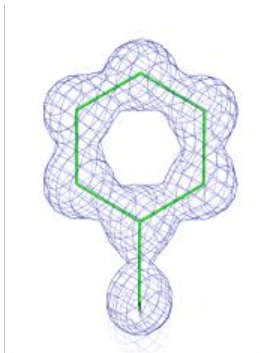


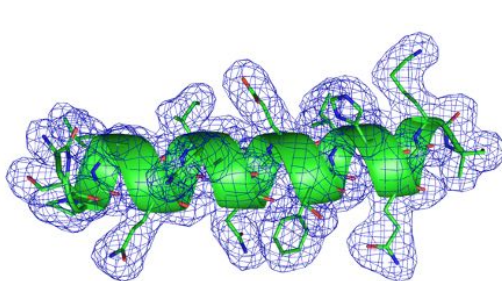
# Advanced (low resolution) restraints in Phenix

Oleg V Sobolev

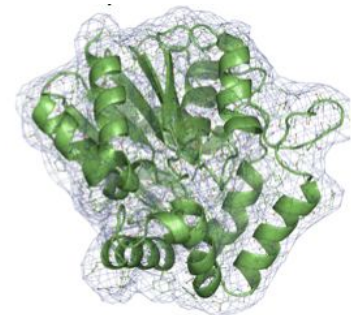
# Restraints in structure refinement



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  $3\text{\AA}$ ) more restraints needed:  
NCS, Secondary Structure,  
Ramachandran, ...

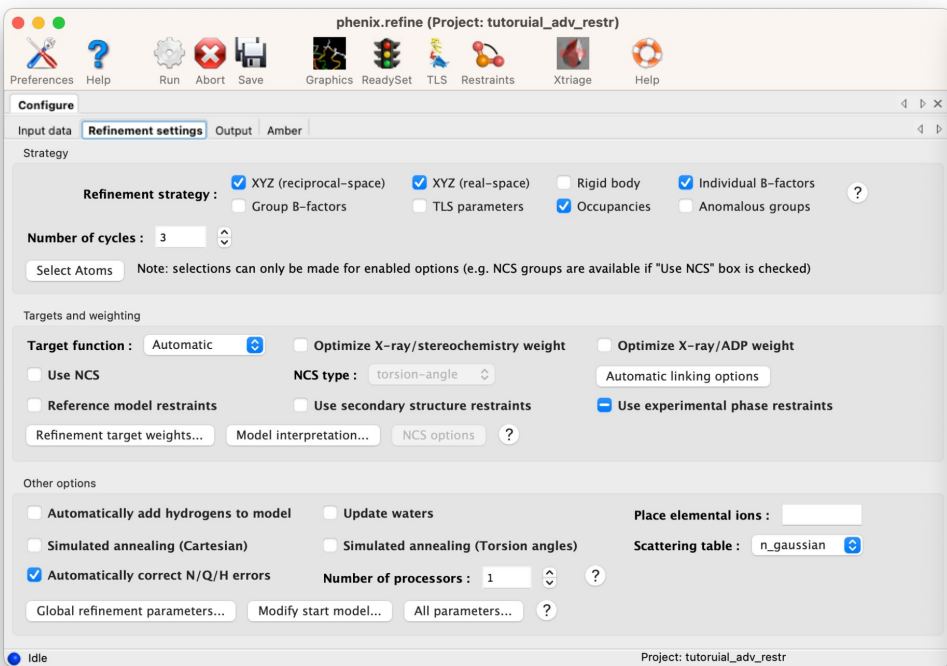
## Restraints for low resolution

- 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

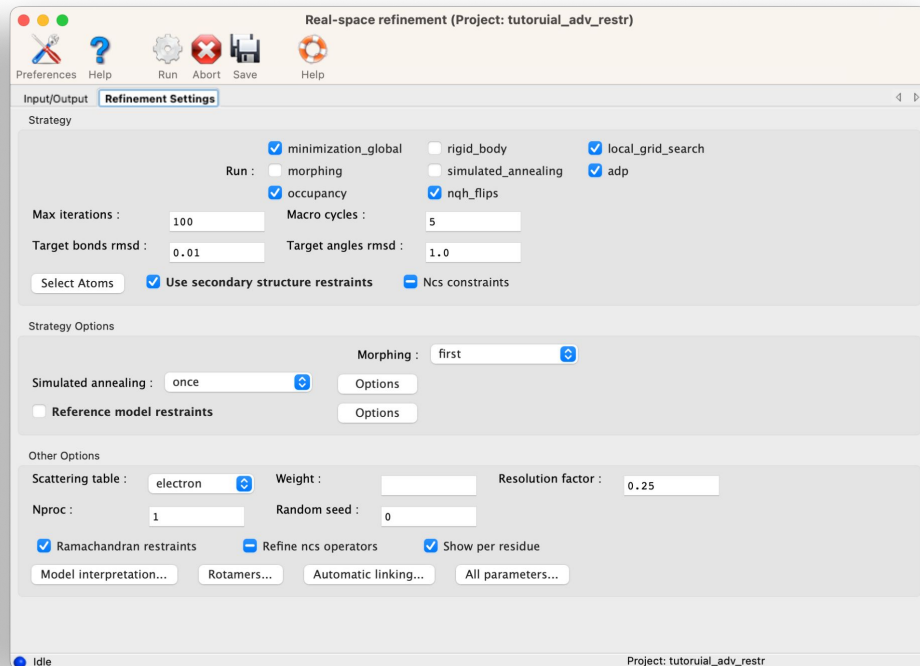
# Difference between phenix.refine and phenix.real\_space\_refine

## 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. '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 active. Under 'Strategy', 'minimization\_global' and 'local\_grid\_search' are checked. 'Run' options include '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

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 'Use secondary structure restraints' checkbox is highlighted with a red circle. Other visible options include 'Use NCS', 'Reference model restraints', and 'Use experimental phase restraints'. The 'Number of cycles' is set to 3.

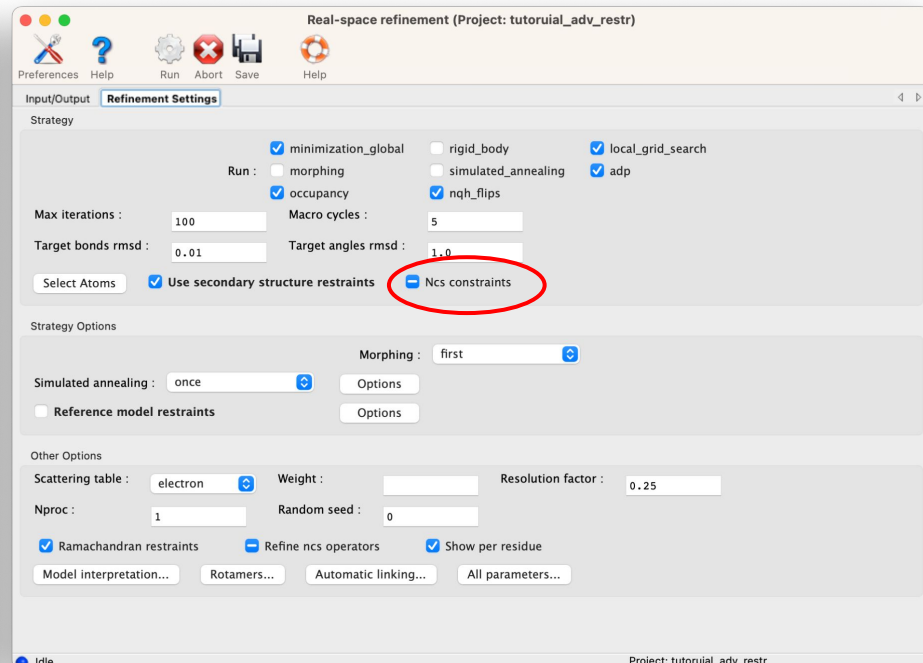
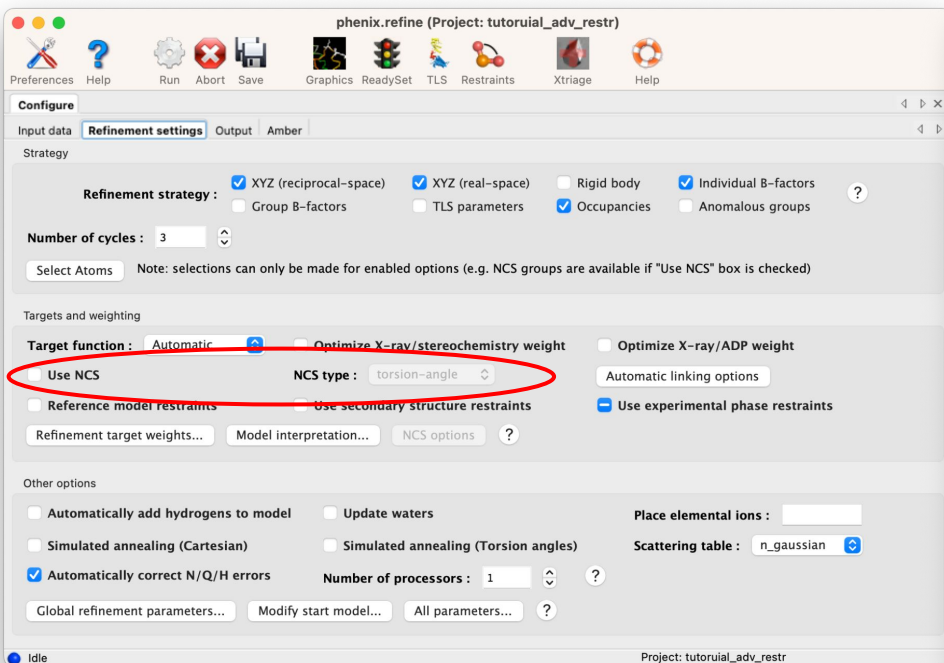
## phenix.real\_space\_refine

The screenshot shows the 'Real-space refinement' GUI window. The 'Refinement Settings' tab is active. In the 'Strategy' section, the 'Use secondary structure restraints' checkbox is highlighted with a red circle. Other visible options include 'minimization\_global', 'rigid\_body', 'local\_grid\_search', 'morphing', 'simulated\_annealing', 'adp', 'occupancy', and 'nqh\_flips'. The 'Max iterations' is set to 100, and 'Target bonds rmsd' is 0.01.

# 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 'Target bonds rmsd' is 0.01.

# phenix.refine vs phenix.real\_space\_refine: ramachandran

## 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 'Model interpretation...' button is circled in red. Other visible options include 'Use secondary structure restraints' and 'Use experimental phase restraints'.

## phenix.real\_space\_refine

The screenshot shows the 'Real-space refinement' GUI window. The 'Refinement Settings' tab is active. In the 'Other Options' section, the 'Ramachandran restraints' checkbox is checked and circled in red. Other visible options include 'Use secondary structure restraints' and 'Ncs constraints'.



# 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?

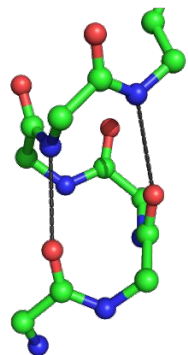
Tell Phenix to establish restraints:

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

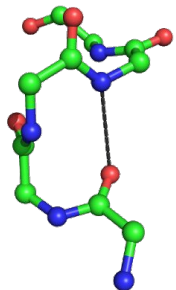
Make sure the restraints are established

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

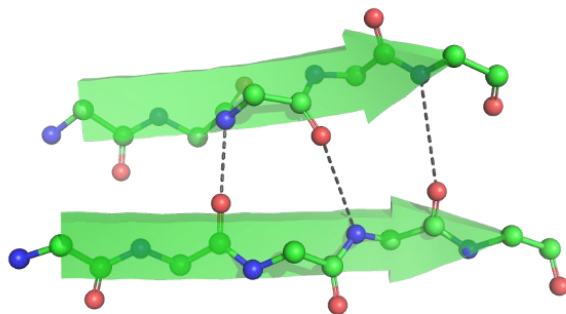
# Secondary structure restraints



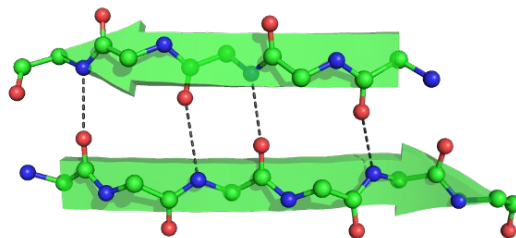
alpha helix



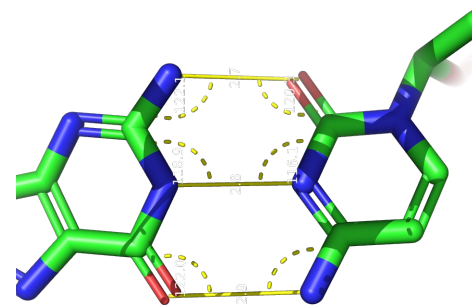
$3_{10}$  helix



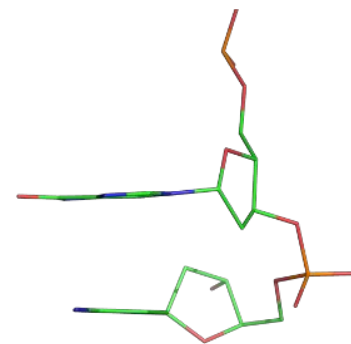
Parallel sheet



antiparallel sheet



Basepair

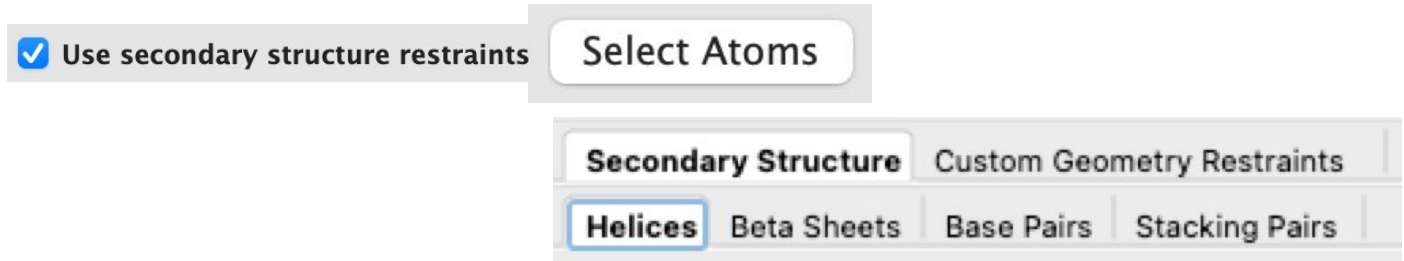


Stacking pair

# Secondary structure restraints how to set

How to set:

- Using GUI



- 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

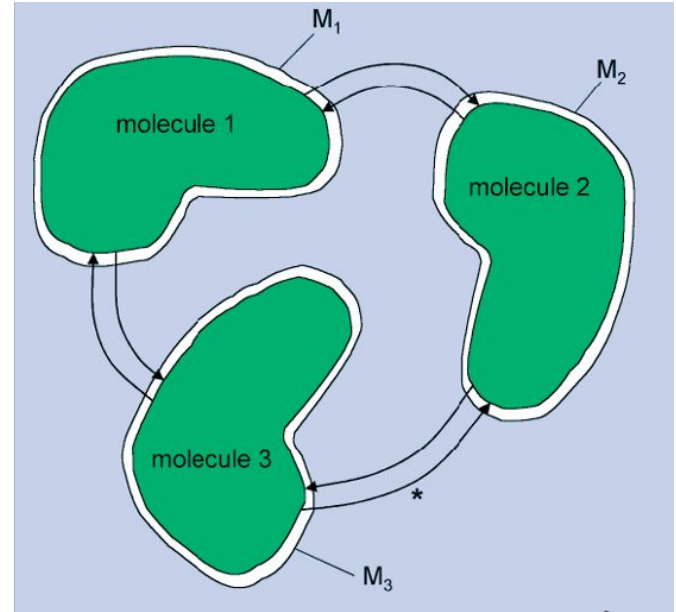
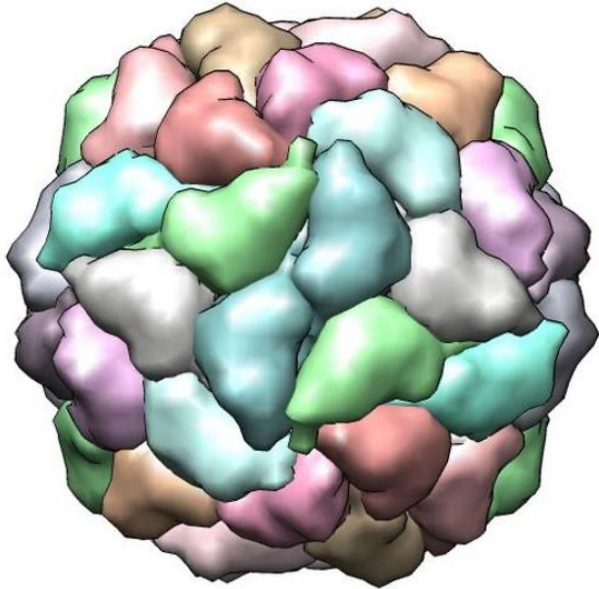
```
Finding SS restraints...
Secondary structure from input PDB file:
  22 helices and 7 sheets defined
  36.1% alpha, 8.0% beta
  0 base pairs and 0 stacking pairs defined.
Time for finding SS restraints: 0.07
Creating SS restraints...
Processing helix chain 'A' and resid 8 through 12
Processing helix 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
```

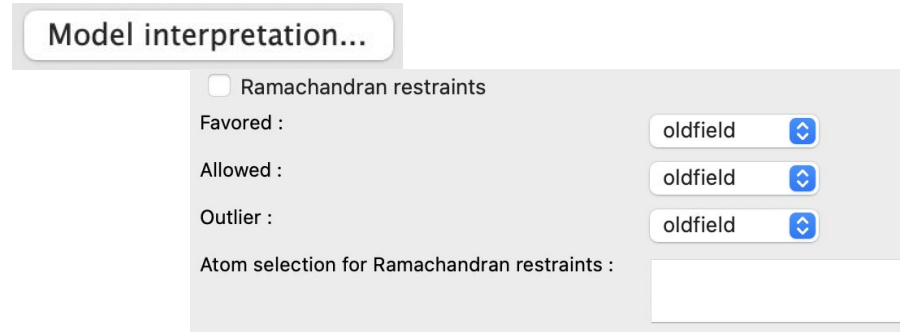
## .geo file

```
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](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](https://phenix-online.org/documentation/reference/secondary_structure.html)

Changing parameters

List of all available keywords

```
refinement:  Range of parameters for structure refinement with phenix.refine
model_building:  Range of options for model building
ligand:  Range of options for ligand fitting
geometry_restraints:  Range of options for geometry restraints
pdb:  Range of options for PDB file handling
```

Tutorial

4:05

Changing custom parameters in phenix.refine

NCS search [https://phenix-online.org/documentation/reference/simple\\_ncs\\_from\\_pdb.html](https://phenix-online.org/documentation/reference/simple_ncs_from_pdb.html)

Thank you.