

# Comparative Modeling and Loop Building in Rosetta 3.2



# Outline

- Introduction to comparative modeling
- General comparative modeling protocol
- Comparative modeling in Rosetta 3.2
- Loop building in Rosetta 3.2: CCD vs. KIC
- Clustering output models
- Analyzing results
- Modeling membrane proteins
- Useful references and websites

# Introduction to Comparative Modeling

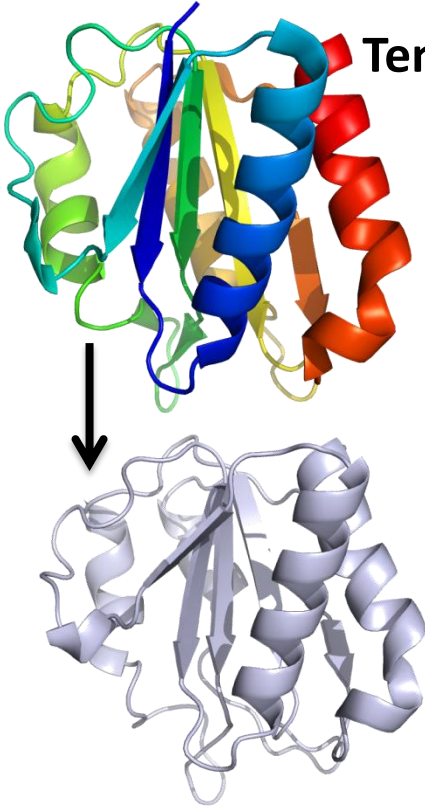
## Target

MKIVYWSGTGNTE<sup>?</sup>MA<sup>?</sup>IAKGIIESGKDVNTI  
NVSDVNIDELLNEDI<sup>?</sup>MGCSAMGDEVLEESEF  
EPFIEEISTKISGK<sup>?</sup>ALFGSYGWGDGKWMRDF  
EERMNGYGCVVET<sup>?</sup>IVQNEPDEAEQDCIEFG  
KKIANI



RCSB **PDB**  
PROTEIN DATA BANK

## Template



**Comparative Modeling:** construction of an atomic-resolution model of a protein with no experimentally determined structure using the 3D structure of a related protein

**Homology Modeling:** modeling a protein based on a template with common evolutionary origin

**Threading:** placing amino acids of the target sequence onto the coordinates of the template structure

## Application of Models

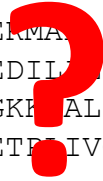
- Predict structure-function relationships
- Predict binding pockets for ligands for structure-based drug design
- Suggest site-directed mutagenesis experiments

**Show Threading Video**

# Identifying Template Structures

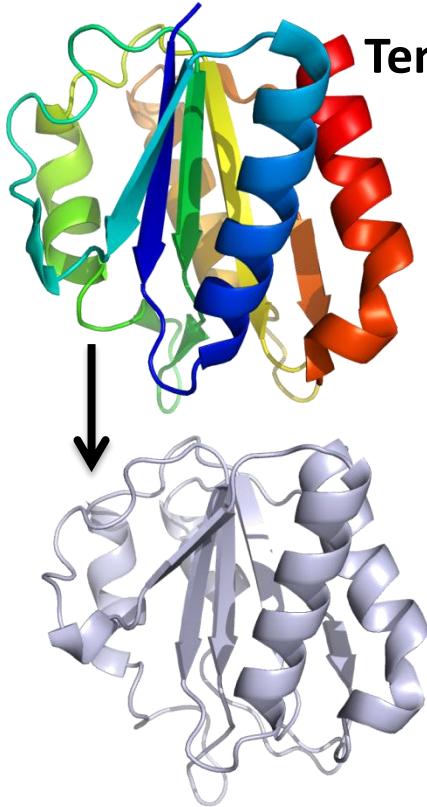
## Target

MKIVYWSGTGNTEPMAIAKGIIESGKDVNTI  
NVSDVNIDELLNEDIIGCSAMGDEVLEESEF  
EPFIEEISTKISGKRALFGSYGWGDGKWMRDF  
EERMNGYGCVVETPIVQNEPDEAEQDCIEFG  
KKIANI



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**PDB**  
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## Template



## Identifying a Suitable Template:

Templates should ideally have >30% sequence identity to the target. There are two approaches to identifying templates:

- 1. Sequence Similarity:** comparing proteins based on amino acid properties alone (BLAST, PSI-BLAST)
- 2. Fold Recognition:** using predicted secondary structure information to detect proteins with similar 3D characteristics (DALI, PHYRE)

# Comparative Modeling Protocol

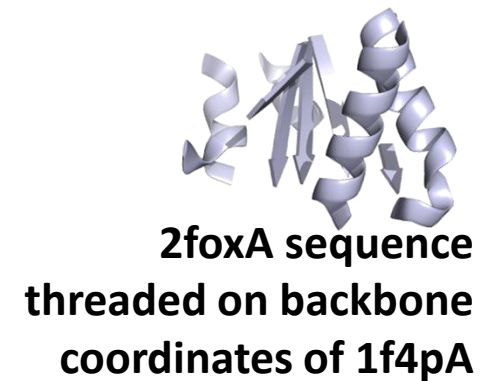
- **Step 1:** Align target sequence with sequence of template structure
- **Step 2:** Thread the target sequence onto the backbone of the template structure
- **Step 3:** Rebuild the loop regions of the model
- **Step 4:** Full-atom refinement of the model
- **Step 5:** Cluster models and analyze your results

```
2foxA 1 --MKIVYWSGTGNTTEKMAELIAKGIIE  
1f4pA 1 PKALIVYGSTTGNTTEYTAETIARELAD
```

# Comparative Modeling Protocol

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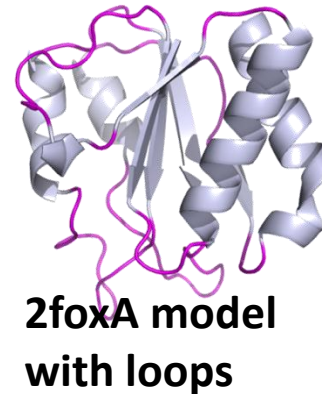
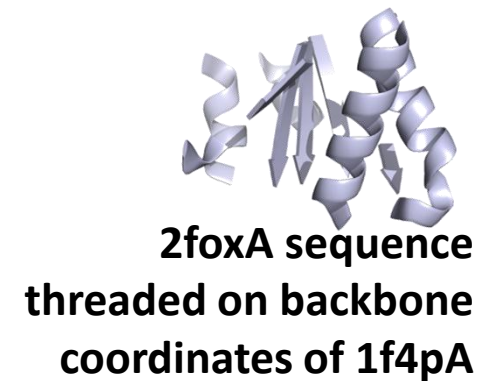
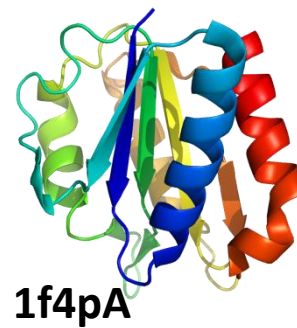
```
2foxA 1 --MKIVYWSGTGNTTEKMAELIAKGIIE  
1f4pA 1 PKALIVYGSTTGNTTEYTAETIARELAD
```



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```
2foxA 1 --MKIVYWSGTGNTTEKMAELIAKGIIE  
1f4pA 1 PKALIVYGSTTGNTTEYTAETIARELAD
```

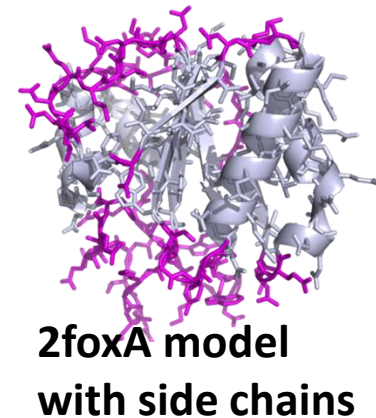
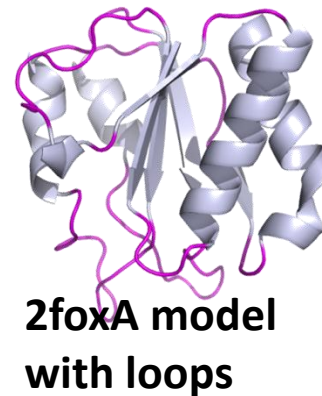
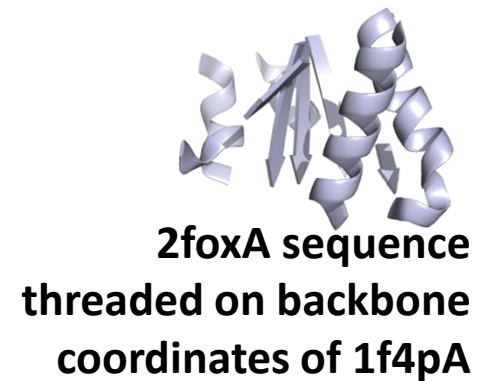
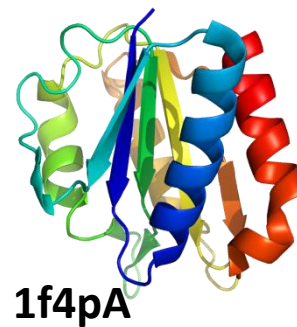




# Comparative Modeling Protocol

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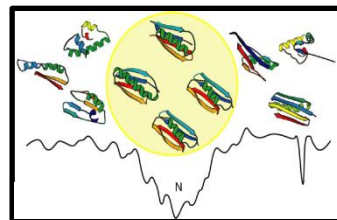
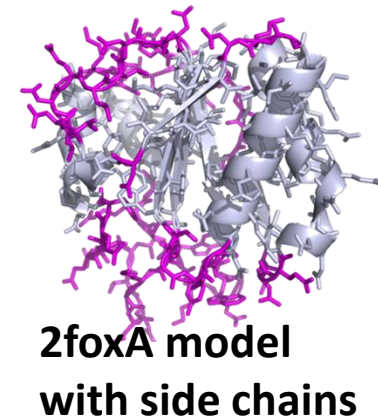
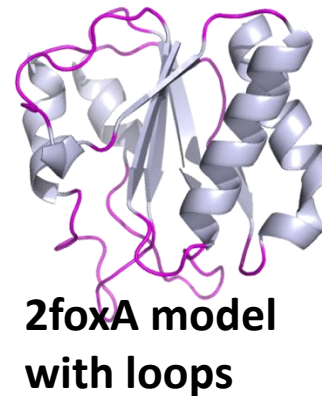
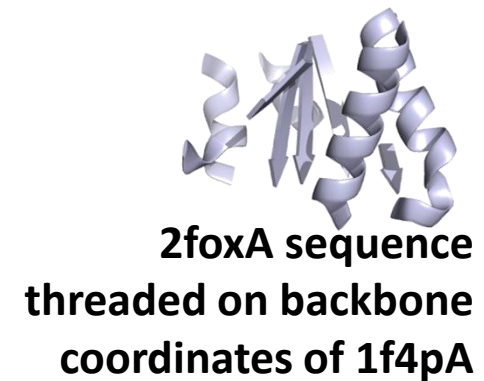
```
2foxA 1 --MKIVYWSGTGNTTEKMAELIAKGIIE  
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1f4pA 1 PKALIVYGSTTGNTTEYTAETIARELAD
```



# Rosetta 3.2 Threading Protocol

## Inputs

1. Target sequence (**2foxA**)
2. Template PDB (**1f4pA**)
3. Fragment files
4. Alignment of target and template sequences
5. Optional: Secondary structure file

## Output

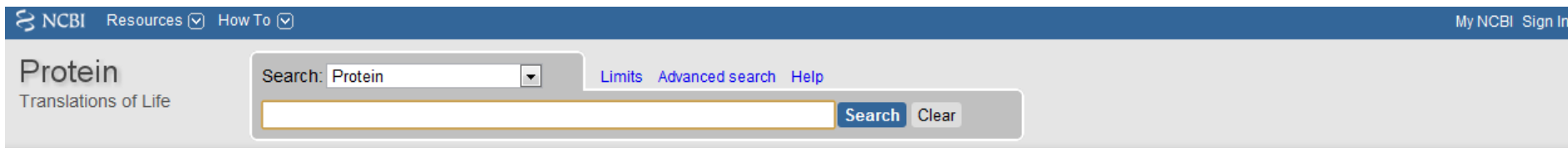
- Silent file containing models and corresponding scores

# 1: Target sequence

Find this file at `$WORKSHOP_ROOT/tutorials/modeling/input_model/2foxA.fasta`

```
>2foxA
```

```
MKIVYWSGTGNTTEKMAELIAKGIIESGKDVNTINVSDVNIDELLNEDILILGCSAMG  
DEVLEESEFEPFIEEISTKISGKKVALFGSYGWGDGKWMRDFEERMNGYGCVVETP  
LIVQNEPDEAEQDCIEFGKKIANI
```



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Protein  
Translations of Life

Search: Protein Limits Advanced search Help

Search Clear



## Protein

The Protein database is a collection of sequences from several sources, including translations from annotated coding regions in GenBank, RefSeq and TPA, as well as records from SwissProt, PIR, PRF, and PDB. Protein sequences are the fundamental determinants of biological structure and function.

<http://www.ncbi.nlm.nih.gov/protein>

# 2: Template PDB

Find this file at `$WORKSHOP_ROOT/tutorials/modeling/input_model/1f4pA.pdb`

MyPDB Hide

Login to your Account  
Register a New Account

Home Hide

News & Publications  
Usage/Reference Policies  
Deposition Policies  
Website FAQ  
Deposition FAQ  
Contact Us  
About Us  
Careers  
External Links  
Sitemap  
New Website Features

Deposition Hide

All Deposit Services  
Electron Microscopy  
X-ray | NMR  
Validation Server  
BioSync Beamline  
Related Tools

Search Hide

[Advanced Search](#)

## A Resource for Studying Biological Macromolecules

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the [wwPDB](#), the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

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### Structural View of Biology



#### Molecule of the Month: Integrin



Our bodies are composed of approximately ten trillion cells, which poses challenging problems for structure and communication. All of these cells must be connected strongly together, to allow us to stand and walk. The infrastructure holding us together, however, must also be malleable enough to allow repairs, to allow us to heal from wounds. These many cells must also communicate with each other, ensuring that each plays its own proper part. Many different molecules in our bodies are involved in this complex infrastructure of connected communication and interaction.

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### Transporter Classification Database Browser

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2011-02-22

### Structural Neighbors



Explore structural neighbors lists to find connections between PDB

# 3: Fragments

Find these files at

*\$WORKSHOP\_ROOT/tutorials/modeling/input\_model/aa2foxA03\_05.200\_v1\_3 and  
\$WORKSHOP\_ROOT/tutorials/modeling/input\_model/aa2foxA09\_05.200\_v1\_3*



[Structure Prediction](#)   [Fragment Libraries](#)   [Alanine Scanning](#)   [DNA Interface Scan](#)  
[\[ Queue \] \[ Submit \]](#)   [\[ Queue \] \[ Submit \]](#)   [\[ Queue \] \[ Submit \]](#)   [\[ Queue \] \[ Submit \]](#)  
[\[ Register / Update \]](#)   [\[ Docs / FAQs \]](#)   [\[ Login \]](#)

## Submit a job to the Fragment Server

\*Please submit one job at a time

### Required

[Registered Username:](#)  or [Registered Email Address:](#)

Target Name:

Paste [Fasta](#)

or Upload [Fasta:](#)  No file chosen

[http://robeta.bakerlab.org/  
fragmentsubmit.jsp](http://robeta.bakerlab.org/fragmentsubmit.jsp)

# 4: Alignment

Find this file at `$WORKSHOP_ROOT/tutorials/modeling/input_model/2foxA.1f4pA.aln`

```
2foxA 1 --MKIVYWSGTGNTEKMAELIAKGIIE
1f4pA 1 PKALIVYGSTTGNTEYTAETIARELAD
```



## Multiple Sequence Alignment by CLUSTALW

CLUSTALW	MAFFT	PRRN
<a href="#">Help</a>		
<b>General Setting Parameters:</b>		
Output Format: <input type="text" value="CLUSTAL"/>		
Pairwise Alignment: <input checked="" type="radio"/> FAST/APPROXIMATE <input type="radio"/> SLOW/ACCURATE		
Enter your <b>sequences</b> (with labels) below (copy & paste): <input checked="" type="radio"/> PROTEIN <input type="radio"/> DNA		
Support Formats: FASTA (Pearson), NBRF/PIR, EMBL/Swiss Prot, GDE, CLUSTAL, and GCG/MSF		
<div style="border: 1px solid #ccc; height: 80px; width: 100%;"></div>		
<b>Or give the file name containing your query</b>		
<input type="button" value="Choose File"/> No file chosen		
<input type="button" value="Execute Multiple Alignment"/> <input type="button" value="Reset"/>		

<http://align.genome.jp/>

# 5: PSIPRED

*Find this file at \$WORKSHOP\_ROOT/tutorials/modeling/input\_model/2foxA.psipred\_ss2*

## The PSIPRED Protein Structure Prediction Server

The PSIPRED Protein Structure Prediction Server aggregates several of our structure prediction methods into one location. Users can submit a protein sequence, perform the prediction of their choice and receive the results of the prediction via e-mail. You may select one of three prediction methods to apply to your sequence:

PSIPRED - a highly accurate method for protein secondary structure prediction

MEMSAT and MEMSAT-SVM - our widely used transmembrane topology prediction method

and one of GenTHREADER, pGenTHREADER and pDomTHREADER - sequence profile based fold recognition methods. [More...](#)

**For queries regarding PSIPRED:** [psipred@cs.ucl.ac.uk](mailto:psipred@cs.ucl.ac.uk)

### Choose Prediction Method

- Predict Secondary Structure (PSIPRED v3.0)
- Predict Transmembrane Topology (MEMSAT3 & MEMSAT-SVM)
- SVM Prediction of TM Topology and Helix Packing (MEMPACK) - **NEW!**
- Fold Recognition (GenTHREADER - quick)
- Fold Recognition (pGenTHREADER - with profiles and predicted secondary structure)
- Fold Recognition (pDomTHREADER - annotates multiple domain on chains)

[Help...](#)

### Input Sequence (single letter amino acid code)

<http://bioinf.cs.ucl.ac.uk/psipred/>



# Options: Input

*Find this file at*

*`$WORKSHOP_ROOT/tutorials/modeling/input_model/comparative_model.options`*

**-run:protocol threading** #call threading protocol

**-in:file:fasta \*.fasta** #target sequence

**-in:file:template\_pdb \*.pdb** #template structure

**-in:file:fullatom** #input will be fullatom

**-in:file:psipred\_ss2 \*.psipred\_ss2** #optional: psipred secondary structure

**-in:file:alignment \*.aln** #input alignment

# Options: Loop Building (CCD)

```
-loops:frag_sizes 9 3 1 #sizes of fragments
-loops:frag_files <fragment files> #fragment files
-loops:remodel quick_ccd #use ccd to remodel loops
-idealize_after_loop_close #idealize structure after closing
loops (structure will have ideal rosetta bond lengths and
angles)
-loops:extended true #force extended on loops (phi-psi angles
set to 180 degrees) independent of loop input file. for
rebuilding loops entirely.
-loops:build_initial true #precede loop modeling with initial
round of removing missing densities and building simple loops
-loops:relax fastrelax #fastrelax loops (5 cycles at default)
-cm:min_loop_size <int> #minimum size of loops to consider for
rebuild
```

# Options: Output

- `-out:nstruct <int>` #number of models to build
- `-out:file:silent_struct_type binary` #output file type
- `-out:file:silent *.out` #output file name
- `-out:file:fullatom` #output file will be fullatom

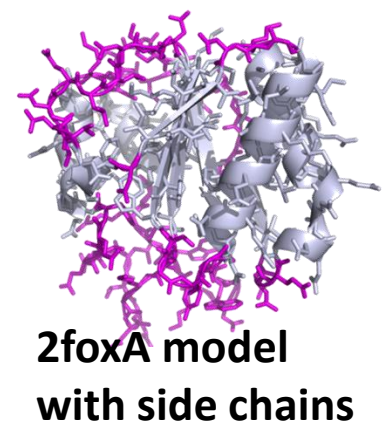
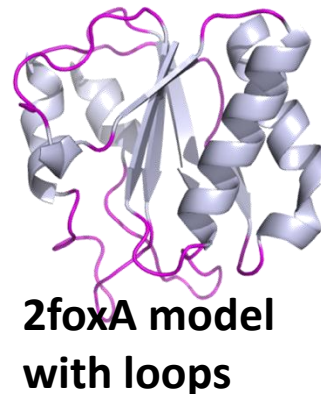
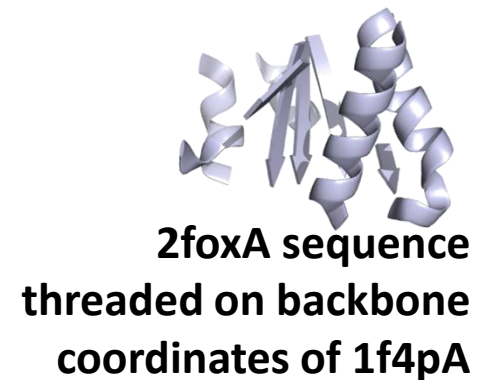
# Running Rosetta

```
$ROSETTA_BIN/minirosetta.$ROSETTA_SUFFIX  
@$WORKSHOP_ROOT/tutorials/modeling/input_model/comparative  
_model.options -database $ROSETTA_DATABASE >&  
$WORKSHOP_ROOT/tutorials/modeling/comparative_model.log &
```

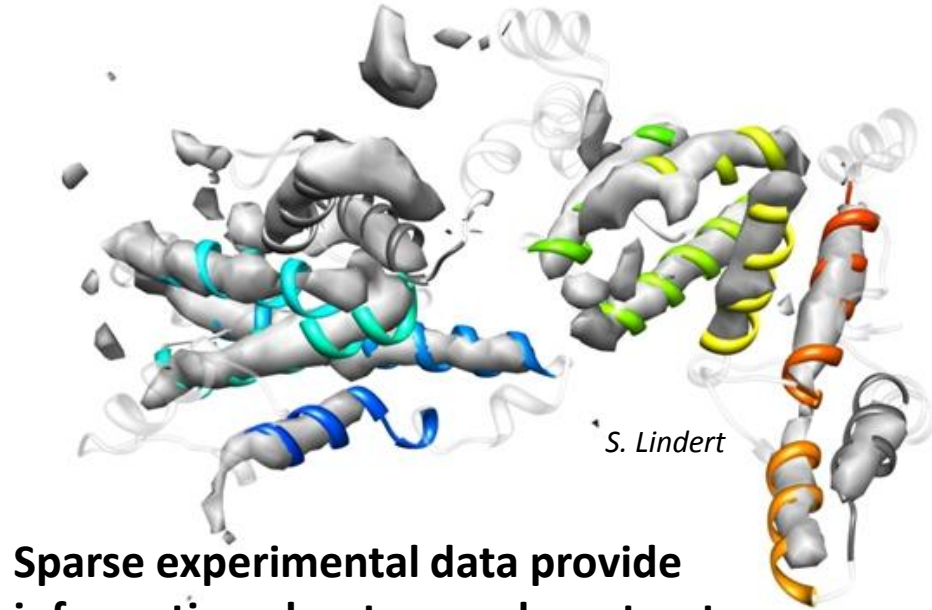
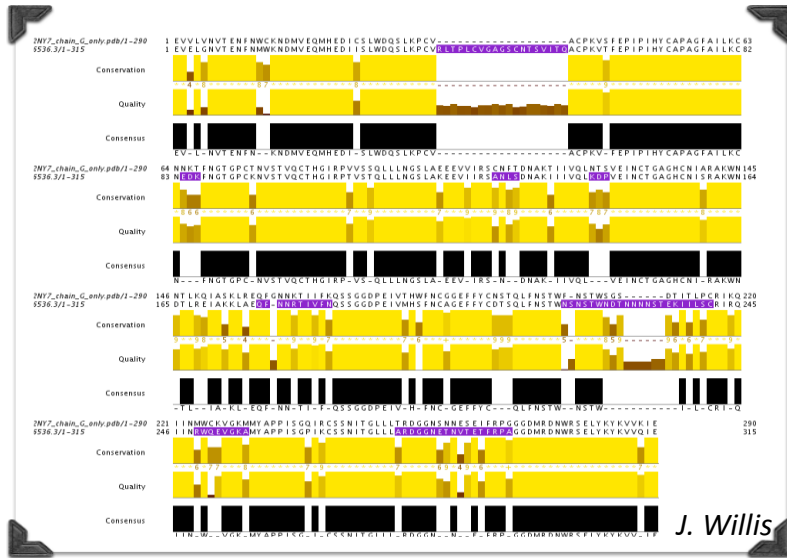
# Behind the Rosetta 3.2 Threading Protocol

- **Step 1:** Align target sequence with sequence of template structure
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```
2foxA 1 --MKIVYWSGTGNTTEKMAELIAKGIIE  
1f4pA 1 PKALIVYGSTTGNTEYTAETIARELAD
```

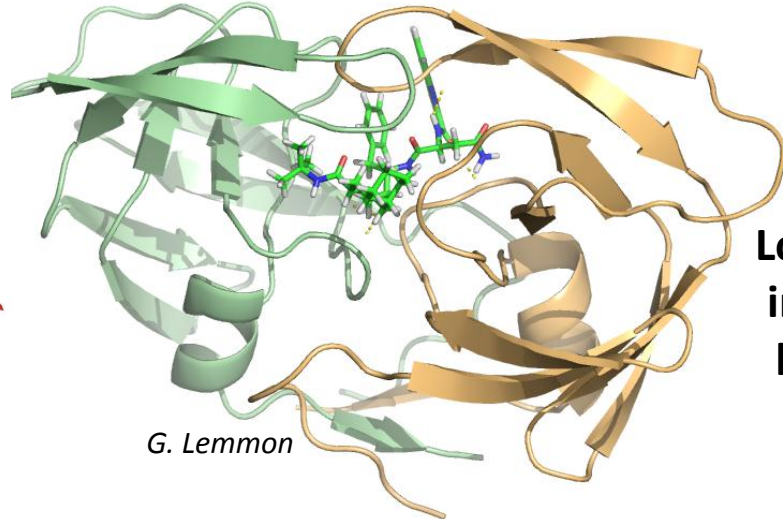
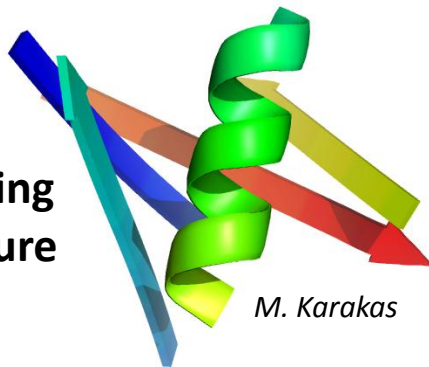


# Why Loop Building is Important



Sparse experimental data provide information about secondary structure elements, but rarely loop conformations

De novo folding requires connecting secondary structure elements



Loops can play an important role in ligand & peptide binding sites

# Comparing Loop Building Methods

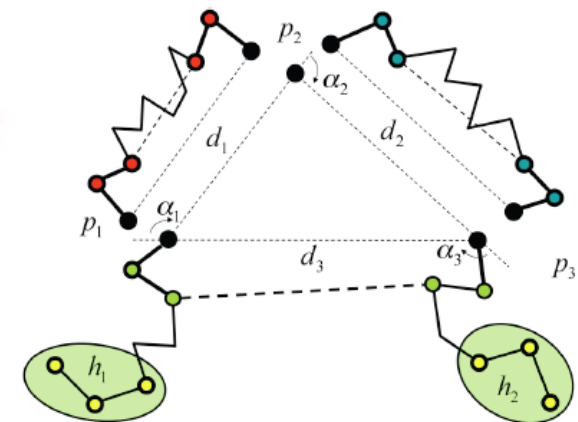
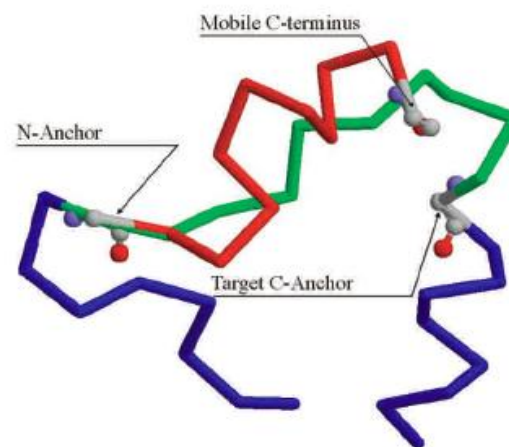
	Cyclic Coordinate Descent (CCD)	Kinematic Loop Closure (KIC)
Speed	★	
Accuracy in recovering crystal structures		★
Accuracy in homology modeling	★	

## Stage 1: Remodel (CCD or KIC)

fast, broad sampling of backbone conformations, centroid

## Stage 2: Refine (KIC)

side-chains are represented in all-atom detail, and together with backbone conformations, evaluated by Rosetta's high-resolution scoring function.



# Rosetta 3.2 Loop Building Protocol

## Inputs

1. Loop File
2. Model PDB with or without loop coordinates

## Output

- Silent file containing models and corresponding scores

## CCD Only

3. Fragment files



# 1: Loop File

Find this file at `$WORKSHOP_ROOT/tutorials/modeling/input_loop/2foxA.loops`

```
LOOP 6 11 0 0 0
```

Column 1	LOOP	The loop file identity tag
Column 2	<integer>	Residue number for starting loop anchor. NOTE: The starting structure must have real coordinates for all residues outside the loop definition, including the loop anchors (residues indicated in the loops file).
Column 3	<integer>	Residue number for ending loop anchor
Column 4	<integer>	Cut point residue number, $\geq$ startRes, $\leq$ endRes. default - let LoopRebuild choose cutpoint
Column 5	<float>	Skip rate (probability between 0 and 1). default - never skip
Column 6	<boolean>	Extend loop. Default false

## Differences for KIC:

- For de novo reconstruction of protein loops, set 'extend loop' field in the loop definition file (the last column) to '1'.

# 2: PDB without loop coordinates

*Find this file at*

`$WORKSHOP_ROOT/tutorials/modeling/input_loop/2foxA_no_loops.pdb`

If you want to completely rebuild your loops, set the loop coordinates of your PDB file to zero with the following script:

```
$WORKSHOP_ROOT/py_protein_utils/scripts/remove_loop_coords.py  
2foxA.loops 2foxA_start_model.pdb 2foxA_no_loops.pdb
```

The script sets loop x-y-z coordinates (as defined by your loop file) to 0.000 and the occupancy column to -1.00, as below:

ATOM	2253	CB	PHE	A	231	0.000	0.000	0.000	-1.00	0.00	C
ATOM	2254	CG	PHE	A	231	0.000	0.000	0.000	-1.00	0.00	C
ATOM	2255	CD1	PHE	A	231	0.000	0.000	0.000	-1.00	0.00	C
ATOM	2256	CD2	PHE	A	231	0.000	0.000	0.000	-1.00	0.00	C
ATOM	2257	CE1	PHE	A	231	0.000	0.000	0.000	-1.00	0.00	C

# Setting up CCD versus KIC

Find these files at `$WORKSHOP_ROOT/tutorials/modeling/input_loop/kic.options` and `$WORKSHOP_ROOT/tutorials/modeling/input_loop/ccd.options`

## Common Options

```
-nstruct <int> #number of models to build. 1000 recommended for
production runs.
-loops:input_pdb *.pdb #starting pdb with loops to rebuild
-loops:loop_file *.loops #loop file
-loops:relax fastrelax #does a minimization of the structure in the
torsion space
-loops:extended #force phi-psi angles to be set to 180 degrees
independent of loop input file (recommended for production runs)
-out:file:silent_struct_type binary #output file type
-out:file:silent *.out #output file name
-out:file:fullatom #output file will be fullatom
```

## Options for CCD

```
-loops:frag_sizes 9 3 1
-loops:frag_files <fragment files>
-loops:remodel quick_ccd
-loops:refine refine_kic
```

## Options for KIC

```
-loops:remodel perturb_kic
-loops:refine refine_kic
-ex1 #Include extra chi1 rotamers
-ex2
```

# Running Rosetta

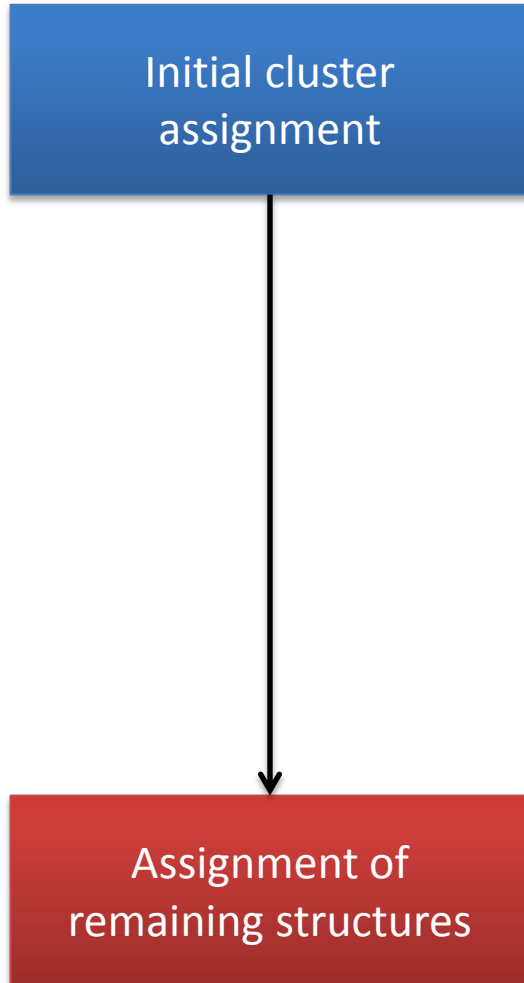
```
$ROSETTA_BIN/loopmodel.$ROSETTA_SUFFIX  
@$WORKSHOP_ROOT/tutorials/modeling/input_loop/ccd.options  
-database $ROSETTA_DATABASE >& $WORKSHOP_ROOT/  
tutorials/modeling/ccd.log &
```

```
$ROSETTA_BIN/loopmodel.$ROSETTA_SUFFIX  
@$WORKSHOP_ROOT/tutorials/modeling/input_loop/kic.options  
-database $ROSETTA_DATABASE >& $WORKSHOP_ROOT/  
tutorials/modeling/kic.log &
```

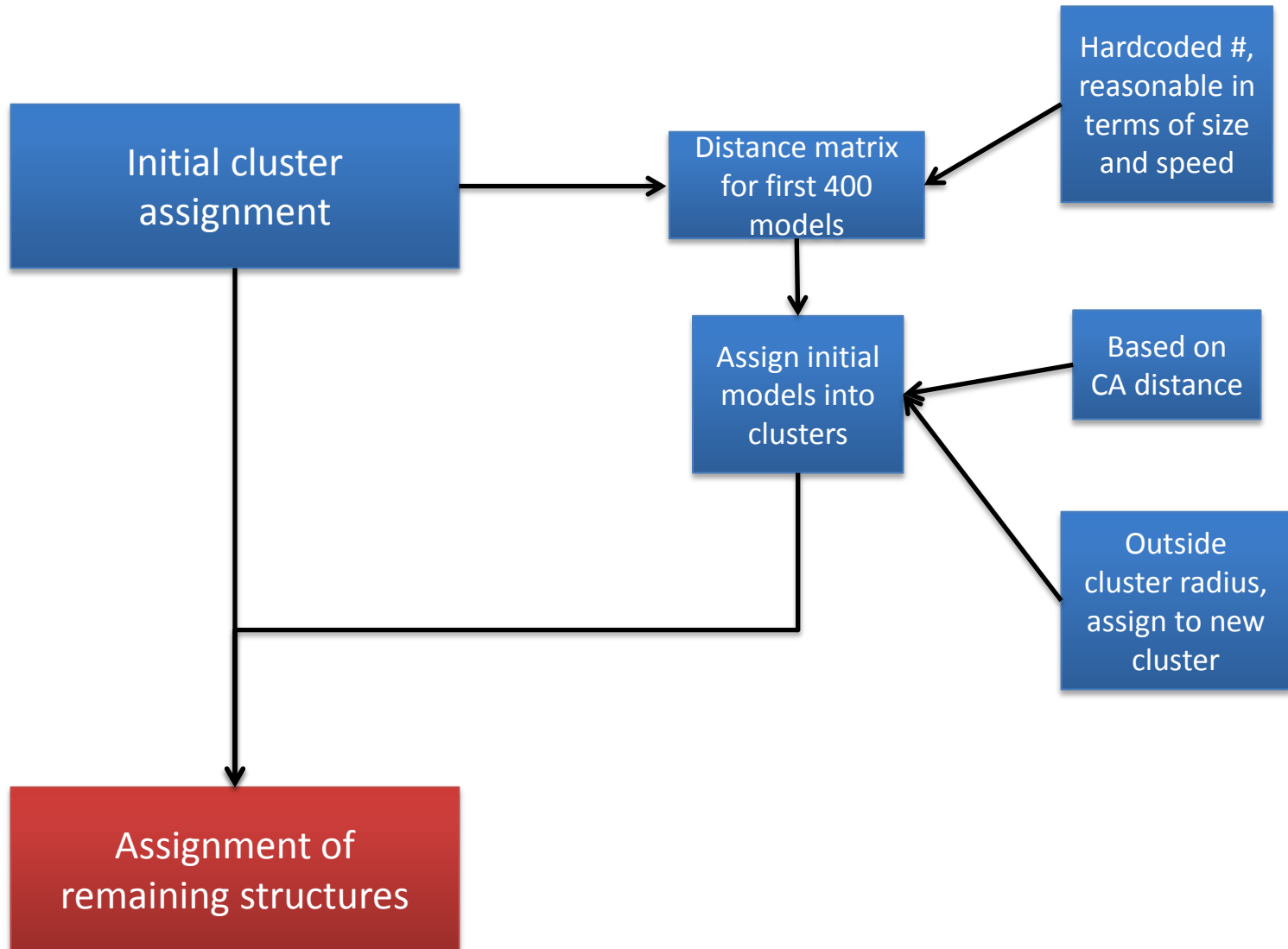
# (Clustering in Rosetta)

- The Rosetta clustering algorithm is slightly unconventional
- Traditional clustering methods require the calculation of a pairwise distance matrix
  - The memory requirements of this method are  $n^2$  where  $n$  is the number of models being clustered
  - For large numbers of models, these methods are therefore impractical

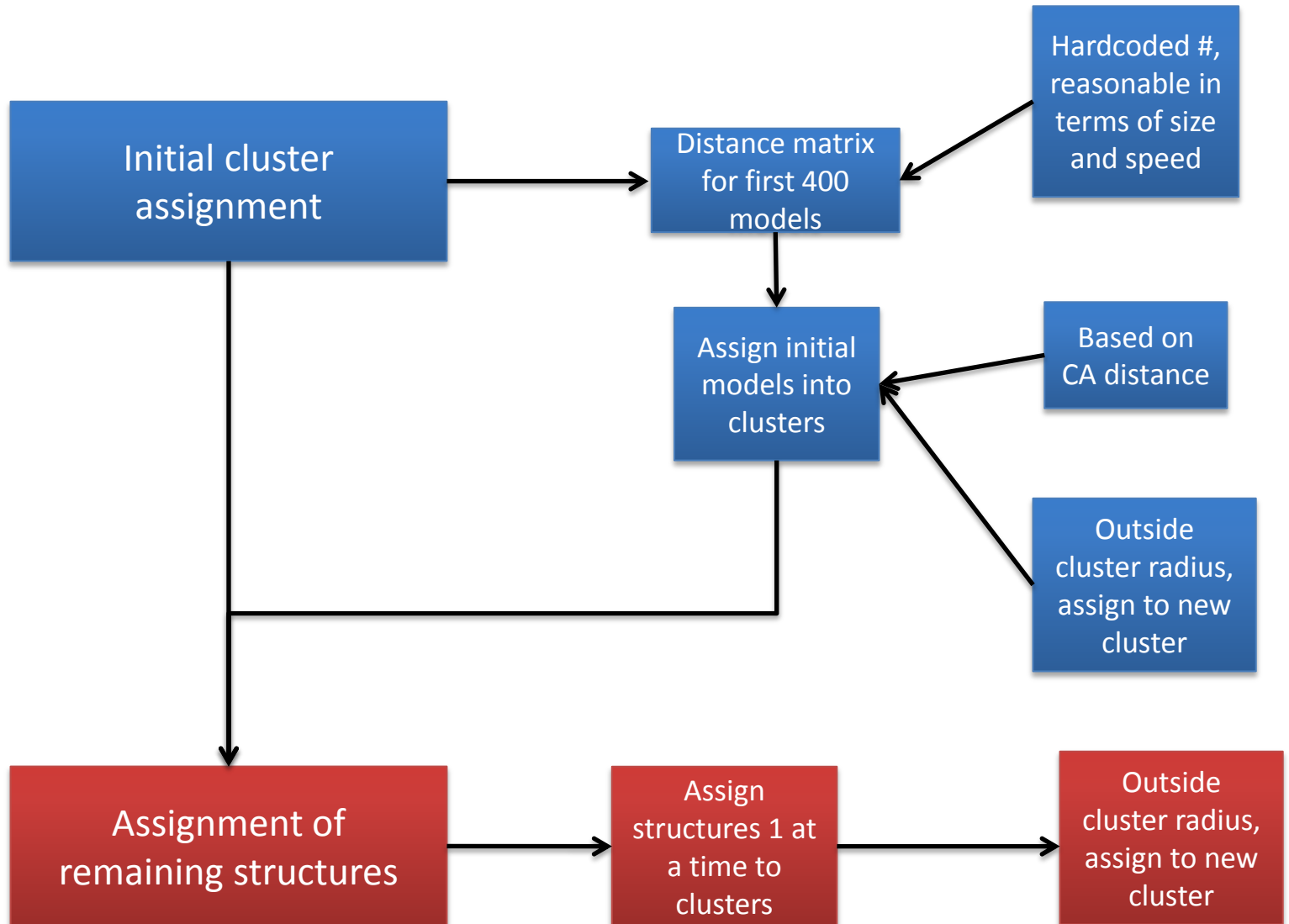
# (Clustering In Rosetta)



# (Clustering In Rosetta)



# (Clustering In Rosetta)





# Clustering : Options

*Find this file at `$WORKSHOP_ROOT/tutorials/modeling/input_cluster/cluster.options`*

- in:file:fullatom** #Read as fullatom input structure
- out:file:silent** #Output silent structures instead of PDBs
- run:shuffle** #Use shuffle mode
- cluster:radius <float>** #Cluster radius in A for RMS clustering or in inverse GDT\_TS for Global Distance Test score clustering. Use "-1" to trigger automatic radius detection
- cluster:exclude\_res <int> [<int> <int> ..]** #Exclude residue numbers from structural comparisons

# Clustering : Running

Before running the cluster application, combine all your silent files:

```
$ROSETTA_BIN/combine_silent.$ROSETTA_SUFFIX  
-database $ROSETTA_DATABASE  
-in:file:silent *.out  
-in:file:silent_struct_type binary  
-out:file:silent cluster_all.out  
-out:file:silent_struct_type binary
```

The clustering python script runs the Rosetta application and outputs summary files:

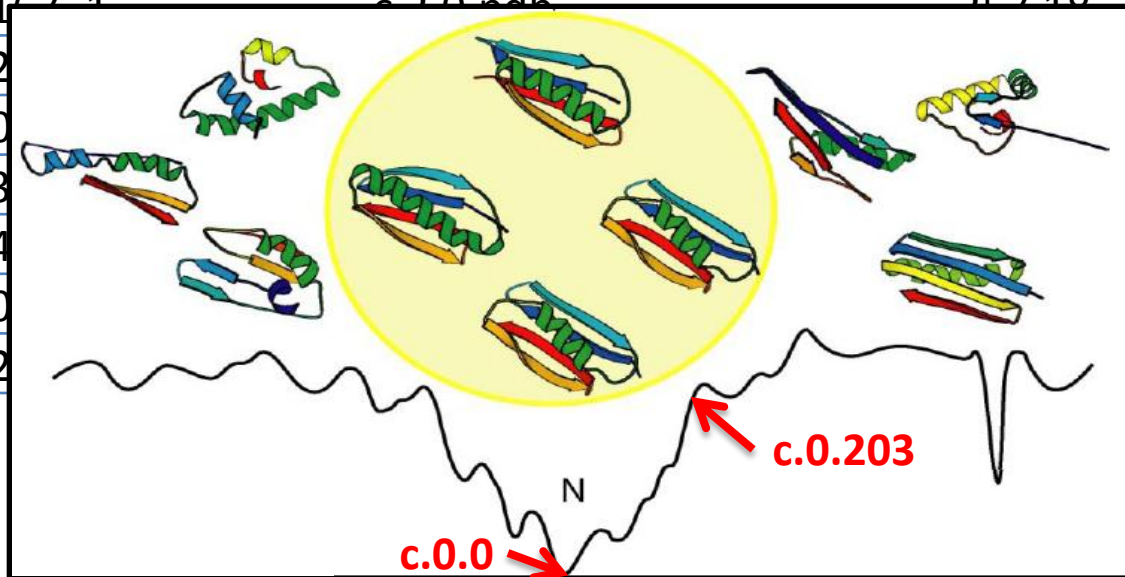
```
python $WORKSHOP_ROOT/py_protein_utils/scripts/clustering.py  
--silent=cluster_all.out  
--rosetta=$ROSETTA_BIN/cluster.$ROSETTA_SUFFIX  
--database=$ROSETTA_DATABASE  
--options=cluster.options  
cluster_summary.txt cluster_histogram.txt
```

# Clustering : Results

cluster number (random)

model number, sorted by energy

Tag	file_name	score	size
S_1F4PA_0410_1	c.0.0.pdb	-267.131	203
S_1F4PA_0356_1	c.6.0.pdb	-252.855	40
S_1F4PA_0036	c.22.0.pdb	-248.465	29
S_1F4PA_0127_1	c.13.0.pdb	-251.634	24
S_1F4PA_0116_1	c.14.0.pdb	-251.295	24
S_1F4PA_0281	c.25.0.pdb	-248.026	24
S_1F4PA_0162	c.29.0.pdb	-245.988	20
S_1F4PA_0167_1	c.2.0.pdb	-257.19	17
S_1F4PA_02			17
S_1F4PA_00			16
S_1F4PA_03			14
S_1F4PA_04			13
S_1F4PA_00			13
S_1F4PA_02			13



# Analyze Your Results: See Workshop 1

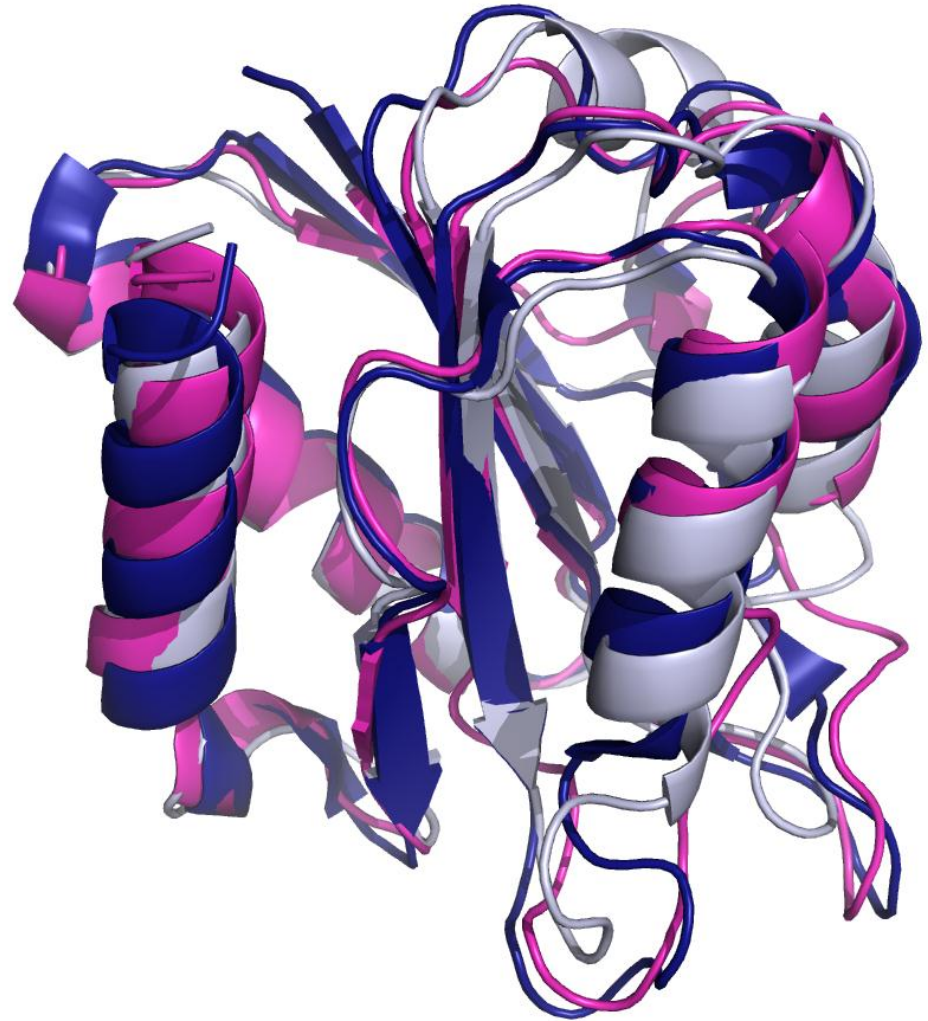
- Generating Score vs. RMSD Plots
- Extracting and Looking at Models
- Looking at Models in PyMol
- Looking at RMSD Distributions

# Results of Comparative Modeling Protocol

2foxA native structure
1f4pA template structure
2foxA model

**RMSD of 2foxA model (c.0.0) to 2foxA native structure:**  
1.376 Angstroms

**RMSD of 2foxA model (c.0.0) to 1f4pA template:**  
1.312 Angstroms



# Comparative Modeling of Membrane Proteins

*\*this protocol is not yet benchmarked, use with caution*

- Generate LIPS and spanfile (see workshop 1)
- Add the following options to the options file:

```
-in:file:spanfile *.span # newly created spanfile  
-in:file:lipofile *.lips4 # newly created lipo file  
-membrane:no_interpolate_Mpair # membrane scoring specification  
-membrane:Menv_penalties # turn on membrane penalty scores  
-score:weights membrane_highres_Menv_smooth.wts
```

- Run protocol as before

# It's Your Turn!

Three stand-alone tutorials are included in your worksheet:

- Comparative Modeling
- Loop Building (CCD and KIC)
- Clustering

## Tips:

- BLUE text means that these files and/or this information is provided.
- RED text means that this material will NOT be conducted during the workshop
- Change all environment variables (anything starting with a \$) to your local paths
- If you want to try making files that already exist (e.g., input files), write them to a new directory!
- See Tutorial 1 (De Novo Folding) for more details on analyzing your results.

# References

- **Rosetta 3.2 User Guide**

[http://www.rosettacommons.org/manuals/archive/rosetta3.2\\_user\\_guide/comparative\\_modeling.html](http://www.rosettacommons.org/manuals/archive/rosetta3.2_user_guide/comparative_modeling.html)

- **Comparative Modeling**

[http://www.rosettacommons.org/manuals/archive/rosetta3.2\\_user\\_guide/comparative\\_modeling.html](http://www.rosettacommons.org/manuals/archive/rosetta3.2_user_guide/comparative_modeling.html)

Raman, S., Vernon, R., Thompson, J., Tyka, M., Sadreyev, R., Pei, J., Kim, D., et al. (2009). Structure prediction for CASP8 with all-atom refinement using Rosetta. *Proteins*, 77 Suppl 9, 89-99.

- **Loop Building**

[http://www.rosettacommons.org/manuals/archive/rosetta3.2\\_user\\_guide/ccd\\_loop\\_modeling.html](http://www.rosettacommons.org/manuals/archive/rosetta3.2_user_guide/ccd_loop_modeling.html)

Wang, C., Bradley, P., & Baker, D. (2007). Protein-Protein Docking with Backbone Flexibility. *Journal of Molecular Biology*, 373(2), 503-519.

Mandell, D. J., Coutsias, E. A., & Kortemme, T. (2009). Sub-angstrom accuracy in protein loop reconstruction by robotics-inspired conformational sampling. *Nat Meth*, 6(8), 551-552.

- **Clustering**

[http://www.rosettacommons.org/manuals/archive/rosetta3.2\\_user\\_guide/cluster\\_commands.html](http://www.rosettacommons.org/manuals/archive/rosetta3.2_user_guide/cluster_commands.html)

- **Modeling Membrane Proteins**

[http://www.rosettacommons.org/manuals/archive/rosetta3.2\\_user\\_guide/membrane\\_abinitio.html](http://www.rosettacommons.org/manuals/archive/rosetta3.2_user_guide/membrane_abinitio.html)