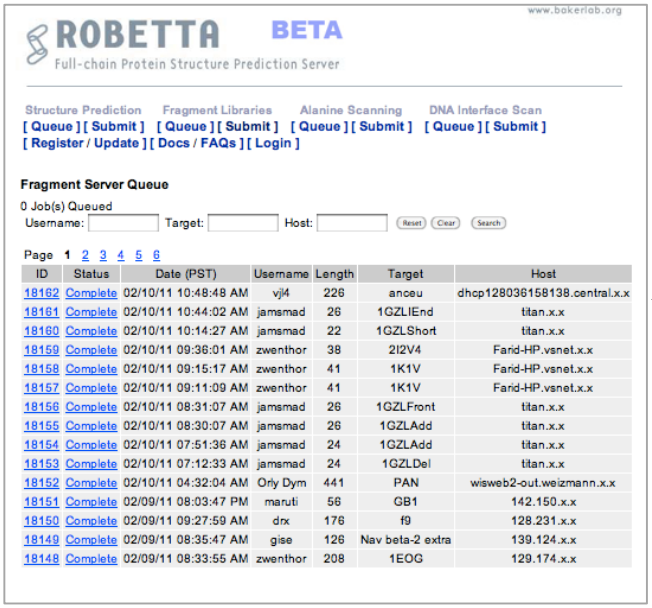
- Keep the best N fragments
- Default = 200 per sequence
- Write to fragment files

Making fragments with Robetta

http://robetta.bakerlab.org/







ID	Status	Date (PST)	Username	Length	Target	Host
18162	Complete	02/10/11 10:48:48 AM	vjl4	226	anceu	dhcp128036158138.central.x.x
18161	Complete	02/10/11 10:44:02 AM	jamsmad	26	1GZLIEnd	titan.x.x
18160	Complete	02/10/11 10:14:27 AM	jamsmad	22	1GZLShort	titan.x.x
18159	Complete	02/10/11 09:36:01 AM	zwenthor	38	2I2V4	Farid-HP.vsnet.x.x
18158	Complete	02/10/11 09:15:17 AM	zwenthor	41	1K1V	Farid-HP.vsnet.x.x
18157	Complete	02/10/11 09:11:09 AM	zwenthor	41	1K1V	Farid-HP.vsnet.x.x
18156	Complete	02/10/11 08:31:07 AM	jamsmad	26	1GZLFront	titan.x.x
18155	Complete	02/10/11 08:30:07 AM	jamsmad	26	1GZLAdd	titan.x.x
18154	Complete	02/10/11 07:51:36 AM	jamsmad	24	1GZLAdd	titan.x.x
18153	Complete	02/10/11 07:12:33 AM	jamsmad	24	1GZLDel	titan.x.x
18152	Complete	02/10/11 04:32:04 AM	Orly Dym	441	PAN	wisweb2-out.weizmann.x.x
18151	Complete	02/09/11 08:03:47 PM	maruti	56	GB1	142.150.x.x
18150	Complete	02/09/11 09:27:59 AM	drx	176	f9	128.231.x.x
18149	Complete	02/09/11 08:35:47 AM	gise	126	Nav beta-2 extra	139.124.x.x
18148	Complete	02/09/11 08:33:55 AM	zwenthor	208	1EOG	129.174.x.x

Making fragments with make_fragments.pl

- If you are an industry user, you will not have access to Robetta
- You can use the make_fragments.pl script, which is discussed in the tutorial
- This script will need to be modified to work with your own environment

Setting up options for folding

```
-in
    -file
        -native <native PDB file>                                  # native PDB file (optional)
        -fasta <primary sequence in FASTA format>      # or command line: -in:file:fasta
        -frag3 <3mer fragment file>                                # protein 3-residue fragments file
        -frag9 <9mer fragment file>                                # protein 9-residue fragments file
-psipred_ss2 <PSIPRED secondary structure prediction file>   # psipred_ss2 secondary structure
                                                                     definition file (required for -use_filters)
-broker
    -setup <topology broker file>                                  # topology broker file
```

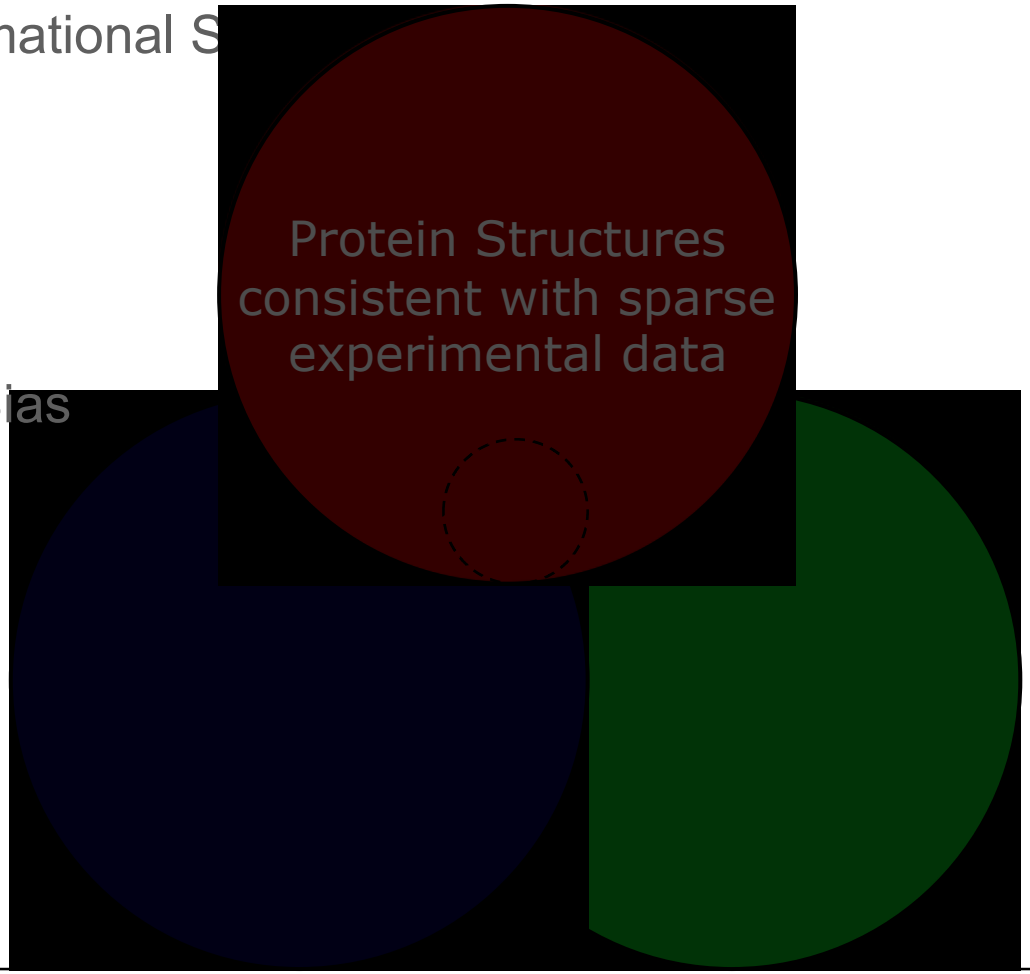
```
-abinitio
    -increase_cycles 10     # Increase the number of cycles at each stage in AbinitioRelax by this factor
    -rg_reweight 0.5        # Reweight contribution of radius of gyration to total score by this factor
    -rsd_wt_helix 0.5       # Reweight env, pair, and cb scores for helix residues by this factor
    -rsd_wt_loop 0.5        # Reweight env, pair, and cb scores for loop residues by this factor
    -relax                  # At the end of de novo folding, do a relax step
    -use_filters true       # Use radius of gyration (RG), contact-order, and sheet filters. This option
conserves computing by not continuing with refinement if a filter fails. A caveat is that for some
sequences, a large percentage of models may fail a filter. The filters are meant to identify models with
non-protein like features
-relax
    -fast           # Type of relax protocol.  This has been shown to be the best deal for speed and
                      robustness.
```

Setting up options for folding cont.

```
-run
      -reinitialize_mover_for_each_job  # Job distributor generates fresh copy of its mover
                                          before each apply (once per job)
-protocol broker                        # run the topology_broker protocol for de novo folding
-score
      -find_neighbors_3dgrid            # Use a 3D lookup table for doing neighbor calculations. For
                                          spherical, well-distributed conformations
-evaluation
      -rmsd <file to compute RMSD against> <column name> <file defining residues over which to
compute RMSD>                           # compute CA-RMSD for model comparing to native structure
-out
      -output                           # use this to tell Rosetta you actually want output
      -nstruct 1                        # how many structures do you want to generate?
      -file
            -silent <silent output file>  # full path to silent file output
            -silent_struct_type binary    # we want binary silent files
            -scorefile <scorefile>        # full path to scorefile
-overwrite                              # overwrite any existing output with the same name you may
                                          have generated
```

Why combine experimental restraints with Rosetta?

- Complete Conformational S
- Local Sequence Bias



- Energy Evaluation of non-Local Interactions

Figure from Jens Meiler

Adding experimental restraints

```
-fold_cst                    # use FoldConstraints protocol
        -force_minimize      # minimize in FoldConstraints protocol
-constraints
        -cst_file <file>     # path to your cst file
        -cst_weight 4        # factor by which to multiply cst score
        -cst_fa_file <file> # path to your cst file
        -cst_fa_weight 4     # factor by which cst score multiplied by
        -epr_distance        # Use RosettaEPR knowledge-based potential
```

Constraint info					Constraint Function info				
<cst type>	<atom1>	<res1>	<atom2>	<res2>	<cst_func>	<RosettaEPR>	<Dcb>	<weight>	<bin>
AtomPair	CB	32	CB	36	SPLINE	EPR_DISTANCE	16.0	1.0	0.5
AtomPair	CB	59	CB	74	SPLINE	EPR_DISTANCE	19.0	1.0	0.5
AtomPair	CB	62	CB	71	SPLINE	EPR_DISTANCE	19.0	1.0	0.5

Formatting the constraints file

- There are *constraint* types and *function* types
  - **Constraint types:** AtomPair, Angle, Dihedral, etc.
  - **Function types:** Bounded, Spline, Harmonic, Gaussian, etc.
- Each constraint you define is scored individually, and the total constraint score is the sum of all individual scores
- Each constraint can have its own constraint type and function type.
  - In some cases, like when using Spline function, each constraint can have its own weight

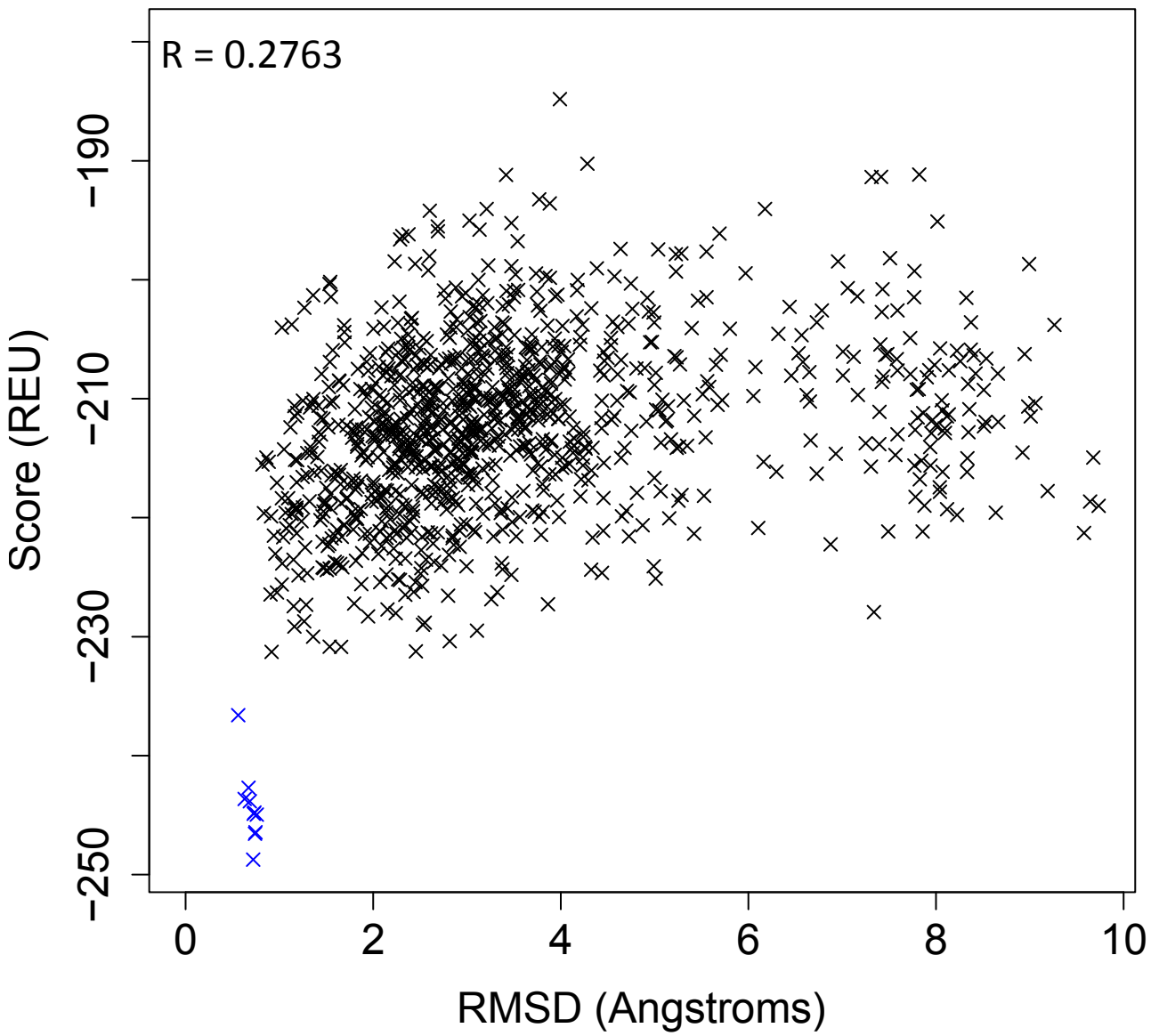
Initiating *de novo* folding

```
minirosetta.default.linuxgccrelease
@2LZM_broker.options -database
path_to/Rosetta/database/
```

- Minirosetta calls many protocols, including abinitio

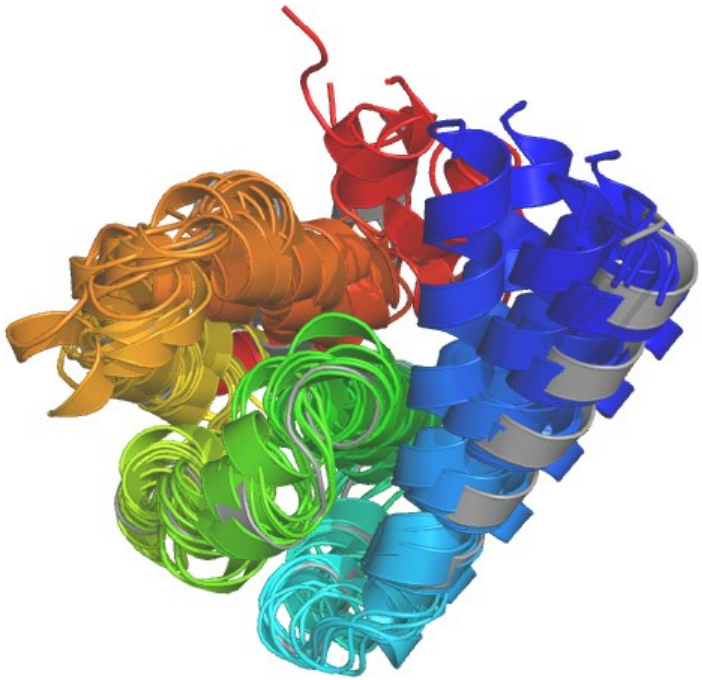
Assessing model quality: score vs. RMSD

- Determine how well Rosetta energy correlates with model quality (RMSD, MaxSub, etc.)
- However this requires a native structure (e.g., crystal structure) or a homolog to which you'd like to compare the structure

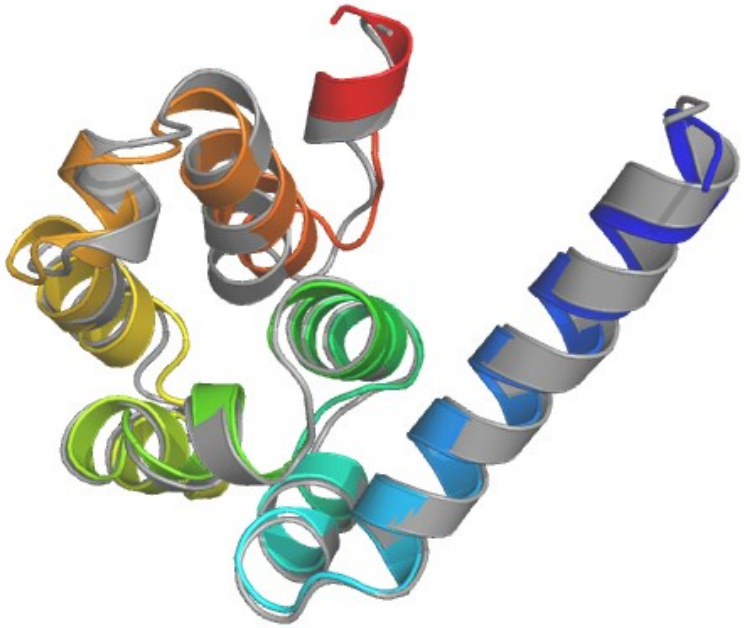

2LZM_score_vs_rmsd_to_native
R = 0.2763
Score (REU)
−190
−210
−230
−250
0
2
4
6
8
10
RMSD (Angstroms)

Assessing model quality: PyMol

Run: `pymol *.pdb`



Top 10 Scoring



Best Scoring

Assessing model quality: restraints

- Filter by constraint score to look at models that satisfy experimental data
- Plot constraint score vs. RMSD, total score vs. constraint score, etc. to identify correlation of constraint score with total energy of model
- Identify constraint violations in your model, how big the violations are, etc.

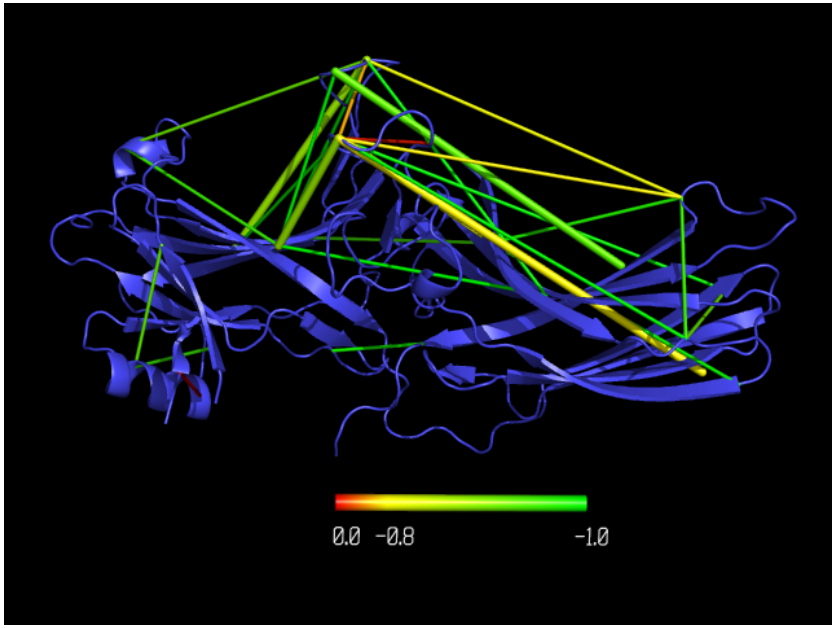


Figure courtesy of Nathan Alexander

Conclusions

- Rosetta *de novo* folding performs best with small proteins (80-100 residues)
- Folding larger, more complex proteins requires more information
- Experimentally derived knowledge can be provided in the form of restraints to improve model quality

Additional resources

- **Rosetta User's Guide:**
  - http://www.rosettacommons.org/docs/latest
- **Protein Structure Prediction Using Rosetta**
  - Rohl, Strauss, Misura and Baker, *Methods Enzymol.,* 2004
- **Using restraints in Rosetta**
  - Rohl, *Methods Enzymol*., 2005
  - Raman *et al*., *Science*, 2010
  - Hirst *et al*., *J. Struct. Biol.*, 2011.
- **Constraint File Instructions**
  - https://www.rosettacommons.org/docs/latest/rosetta_basics/file_types/constraint-file

Today's Tutorial

- Bacteriophage T4 lysozyme
  - Small, soluble, globular protein
  - PDBID: 2LZM
- Tutorial 1 is a *de novo* folding benchmark experiment
  - *Reminder: The Robetta queue will be long, copy the fragment files from the input_files directory*
- Tutorial 2 adds experimental restraints