DireX Tutorials

Download DireX and Tutorial files from:

https://www.simtk.org/home/direx/

Further Information
(Documentation and Tutorial):
http://www.schroderlab.org/software/direx/

All results are part of the tutorial files, e.g. *direx-tutorials/ef-2/results/*

1) Kinked Helix at Low Resolution
   Simple toy example
2) Kinked Helix at High Resolution
   Sidechain fitting
3) Adenylate-Kinase
   Rather simple two-domain protein
4) Resolving Clashes
   when fitting multiple components
5) Occupancy Refinement
   to account for reduced/missing density
6) Extensive Sampling of Backbone Conformations

DireX Tutorials

parameter file

DireX is a command line program:

```
$> direx -pdb input.pdb  -map density.mrc -o conf.pdb -f refine.par
```

Make sure the executable is in the PATH.
e.g. for bash:

```
$>  export PATH=$PATH:$HOME/direx-0.6.2/direx
```

you may want to put this line into ~/.bashrc

Typical usage is iteration over:

- Edit parameter file in text editor
- Run direx (type ‘./run.sh’)
- Look at results with e.g. VMD or Chimera

DIREX Parameter File

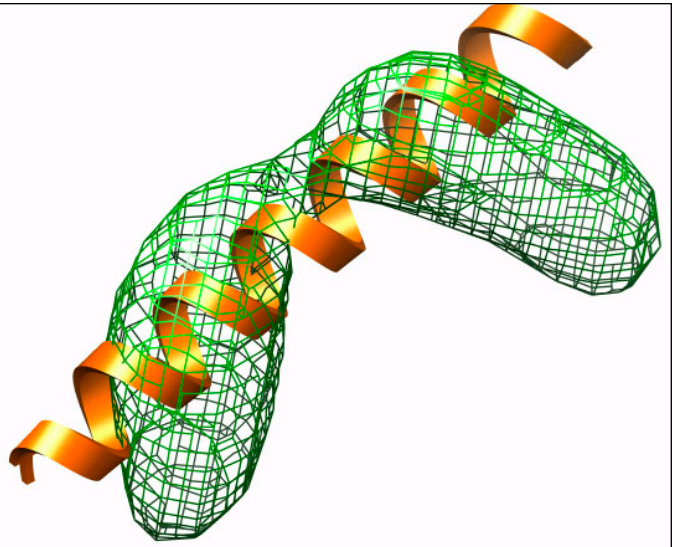
Generate a template parameter file with default values:

```
direx -of all.par [-v]
```

- General options
- Coordinate Perturbation
  - Tirion
  - Advanced perturbation
- DEN
- Density map
  - Occupancy Refinement
- Distance Restraints
- Position Restraints
- NCS Restraints
- Others, including experimental and weird parameters

Kinked Helix at Low Resolution

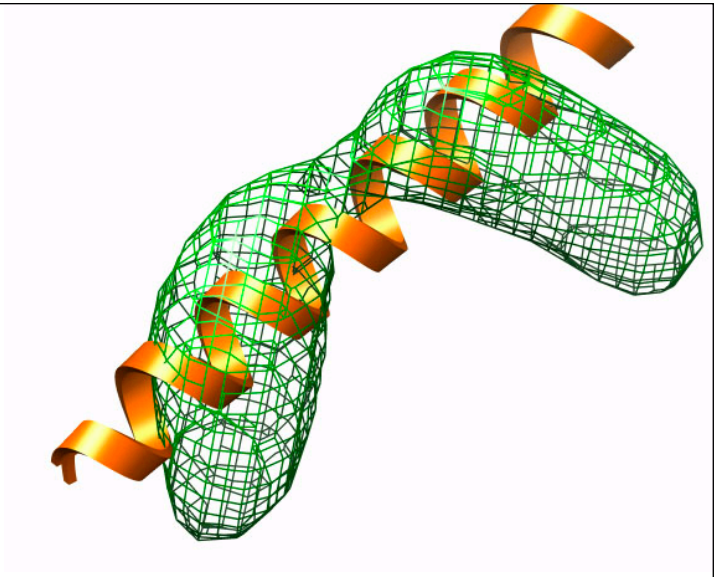
direx-0.6-tutorials/kinked-helix-lores/



```
#!/bin/bash

# Generate density from "target.pdb"
direx -f mkdensity.par -pdb target.pdb        \
   -o mkdensity.pdb -omap kinked-density.mrc \
   -map self -map_apix 2.0


# Run refinement starting from extended-helix.pdb
direx -f refine.par -pdb extended-helix.pdb   \
  -o conf.pdb -omap model.mrc                 \
  -map kinked-density.mrc -ox traj.xtc        \
  -mapcc mapcc.dat
```

Kinked Helix at Low Resolution

direx-0.6-tutorials/kinked-helix-lores/



General

```
nsteps      = 100
annealing   = 100
pert_fac = 0.02
```

DEN

```
use_den = yes
den_no_ratio = 3.0
den_strength = 0.4
den_upper = 15.0
den_lower = 3.0
den_gamma = 0.1
den_const_dist = 0.1
den_break_dist = 0.5
```

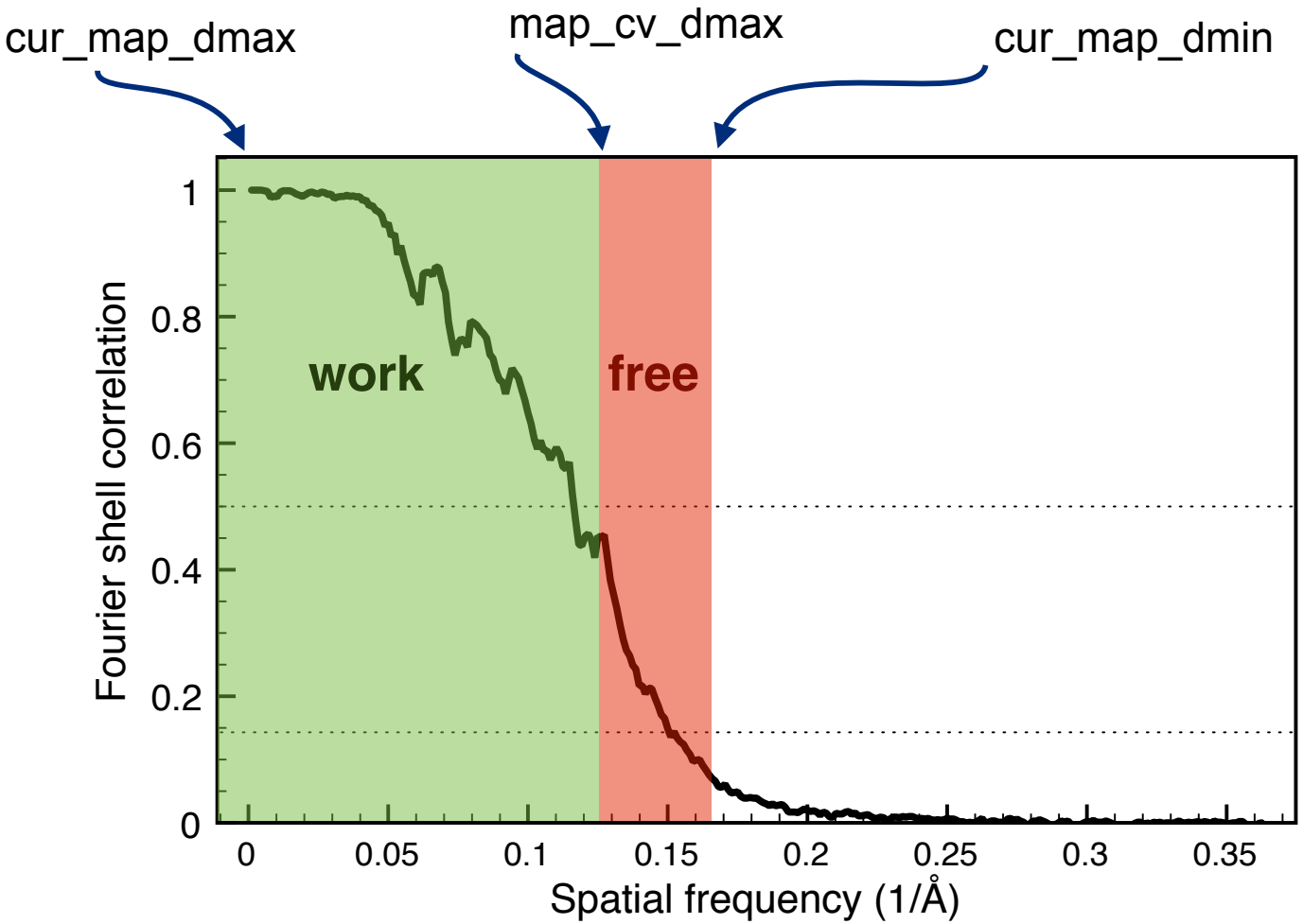
Density Map

```
map_strength = 0.03
cur_map_kernel = gaussian
```

set model map resolution

```
cur_map_dmin = 8.0
cur_map_dmax = 200.0
map_cv_dmin = 10.0
```

A note on choosing the free interval

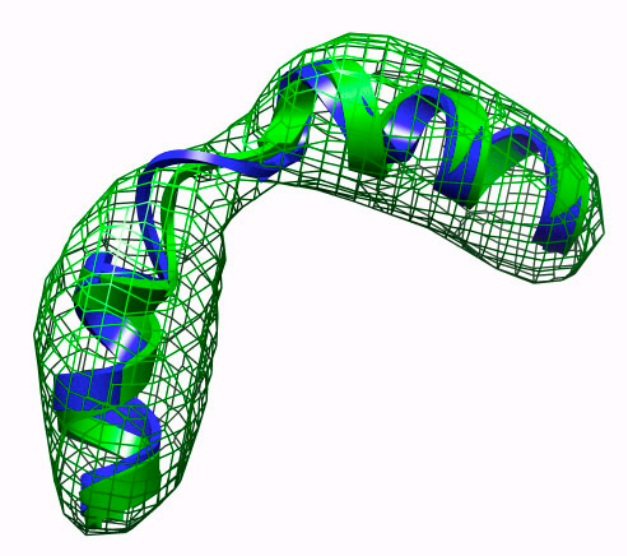


- Initial C_{free} value should be about 0.1
- Final C_{free} does not to be higher than 0.2

in other words: large enough to significant, but not much larger

Kinked Helix at Low Resolution

direx-0.6-tutorials/kinked-helix-lores/



Look at results:

Watch fitting trajectory:

```
$> vmd -f extended-helix.pdb traj.xtc  -m kinked-density.mrc
```

Final structure is `conf.pdb`

Model density map is `model.mrc`

```
$> chimera conf.pdb model.mrc kinked-density.mrc
```

DireX Output

The *mapcc.dat* file:

```
Step #       Cwork       Cfree         RealR     RealRfree      R       Rfree
Cwork(mask)     Cfree(mask)    RealR(mask)   RealRfree(mask)    AvgMapValue
          1         0.62261   0.10071    0.23258   0.81701    0.53514   0.72517
0.59635   0.09765    0.22958   0.82224   1.16953e+01
...
```

12 columns:

1) Iteration step number
2) **Cwork**, map cross-correlation of model and EM map.
3) **Cfree**, as Cwork but for free maps
4) **RealR-**value of the work maps
5) **RealR-**value of the free maps
6) **R** value, (reciprocal), compares only Fourier amplitudes
7) **Rfree**, R value of the free maps
8) **Cwork(mask)** computed only in the region of the map where atoms are
9) **Cfree(mask)** as Cwork but for the free maps
10) **average density** value at the location of atoms.

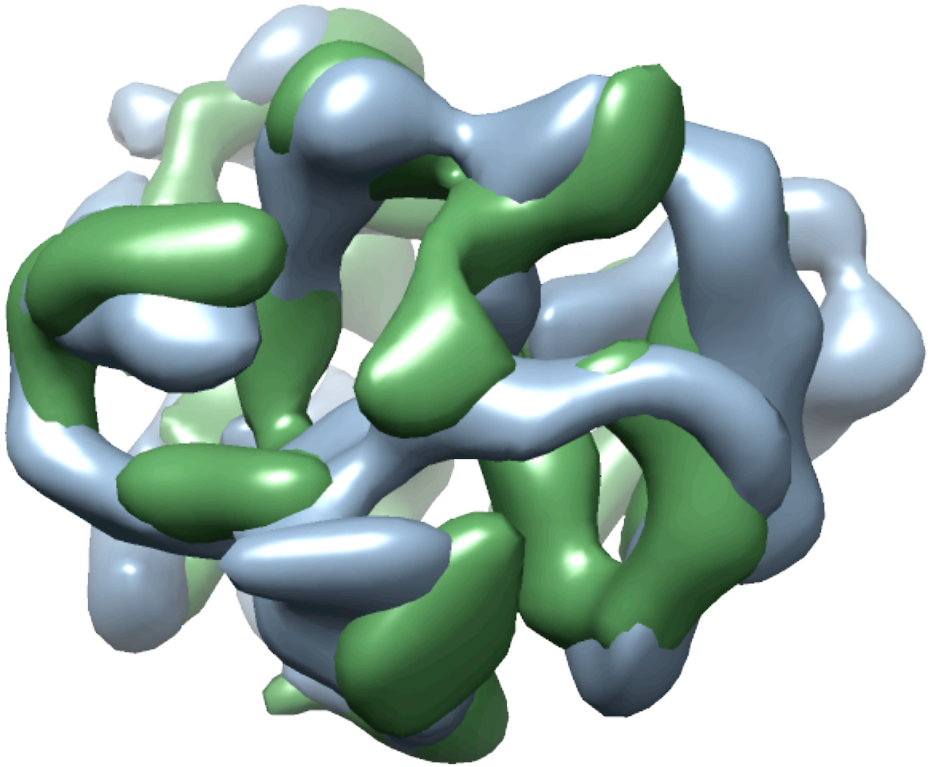
DireX Output

Write out the model map with `-omap model.mrc`

This helps to visualize how well the model fits to the EM map.

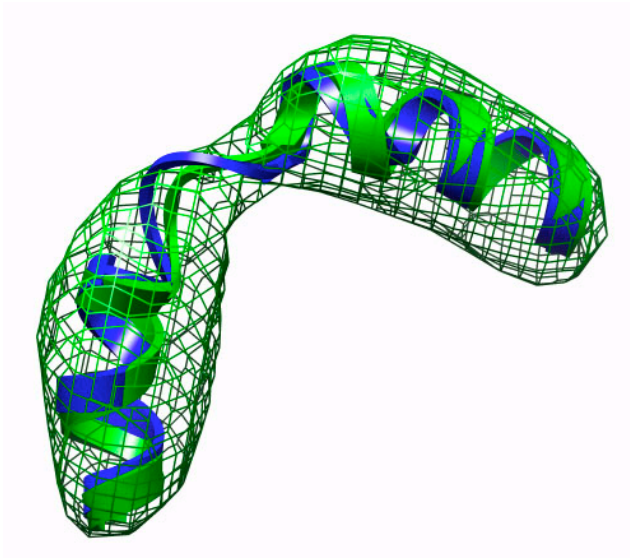
EM density

Model density

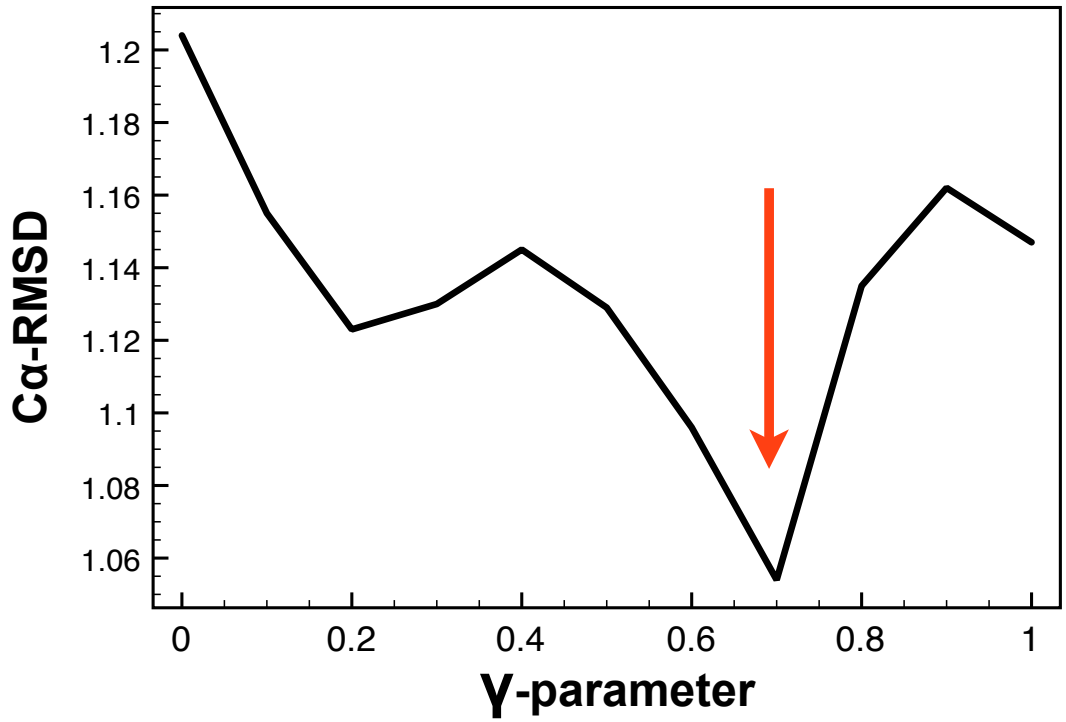


Kinked Helix at Low Resolution

direx-0.6-tutorials/kinked-helix-lores/



Optimizing the γ-parameter

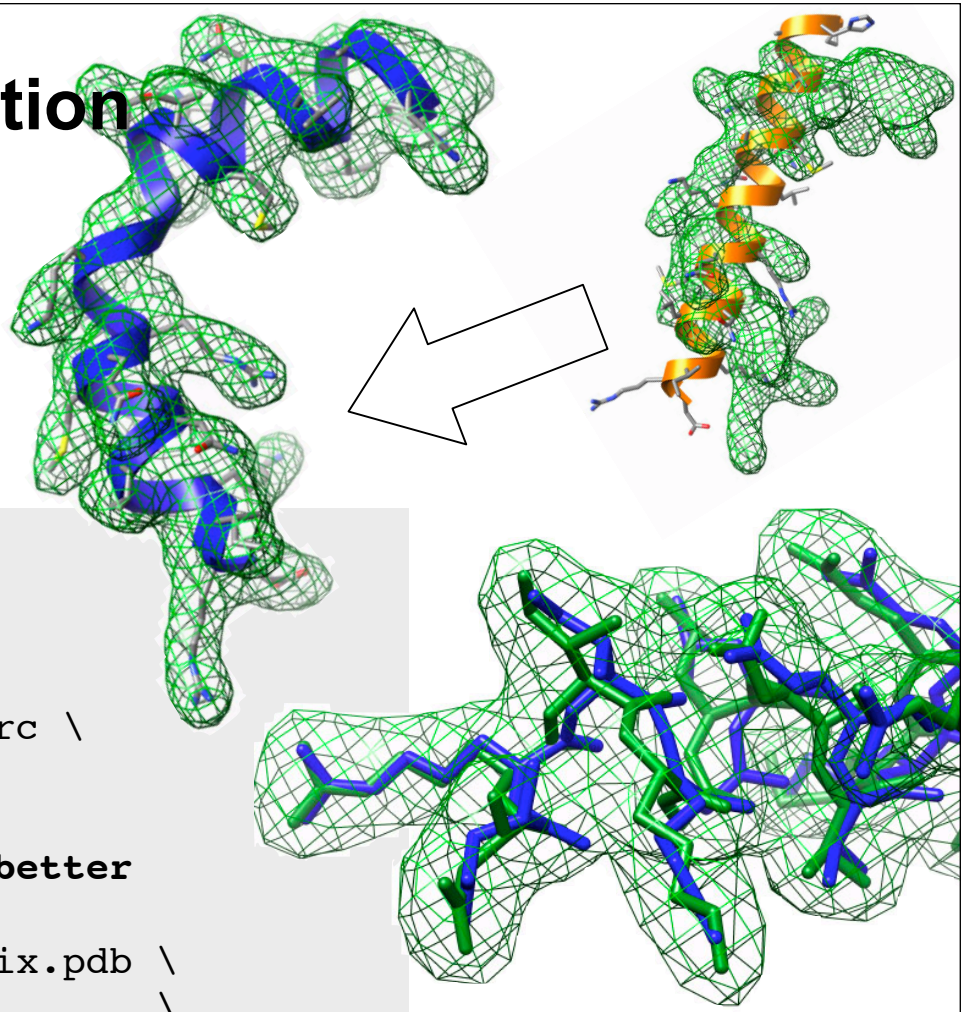


In a realistic application: Optimal γ-parameter is obtained by cross-validation, i.e. best C_{free} value.

Kinked Helix at High Resolution

direx-0.6-tutorials/kinked-helix-hires/

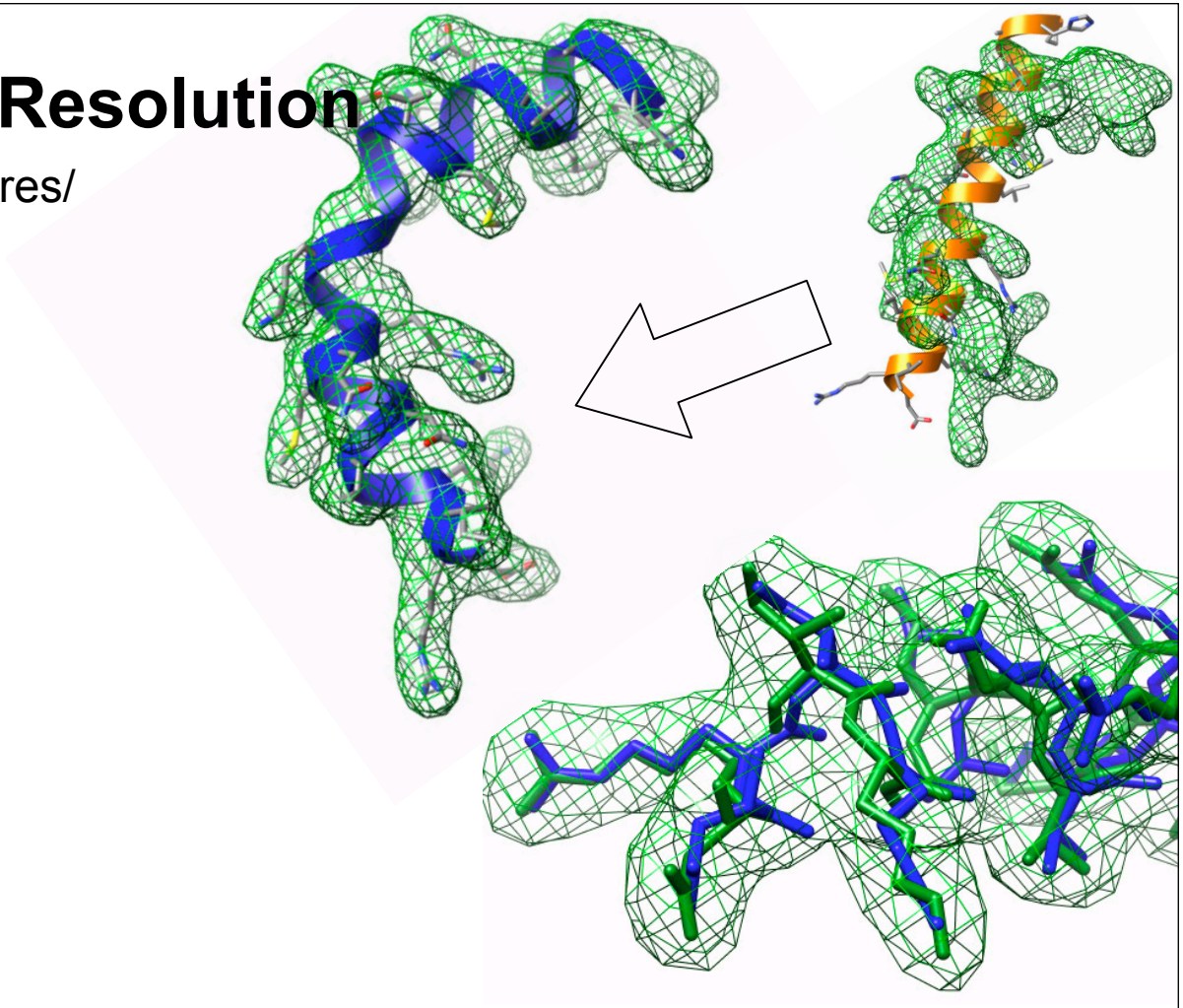
To help convergence, we use a two-step protocol, fitting first at low-resolution



```
#!/bin/bash

# Make density
direx -f mkdensity.par -pdb target.pdb  \
  -o mkdensity.pdb -omap kinked-density.mrc \
  -map generate -map_apix 1.0

# Run refinement at lower resolution for better
# convergence
direx -f refine-low.par -pdb extended-helix.pdb \
   -o conf-low.pdb -omap model-low.mrc          \
   -map kinked-density.mrc -ox traj-low.xtc     \
   -mapcc mapcc-low.dat

# Run refinement at higher resolution
direx -f refine-high.par -pdb extended-helix.pdb   \
   -p conf-low.pdb -refden extended-helix.pdb      \
   -o conf-high.pdb -omap model-high.mrc           \
   -map kinked-density.mrc -ox traj-high.xtc  -mapcc
mapcc-high.dat
```

Kinked Helix at High Resolution

direx-0.6-tutorials/kinked-helix-hires/



First step: Low-resolution

```
nsteps      = 200
annealing     = 200
pert_fac      = 0.5
```

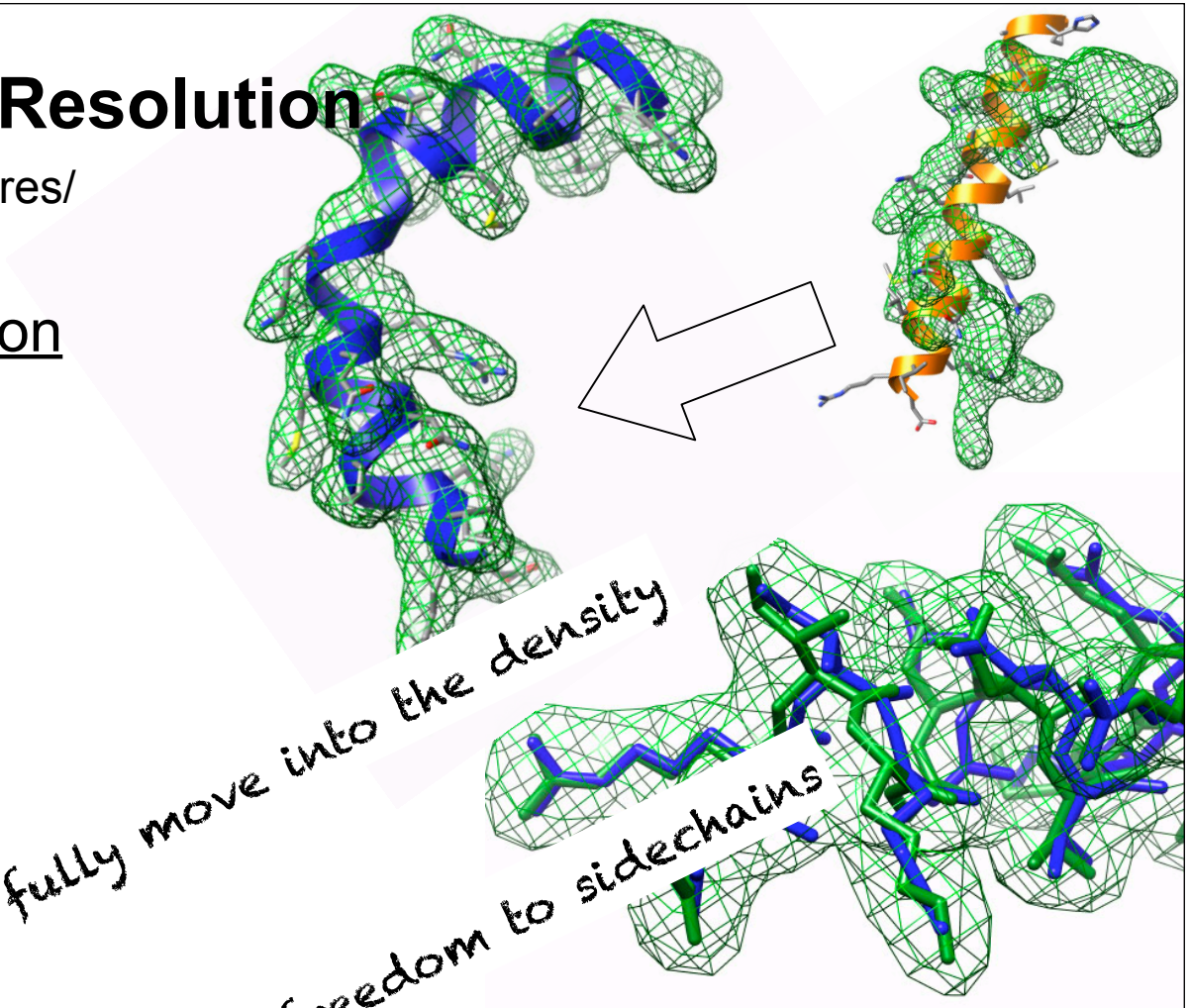
```
den_strength = 0.4
den_gamma = 0.5
den_upper = 10.0
den_lower = 3.0
den_resid_range = 10
den_sidechain = 1.0
```

set resolution to 6 Å

```
cur_map_dmin = 3.0
map_cv_dmin = 6.0
cur_map_dmax = 200.0
```

Kinked Helix at High Resolution

direx-0.6-tutorials/kinked-helix-hires/



Second step: High-resolution

```
nsteps      = 400
annealing     = 400
pert_fac      = 0.0
```

```
den_strength = 0.4
den_gamma = 1.0
den_upper = 10.0
den_lower = 3.0
den_resid_range = 10
den_sidechain = 0.2
```

fully move into the density

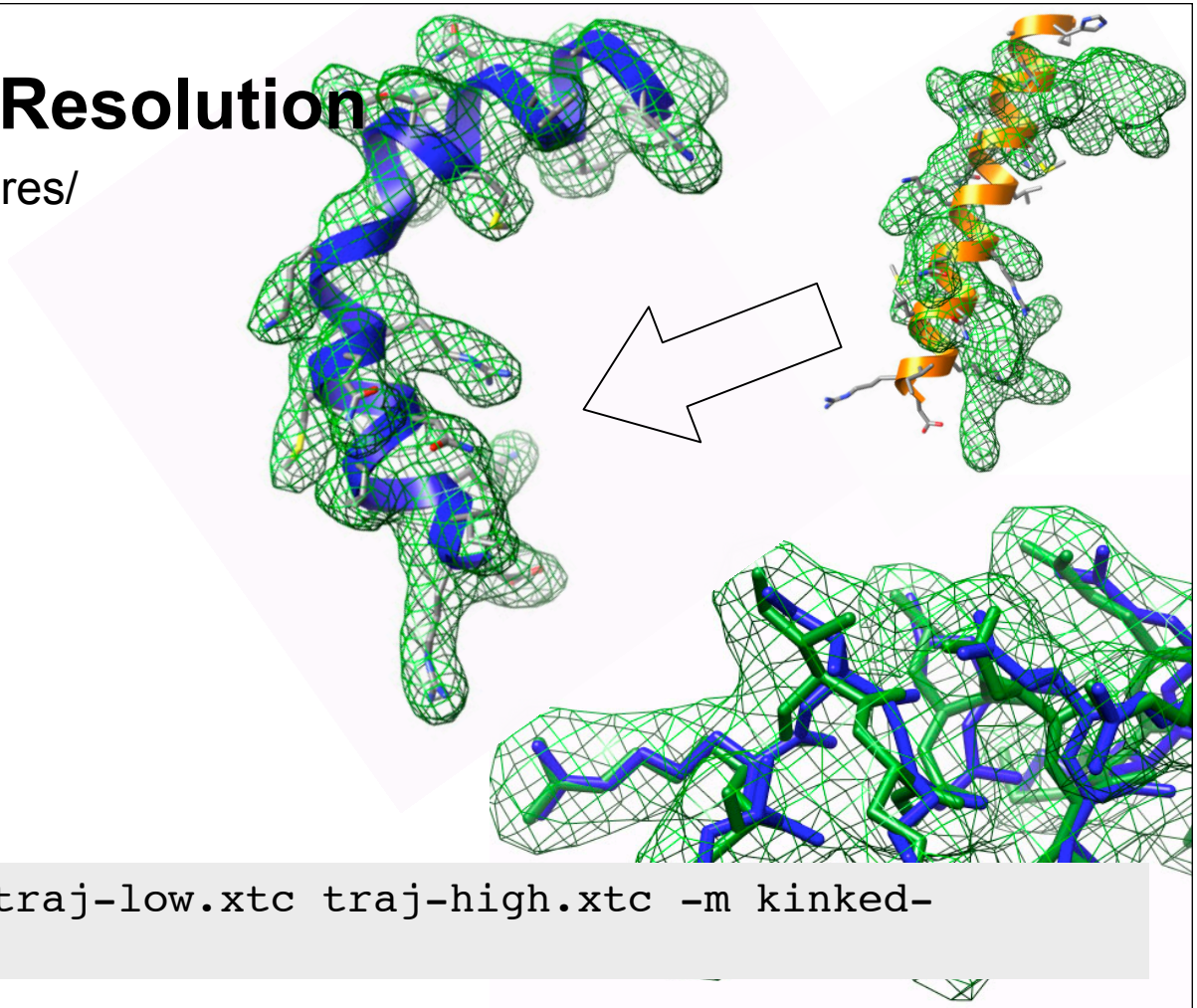
give more freedom to sidechains

Set resolution to about 3 Å

```
cur_map_dmin = 3.0
map_cv_dmin = 3.3
cur_map_dmax = 200.0
```

Kinked Helix at High Resolution

direx-0.6-tutorials/kinked-helix-hires/



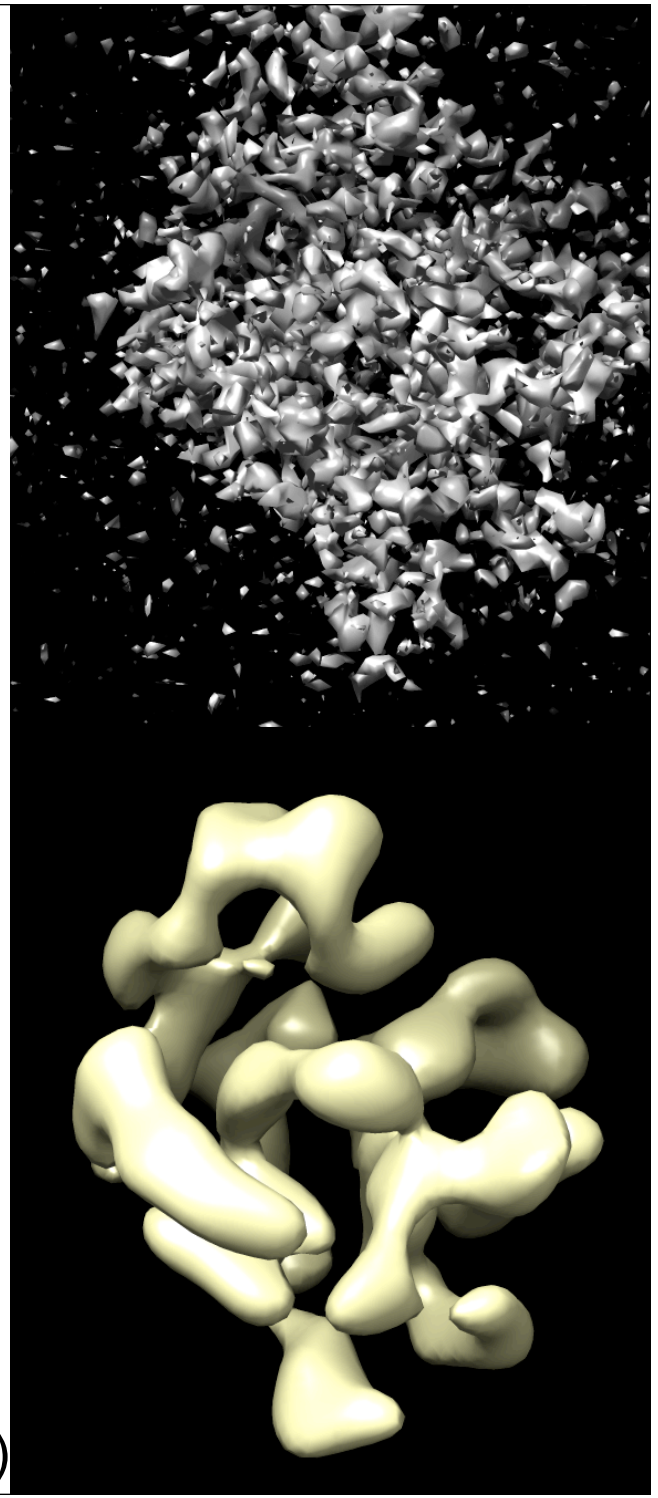
Look at results:

```
vmd -f extended-helix.pdb traj-low.xtc traj-high.xtc -m kinked-
density.mrc
```

Final structure is `conf-high.pdb`

Adenylate Kinase

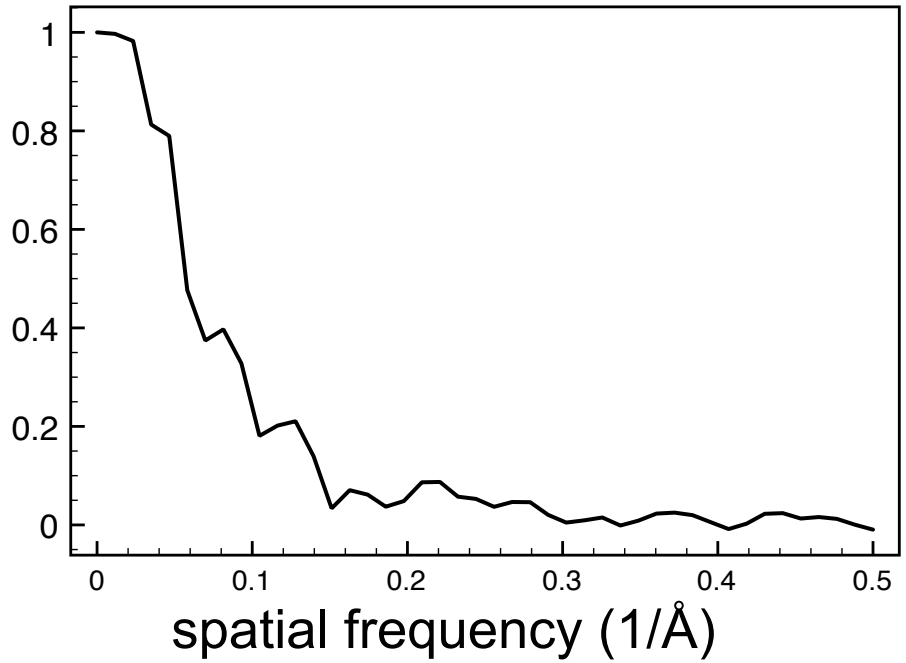
original reconstruction
(1ake-7A.mrc)



<u>A more realistic example:</u>

3D reconstruction from noisy projection images
(with perfect Euler angles, though!)

Fourier Shell Correlation (FSC)



filtered to 7Å
(1ake-7A-filt7A.mrc)

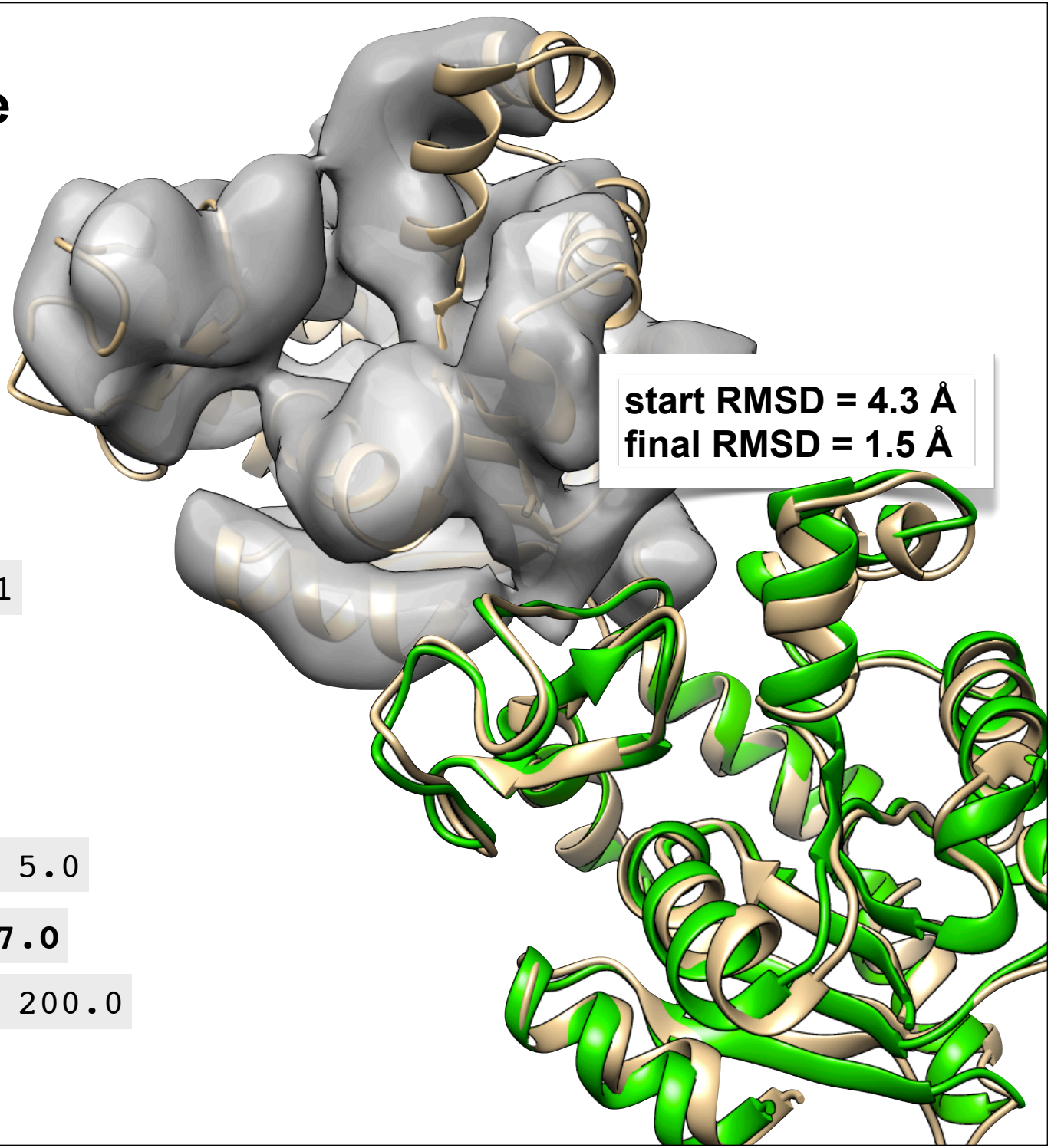
Adenylate Kinase

DEN parameters

```
den_strength = 0.4
den_upper = 10.0
den_lower = 3.0
den_resid_range = -1
den_gamma = 0.6
```

Density parameters

```
cur_map_dmin = 5.0
map_cv_dmin = 7.0
cur_map_dmax = 200.0
```

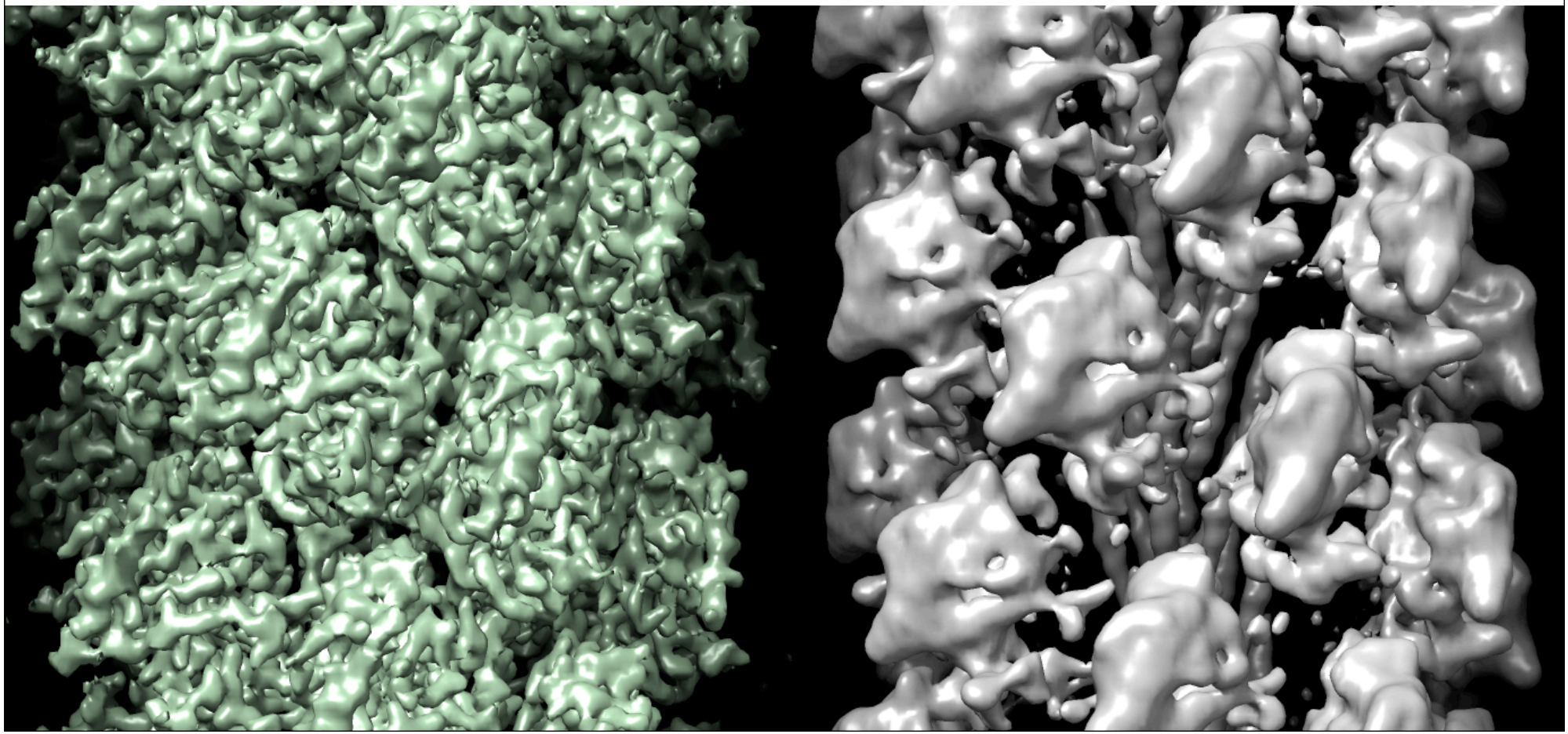


A note on preparing the EM density

Applying the model radial structure factor often improves the EM reconstruction

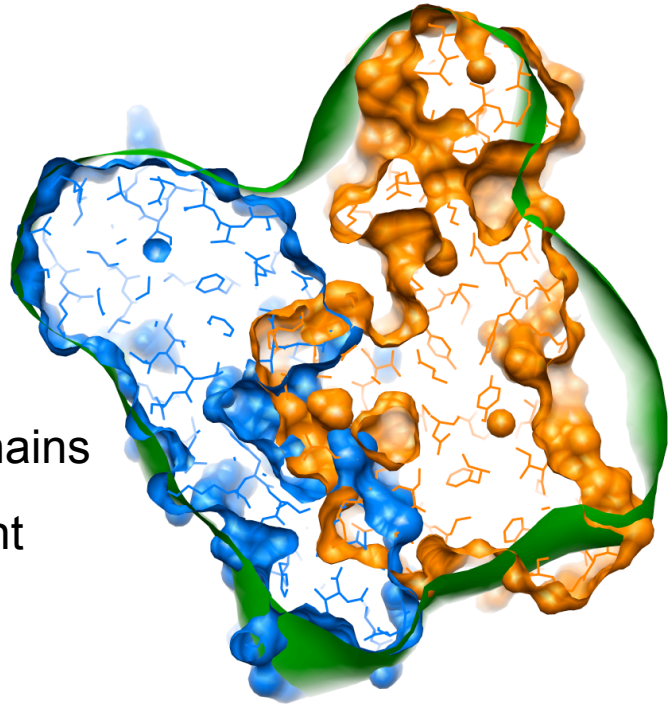
```
$> e2proc3d.py  model-map.mrc junk.mrc --calcsf=sf.dat
$> e2proc3d.py  em.mrc em-sf.mrc  --setsf=sf.dat
```

then possibly sharpen the map with negative B-factor.



Resolve Clashes

direx-0.6-tutorials/resolve-clashes/



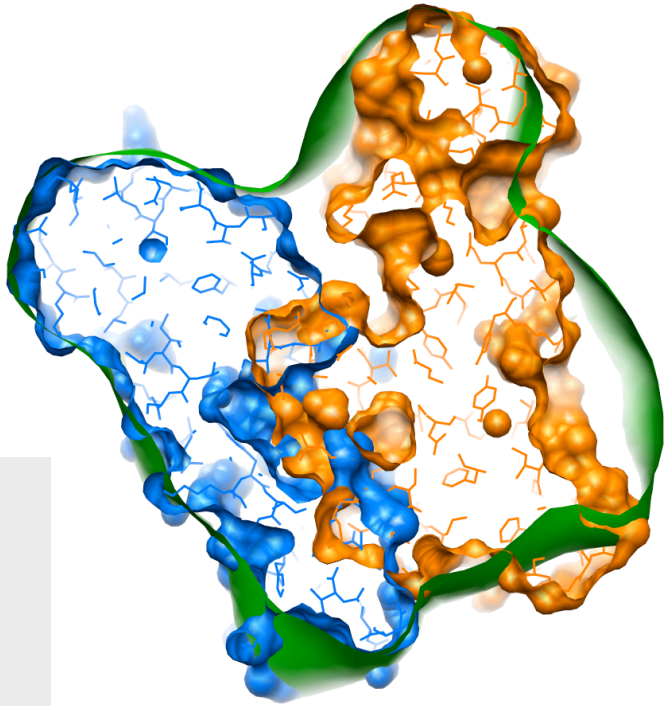
- Prevent CONCOORD algorithm from defining distance restraints between overlapping models
- Add repulsive forces between atoms from overlapping chains
- Set resid_range = 100 (i.e. no restraints between different chains

2-step protocol:

```
# Run refinement allowing chain overlap
# with chain repelling forces added to resolve clashes.
direx -f refine-1.par -pdb separate-chains.pdb -p clash.pdb \
  -o conf-1.pdb -omap model-1.mrc -map dimer-density.mrc -ox traj-1.xtc\
  -refden separate-chains.pdb -mapcc mapcc-1.dat

# Regular refinement
direx -f refine-2.par -pdb separate-chains.pdb -p current-1.pdb \
   -o conf-2.pdb -ox traj-2.xtc -map dimer-density.mrc          \
   -refden separate-chains.pdb -omap model-2.mrc -mapcc mapcc-2.dat
```

Resolve Clashes

direx-0.6-tutorials/resolve-clashes/



Parameters (*Misc options*)

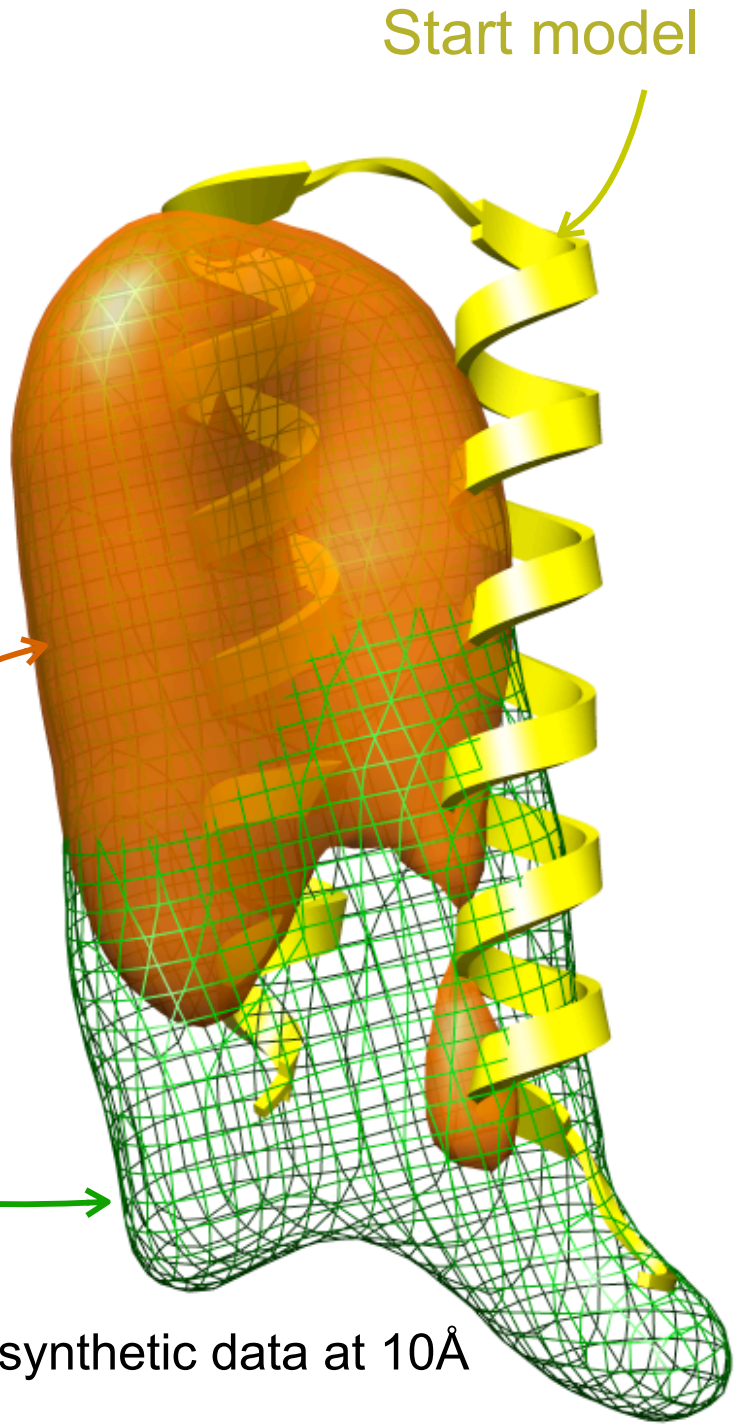
```
# >0 means atoms repel each other.
# <0 means atoms attract each other.
repel_shift = 0.01


# Use interchain concoord
# if "no", chains are allowed to overlap.
interchain_concoord = no
```

Occupancy Refinement

direx-0.6-tutorials/occ-refine/

Make sure all occupancy values are set to 1.0 in the starting PDB file !

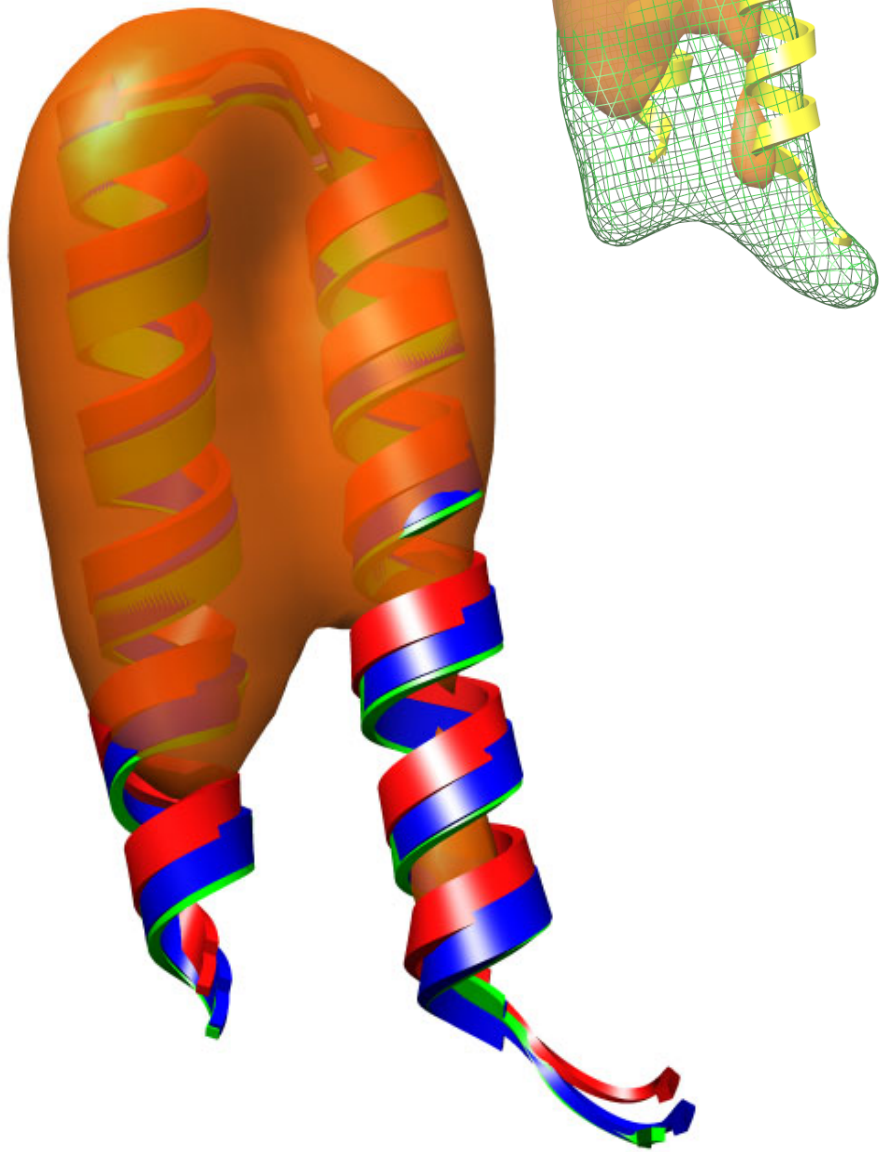
```
direx -f refine.par -pdb ../start.pdb
  -o conf.pdb -map ../map-10A-occ.mrc
  -omap model.mrc -ox traj.xtc
```



Occupancy Refinement

direx-0.6-tutorials/occ-refine/

RMSD to target:
Standard = 1.6 Å
With occ_ref = 0.2 Å



Parameters:

```
compute_map_use_occ = yes
```

```
map_refine_occ = yes
```

```
map_refine_occ_damp = 0.0001
```

```
map_occ_restraint_cycles = 1
```

Use one occ-value for whole residue:

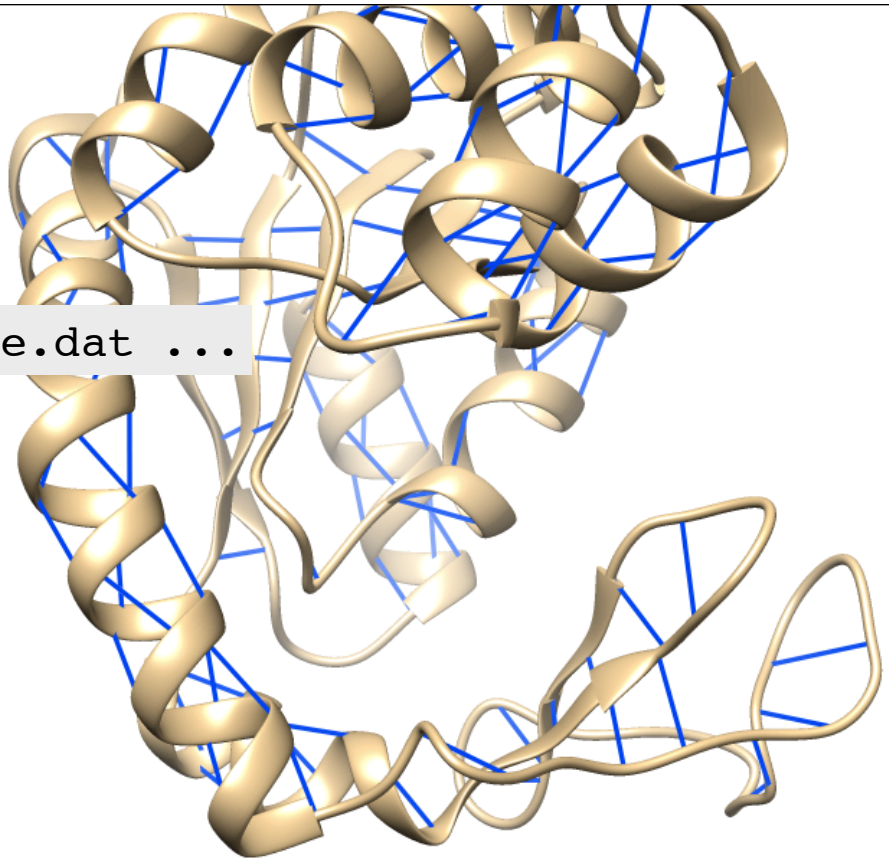
```
map_group_refine_occ = yes
```

```
map_bfac_as_occ = yes
```

To limit number of additional parameters use occupancy restraints:

```
map_occ_restraint_lambda = 0.01
```

Distance Restraints



Command line:

```
$> direx -pdb input.pdb  -expd disre.dat ...
```

Parameters:

```
expd_strength = 0.5
```

```
expd_const_dist = 1.0
```

disre.dat:

```
2450
 83   87    6.586872   1.0 1.0
134  142   11.959764   1.0 1.0
 29   36   10.655318   1.0 1.0
 47   50    5.115659   1.0 1.0
 51   44   11.699368   1.0 1.0
117  119    5.155894   1.0 1.0
 42   52   14.894925   1.0 1.0
 82   88   10.340846   1.0 1.0
114  123   14.310755   1.0 1.0
108   98   15.450371   1.0 1.0
...
```

Tip: Get list of H-bonds from Chimera:

- Tools->Structure Analysis->FindHBond
- Check box “Write information to file”
- in Save File Dialog, choose Naming Style “serial number”
- edit file to match the DireX format (see left)

NCS Restraints

Non-crystallographic symmetry

Command line:

```
$> direx -pdb input.pdb  -ncs ncs.dat ...
```

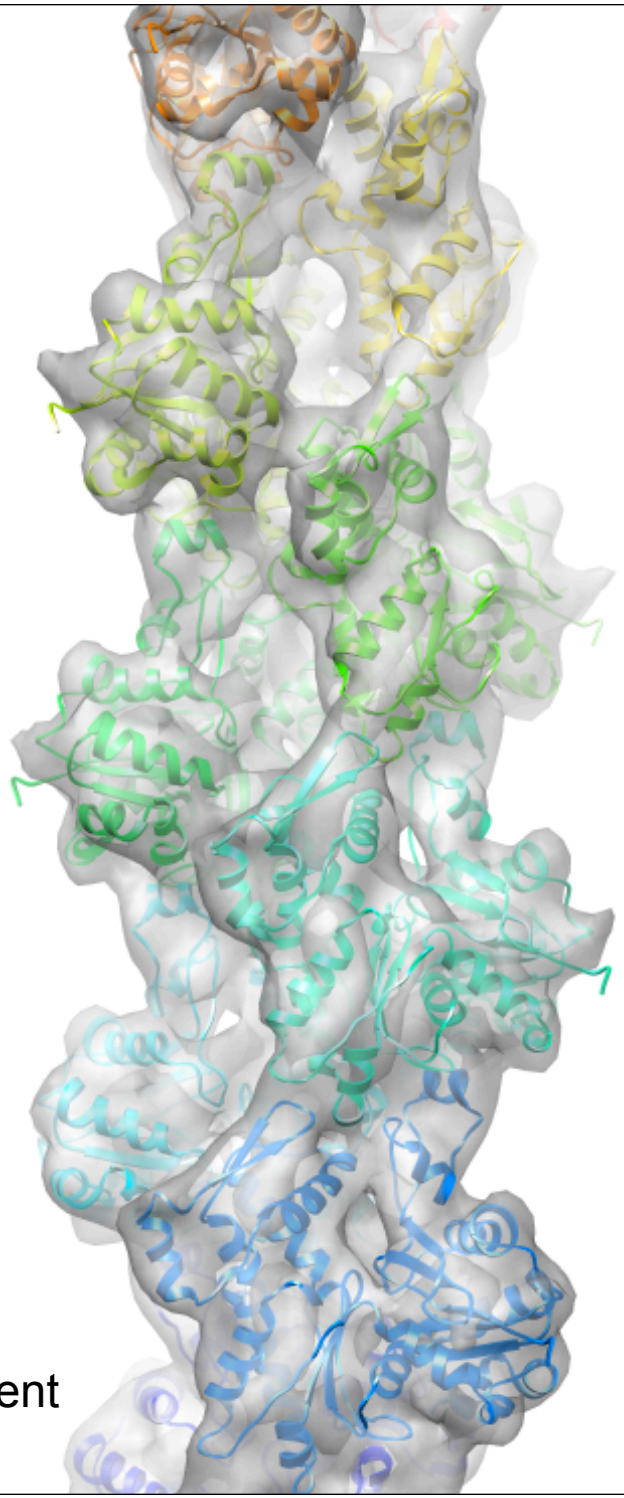
Parameters:

```
n_ncs = 5000
ncs_strength = 0.01
ncs_upper = 15.0
ncs_lower = 3.0
```

The specific symmetry operator is not defined. The restraints just keep all monomers similar without restraining their relative position/orientation

ncs.dat:

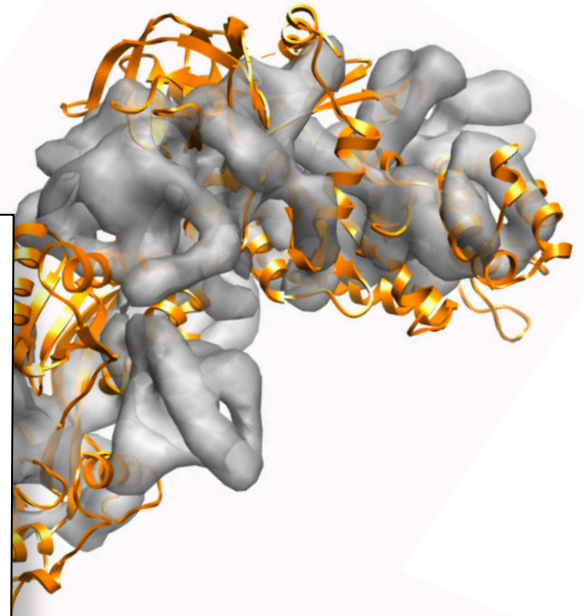
```
3847    14
1
2
3
4
5
6
...
```



Actin filament

Elongation-factor 2

direx-0.6-tutorials/ef-2/



Read secondary structure information

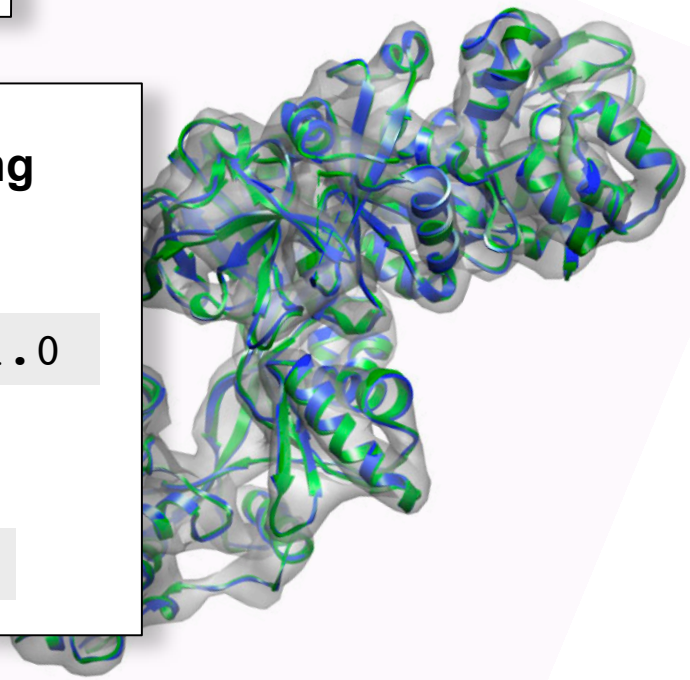
```
$> direx -pdb start-1n0u.pdb -secstr 1n0u.ss
```

File format:

```
[...]
     7     9   A   M   H
     8    10   A   R   H
     9    11   A   S   H
    10    12   A   L   H
    11    13   A   M   H
    12    14   A   D   H
    13    15   A   K
    14    16   A   V   G
    15    17   A   T   G
    16    18   A   N   G
    17    19   A   V   E
    18    20   A   R   E
    19    21   A   N   E
    20    22   A   M   E
    21    23   A   S   E
    22    24   A   V   E
[...]
```

Parameter:

den_secstr_loop = 0.6



Tirion Enhanced Sampling

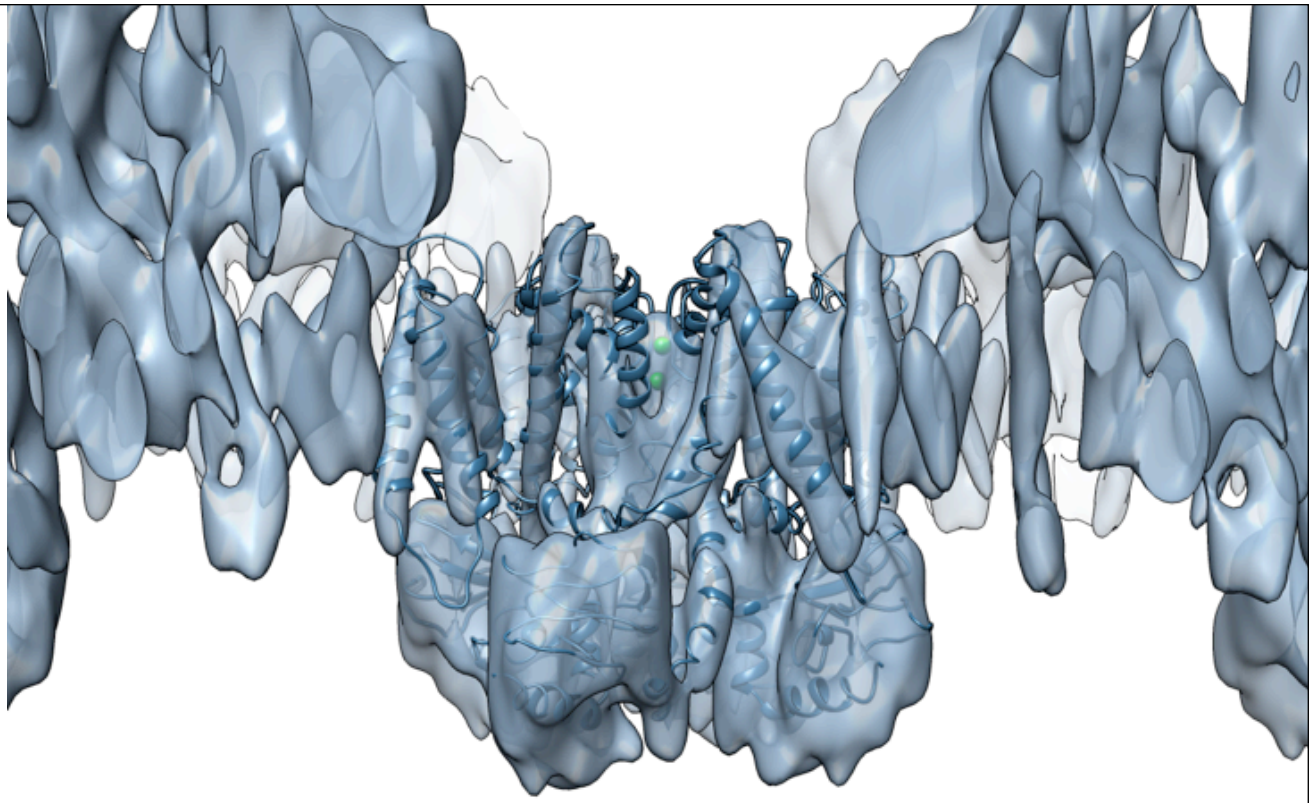
```
tirion_use = yes
```

```
tirion_pert_fac = 1.0
```

```
tirion_lb = 0.5
```

```
tirion_ub = 0.5
```

Anisotropic B-factors



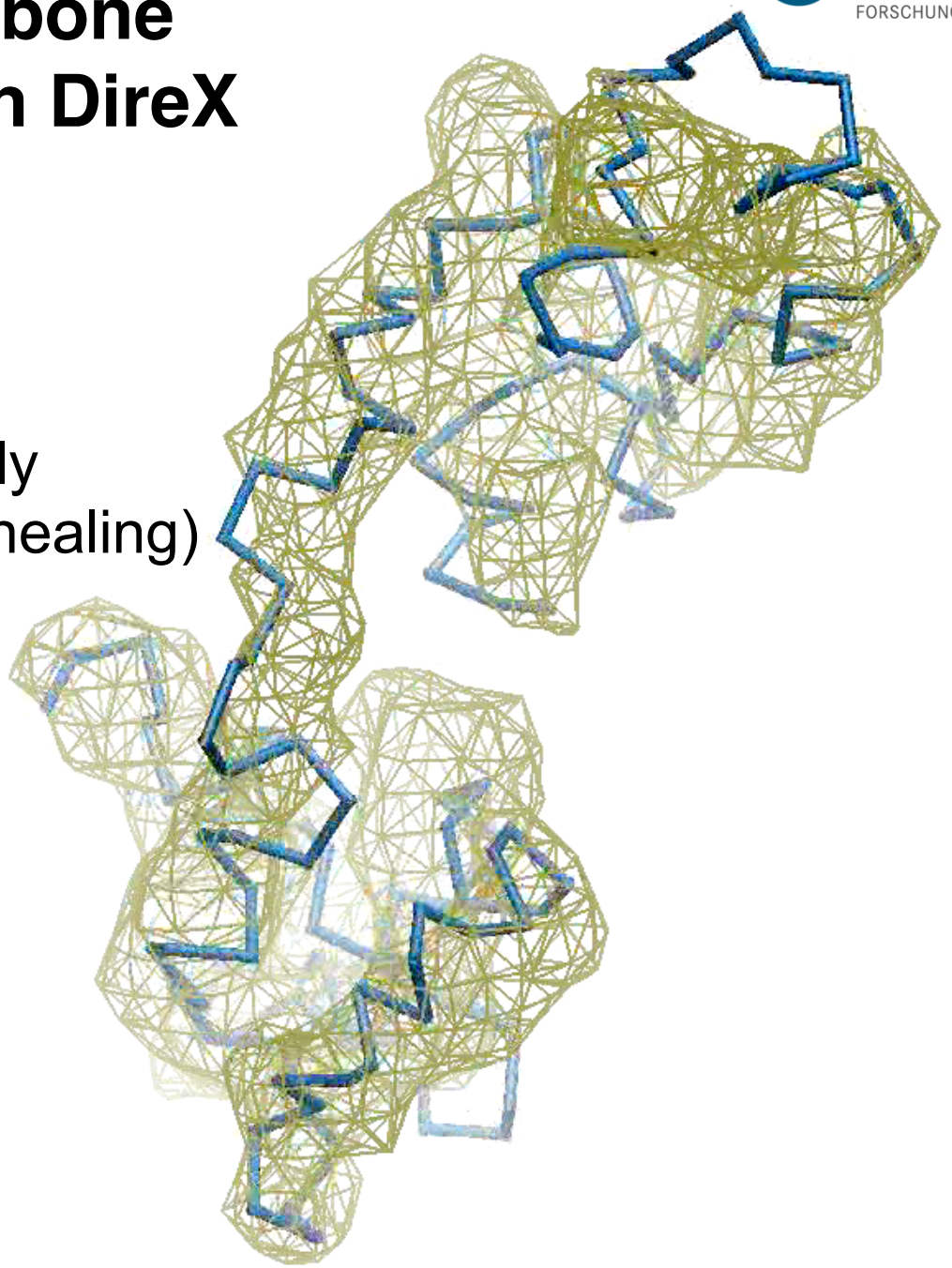
```
Baniso_x = 0.000000
Baniso_y = 0.000000
Baniso_z = 200.000000
```

Sampling the backbone conformations with DireX

DireX does not require
a complete input model

Ca-trace can extensively
sampled (simulated annealing)

Distance restraints
can impose secondary
structure information



JÜLICH
FORSCHUNGSZENTRUM

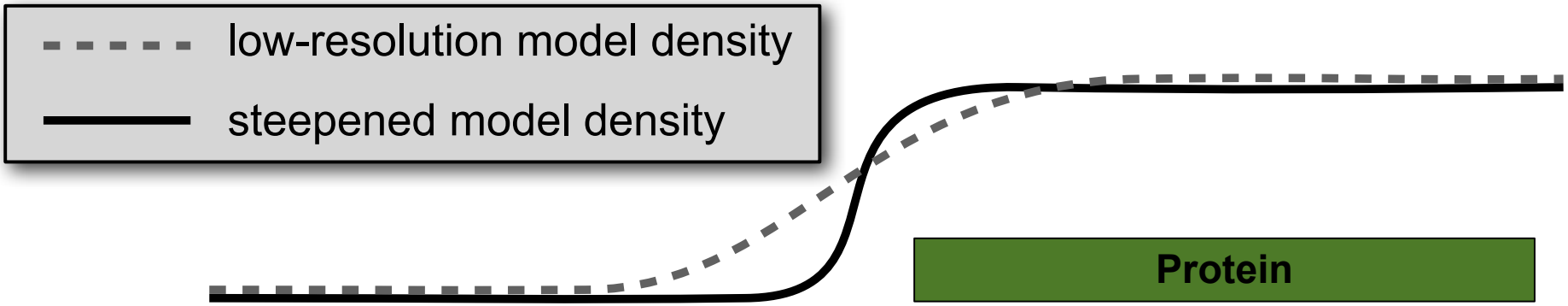
Masking

If your model is not complete.

1) Compute low-resolution model density map (e.g. 30 Å) from your rigidly or roughly fitted model.

2) Steepen the edges of the model density (with *apply-cos-mapping*)
```
$>  apply-cos-mapping model.mrc mask.mrc 0.4 0.3
```

3) use `-fmap mask.mrc` option. The map mask.mrc will then be multiplied to the model map in each iteration (but not to the EM map, that you have to do yourself).



Sharp edges create artifacts in Fourier space !!

Final optimization

DireX uses allowed distance intervals for the bonds, angles, etc.

Therefore after DireX refinement, the geometry is often not perfect.

Usually a energy minimization of the fitted structure is recommended (CNS, NAMD, Gromacs, etc)

DireX Tutorials

Download DireX and Tutorial files from:

https://www.simtk.org/home/direx/

Further information (Documentation and Tutorial description):

http://www.schroderlab.org/software/direx/index.html