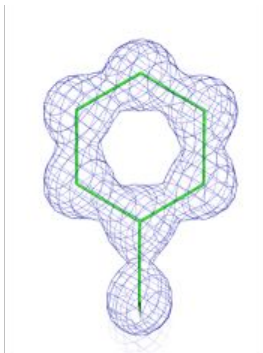


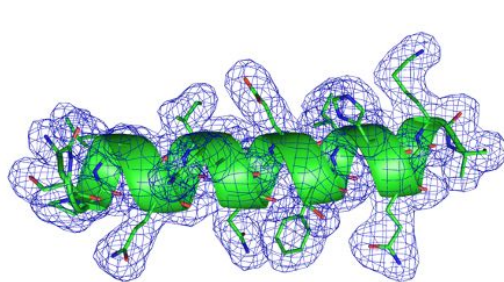
Advanced (low resolution) restraints in Phenix

Oleg V Sobolev

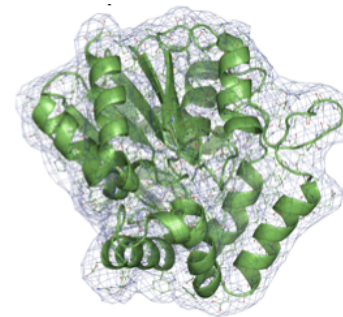
Restraints in structure refinement



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



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



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

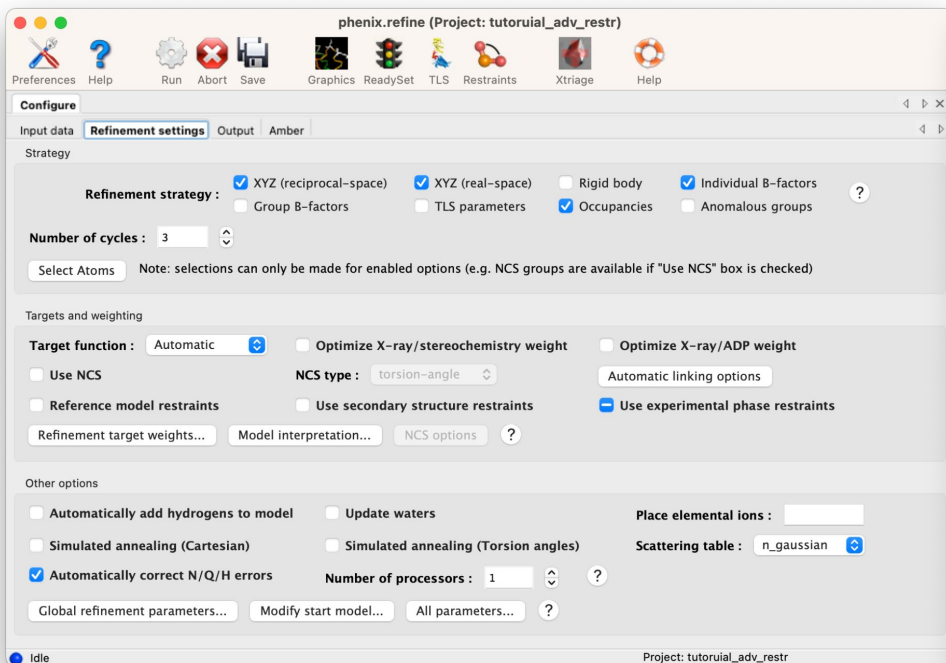
Restraints for low resolution

- 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

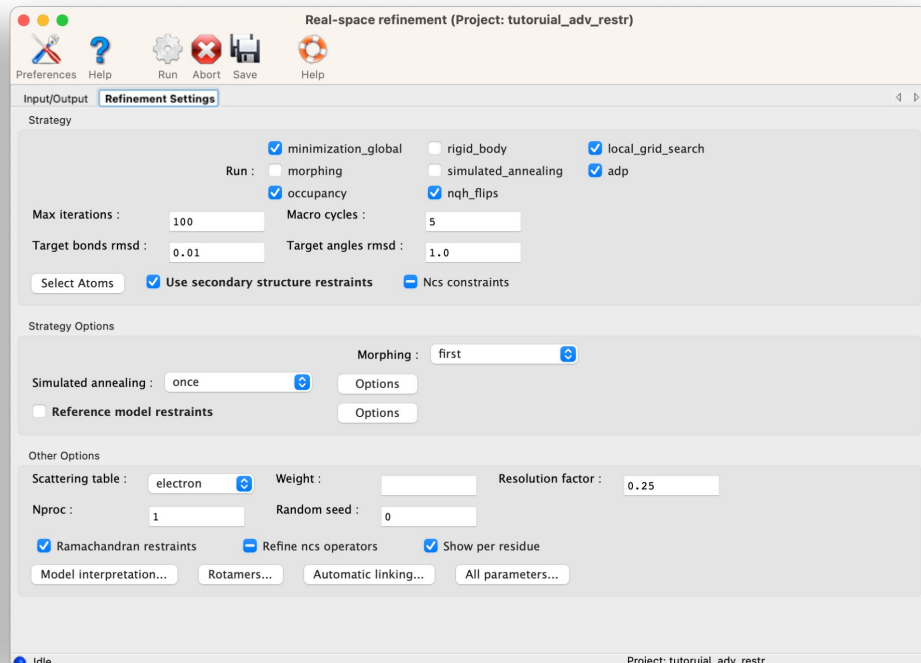
Difference between phenix.refine and phenix.real_space_refine

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. The 'Refinement strategy' section has checkboxes for 'XYZ (reciprocal-space)', 'XYZ (real-space)', 'Rigid body', 'Individual B-factors', 'Group B-factors', 'TLS parameters', 'Occupancies', and 'Anomalous groups'. The 'Number of cycles' is set to 3. The 'Targets and weighting' section includes options for 'Target function' (Automatic), 'Optimize X-ray/stereochemistry weight', 'Optimize X-ray/ADP weight', 'Use NCS', 'Reference model restraints', 'Use secondary structure restraints', and 'Use experimental phase restraints'. The 'Other options' section at the bottom includes checkboxes for 'Automatically add hydrogens to model', 'Update waters', 'Simulated annealing (Cartesian)', 'Simulated annealing (Torsion angles)', 'Automatically correct N/Q/H errors', 'Place elemental ions', 'Scattering table' (n_gaussian), and 'Number of processors' (1).

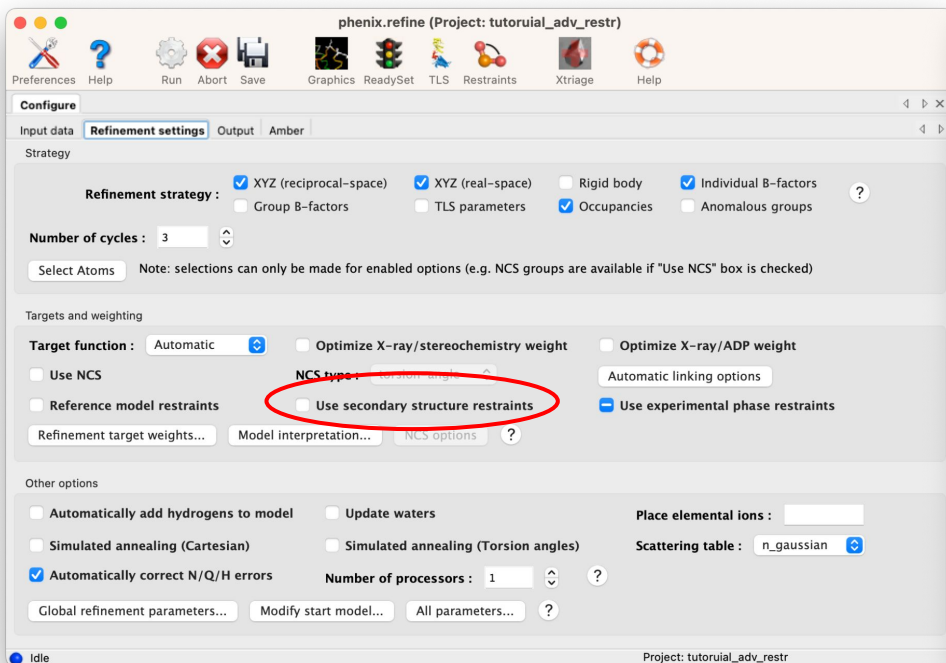
phenix.real_space_refine



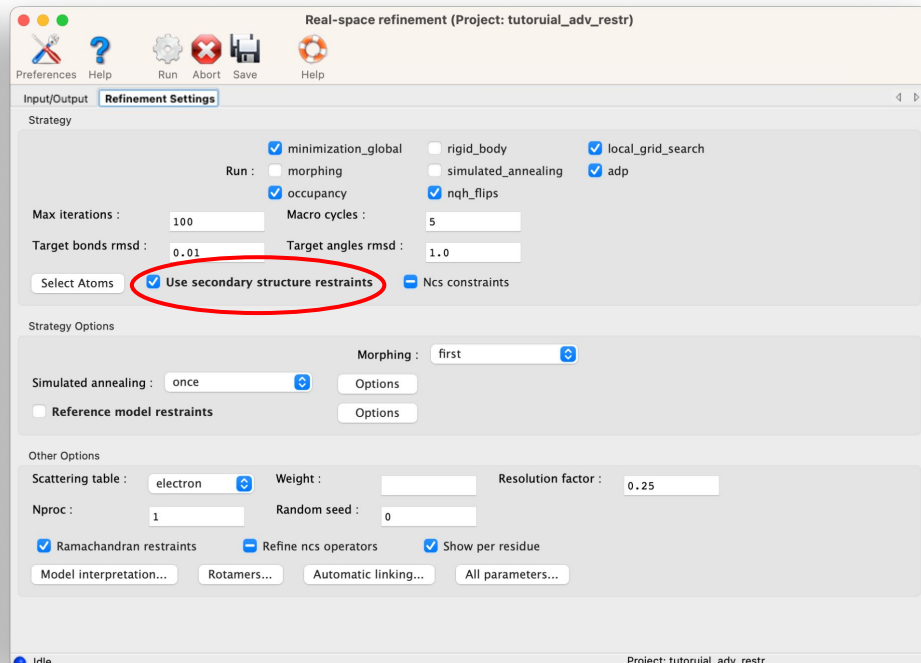
The screenshot shows the 'phenix.real_space_refine' GUI window. The title bar reads 'Real-space refinement (Project: tutorial_adv_restr)'. The 'Refinement Settings' sub-tab is selected. The 'Strategy' section includes checkboxes for 'minimization_global', 'rigid_body', 'local_grid_search', 'morphing', 'simulated_annealing', 'adp', 'occupancy', and 'nqh_flips'. The 'Max iterations' is set to 100, 'Macro cycles' to 5, 'Target bonds rmsd' to 0.01, and 'Target angles rmsd' to 1.0. The 'Select Atoms' button is present, along with checkboxes for 'Use secondary structure restraints' and 'Ncs constraints'. The 'Strategy Options' section includes a 'Morphing' dropdown set to 'first' and buttons for 'Options'. The 'Other Options' section includes 'Scattering table' (electron), 'Weight', 'Resolution factor' (0.25), 'Nproc' (1), 'Random seed' (0), and checkboxes for 'Ramachandran restraints', 'Refine ncs operators', and 'Show per residue'. At the bottom, there are buttons for 'Model interpretation...', 'Rotamers...', 'Automatic linking...', and 'All parameters...'.

phenix.refine vs phenix.real_space_refine: secondary structure

phenix.refine

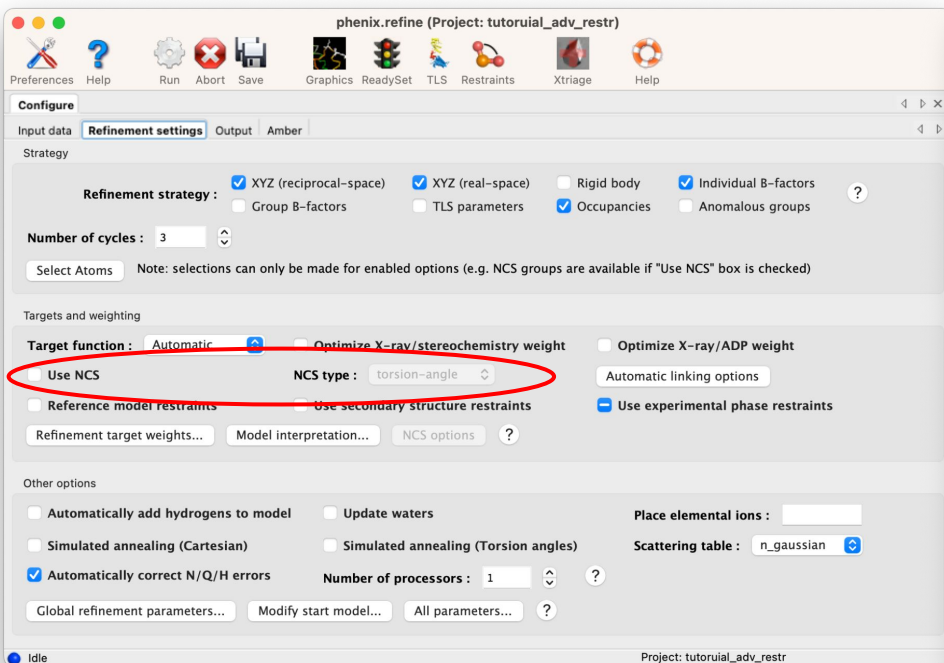


phenix.real_space_refine

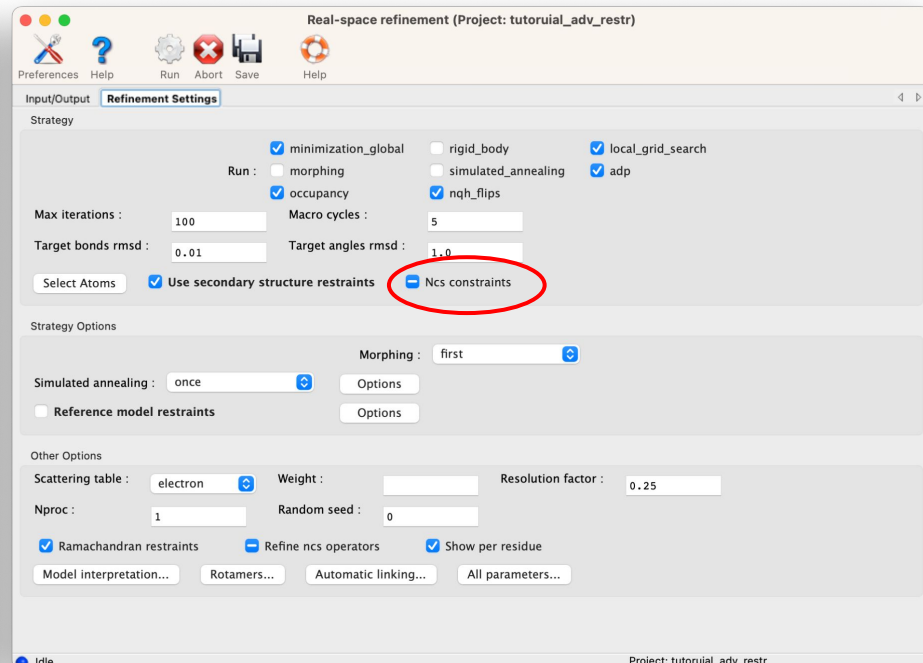


phenix.refine vs phenix.real_space_refine: NCS

phenix.refine



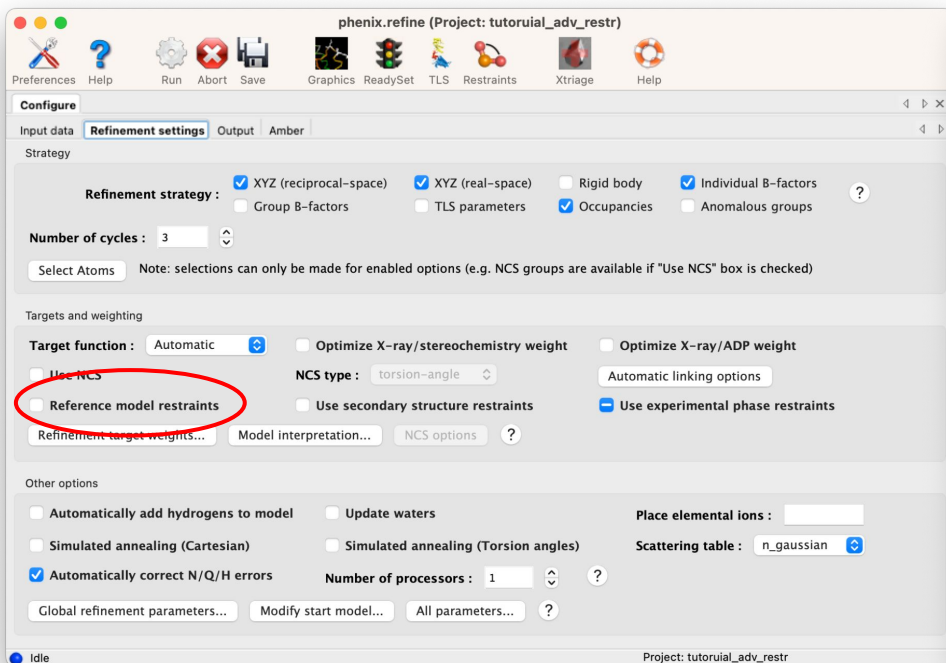
phenix.real_space_refine



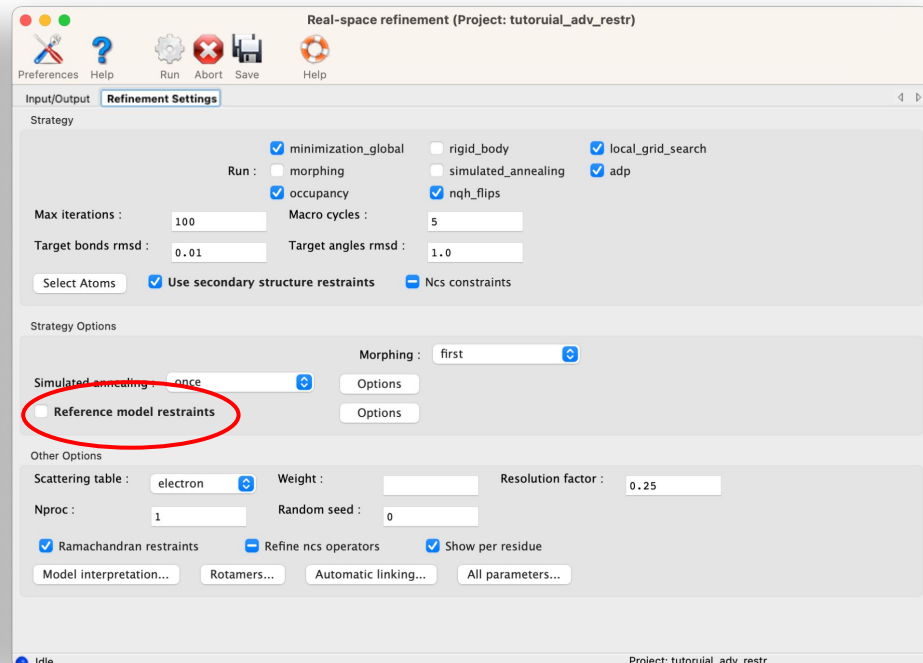
NCS - only constraints in RSR

phenix.refine vs phenix.real_space_refine: reference model

phenix.refine

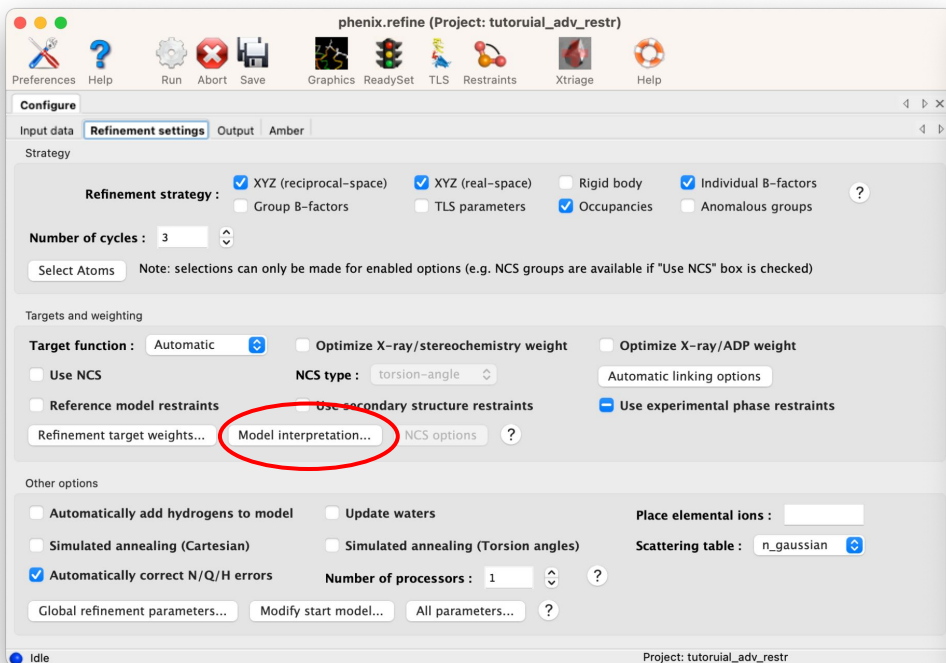


phenix.real_space_refine

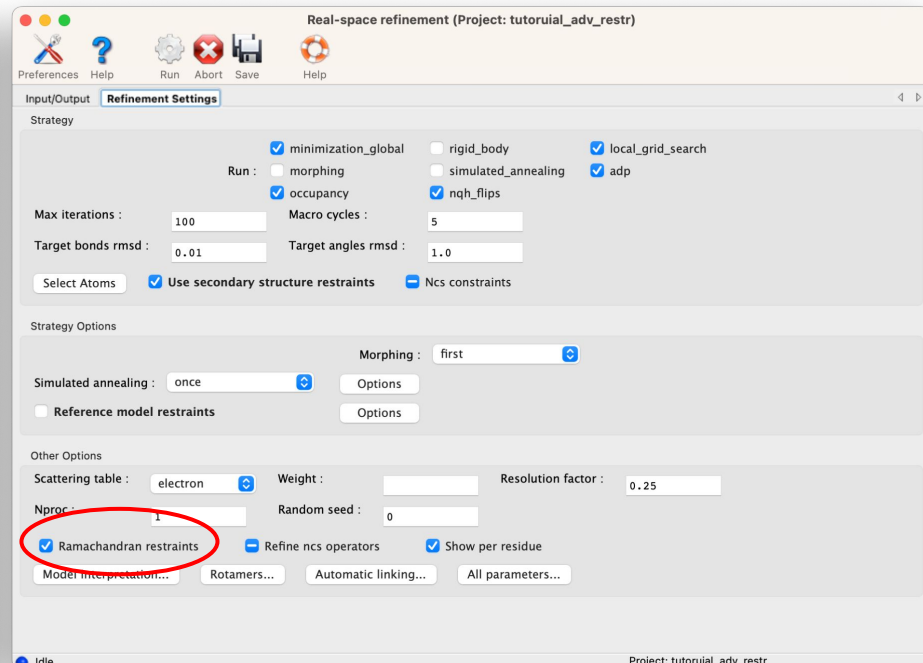


phenix.refine vs phenix.real_space_refine: ramachandran

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?

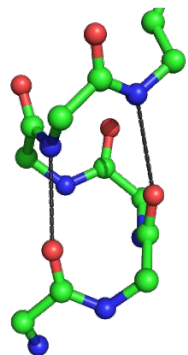
Tell Phenix to establish restraints:

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

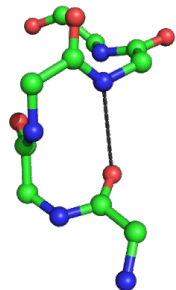
Make sure the restraints are established

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

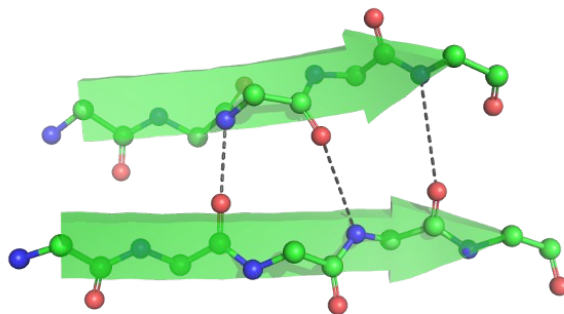
Secondary structure restraints



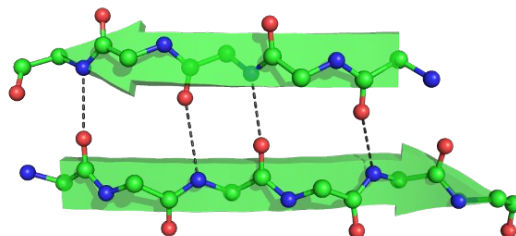
alpha helix



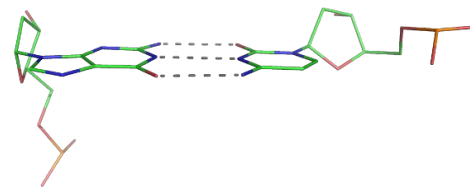
3_{10} helix



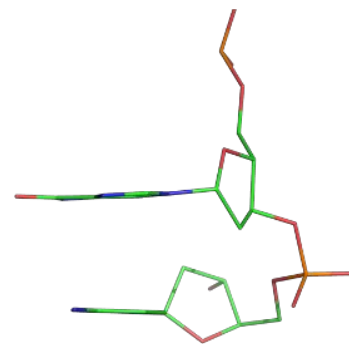
Parallel sheet



antiparallel sheet



Basepair

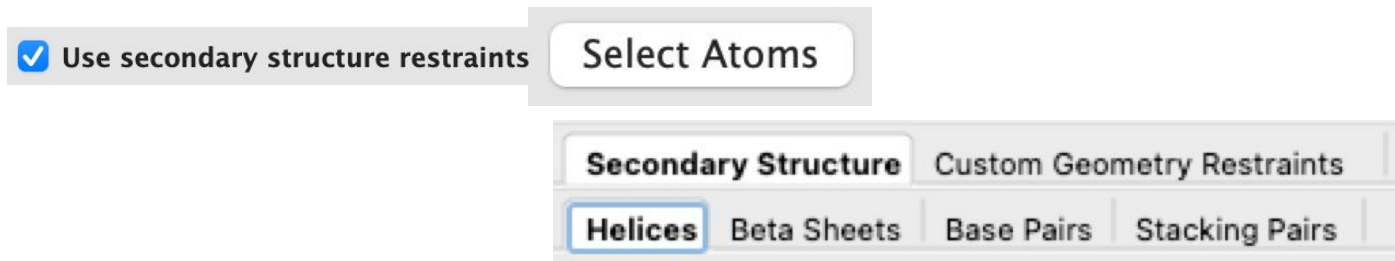


Stacking pair

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

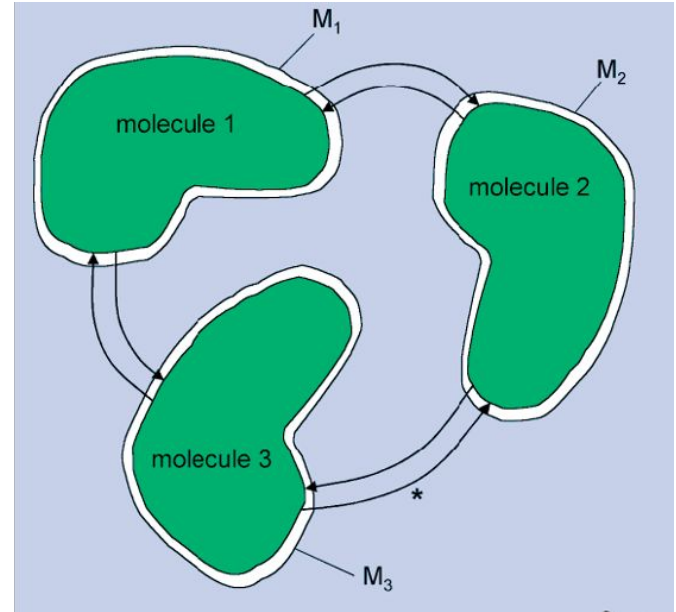
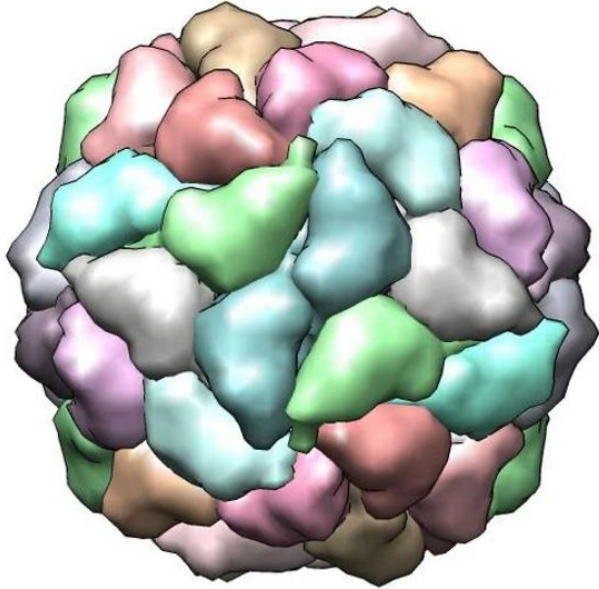
```
Finding SS restraints...
Secondary structure from input PDB file:
  22 helices and 7 sheets defined
  36.1% alpha, 8.0% beta
  0 base pairs and 0 stacking pairs defined.
Time for finding SS restraints: 0.07
Creating SS restraints...
Processing helix chain 'A' and resid 8 through 12
Processing helix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

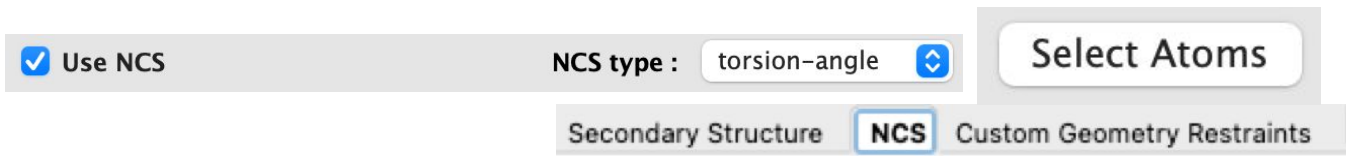


- **Constraints:** molecules 1, 2 and 3 are required to be **identical**
- **Torsion restraints:** molecules 1, 2 and 3 are required to be **similar**
- **Cartesian restraints:** molecules 1, 2 and 3 are required to be **similar**

NCS restraints

How to set:

- Using GUI



The screenshot shows a software interface for setting NCS restraints. It features a horizontal bar with a checked checkbox labeled 'Use NCS'. To the right, a label 'NCS type :' is followed by a dropdown menu currently showing 'torsion-angle'. Further right is a button labeled 'Select Atoms'. Below this bar is a tabbed interface with three tabs: 'Secondary Structure', 'NCS' (which is highlighted with a blue border), and '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

☒ Reference model restraints

All parameters...

Reference model

Reference group...

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

Reference group...

Reference group (1)

Selection in the reference model : View/pick... ?

Selection in the refined model : View/pick... ?

Add another Delete last

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
```

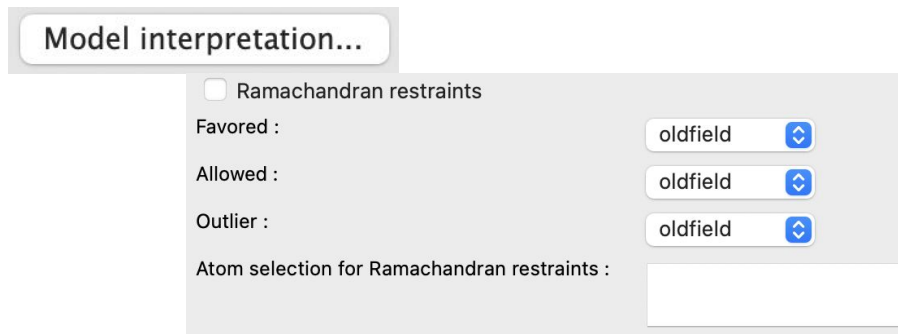
.geo file

```
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



The screenshot shows a dialog box titled "Model interpretation...". Inside, there is a section for "Ramachandran restraints" which is currently unchecked. Below this, there are three rows of settings: "Favored :", "Allowed :", and "Outlier :". Each row has a dropdown menu set to "oldfield" and a blue arrow icon. At the bottom, there is a label "Atom selection for Ramachandran restraints :" followed by an empty text input field.

Model interpretation...

☐ Ramachandran restraints

Favored : oldfield

Allowed : oldfield

Outlier : oldfield

Atom selection for Ramachandran restraints :

- 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 "  
<...>
```

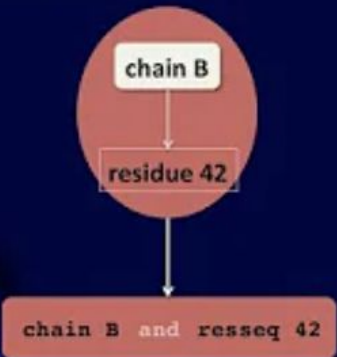
```
Ramachandran plot restraints (Emsley): 0  
Sorted by residual:
```

```
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



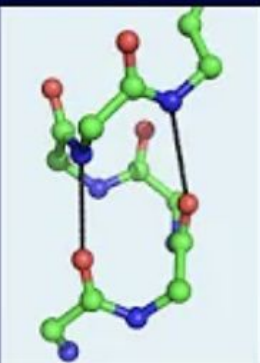
Tutorial

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.

Secondary structure restraints

Proteins

Helix sheet

NA

Basepair

Stacking pair

Custom covalent geometry restraints

Types:

- Bond - supports bonds over symmetry.
- Angle
- Dihedral
- Planarity
- Parallelity

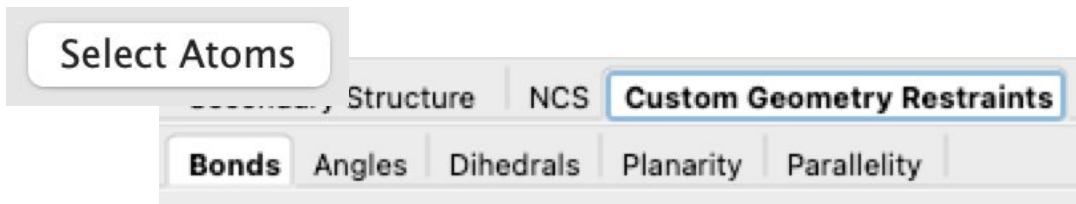
Actions:

- Add
- Delete
- Change

Custom covalent geometry restraints how to set

How to set:

- Using GUI: “Select atoms”



- Using parameter file

Custom covalent geometry restraints how to check

Log file

```
Finding SS restraints...  
Secon
```

```
d\gss\das\das15A.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="adfsandfasfudfasf
```

```
" O    TYR A 39 "
```

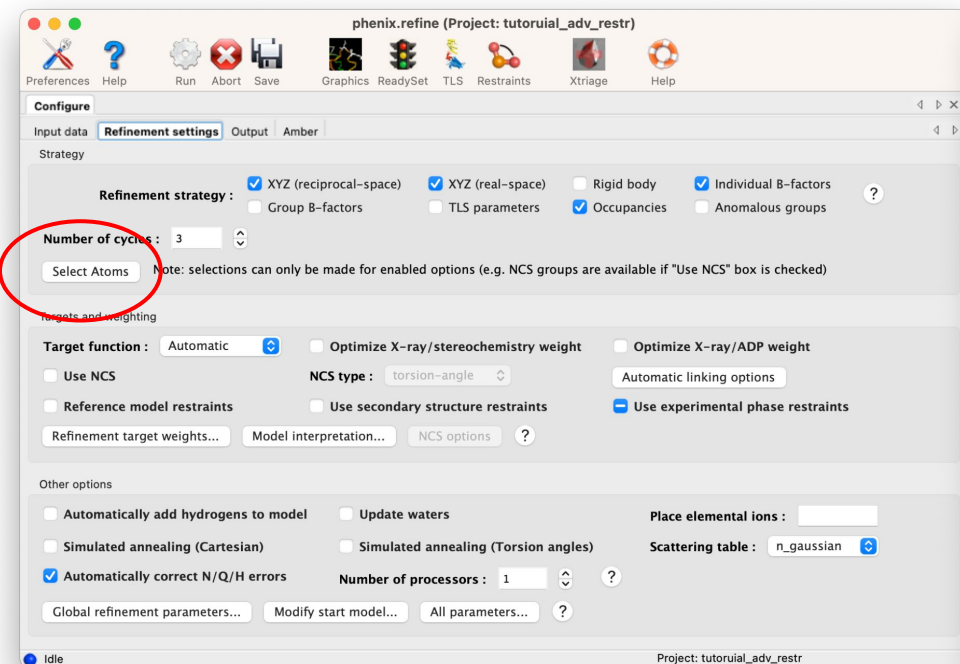
Difference between phenix.refine and phenix.real_space_refine

Both programs use the same machinery to establish restraints:

- Covalent geometry;
- Ligands;
- Additional restraints like
 - “Edits” - new bonds/angles/planes ...
 - Secondary structure
 - NCS
 - Reference model
 - Ramachandran

Difference between phenix.refine and phenix.real_space_refine

phenix.refine



phenix.real_space_refine

