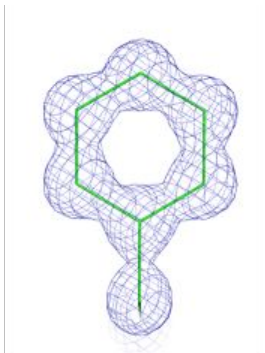


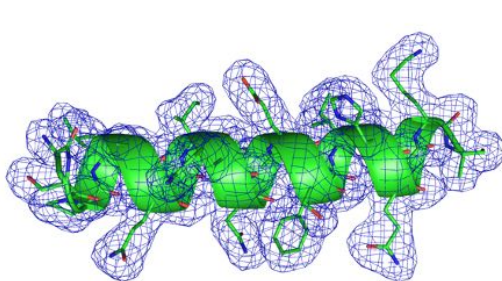
Advanced (low resolution) restraints in Phenix

Oleg V Sobolev

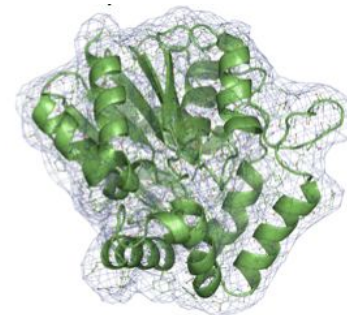
Restraints in structure refinement



At ultra-high resolution ($<1\text{\AA}$) an unrestrained refinement sometimes may be possible.



At 'typical' resolutions ($1-3\text{\AA}$) *standard* restraints are necessary:
covalent bond,
angles, etc



At lower resolution (lower than 3\AA) more restraints needed:
NCS, Secondary Structure,
Ramachandran, ...

Restraints for low resolution

- Secondary structure
- NCS
 - Torsion (X-ray only)
 - Cartesian (=global) (X-ray only)
 - Constraints
- Reference model
 - Torsion
 - Coordinate (=cartesian)
- Ramachandran

Both `phenix.refine` and `phenix.real_space_refine` use (almost) the same machinery to establish restraints

Difference between phenix.refine and phenix.real_space_refine

phenix.refine

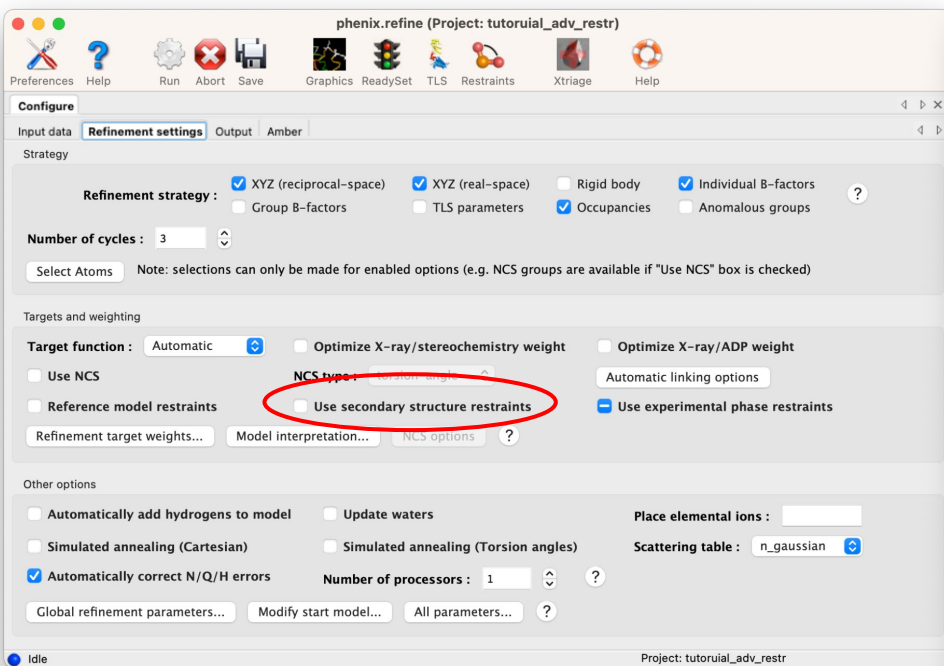
The screenshot shows the 'phenix.refine' GUI window. The title bar reads 'phenix.refine (Project: tutorial_adv_restr)'. The 'Configure' tab is active, with the 'Refinement settings' sub-tab selected. Under 'Strategy', the 'Refinement strategy' section has 'XYZ (reciprocal-space)', 'XYZ (real-space)', and 'Individual B-factors' checked. The 'Number of cycles' is set to 3. The 'Targets and weighting' section has 'Automatic' selected for the target function, and 'Use experimental phase restraints' is checked. The 'Other options' section has 'Automatically correct N/Q/H errors' checked and 'Number of processors' set to 1.

phenix.real_space_refine

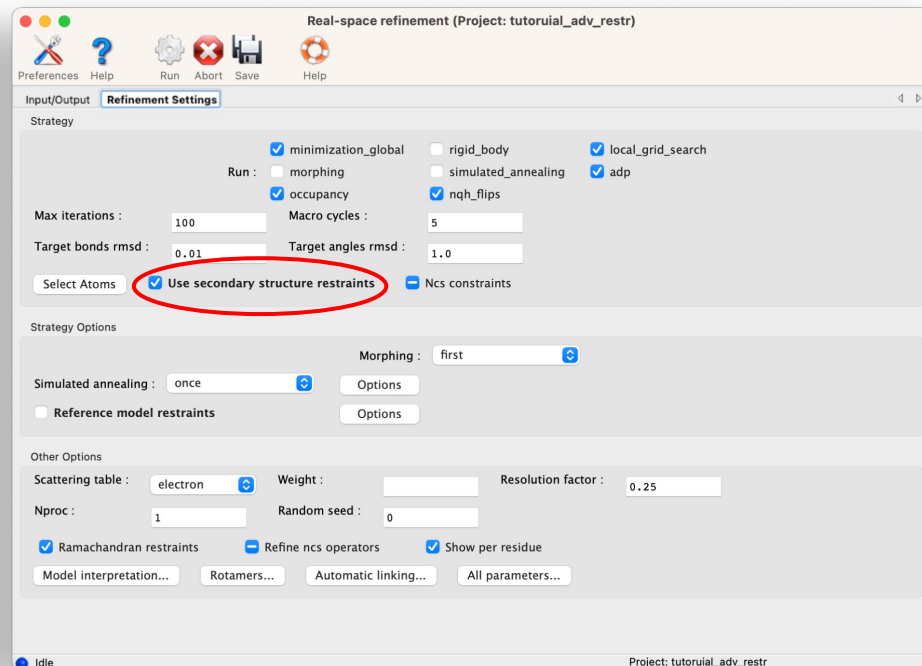
The screenshot shows the 'phenix.real_space_refine' GUI window. The title bar reads 'Real-space refinement (Project: tutorial_adv_restr)'. The 'Refinement Settings' sub-tab is selected. Under 'Strategy', 'minimization_global' and 'local_grid_search' are checked. The 'Run' section has 'occupancy' and 'nqh_flips' checked. 'Max iterations' is 100, 'Macro cycles' is 5, 'Target bonds rmsd' is 0.01, and 'Target angles rmsd' is 1.0. 'Use secondary structure restraints' is checked. Under 'Strategy Options', 'Morphing' is set to 'first'. Under 'Other Options', 'Scattering table' is 'electron' and 'Resolution factor' is 0.25. 'Ramachandran restraints' and 'Show per residue' are checked.

phenix.refine vs phenix.real_space_refine: secondary structure

phenix.refine



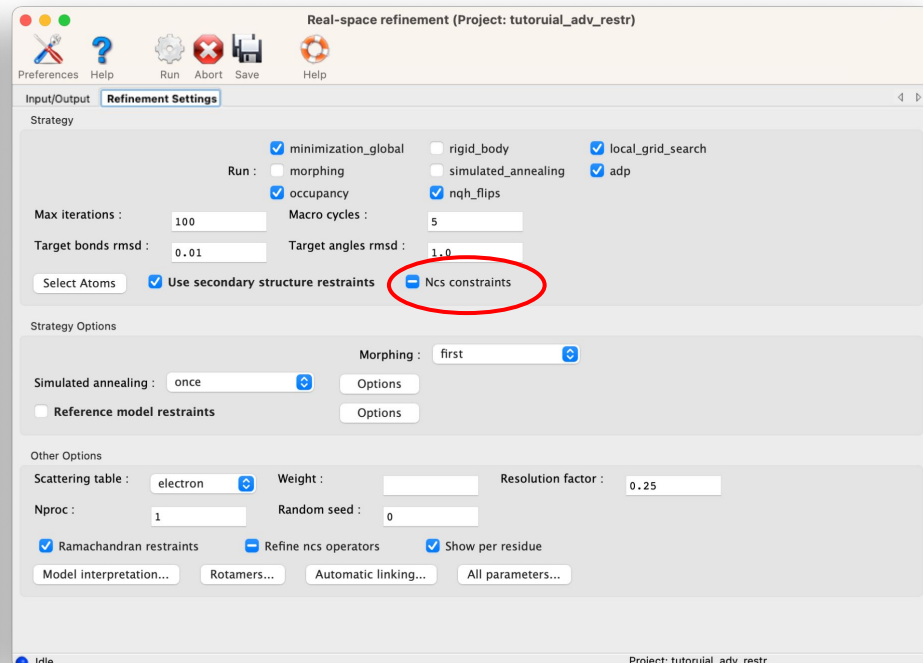
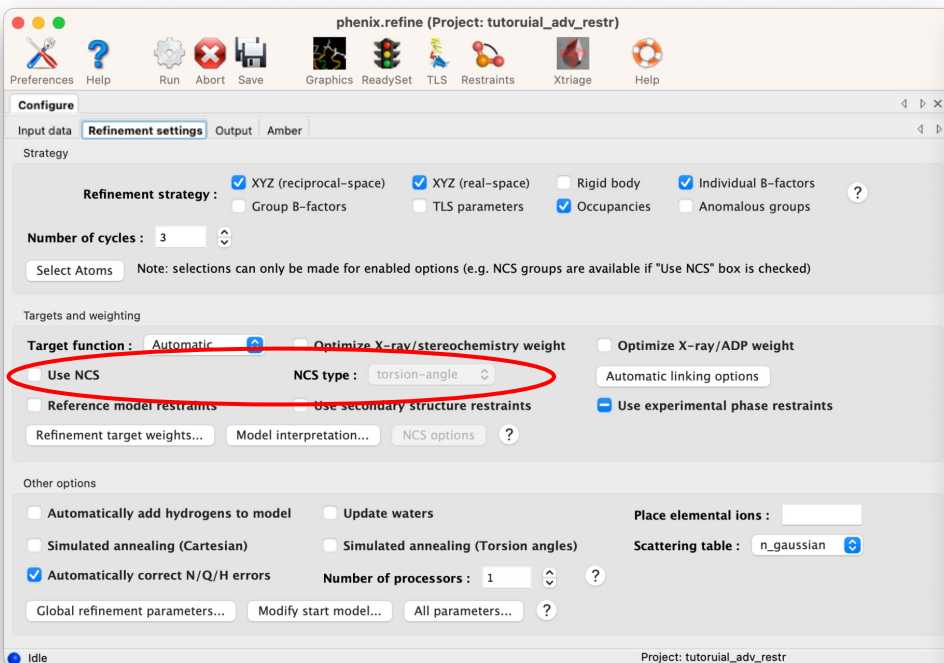
phenix.real_space_refine



phenix.refine vs phenix.real_space_refine: NCS

phenix.refine

phenix.real_space_refine



NCS - only constraints in RSR

phenix.refine vs phenix.real_space_refine: reference model

phenix.refine

The screenshot shows the 'phenix.refine' GUI window. The 'Refinement settings' tab is active. Under 'Refinement strategy', the 'XYZ (real-space)' option is selected. In the 'Targets and weighting' section, the 'Reference model restraints' checkbox is circled in red. Other visible options include 'Use NCS', 'Use secondary structure restraints', and 'Use experimental phase restraints'. The 'Number of cycles' is set to 3.

phenix.real_space_refine

The screenshot shows the 'Real-space refinement' GUI window. The 'Refinement Settings' tab is active. In the 'Strategy Options' section, the 'Reference model restraints' checkbox is circled in red. Other visible options include 'minimization_global', 'local_grid_search', 'adp', and 'Use secondary structure restraints'. The 'Max iterations' is set to 100 and the 'Resolution factor' is 0.25.

phenix.refine vs phenix.real_space_refine: ramachandran

phenix.refine

phenix.refine (Project: tutorial_adv_restr)

Preferences Help Run Abort Save Graphics ReadySet TLS Restraints Xtriage Help

Configure Input data Refinement settings Output Amber

Strategy

Refinement strategy: XYZ (reciprocal-space) XYZ (real-space) Rigid body Individual B-factors
 Group B-factors TLS parameters Occupancies Anomalous groups

Number of cycles: 3

Select Atoms Note: selections can only be made for enabled options (e.g. NCS groups are available if "Use NCS" box is checked)

Targets and weighting

Target function: Automatic Optimize X-ray/stereochemistry weight Optimize X-ray/ADP weight

Use NCS NCS type: torsion-angle Automatic linking options

Reference model restraints Use secondary structure restraints Use experimental phase restraints

Refinement target weights... **Model interpretation...** NCS options ?

Other options

Automatically add hydrogens to model Update waters Place elemental ions:

Simulated annealing (Cartesian) Simulated annealing (Torsion angles) Scattering table: n_gaussian

Automatically correct N/Q/H errors Number of processors: 1

Global refinement parameters... Modify start model... All parameters...

Project: tutorial_adv_restr

phenix.real_space_refine

Real-space refinement (Project: tutorial_adv_restr)

Preferences Help Run Abort Save Help

Input/Output Refinement Settings

Strategy

minimization_global rigid_body local_grid_search
Run: morphing simulated_annealing adp

occupancy nqh_flips

Max iterations: 100 Macro cycles: 5

Target bonds rmsd: 0.01 Target angles rmsd: 1.0

Select Atoms Use secondary structure restraints Ncs constraints

Strategy Options

Morphing: first

Simulated annealing: once Options

Reference model restraints Options

Other Options

Scattering table: electron Weight: Resolution factor: 0.25

Nproc: 1 Random seed: 0

Ramachandran restraints Refine ncs operators Show per residue

Model interpretation... Rotamers... Automatic linking... All parameters...

Project: tutorial_adv_restr

General considerations

Figure out proper restraints:

- Do I have a source of information?
- Was my map symmetrized?
- Does my model have NCS?
- Do I have good enough data to reasonably expect to see difference in NCS copies?

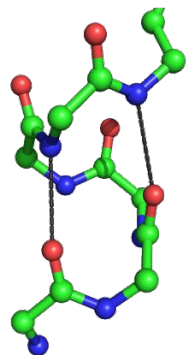
Tell Phenix to establish restraints:

- Click in the GUI
- Prepare (save) parameter file for later use:

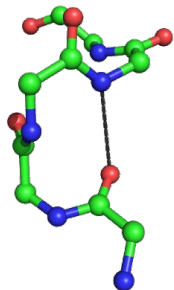
Make sure the restraints are established

- Check the proper locations in .log or .geo file.

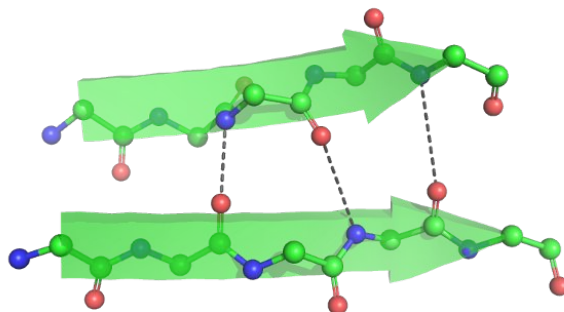
Secondary structure restraints



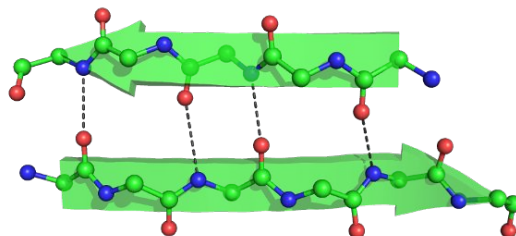
alpha helix



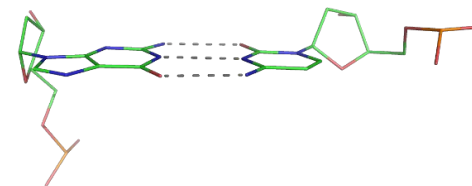
3_{10} helix



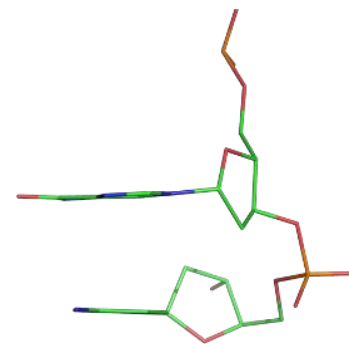
Parallel sheet



antiparallel sheet



Basepair



Stacking pair

Secondary structure restraints how to set

How to set:

- Using GUI

Use secondary structure restraints

Select Atoms

Secondary Structure

Custom Geometry Restraints

Helices

Beta Sheets

Base Pairs

Stacking Pairs

- Using parameter file

- Can be prepared in advance in the GUI or command-line
(phenix.secondary_structure_restraints)

Secondary structure restraints how to check

Log file

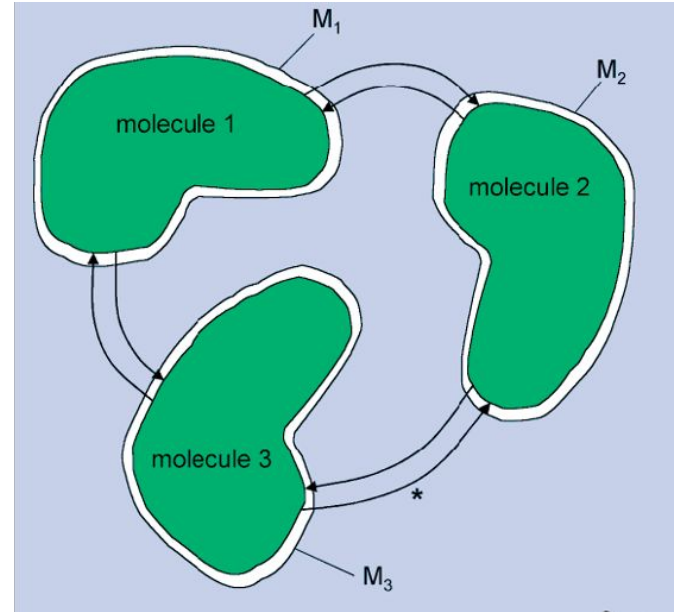
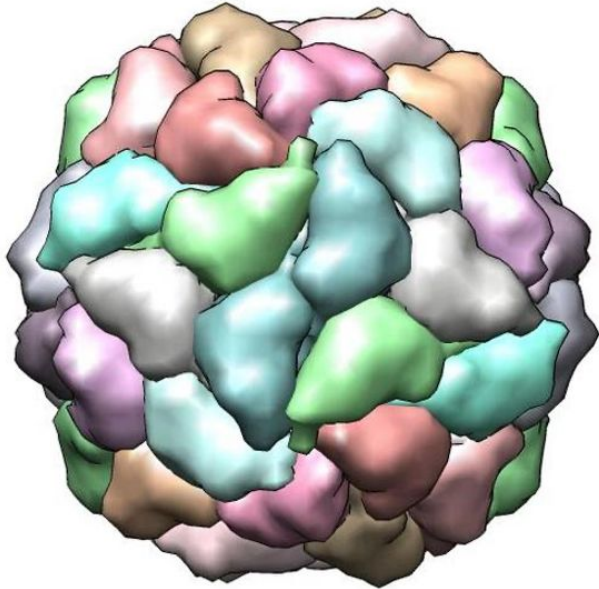
```
Finding SS restraints...
Secondary structure from input PDB file:
  22 helices and 7 sheets defined
  36.1% alpha, 8.0% beta
  0 base pairs and 0 stacking pairs defined.
Time for finding SS restraints: 0.07
Creating SS restraints...
Processing helix chain 'A' and resid 8 through 12
Processing helix chain 'A' and resid 57 through 69
Processing helix chain 'A' and resid 100 through 105
Processing helix chain 'A' and resid 106 through 109
Processing helix chain 'A' and resid 112 through 132
  removed outlier: 3.914A pdb=" N   PHE A 119 " -->
pdb=" O   GLU A 115 " (cutoff:3.500A)
  removed outlier: 3.678A pdb=" N   GLN A 122 " -->
pdb=" O   GLU A 118 " (cutoff:3.500A)
  removed outlier: 3.758A pdb=" N   ASN A 125 " -->
pdb=" O   LYS A 121 " (cutoff:3.500A)
  removed outlier: 3.578A pdb=" N   TYR A 129 " -->
pdb=" O   ASN A 125 " (cutoff:3.500A)
  removed outlier: 4.115A pdb=" N   LEU A 130 " -->
pdb=" O   GLY A 126 " (cutoff:3.500A)
Processing helix chain 'A' and resid 141 through 145
```

.geo file

```
Bond-like restraints: 120
Sorted by residual:
bond pdb=" O   CYS A  30 "
      pdb=" N   TYR A  39 "
      ideal model delta   sigma   weight residual
      2.900 2.225 0.675 5.00e-02 4.00e+02 1.82e+02
bond pdb=" O   ILE B 209 "
      pdb=" N   GLY B 213 "
      ideal model delta   sigma   weight residual
      2.900 2.230 0.670 5.00e-02 4.00e+02 1.80e+02
bond pdb=" O   THR A 112 "
```

```
Secondary Structure restraints around h-bond angle
restraints: 312
Sorted by residual:
angle pdb=" C   MET A 200 "
      pdb=" O   MET A 200 "
      pdb=" N   GLY A 204 "
      ideal model delta   sigma   weight residual
      155.00 107.20 47.80 5.00e+00 4.00e-02 9.14e+01
angle pdb=" C   TYR A  39 "
      pdb=" O   TYR A  39 "
```

NCS (internal symmetry)



- **Constraints:** molecules 1, 2 and 3 are required to be **identical**
- **Torsion restraints:** molecules 1, 2 and 3 are required to be **similar**
- **Cartesian restraints:** molecules 1, 2 and 3 are required to be **similar**

NCS restraints

How to set:

- Using GUI

Use NCS

NCS type : torsion-angle



Select Atoms

Secondary Structure

NCS

Custom Geometry Restraints

- Using parameter file

NCS restraints how to check

Log file

```
===== Process input NCS or/and find new NCS
=====
```

```
Number of NCS groups: 1
```

```
refinement.pdb_interpretation.ncs_group {
  reference = chain 'A'
  selection = chain 'B'
}
```

```
Not restraining NCS-related b-factors:
refinement.ncs.b_factor_weight = 0.0
```

```
Determining NCS matches...
```

```
-----
Torsion NCS Matching Summary:
THR A 2 <=> THR B 2
VAL A 3 <=> VAL B 3
PHE A 4 <=> PHE B 4
ARG A 5 <=> ARG B 5
GLN A 6 <=> GLN B 6
GLU A 7 <=> GLU B 7
```

.geo file

```
NCS torsion angle restraints: 2298
  sinusoidal: 0
  harmonic: 2298
Sorted by residual:
dihedral  pdb=" CB  ARG B  54  "
          pdb=" CG  ARG B  54  "
          pdb=" CD  ARG B  54  "
          pdb=" NE  ARG B  54  "
          ideal  model  delta  harmonic  sigma  weight
residual
-179.55 -51.59 -127.96 0 2.50e+00 1.60e-01
3.60e+01
dihedral  pdb=" N  ARG B  63  "
          pdb=" CA ARG B  63  "
          pdb=" CB ARG B  63  "
          pdb=" CG ARG B  63  "
          ideal  model  delta  harmonic  sigma  weight
residual
201.50 84.68 116.82 0 2.50e+00 1.60e-01
3.60e+01
dihedral  pdb=" CG ARG B  63  "
          pdb=" CD ARG B  63  "
```

NCS restraints user-supplied how to check

Log file

```
Validating user-supplied NCS groups...
```

```
  Validating:
```

```
ncs_group {  
  reference = "chain A"  
  selection = "chain B"  
}
```

```
  OK. All atoms were included in validated selection.
```

```
Found NCS groups:
```

```
ncs_group {  
  reference = chain 'A'  
  selection = chain 'B'  
}
```


Reference model restraints

How to set:

- Using GUI
- Using parameter file:
 - any number of reference files, any match of chains

Reference model restraints

All parameters...

Reference model

Reference group...

Reference group...

Reference group (1)

Selection in the reference model : ?

Selection in the refined model : ?

User level: Basic

Reference model how to check

Log file

```
*** Adding Reference Model Restraints (torsion) ***

reference file:
/Users/oleg/Documents/phenix/testing/GUI/adv_restr_tutorial_files/4pf4.pdb
Model:           Reference:
-----
Reference Model Matching Summary:

reference file:
/Users/oleg/Documents/phenix/testing/GUI/adv_restr_tutorial_files/4pf4.pdb

Model:           Reference:
THR A    2 <=====> THR A    2
VAL A    3 <=====> VAL A    3
PHE A    4 <=====> PHE A    4
ARG A    5 <=====> ARG A    5
GLN A    6 <=====> GLN A    6
```

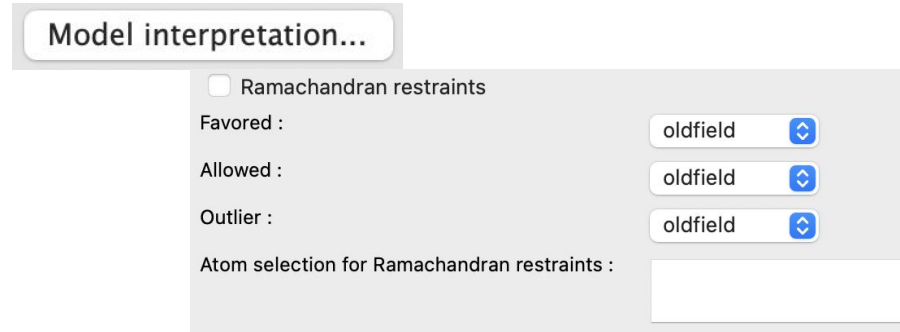
.geo file

```
Reference torsion angle restraints: 2516
  sinusoidal: 0
  harmonic: 2516
Sorted by residual:
dihedral  pdb=" CG  ARG A  5  "
           pdb=" CD  ARG A  5  "
           pdb=" NE  ARG A  5  "
           pdb=" CZ  ARG A  5  "
           ideal  model  delta  harmonic  sigma  weight
residual
169.13 -92.48 -98.39 0 1.00e+00 1.00e+00
2.25e+02
dihedral  pdb=" CA  TYR A 12  "
           pdb=" CB  TYR A 12  "
           pdb=" CG  TYR A 12  "
           pdb=" CD1 TYR A 12  "
           ideal  model  delta  harmonic  sigma  weight
residual
-77.35 79.53 -156.88 0 1.00e+00 1.00e+00
2.25e+02
dihedral  pdb=" CA  LEU A 19  "
           pdb=" CB  LEU A 19  "
           pdb=" CG  LEU A 19  "
           pdb=" CD1 LEU A 19  "
```

Ramachandran restraints

How to set:

- Using GUI



- Using parameter file

Ramachandran how to check

Log file

```
1096 Ramachandran restraints generated.  
548 Oldfield, 0 Emsley, 548 emsley8k and 0  
Phi/Psi/2.
```

.geo file

```
Ramachandran plot restraints (Oldfield): 548  
Sorted by residual:  
phi-psi angles formed by          residual  
  pdb=" C   THR B 180 "           3.60e+02  
  pdb=" N   PRO B 181 "             
  pdb=" CA  PRO B 181 "             
  pdb=" C   PRO B 181 "             
  pdb=" N   GLU B 182 "             
<...>  
  
Ramachandran plot restraints (Emsley): 0  
Sorted by residual:  
  
Ramachandran plot restraints (emsley8k): 548  
Sorted by residual:  
phi-psi angles formed by          residual  
  pdb=" C   HIS A 73 "            1.00e+01  
  pdb=" N   PRO A 74 "              
  pdb=" CA  PRO A 74 "              
  pdb=" C   PRO A 74 "              
  pdb=" N   ASN A 75 "              
<...>  
Ramachandran plot restraints (phi/psi/2): 0  
Sorted by residual:
```

Additional information

Atom selection syntax

chain B
residue 42
chain B and resseq 42

Tutorial

6:01

Explaining the atom selection syntax

https://phenix-online.org/documentation/reference/atom_selections.html

Secondary Structure Restraints

Tutorial

6:23

How to use secondary structure restraints

https://phenix-online.org/documentation/reference/secondary_structure.html

NCS search https://phenix-online.org/documentation/reference/simple_ncs_from_pdb.html

Thank you.