Structure Refinement at Low Resolution

with

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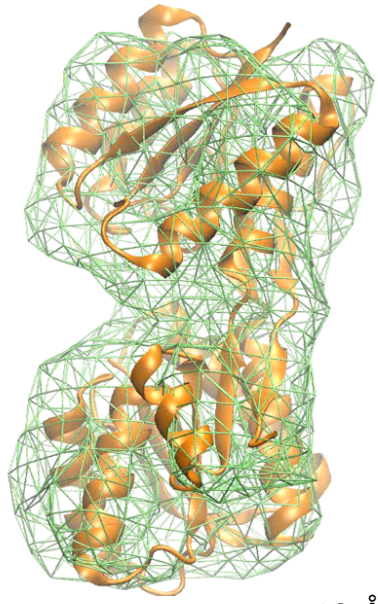
Structure Refinement at Low-resolution

Assume: High-quality starting structure (known structure in different conformation or good homology model)

Standard refinement yields a bad structure

How to make use of prior structural information during the refinement?



10 Å

Deformable Elastic Network (DEN)

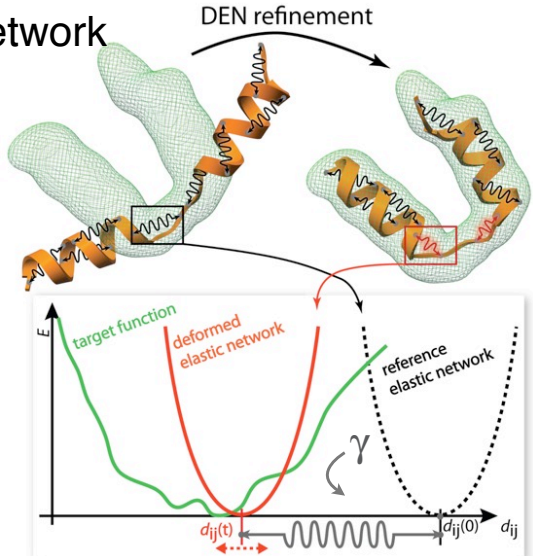
Refine only those degrees of freedom that need to be refined to fit the data, but not more.

Find only the relevant degrees of freedom for which the data actually provide information

Schröder, Brunger & Levitt, *Structure* (2007) **15**:1630

Deformable Elastic Network

The γ-parameter weights between reference model and experimental data



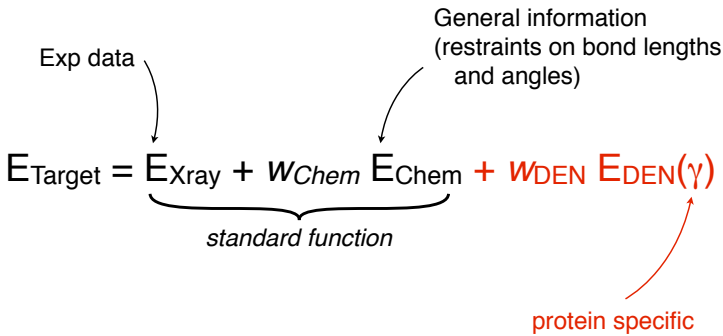
increasing deformability

$\gamma = 0$ **Reference model**

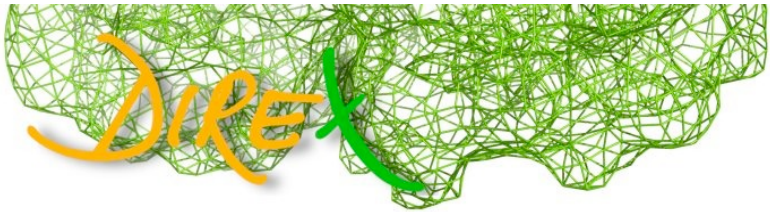
$\gamma = 1$ **Experimental restraints**

Deformable Elastic Network

Target function for minimization



w_{DEN} and γ need to be optimized



Real-space Refinement for X-ray Crystallography and Cryo-EM

- Geometry-based conformational sampling (based on Concoord)
- Forces
  1. Density Map
  2. Deformable Elastic Network (DEN)
  3. Distance restraints
  4. Position restraints

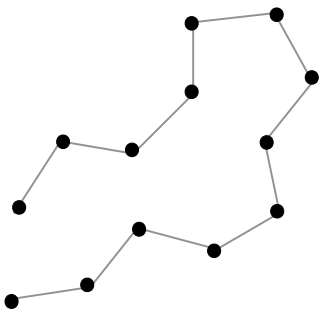
http://simtk.org/home/direx/
http://www.schroderlab.org/software/direx/

DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)

1. Initial model



DireX: Geometry-based conformational sampling

based on CONCOORD

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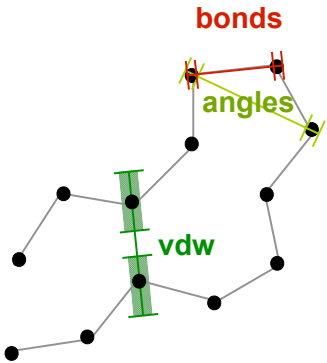

1. Initial model

2. Generate list of
   distance restraints (intervals)

DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)

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   distance restraints (intervals)

3. Perturb coordinates

DireX: Geometry-based conformational sampling

based on CONCOORD

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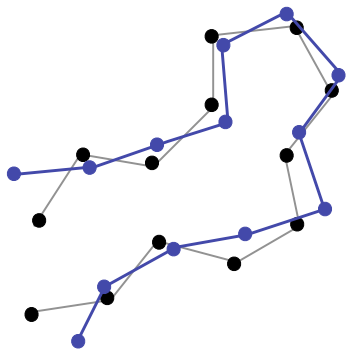


1. Initial model

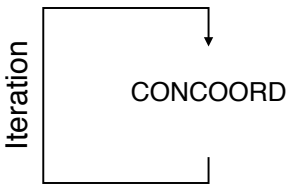
2. Generate list of
   distance restraints (intervals)

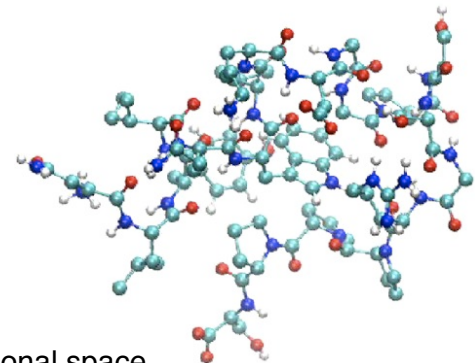
3. Perturb coordinates

4. use CONCOORD algorithm to obtain
   a new structure which also obeys
   all distance restraints

CONCOORD: correct distances
iteratively in a random order

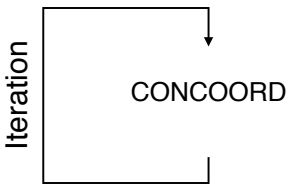
DireX: Geometry-based Conformational Sampling

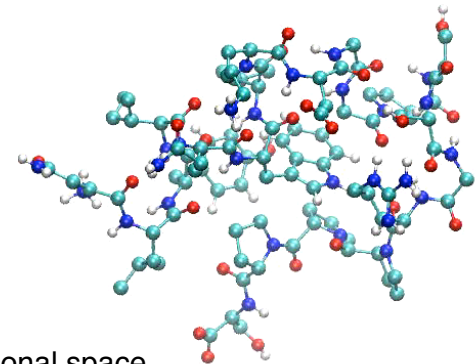




Random walk through conformational space
while maintaining correct stereochemistry
and avoiding atom clashes

DireX: Geometry-based Conformational Sampling



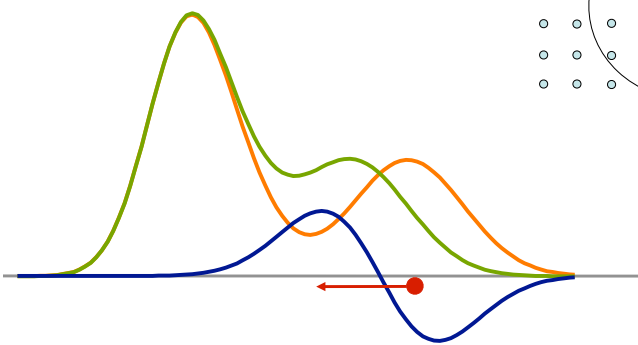


Random walk through conformational space
while maintaining correct stereochemistry
and avoiding atom clashes

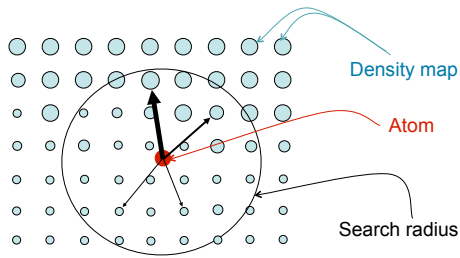
DireX: Forces derived from a density map

$$\rho_{difference}(x) = \rho_{target}(x) - \lambda\, \rho_{model}(x)$$

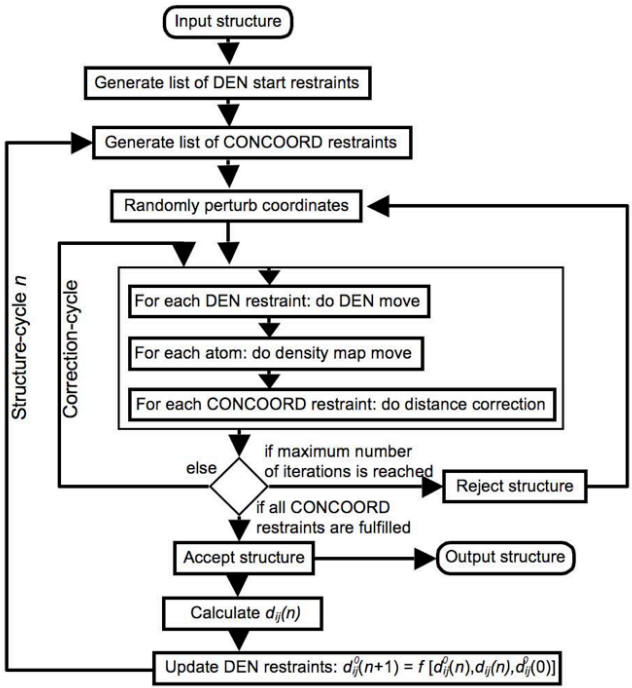
Target density
Model density
Difference



Stochastic gradient to move atoms into high difference-density regions

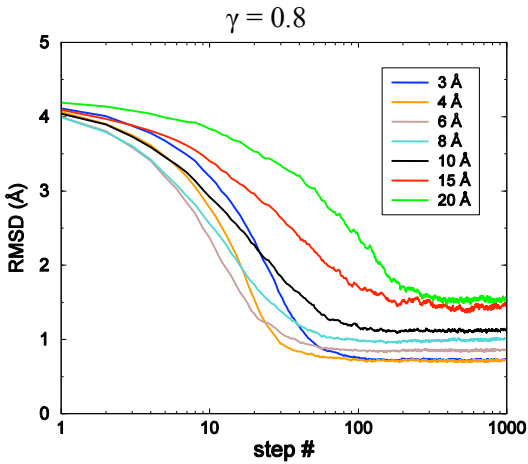


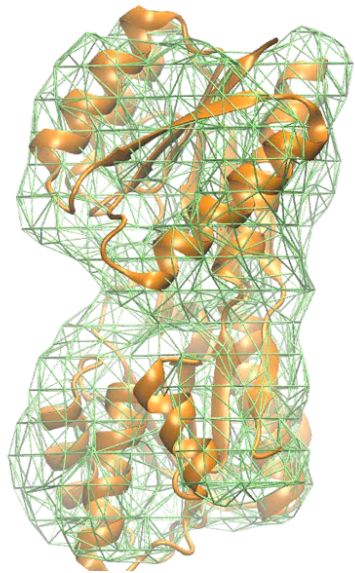
For each atom:
average over 10 randomly chosen vectors weighted by density difference


Input structure
Generate list of DEN start restraints
Generate list of CONCOORD restraints
Randomly perturb coordinates
Structure-cycle n
Correction-cycle
For each DEN restraint: do DEN move
For each atom: do density map move
For each CONCOORD restraint: do distance correction
else
if maximum number of iterations is reached
Reject structure
if all CONCOORD restraints are fulfilled
Accept structure
Output structure
Calculate $d_{ij}(n)$
Update DEN restraints: $d_{ij}^{0}(n+1) = f\,[d_{ij}^{0}(n), d_{ij}(n), d_{ij}^{b}(0)]$

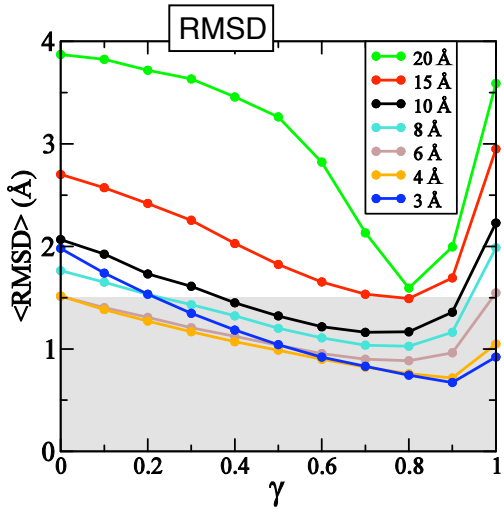
Test system: Ribose-binding protein

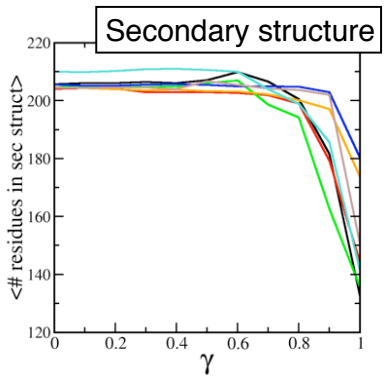
DEN





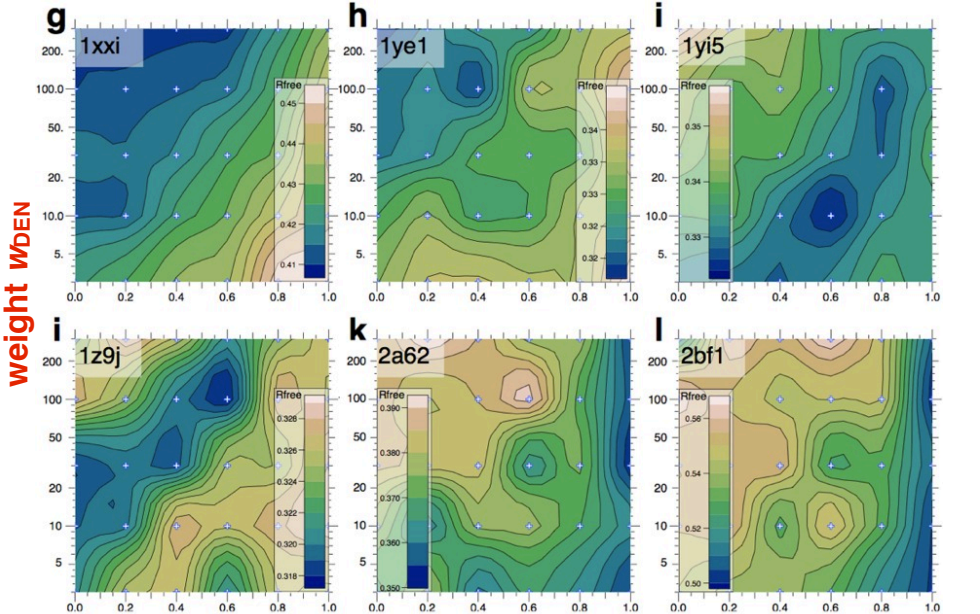
Deformable Elastic Network: Effect of γ





Parameter optimization

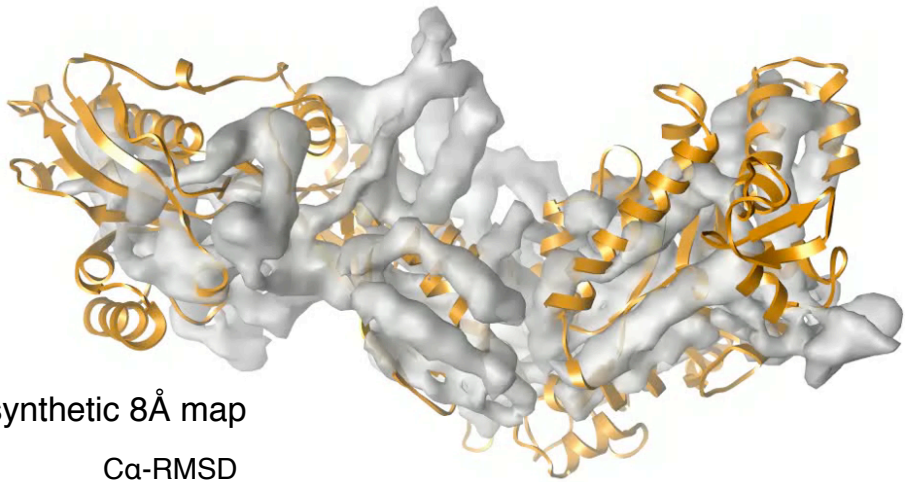
$$E_{Target} = E_{Xray} + w_{Chem}E_{Chem} + w_{DEN}E_{DEN}(\gamma)$$



example from X-ray crystallography

DireX

Example: Elongation Factor 2 (EF-2)



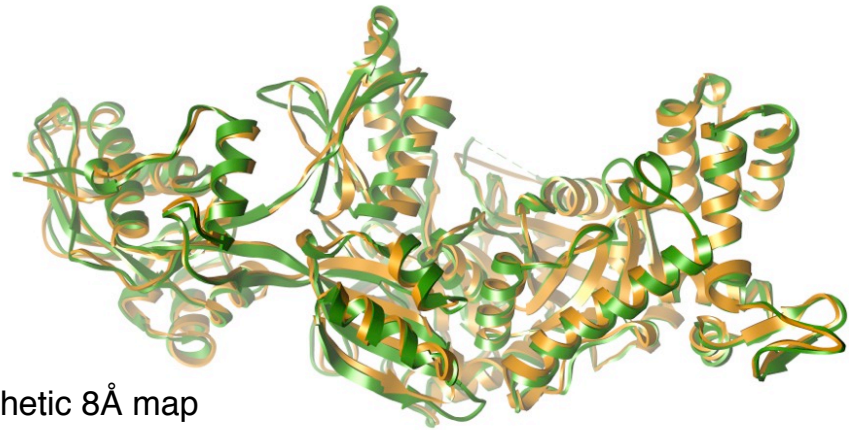
synthetic 8Å map

	Cα-RMSD
initial	13.6 Å
final	0.8 Å

6300 atoms, 14 steps/min
(3.5 hrs for 3000 steps)

DireX

Example: Elongation Factor 2 (EF-2)



synthetic 8Å map

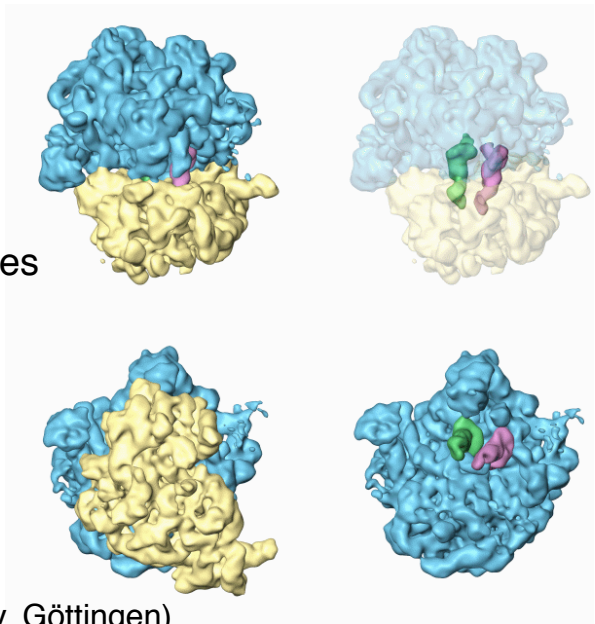
	Cα-RMSD
initial	13.6 Å
final	0.8 Å

6300 atoms, 14 steps/min
(3.5 hrs for 3000 steps)

Ribosome

2 million single-particle images sorted into 50 conformational substates

Resolution 8 - 15 Å

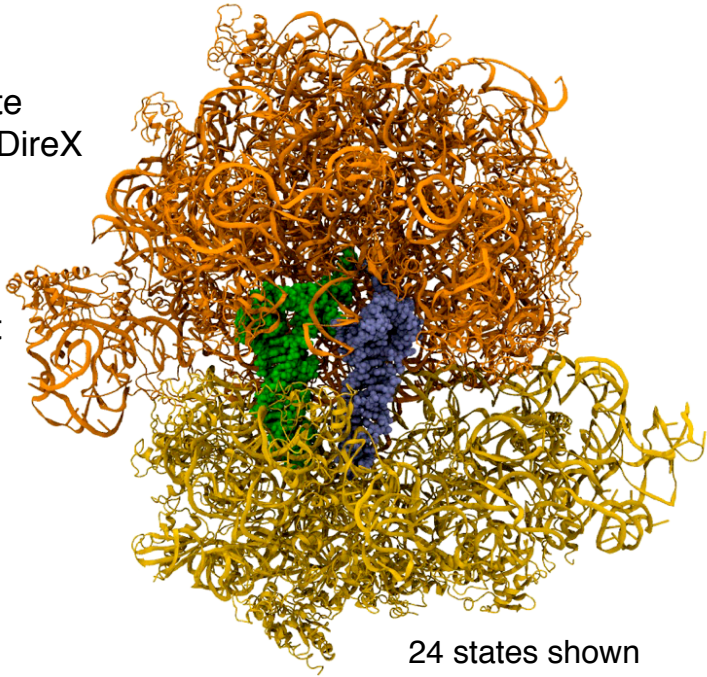


In collaboration with
Holger Stark's lab
(MPI Biophysical Chemistry, Göttingen)

Fischer, Konevega, Wintermeyer, Rodnina & Stark (2010) Nature **466**: 329-333

Ribosome

Refined each state separately using DireX



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
4) Ribose-binding Protein
   Rather simple two-domain protein
5) Elongation-factor 2
   A little more complicated case
6) Resolving Clashes
   when fitting multiple components
7) Occupancy Refinement
   to account for reduced/missing density

DireX Tutorials

parameter file

DireX is a command line program:

```
$> direx -pdb input.pdb  -map density.mrc  -op out  -f refine.par
```

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

```
$>  export PATH=$PATH:$HOME/direx-0.4/direx
```

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

Typical usage is iteration over:

- Edit parameter file in text editor
- Run direx (start 'run.sh')
- Look at results with e.g. VMD or Chimera

DIREX Parameter File

Generate a template parameter file with most important default values:

```
direx -of all.par
```

with all default values:

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

General options

Coordinate Perturbation
- Tirion
- Advanced perturbation

DEN

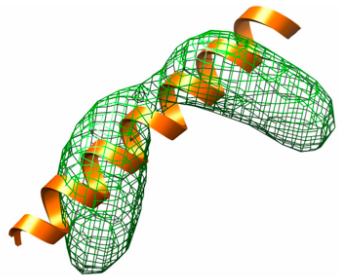
Density map
- Occupancy Refinement

Distance Restraints
Position Restraints
NCS Restraints

Others, including experimental and weird parameters

Kinked Helix at Low Resolution

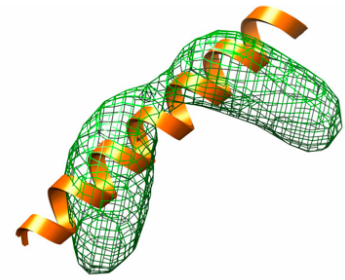
direx-tutorials/kinked-helix-lores/



```
#!/bin/bash

# Make density
direx -f mkdensity.par -pdb target.pdb
  -cur cur_mkdensity.pdb -curmap kinked-density.mrc
  -map self -map_apix 2.0

rm cur_mkdensity.pdb

# Run refinement
direx -f refine.par -pdb extended-helix.pdb
  -cur current.pdb -curmap curmap.mrc
  -map kinked-density.mrc -ox traj.xtc -mapcc
mapcc-1.dat

# Run minimization
direx -f min.par -pdb extended-helix.pdb -p
current.pdb
  -curmap curmap-min.mrc -cur current-min.pdb
  -map kinked-density.mrc -ox traj-min.xtc
  -refden extended-helix.pdb -mapcc mapcc-min.dat
```

Kinked Helix at Low Resolution

direx-tutorials/kinked-helix-lores/



General

```
nsteps     = 200
pert_fac = 0.1
```

DEN

```
use_den = yes
den_no = 412
den_strength = 0.3
den_upper = 15.0
den_lower = 3.0
den_gamma = 0.8
den_kappa = 0.4
```

Density Map

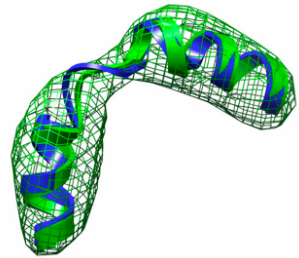
```
map_strength = 0.030
cur_map_kernel = gaussian
```

set model map resolution

```
cur_map_sig = 3.0
cur_map_kernel_rad = 10.0
```

Kinked Helix at Low Resolution

direx-tutorials/kinked-helix-lores/



“Minimization”

```
sampling_mode = gradient [or concoord]
```

```
concoord_damp = 1.0
```

```
gradient_damp = 0.1
```

```
pert_fac = 0.0
```

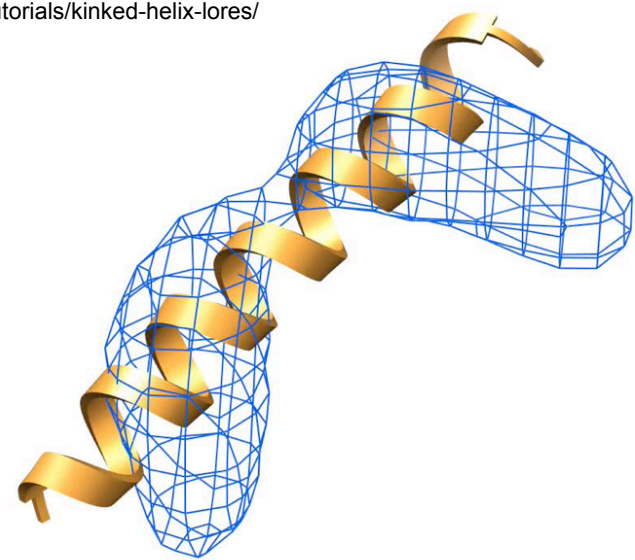
Look at results:

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

Final structure is `current-min.pdb`

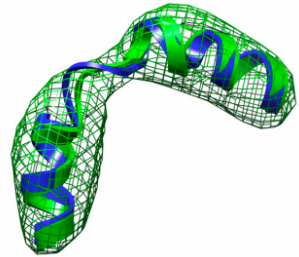
Kinked Helix at Low Resolution

direx-tutorials/kinked-helix-lores/

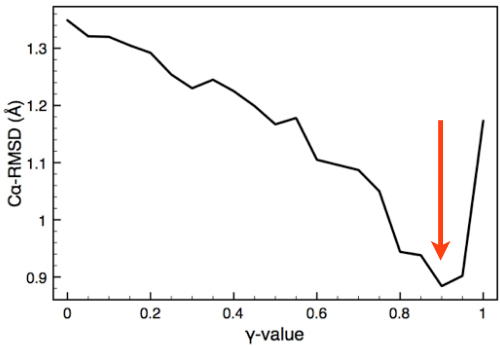


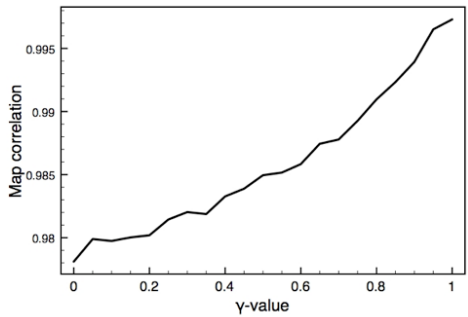
Kinked Helix at Low Resolution

direx-tutorials/kinked-helix-lores/



Optimizing the γ-parameter





Kinked Helix at High Resolution

direx-tutorials/kinked-helix-hires/

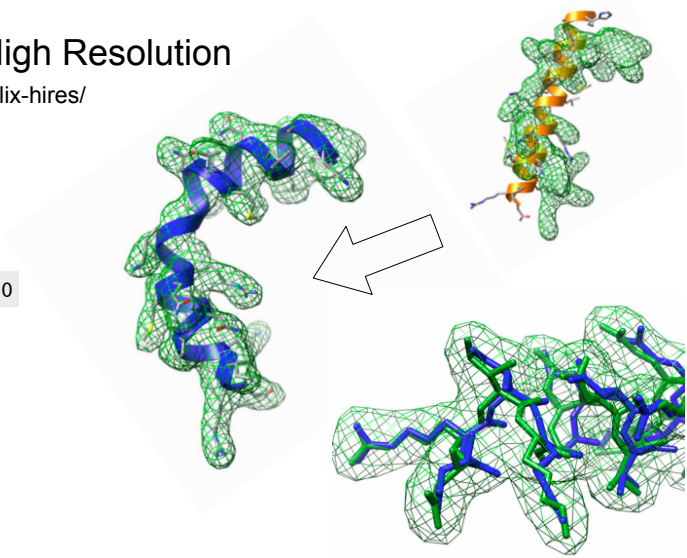
```
nsteps      = 1000
```

```
den_no = 800
```

```
den_sidechain = 0.0
```

set resolution to about 3 Å

```
cur_map_sig = 1.0
```



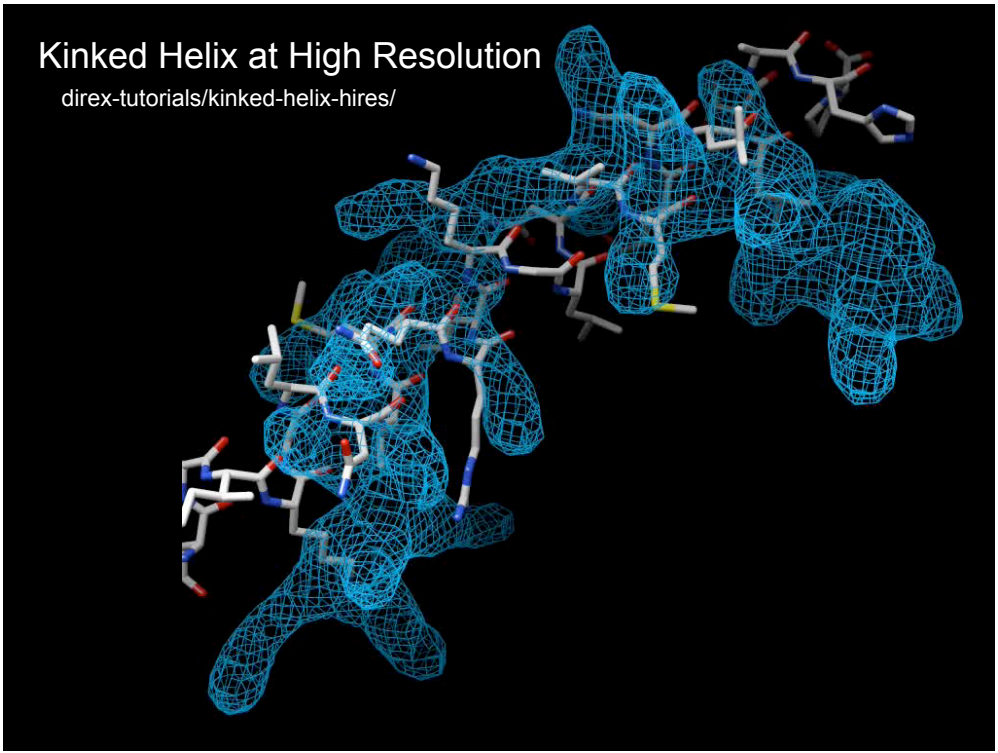
Look at results:

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

Final structure is `current-min.pdb`

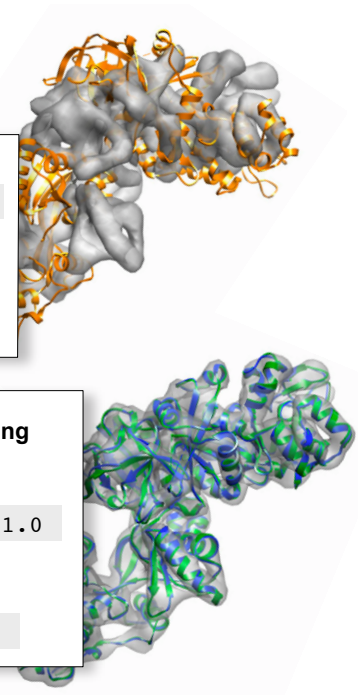
Kinked Helix at High Resolution

direx-tutorials/kinked-helix-hires/



Elongation-factor 2

direx-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:

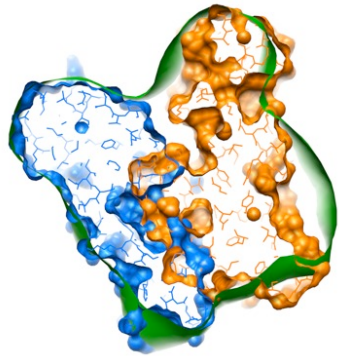
den_secstr_loop = 0.6

Tirion Enhanced Sampling

```
tirion_use = yes
tirion_pert_fac = 1.0

tirion_lb = 0.5
tirion_ub = 0.5
```

Resolve Clashes

direx-tutorials/resolve-clashes/



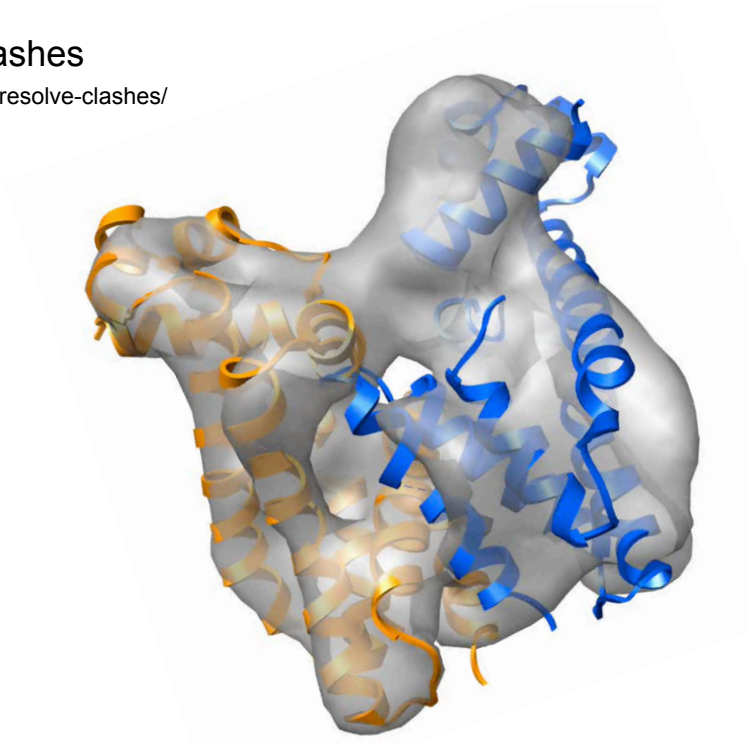
- Prevent CONCOORD algorithm from defining distance restraints between overlapping models
- Add repulsive forces between atoms from overlapping chains

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 -cur
current-1.pdb -curmap curmap-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 -cur
current-2.pdb -ox traj-2.xtc -map dimer-density.mrc -refden separate-
chains.pdb -curmap curmap-2.mrc -mapcc mapcc-2.dat
```

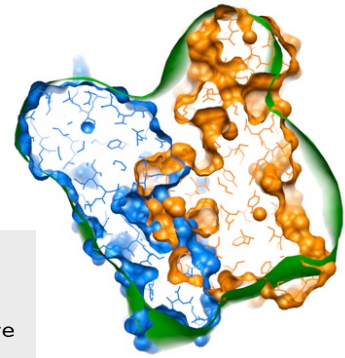
Resolve Clashes

direx-tutorials/resolve-clashes/



Resolve Clashes

direx-tutorials/resolve-clashes/



Parameters (*Misc options*)

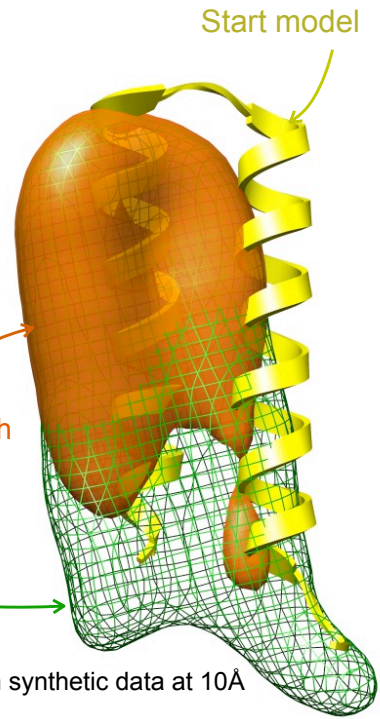
```
# >0 means atoms repel each other.
# <0 means atoms attract each other.
repel_shift = 0.01
# Number of steps during which restraints are
# scaled down from 1 to 0
repel_damp = 10

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

Occupancy Refinement

direx-tutorials/occ-refine/

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

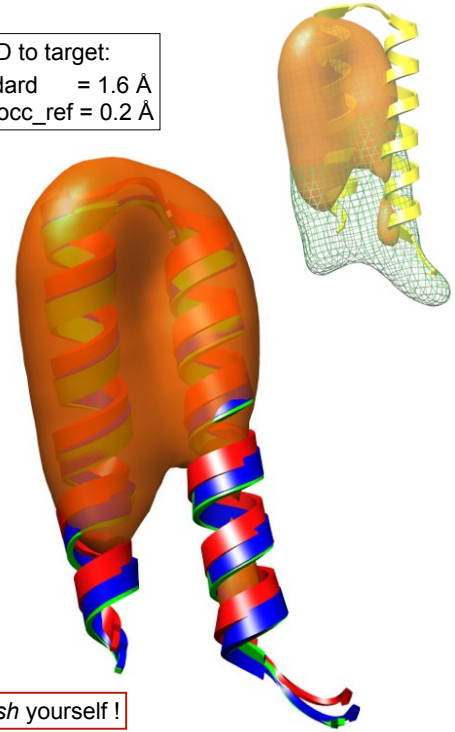
```
direx -f refine.par -pdb ../start.pdb
  -cur current.pdb -map ../map-10A-occ.mrc
  -curmap curmap.mrc -ox traj.xtc
```



Occupancy Refinement

direx-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 = 0.003
```

Use one occ-value for whole residue:

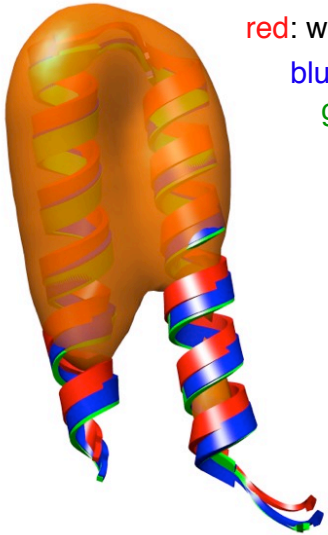
```
map_group_refine_occ = yes
```

```
map_bfac_as_occ = yes
```

To limit number of additional parameters use occupancy restraints:

```
map_occ_restraint_lambda = 0.00
```

Output in */results* are wrong, you should run *run.sh* yourself !



red: without Occ refinement the structure is shifted

blue: with Occ refinement

green: target (correct) structure

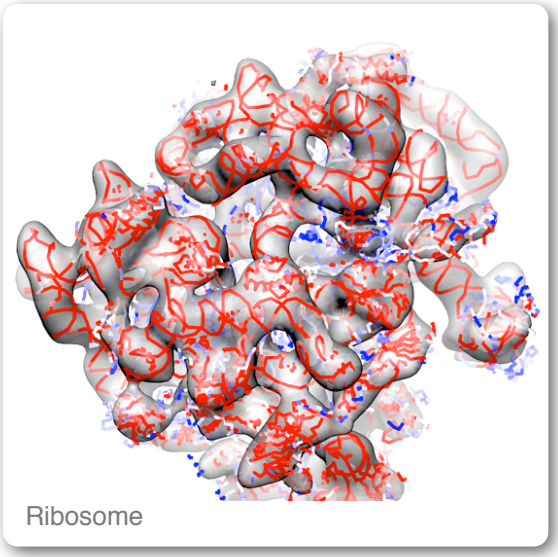
RMSD to target structure

1.6 Å without Occ. refinement

0.3 Å with Occ. refinement

Map correlation 0.93

Map correlation 0.99



Ribosome

NCS Restraints

Non-crystallographic symmetry

Command line:

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

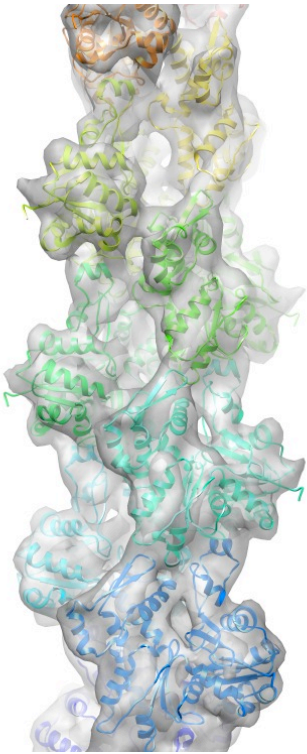
Parameters:

```
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
...
```



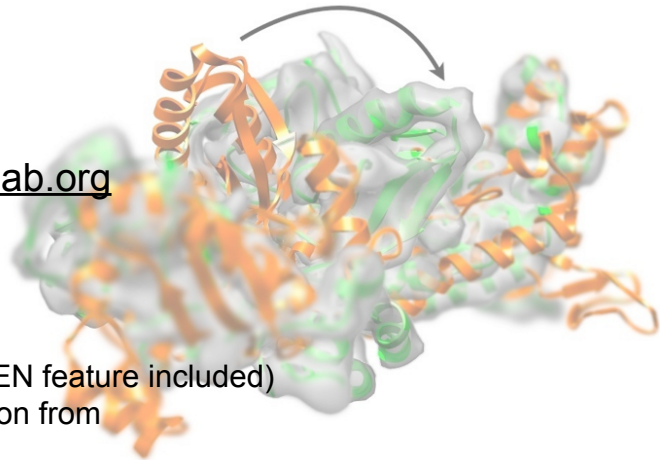
Actin filament

To download DireX and Tutorial files:

www.simtk.org/home/direx

or visit

www.schroderlab.org



CNS 1.3 (with DEN feature included)
will be available soon from

http://cns-online.org/v1.3/