

De novo Protein folding

Rosetta Workshop

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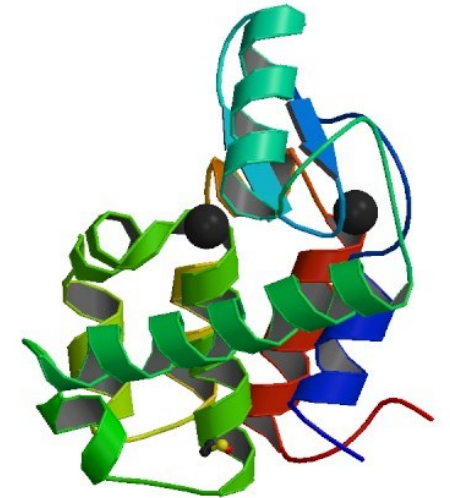
<http://www.meilerlab.org>

De novo Protein folding

- Determination of protein structure from sequence data alone
- No template structures, unlike homology modelling
- Useful for small, simple proteins (<200 residues)

MNIFEMLRIDEGLRLKIYK
DTEGYTIGIGHLLTKSPS
LNASKSELDKAIGRNTNGV
ITKDEAEKLFNQDVDAVR
GILRNAKLKPVYDSLDAVR
RAALINMVFQMGETGVAGF
TNSLRMLQQKRWDEAAVNL
AKSRWYNQTPNRAKRVITT
FRTGTWDAYKNL

Primary Sequence



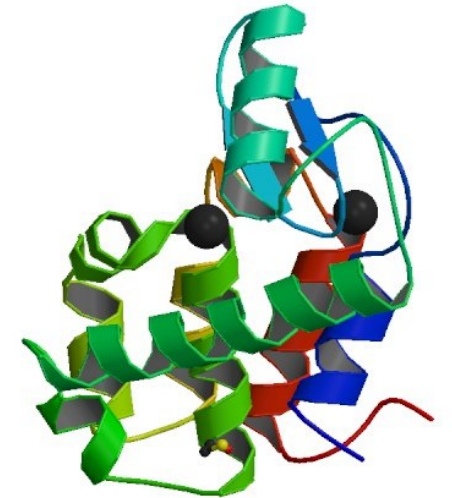
Tertiary Structure

De novo Protein folding

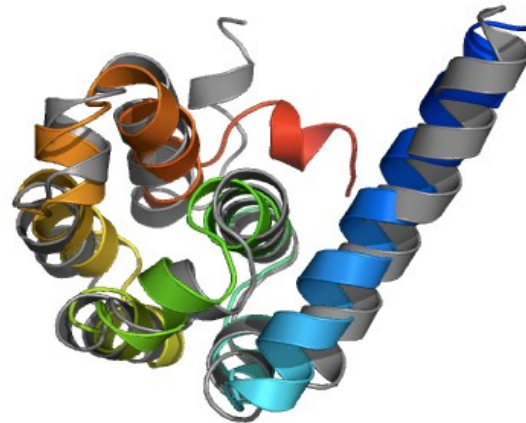
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Primary Sequence

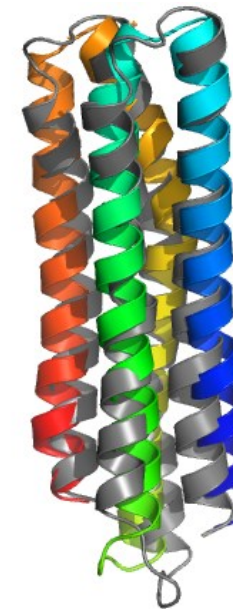


Tertiary Structure



Small, globular,
soluble proteins

T4-lysozyme
C-terminal domain



Small, simple
membrane proteins

V-type Na⁺ ATP
synthase subunit



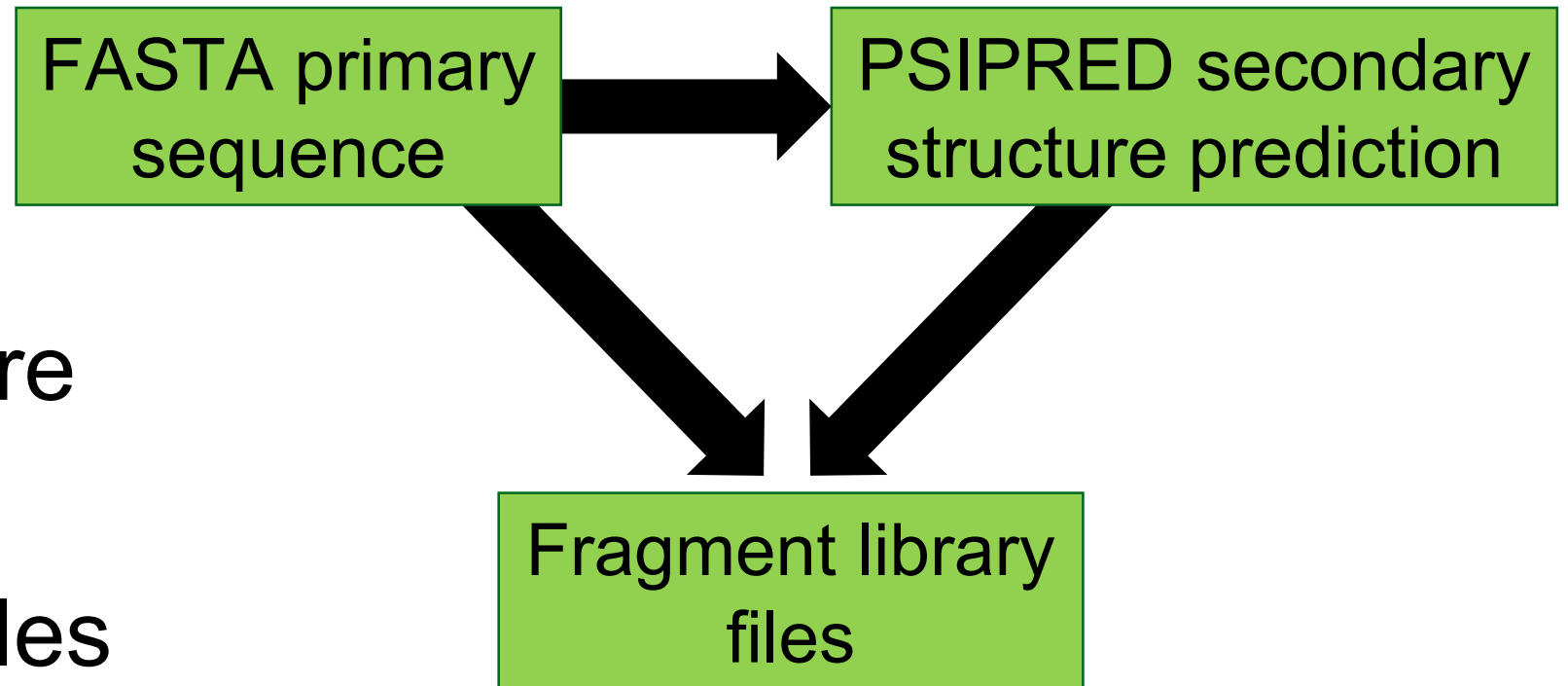
...but not large,
complex proteins

Rhodopsin

Inputs

- **Necessary:**

- FASTA primary sequence
- Secondary structure prediction
- Fragment library files
- Options file

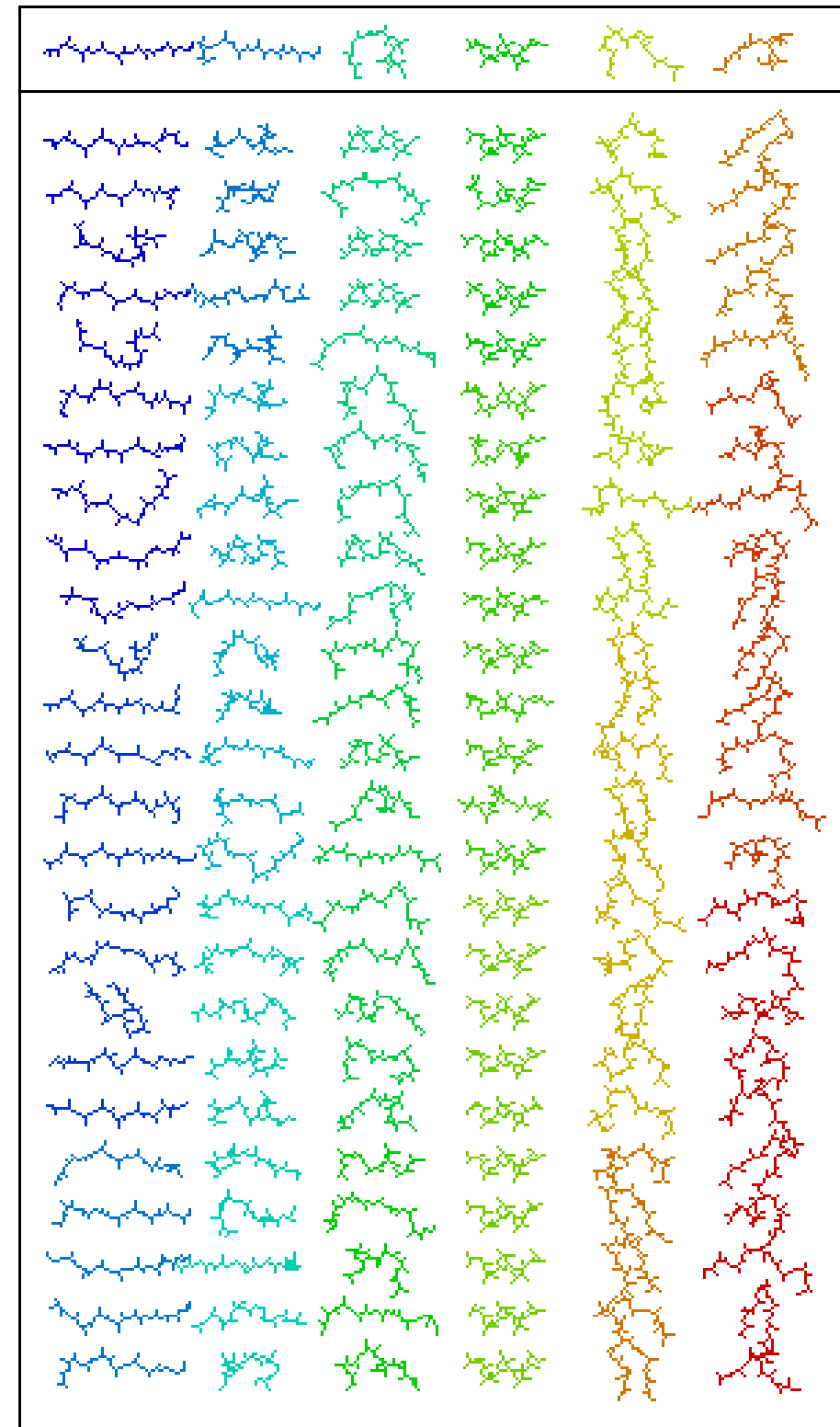


- **Optional:**

- Native structure (PDB)
- Constraints file

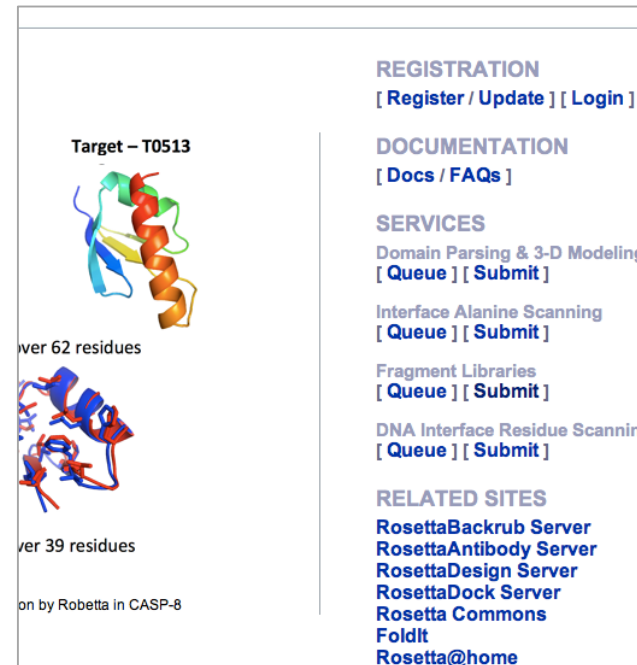
Inputs: fragment library files

- Peptide sequences generated from PDB: either 3 or 9 residues long
- Introduced to backbone to change geometry of protein
- High-quality fragments identified prior to application
- Can be selected using NMR data



Getting fragments: Robetta

- Online server for fragment selection, two simultaneous jobs per user
- Academic users only
- Alternative: perl script that is environment-specific (make_fragments.pl, discussed in the tutorial)



<http://robetta.bakerlab.org/>

The screenshot shows the 'Fragment Server Queue' page on the Robetta website. It includes a search bar with fields for Username, Target, and Host, and buttons for Reset, Clear, and Search. Below the search bar, there is a table with columns: ID, Status, Date (PST), Username, Length, Target, and Host. The table lists several completed jobs.

ID	Status	Date (PST)	Username	Length	Target	Host
18162	Complete	02/10/11 10:48:48 AM	vji4	226	anceu	dhcp128036156
18161	Complete	02/10/11 10:44:02 AM	jamsmad	26	1GZLIEnd	titan
18160	Complete	02/10/11 10:14:27 AM	jamsmad	22	1GZLShort	titan
18159	Complete	02/10/11 09:36:01 AM	zwenhor	38	2I2V4	Farid-HP.
18158	Complete	02/10/11 09:15:17 AM	zwenhor	41	1K1V	Farid-HP.
18157	Complete	02/10/11 09:11:09 AM	zwenhor	41	1K1V	Farid-HP.
18156	Complete	02/10/11 08:31:07 AM	jamsmad	26	1GZLFront	titan
18155	Complete	02/10/11 08:30:07 AM	jamsmad	26	1GZLAdd	titan
18154	Complete	02/10/11 07:51:36 AM	jamsmad	24	1GZLAdd	titan
18153	Complete	02/10/11 07:12:33 AM	jamsmad	24	1GZLDel	titan
18152	Complete	02/10/11 04:32:04 AM	Only Dym	441	PAN	wisweb2-out.v
18151	Complete	02/09/11 08:03:47 PM	maruti	56	GB1	142.1
18150	Complete	02/09/11 09:27:59 AM	drx	176	f9	128.2
18149	Complete	02/09/11 08:35:47 AM	gise	126	Nav beta-2 extra	139.1
18148	Complete	02/09/11 08:33:55 AM	zwenhor	208	1EOG	129.1

The screenshot shows the 'Submit a job to the Fragment Server' form on the Robetta website. It includes a 'Required' section with fields for 'Registered Username' or 'Registered Email Address' (with the example 'stephanie.j.hirst@vanderbi'), 'Target Name' (with the example '2LZM_'), and a 'Paste Fasta' section with a text area containing a protein sequence. There is also an 'Upload Fasta' option with a 'Choose File' button. An 'Optional' section includes an 'Identifier' field (with the example '2LZM_'), an 'Exclude Homologues' checkbox, and links for 'Rosetta NMR' (with a 'Choose File' button), 'Chemical Shifts' (with a 'Choose File' button), 'NOE Constraints' (with a 'Choose File' button), and 'Dipolar Constraints' (with a 'Choose File' button). A 'Submit' button is at the bottom right.

Options for AbInitioRelax

options.txt (1/2)

```
-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      # Discontinues refinement if a filter fails

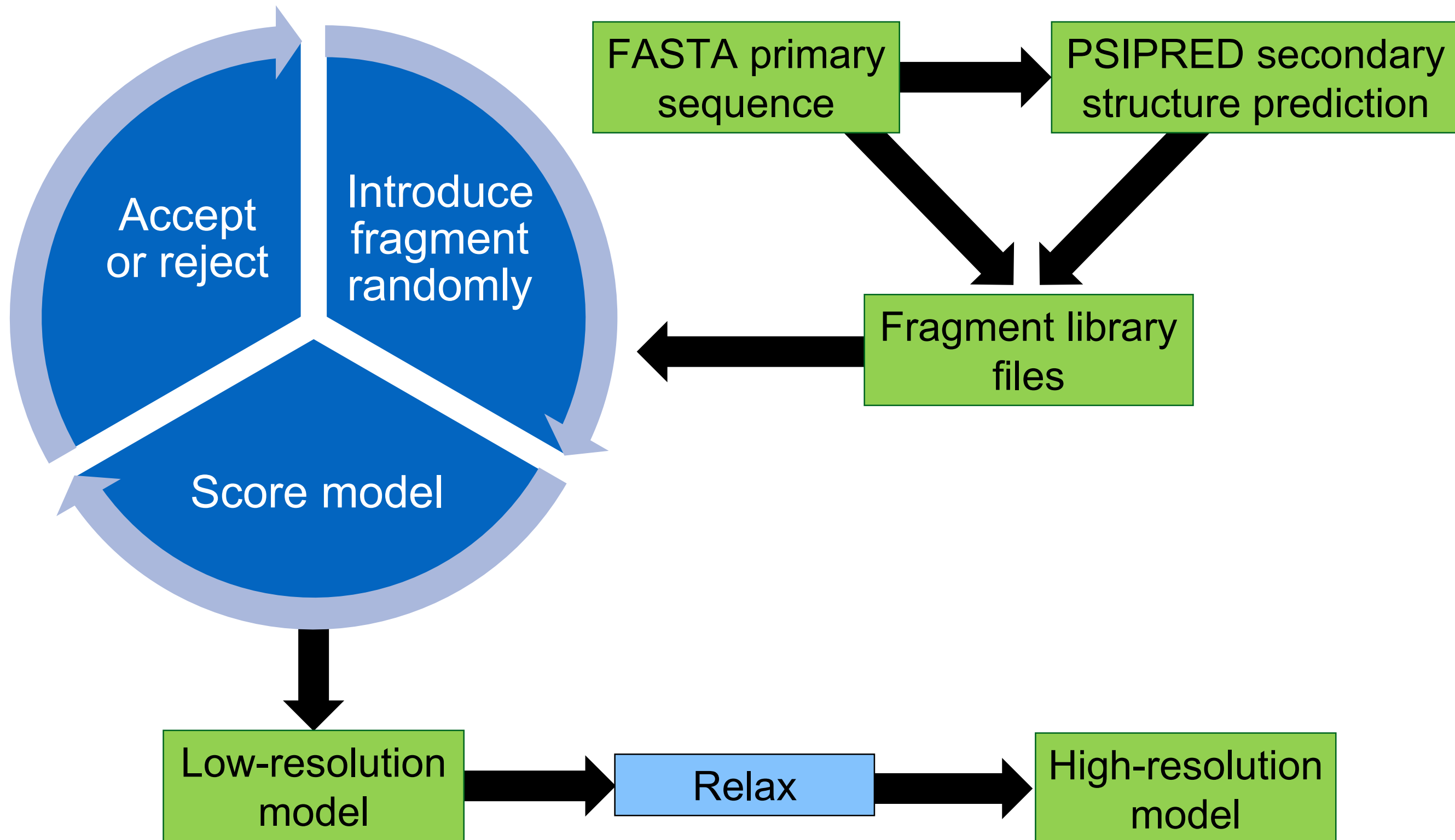
-relax
  -fast                  # Type of relax protocol. This has been shown to be the best deal for speed and
                        robustness.
```

Options for AbInitioRelax

options.txt (2/2)

```
-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
```

Running AbInitioRelax



Adding experimental data

options_cst.txt

```
-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
```

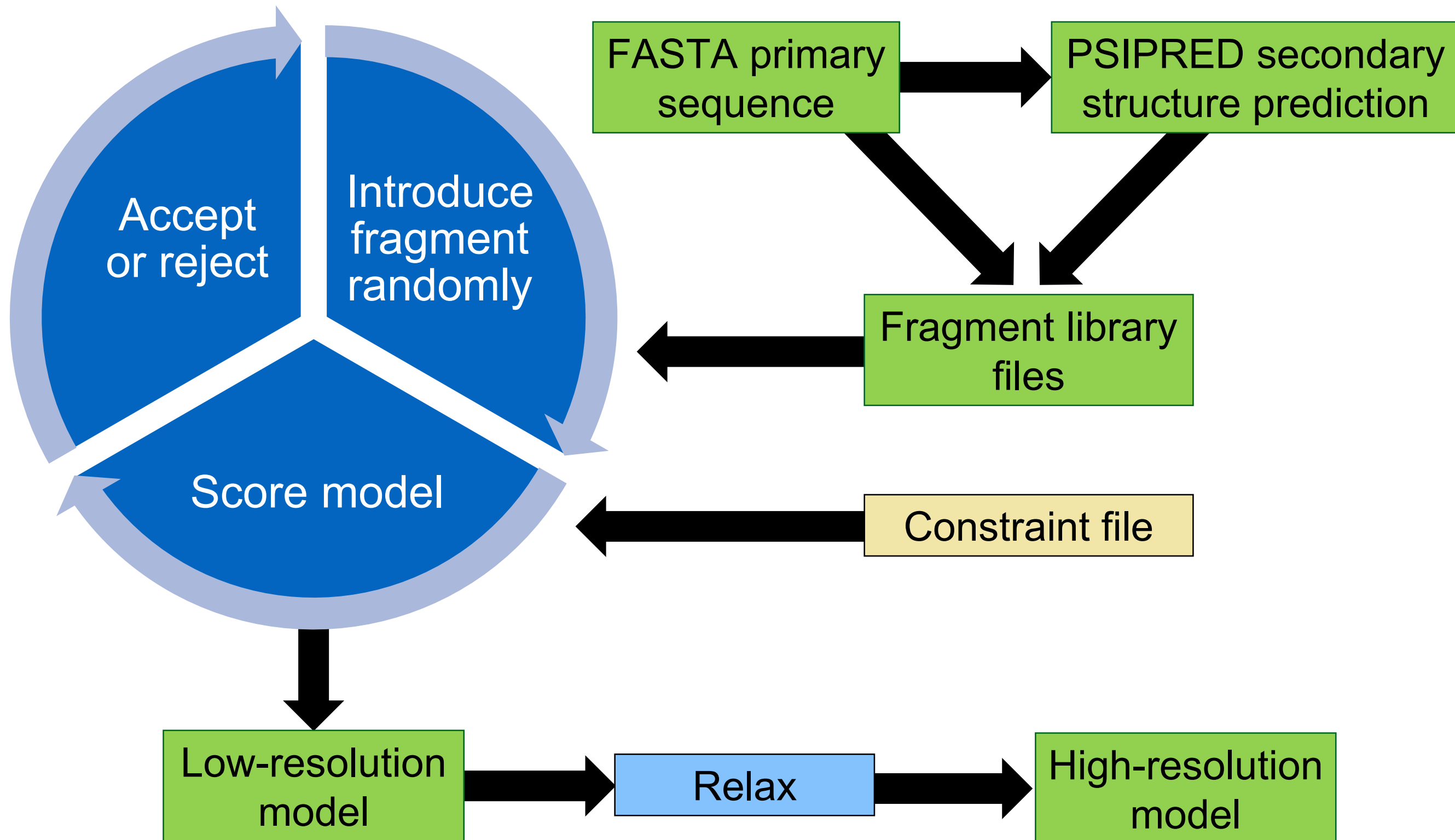
Constraint info

Constraint Function info

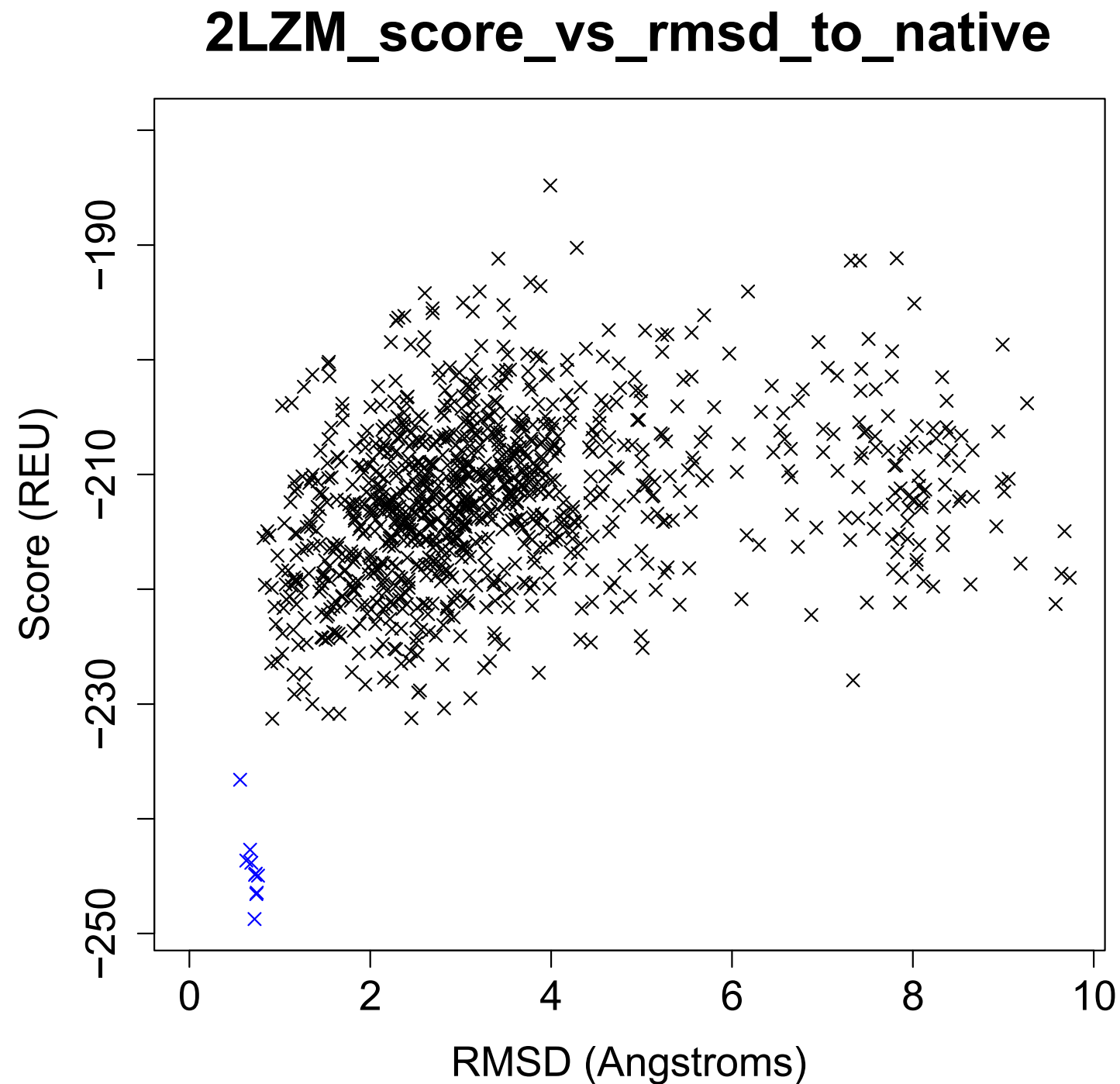
<cst_type>	<atom1>	<res1>	<atom2>	<res2>	<cst_func>	<RosettaEPR>	<Dcb>	<weight>	<bin>
AtomPair	CB	32	CB	36	SPLINE	EPR_DISTANCE	16.0	1.0	0.5
AtomPair	CB	59	CB	74	SPLINE	EPR_DISTANCE	19.0	1.0	0.5
AtomPair	CB	62	CB	71	SPLINE	EPR_DISTANCE	19.0	1.0	0.5

- Constraint type (AtomPair, Angle, etc.)
- Function type (Gaussian, spline, etc.)

Running AbInitioRelax

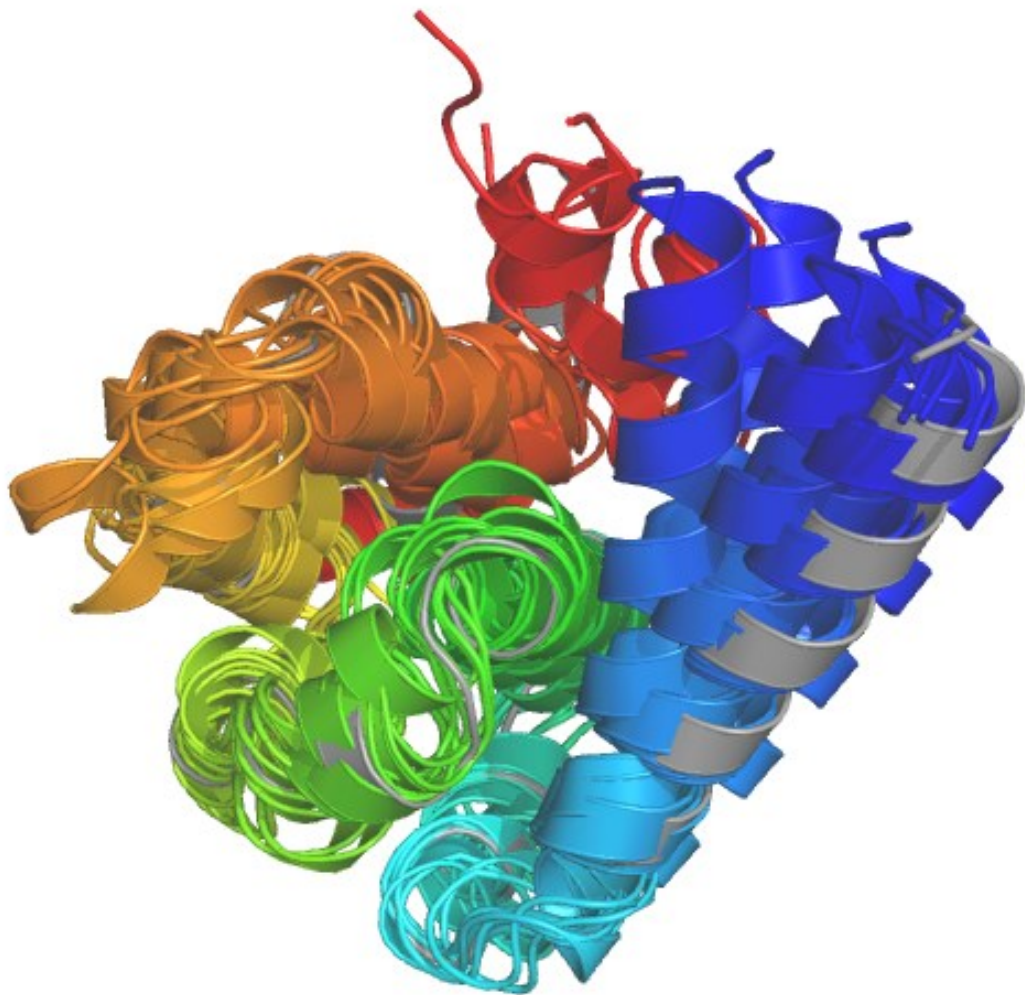


Evaluating model quality

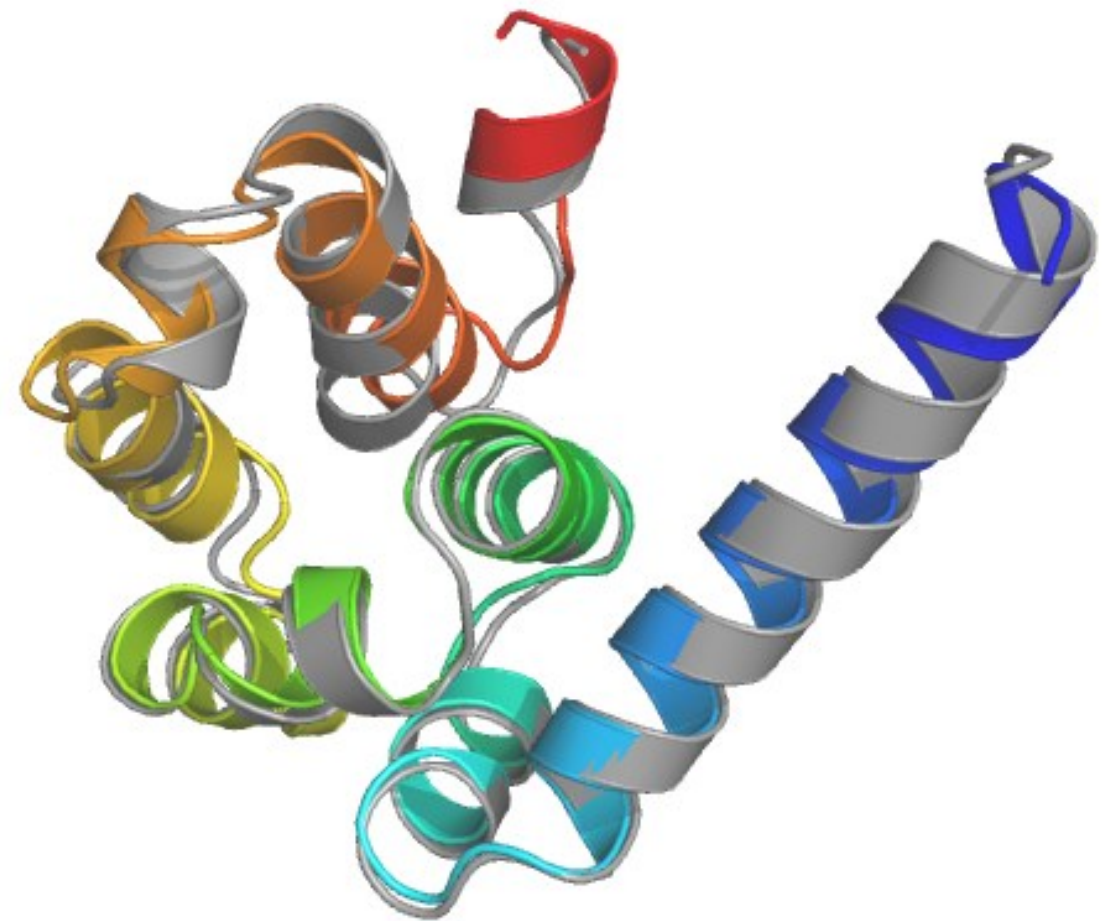


Evaluating model quality

Run: `pymol *.pdb`



Top 10 Scoring



Best Scoring

Conclusions and resources

- Rosetta AbInitio useful for small protein structure prediction
- Experimental data can improve prediction outcomes
- Additional resources:

[4] Protein Structure Prediction Using Rosetta

By CAROL A. ROHL, CHARLIE E. M. STRAUSS, KIRA M. S. MISURA, and
DAVID BAKER

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Structure
Article

Cell
PRESS

De Novo High-Resolution Protein Structure Determination from Sparse Spin-Labeling EPR Data

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