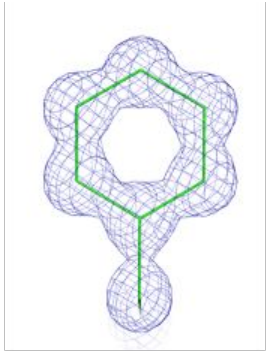
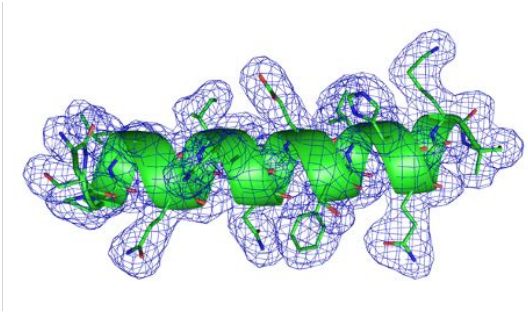
Advanced (low resolution) restraints in Phenix

Oleg V Sobolev

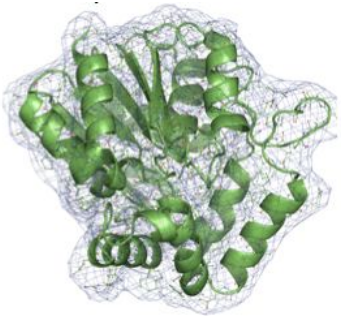
Restraints in structure refinement



At ultra-high resolution (<1Å) an unrestrained refinement sometimes may be possible.



At ‘typical’resolutions (1-3Å) *standard* restraints are necessary: covalent bond, angles, etc



At lower resolution (lower than 3Å) more restraints needed: NCS, Secondary Structure, Ramachandran, …

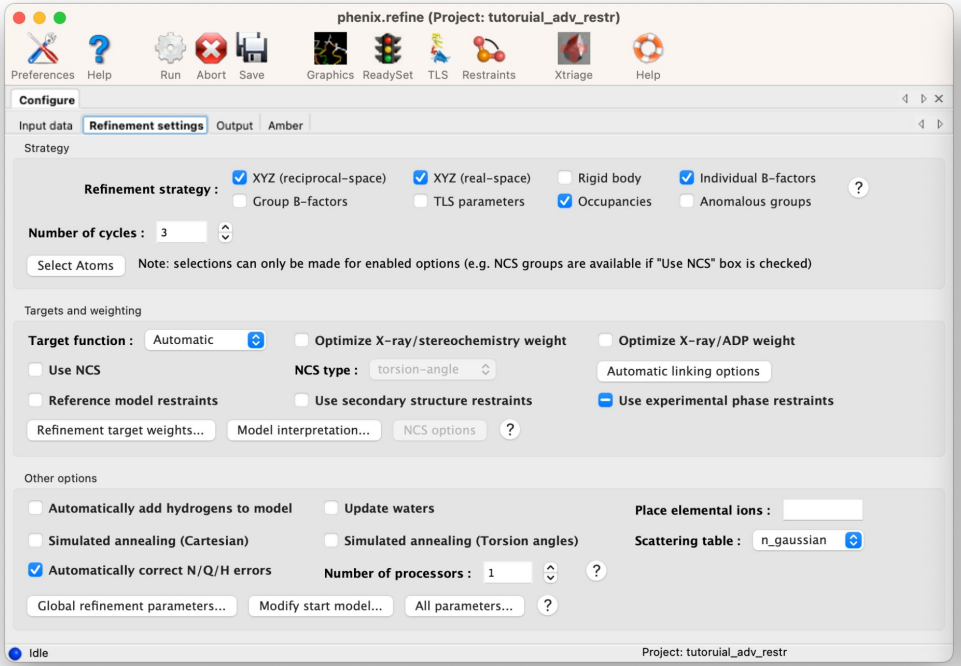
Restraints for low resolution

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

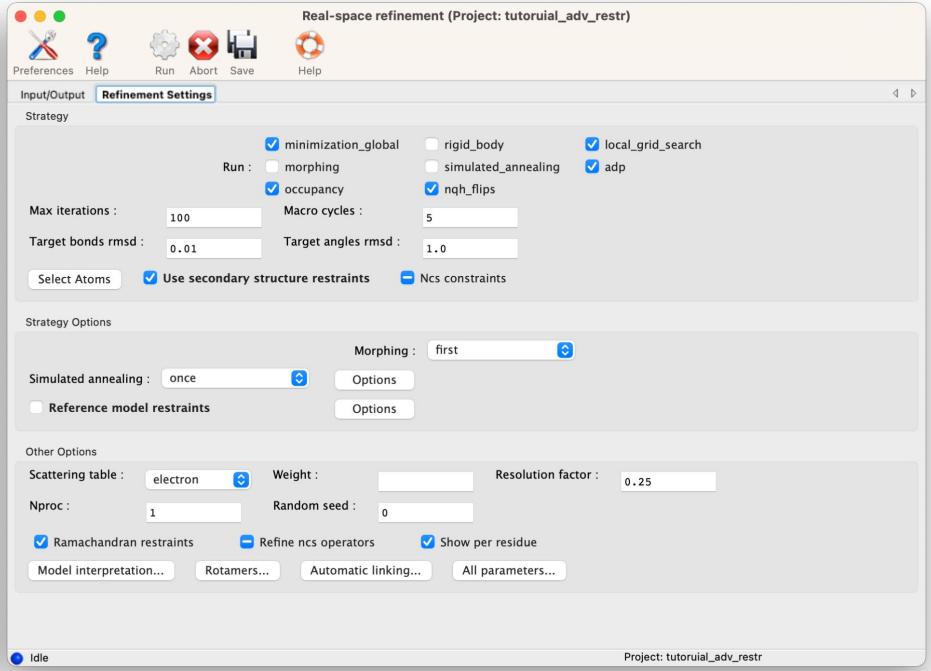
Both phenix.refine and phenix.real_space_refine use (almost) the same machinery to establish restraints

Difference between phenix.refine and phenix.real_space_refine

phenix.refine

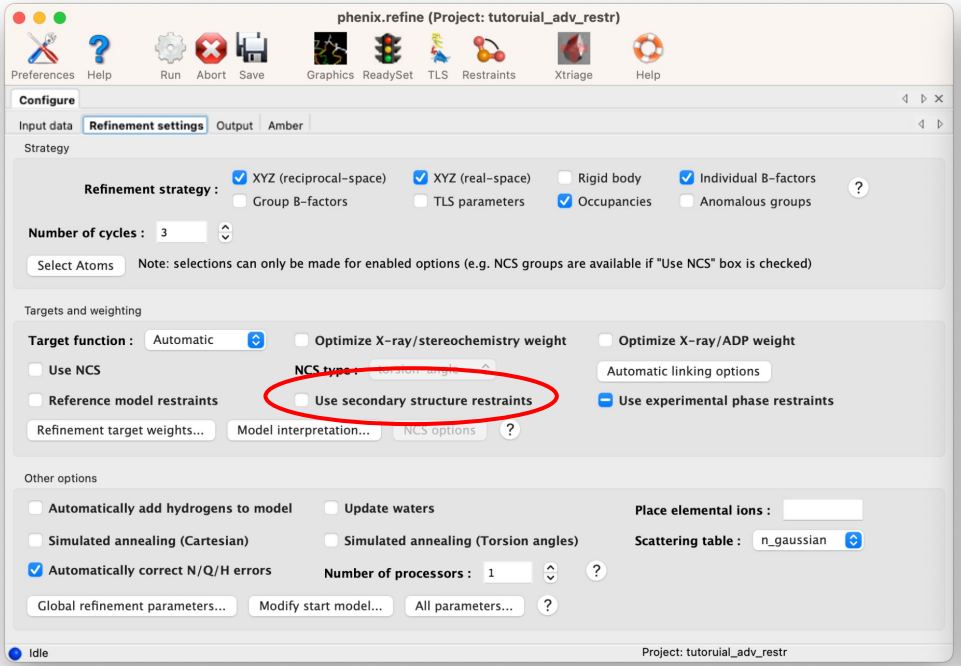


phenix.real_space_refine

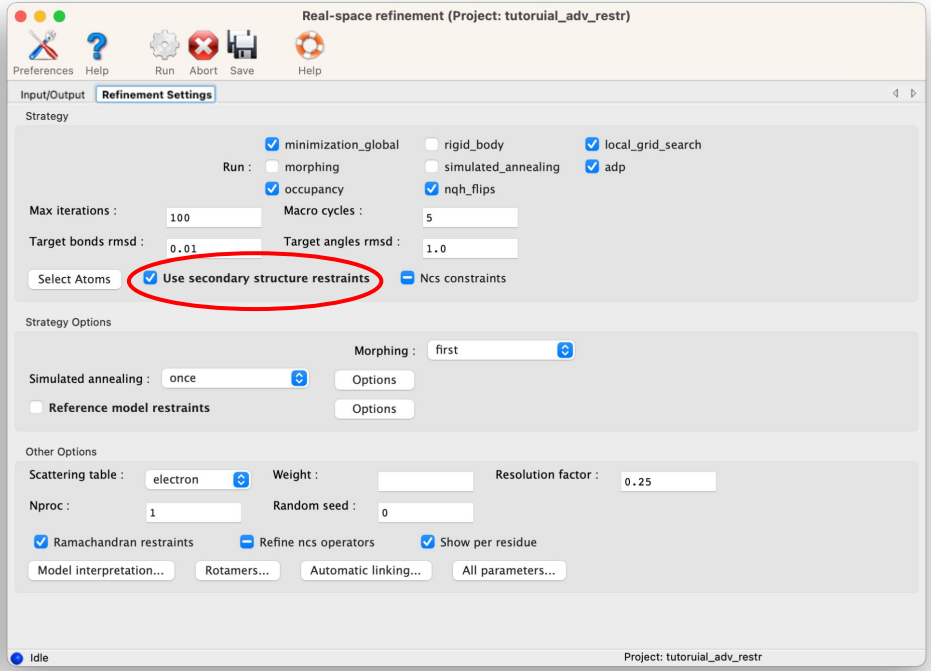


phenix.refine vs phenix.real_space_refine: secondary structure

phenix.refine

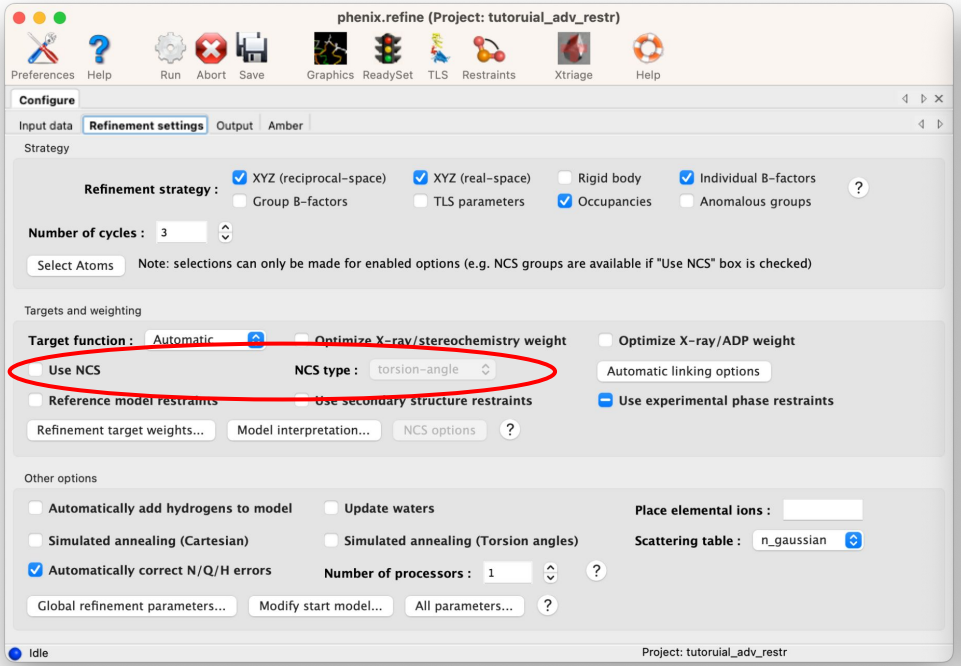


phenix.real_space_refine

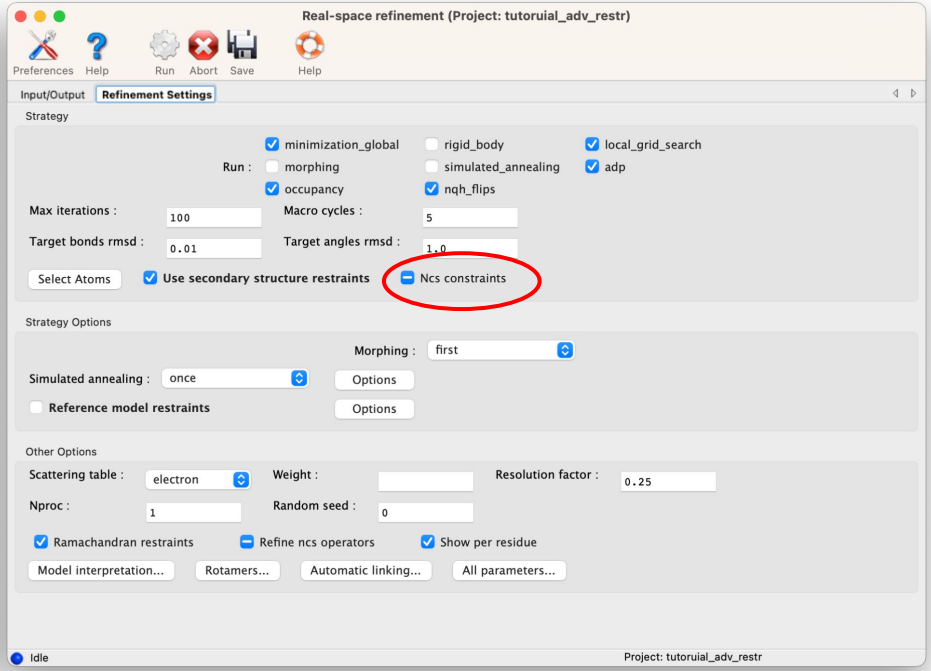


phenix.refine vs phenix.real_space_refine: NCS

phenix.refine



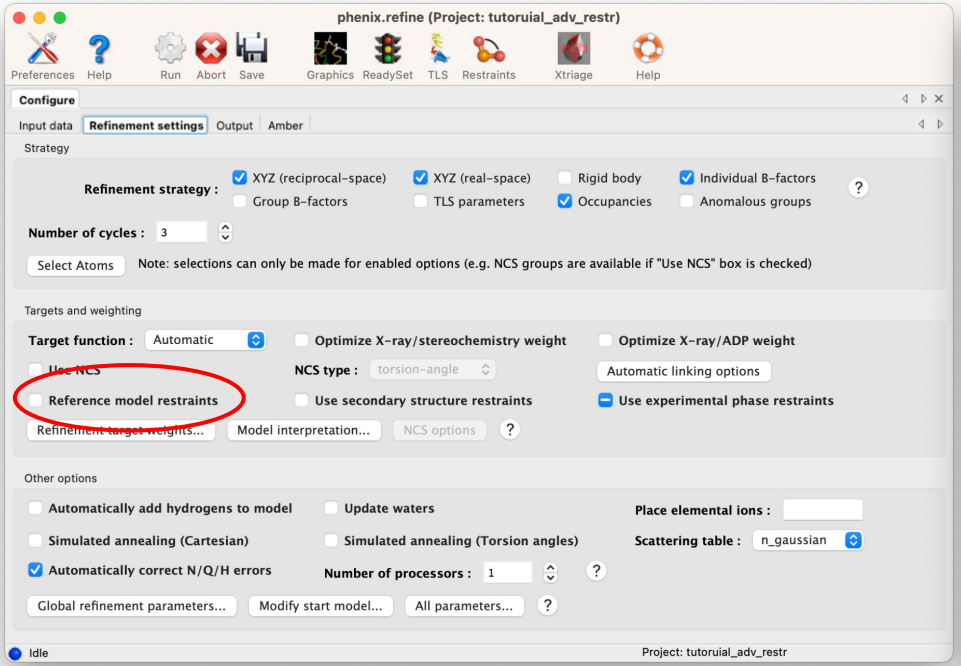
phenix.real_space_refine



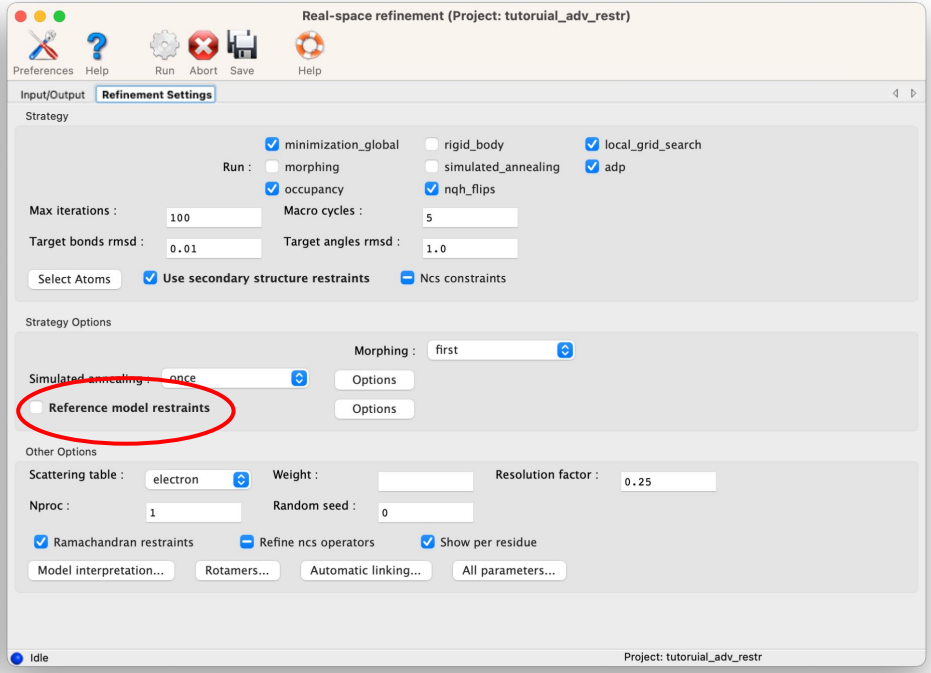
NCS - only constraints in RSR

phenix.refine vs phenix.real_space_refine: reference model

phenix.refine

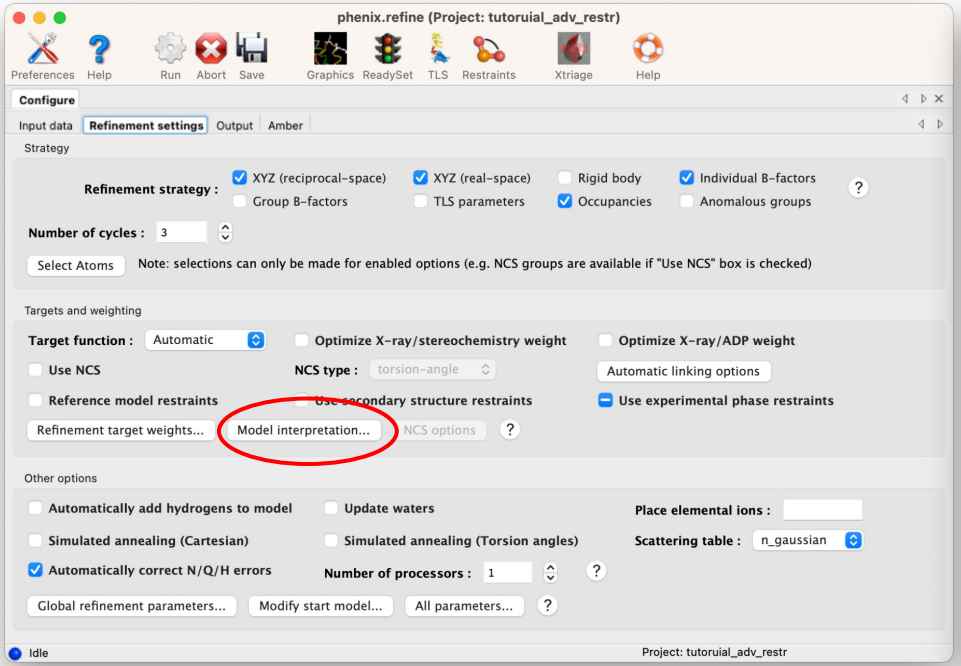


phenix.real_space_refine

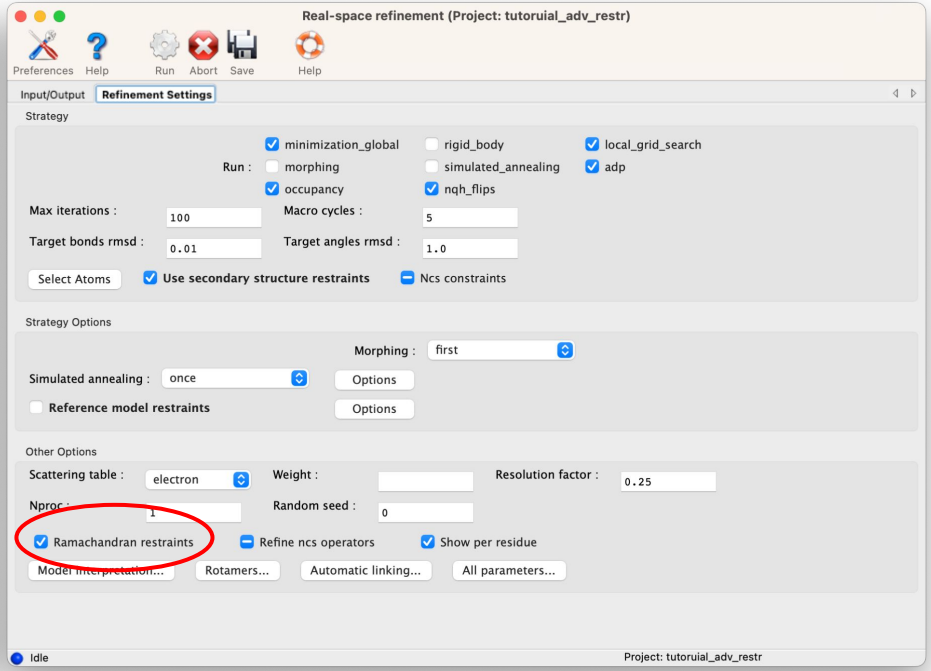


phenix.refine vs phenix.real_space_refine: ramachandran

phenix.refine



phenix.real_space_refine



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?

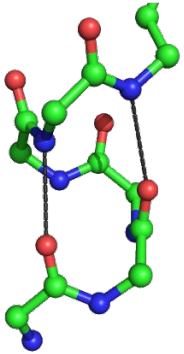
Tell Phenix to establish restraints:

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

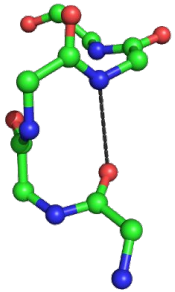
Make sure the restraints are established

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

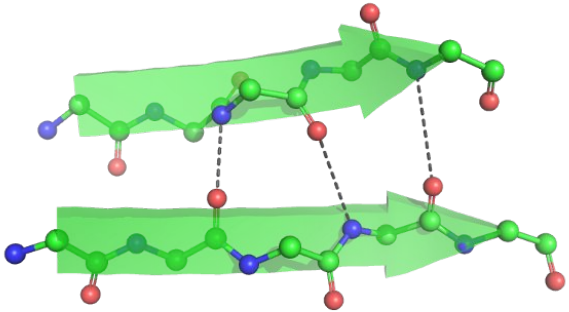
Secondary structure restraints



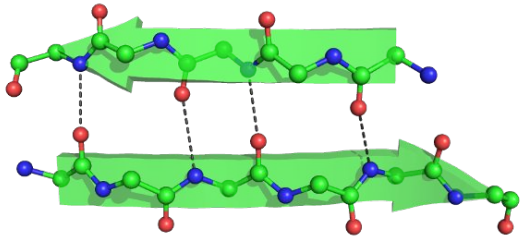
alpha helix



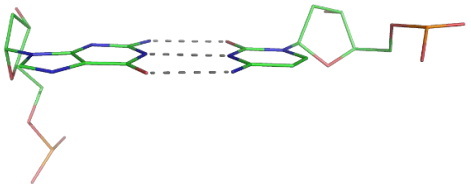
3_{10} helix



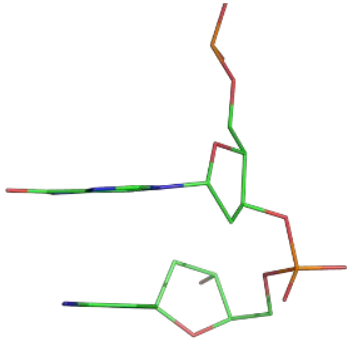
Parallel sheet



antiparallel sheet



Basepair

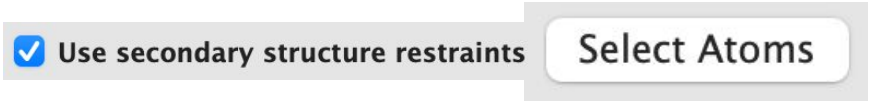


Stacking pair

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

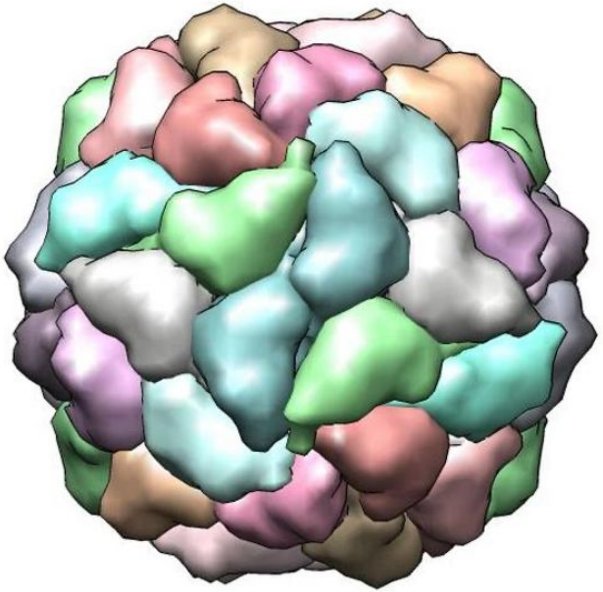
Log file

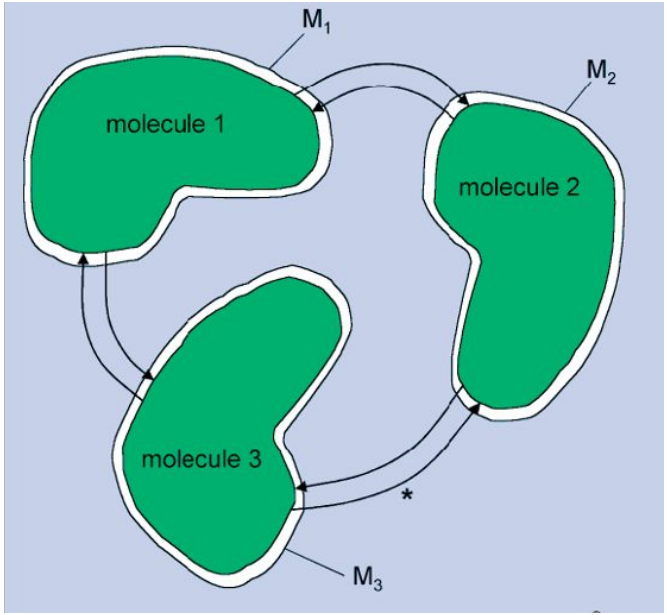
```
  Finding SS restraints...
    Secondary structure from input PDB file:
      22 helices and 7 sheets defined
      36.1% alpha, 8.0% beta
      0 base pairs and 0 stacking pairs defined.
    Time for finding SS restraints: 0.07
  Creating SS restraints...
    Processing helix  chain 'A' and resid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



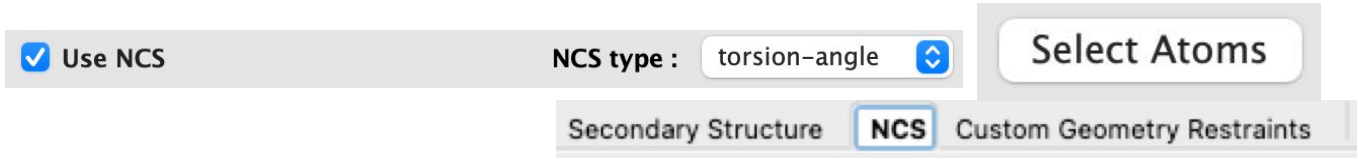


- **Constraints**: molecules 1, 2 and 3 are required to be identical
- **Torsion restraints**: molecules 1, 2 and 3 are required to be similar
- **Cartesian restraints**: molecules 1, 2 and 3 are required to be similar

NCS restraints

How to set:

- Using GUI



- 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

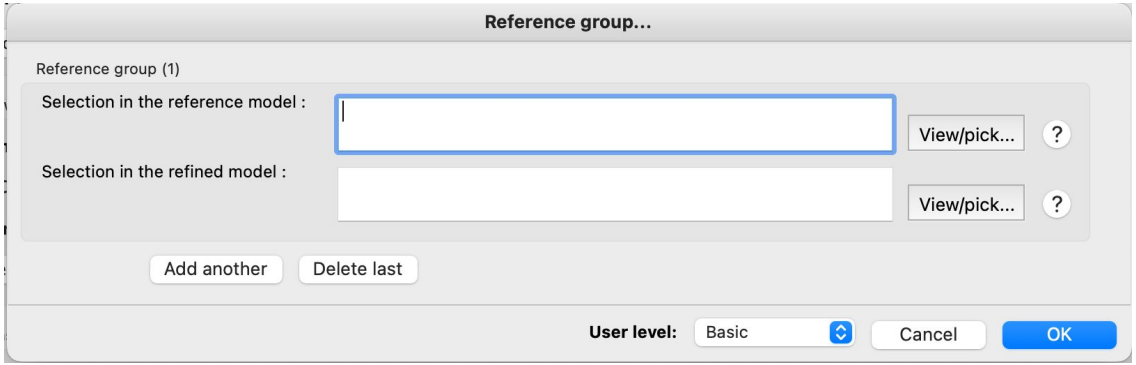
Log file

```
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 how to check

Log file

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

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

reference file:
/Users/oleg/Documents/phenix/testing/GUI/adv_restr_tuto
rial_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

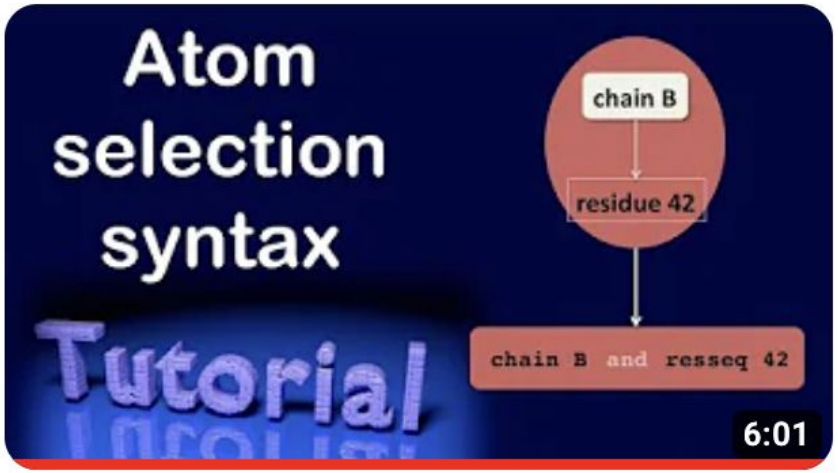
Log file

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

.geo file

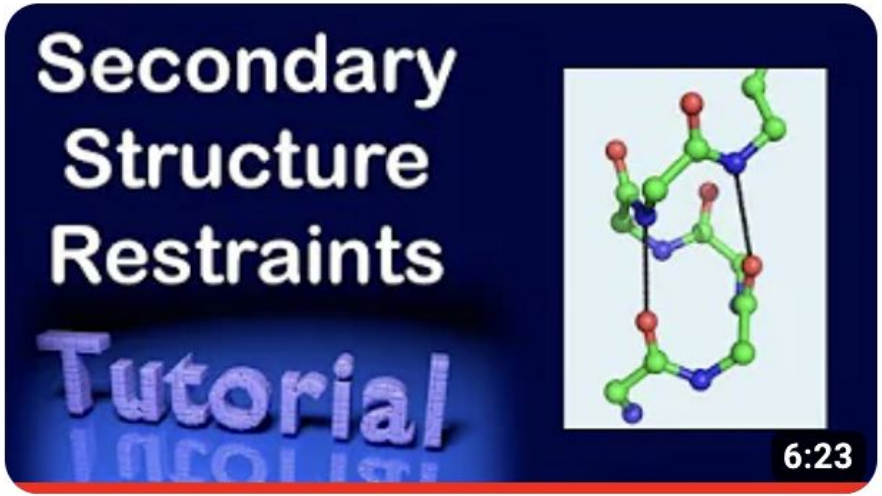
```
Ramachandran plot restraints (Oldfield): 548
Sorted by residual:
phi-psi angles formed by                residual
    pdb=" C   THR B 180 "               3.60e+02
    pdb=" N   PRO B 181 "
    pdb=" CA  PRO B 181 "
    pdb=" C   PRO B 181 "
    pdb=" N   GLU B 182 "
<...>

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



Explaining the atom selection syntax

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



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.