



◆ CCP4 + PHENIX

# Phaser: Molecular Replacement

*CCP4 Study Weekend*

*7 January 2010*



# Phaser

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Phaser is a program for:

1. molecular replacement
2. experimental phasing
3. normal-mode analysis



# Phaser

## ARCIMBOLDO: Crystallographic Ab Initio protein solution far below atomic resolution

Dayte D. Rodríguez Martínez, Christian Grosse, Sebastian Himmel, César González, Iñaki M. de Ilarduya, Stefan Becker, George M. Sheldrick and Isabel Usón

Ab Initio macromolecular phasing has been so far limited to small proteins at atomic resolution ( $1.2\text{\AA}$  or better unless heavy atoms are present). We present a general method for  $2\text{\AA}$  data, based on combination of location of model fragments like small  $\alpha$ -helices with ***PHASER*** and density modification with ***SHELXE***, supported by a grid of computers running ***CONDOR***.

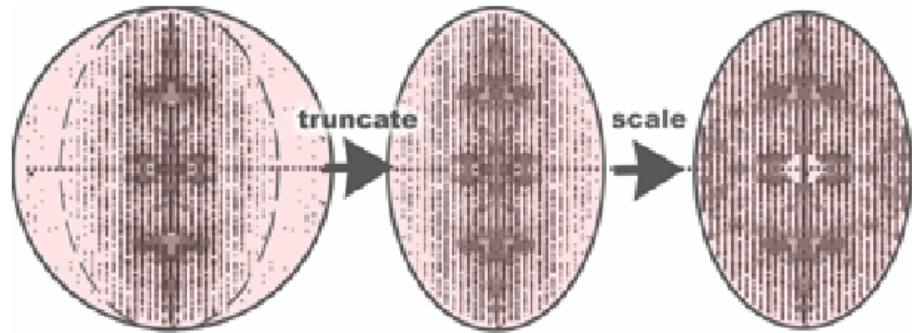
If you want to test our method, and already have login and password, please click below.

[Download ARCIMBOLDO](#)





# Phaser



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### Reference

[M. Strong, M.R. Sawaya, S. Wang, M. Phillips, D. Cascio, D. Eisenberg, \*Proc Natl Acad Sci USA\*. \*\*103\*\*, 8060-8-65, 2006.](#)



# Phaser



Low graphics Help

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## NEWS

**LIVE** BBC NEWS CHANNEL

Page last updated at 07:20 GMT, Thursday, 19 November 2009

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## Lab worms are stunned by 'phaser'

By Jason Palmer  
Science and technology reporter, BBC News

**Scientists have shown off an effect not unlike that of the "phasers" in the show Star Trek - but it only works on tiny worms called nematodes.**

They used a special molecule that, when exposed to ultraviolet (UV) light, changes its shape.

When the worms were fed this molecule and then exposed to UV light, they exhibited paralysis.



Under UV light, the worms turned blue and were paralysed

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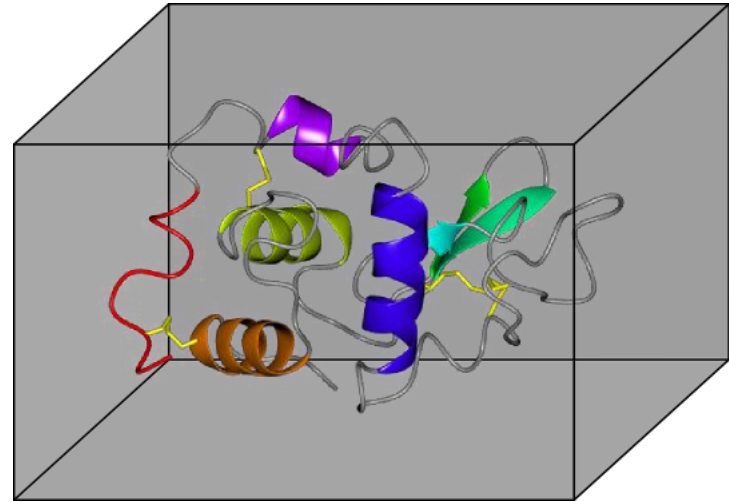
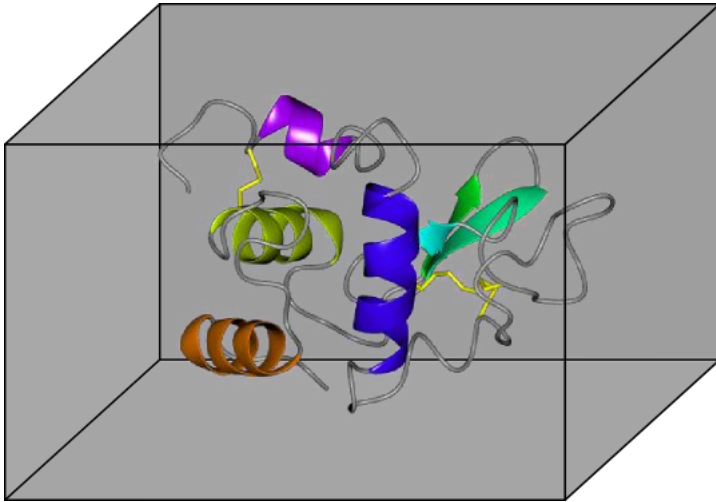
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# Idea

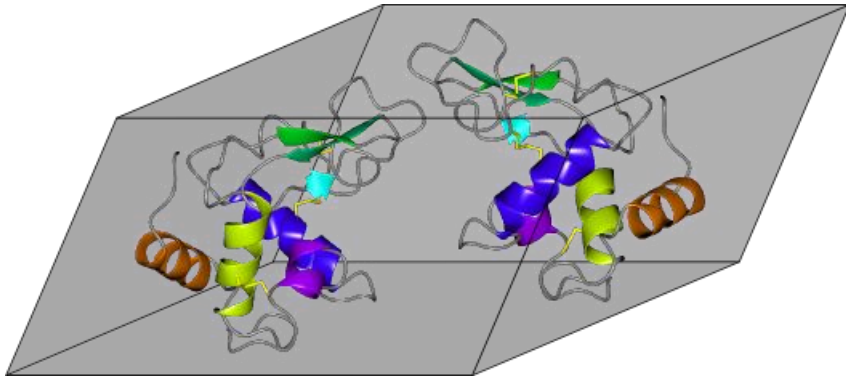


| <b>h</b> | <b>k</b> | <b>l</b> | <b>F</b> | <b><math>\phi</math></b> |
|----------|----------|----------|----------|--------------------------|
| 0        | 0        | 1        | 12.6     | 123                      |
| 0        | 0        | 2        | 2.1      | 12                       |
| 0        | 0        | 3        | 69.9     | 287                      |

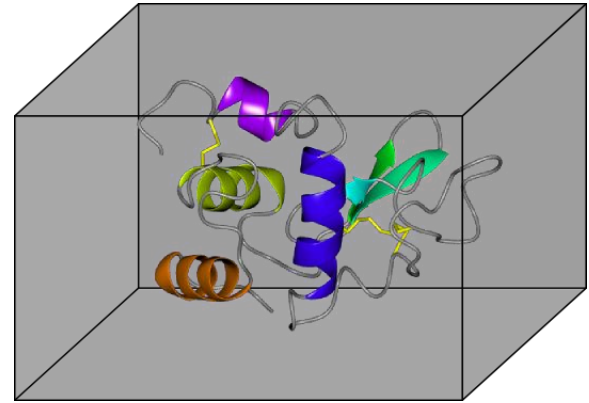
| <b>h</b> | <b>k</b> | <b>l</b> | <b>F</b> | <b><math>\phi</math></b> |
|----------|----------|----------|----------|--------------------------|
| 0        | 0        | 1        | 10.4     | 113                      |
| 0        | 0        | 2        | 3.5      | 18                       |
| 0        | 0        | 3        | 57.2     | 265                      |



# Problem



Real crystal form



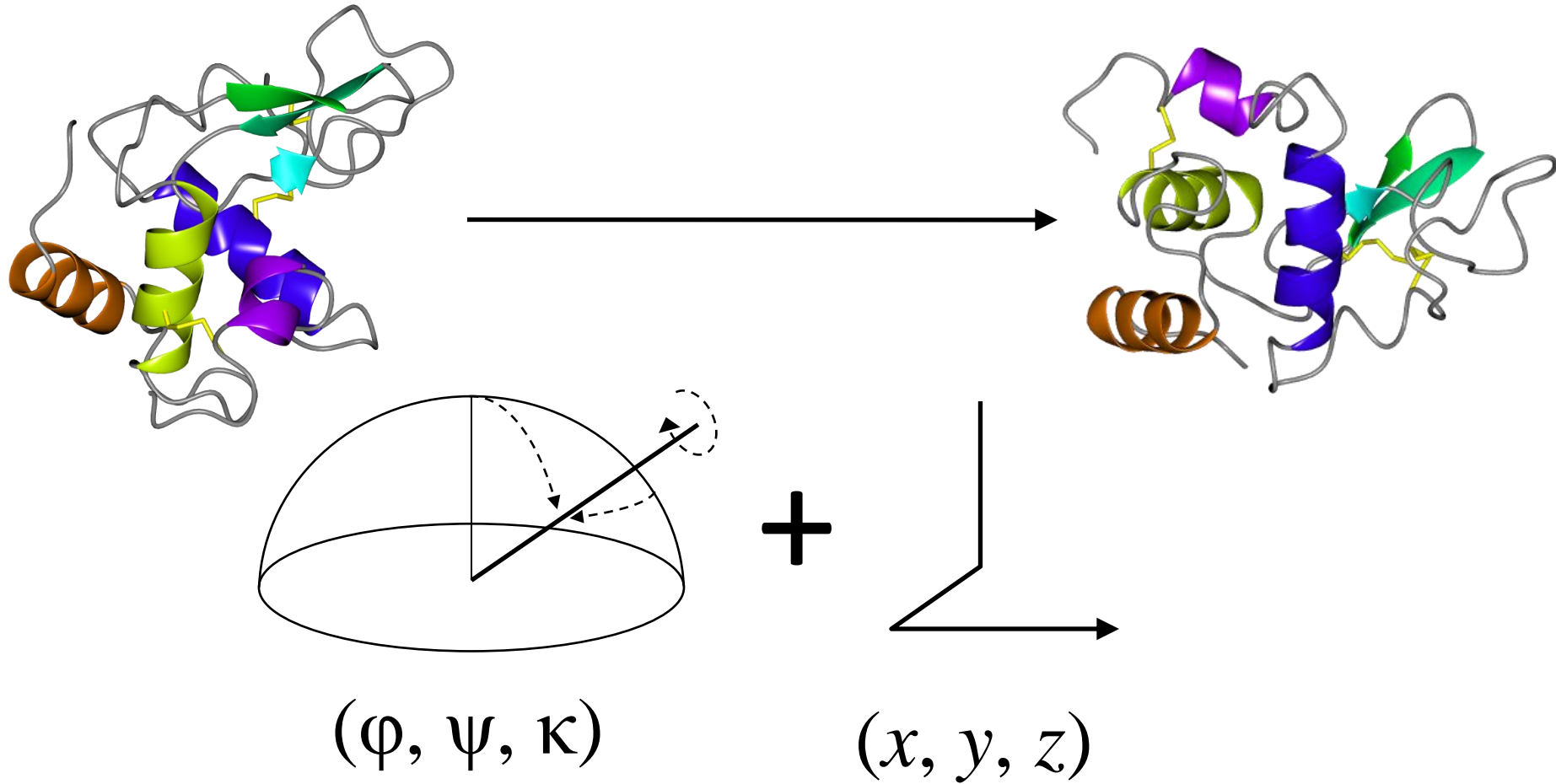
Imaginary crystal form



$$\mathcal{R}(\phi, \psi, \kappa, x, y, z)$$



# Separability



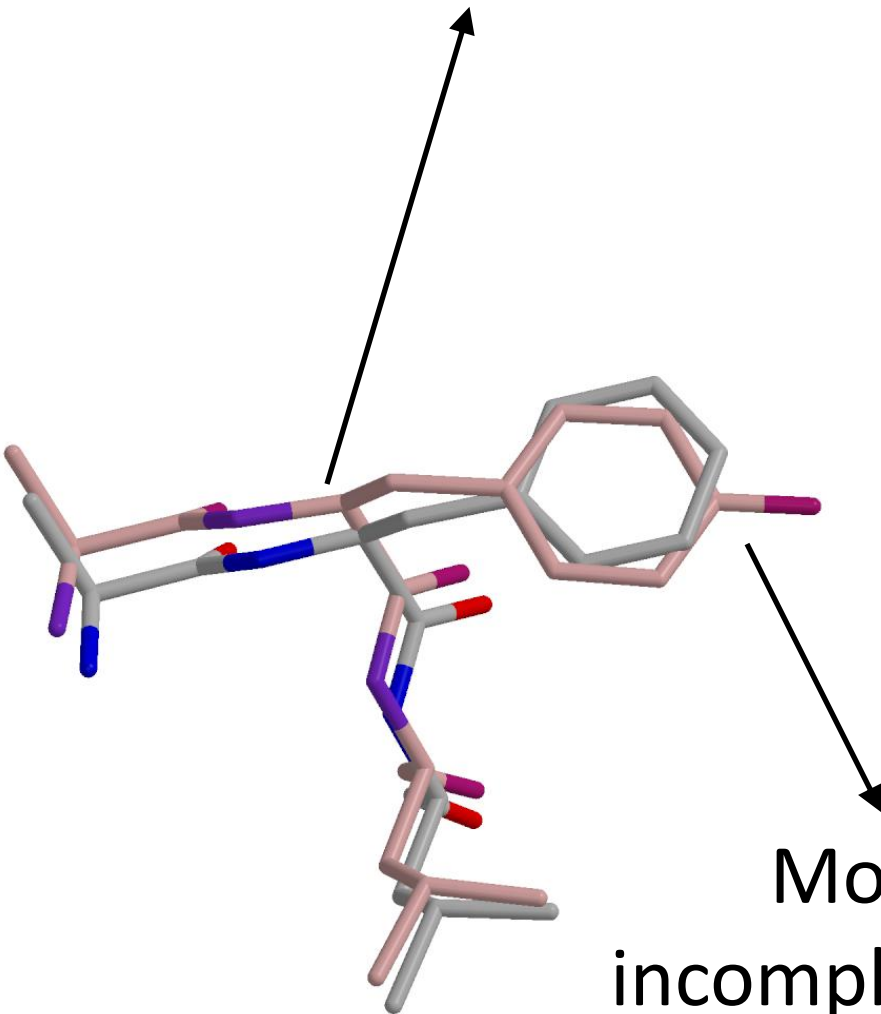
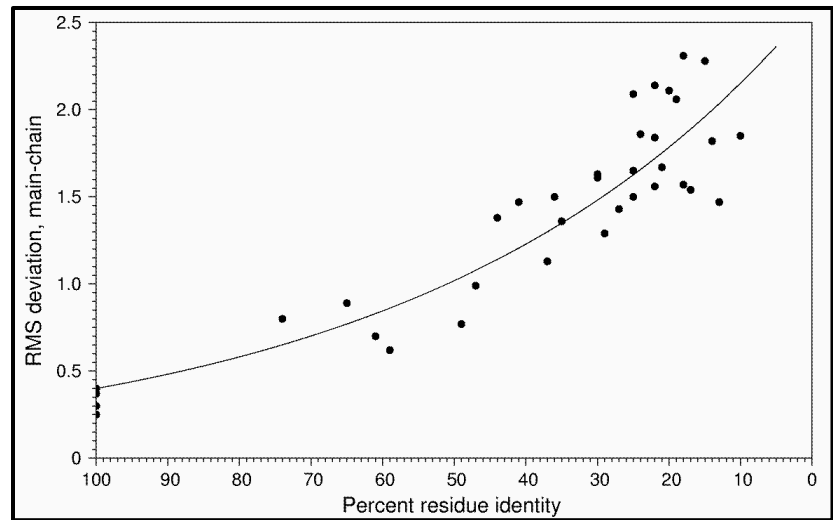




# Model error

Position errors

Calculate from  
sequence identity  
(Chothia & Lesk)



Calculate from unit  
cell composition

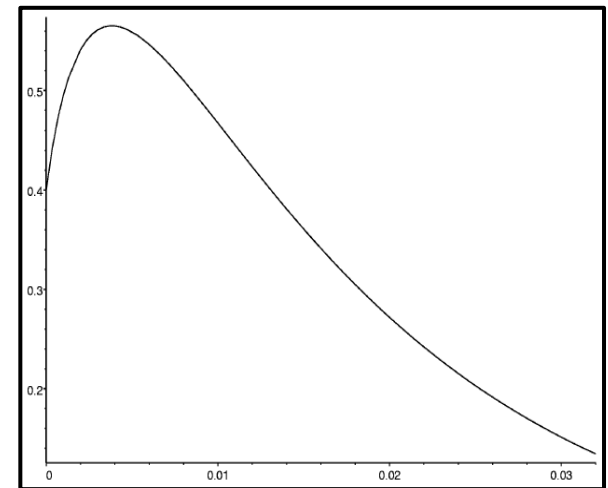


# Likelihood weighting

Position errors



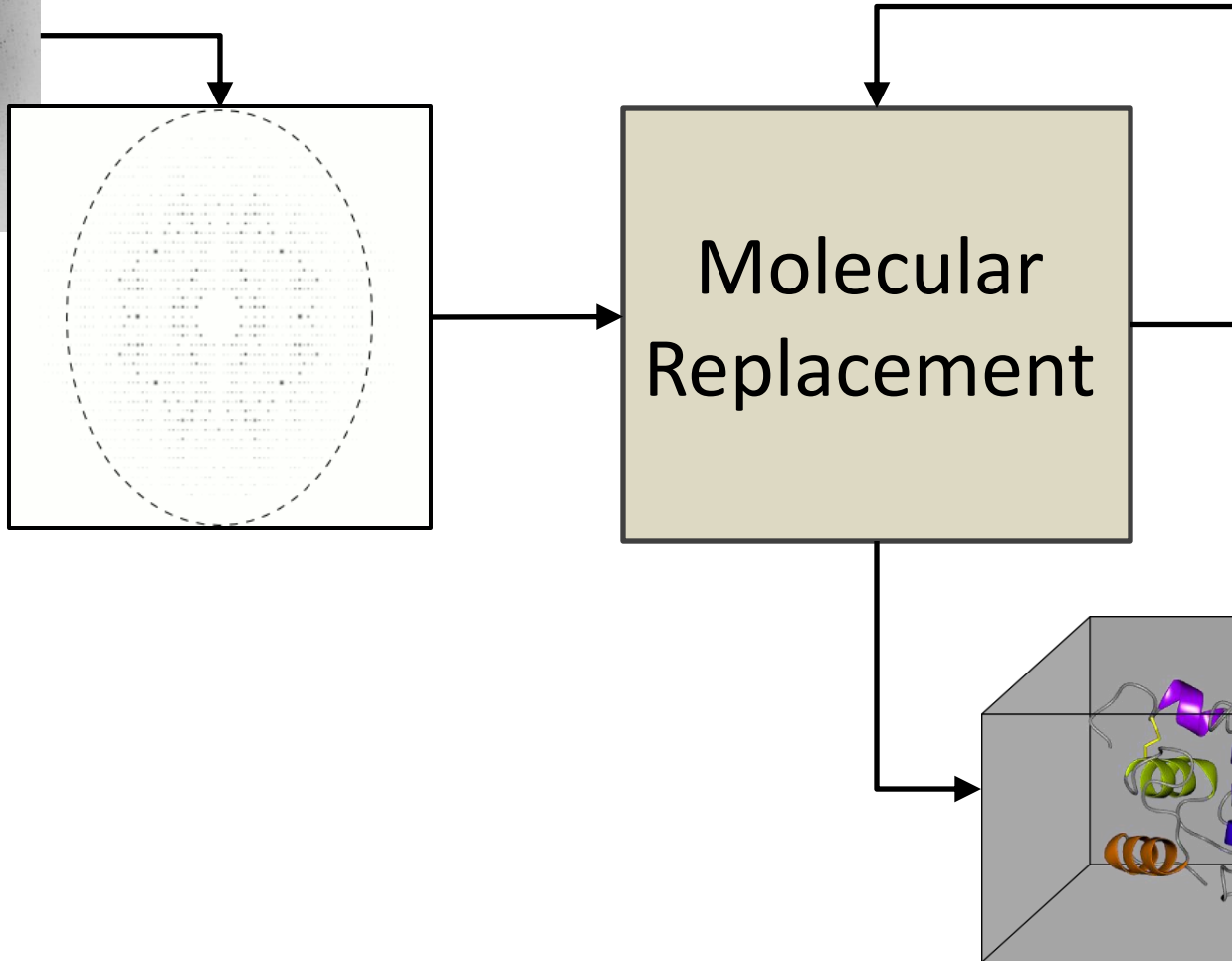
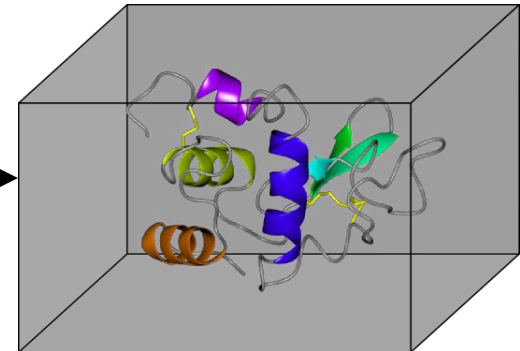
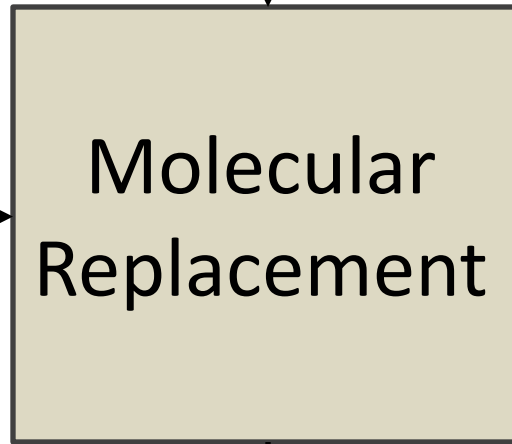
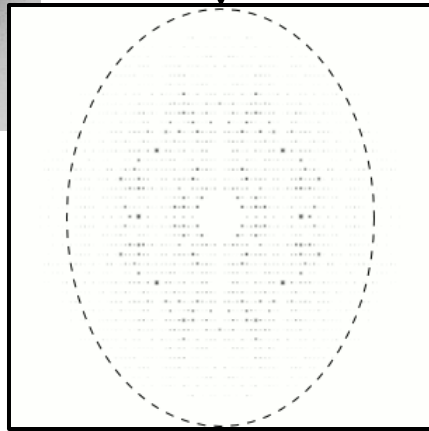
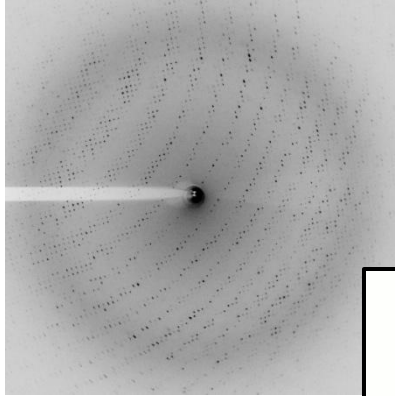
Incompleteness



Optimal resolution-  
dependent weights

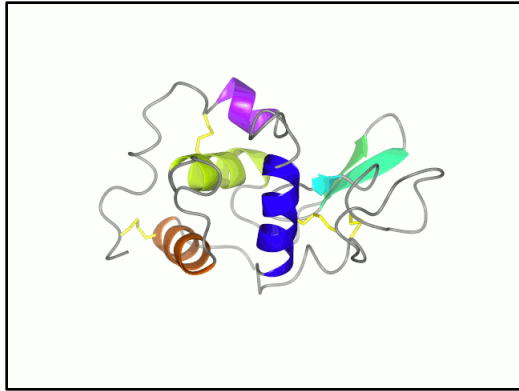


# Phaser Workflow

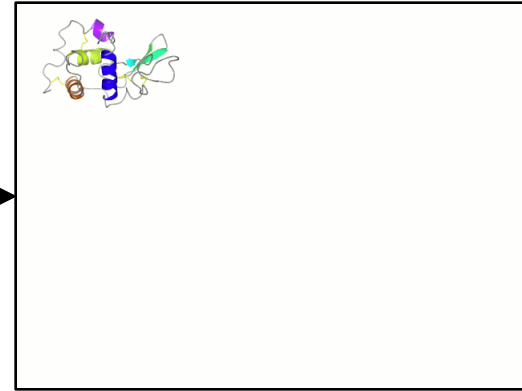




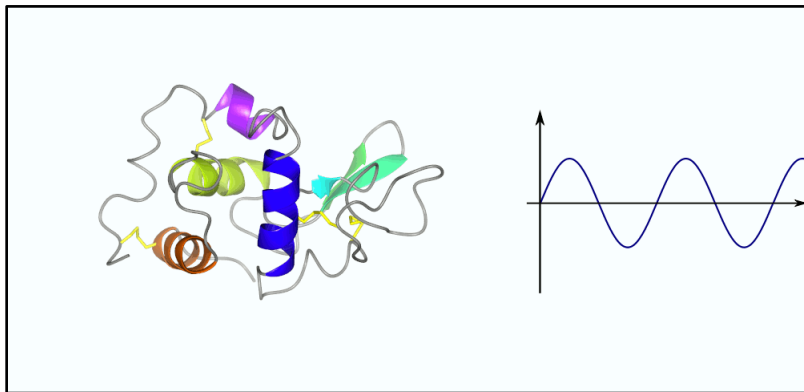
# Molecular Replacement Workflow



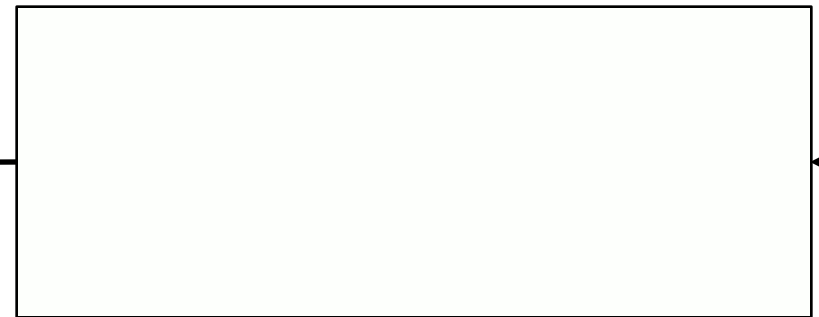
Rotation search



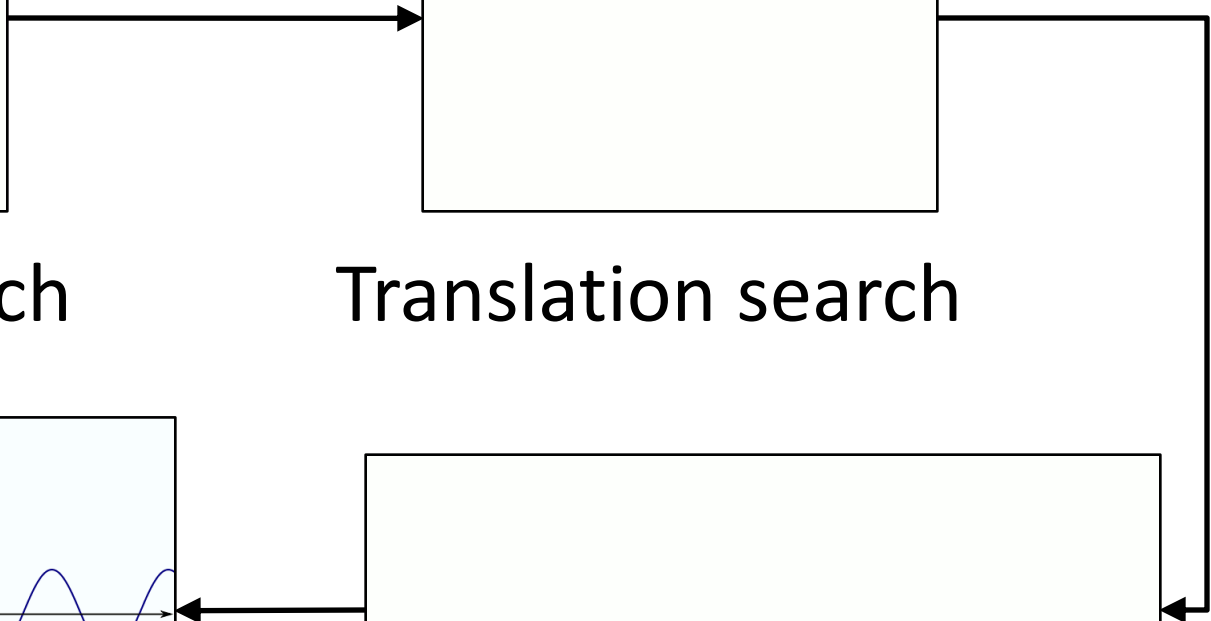
Translation search



Refinement



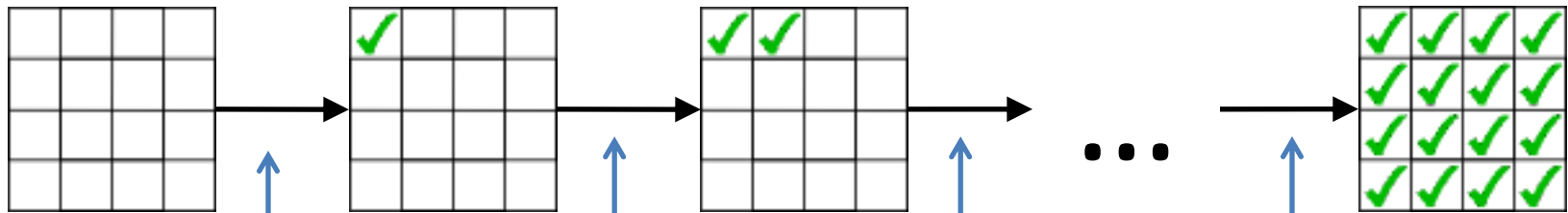
Packing test





# Brute Search

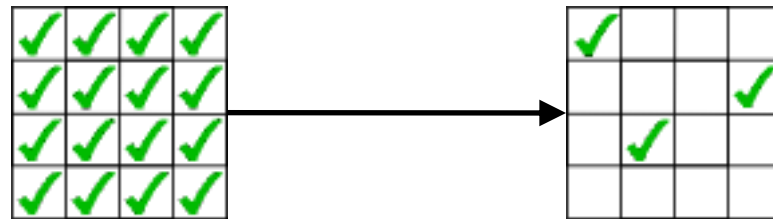
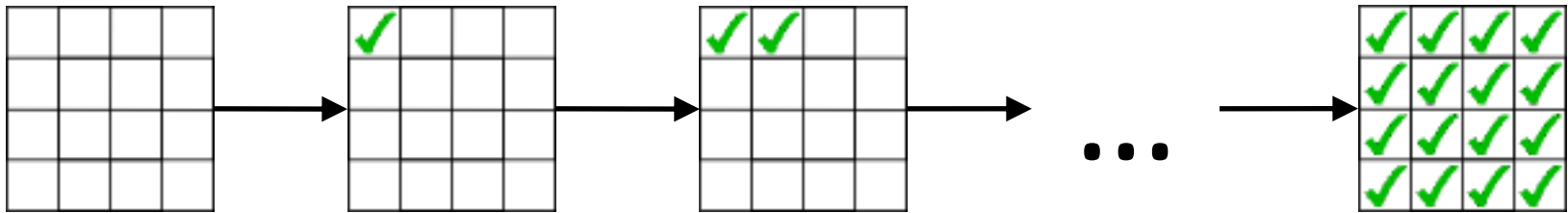
Calculate likelihood for each gridpoint





# Brute Search

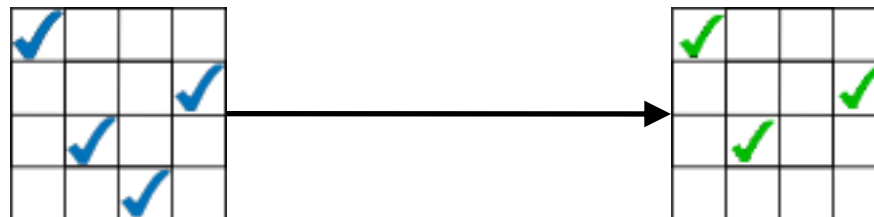
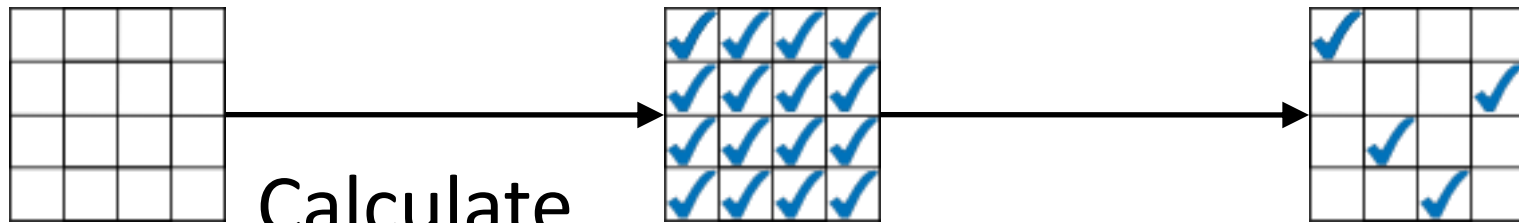
Calculate likelihood for each gridpoint



Find peaks



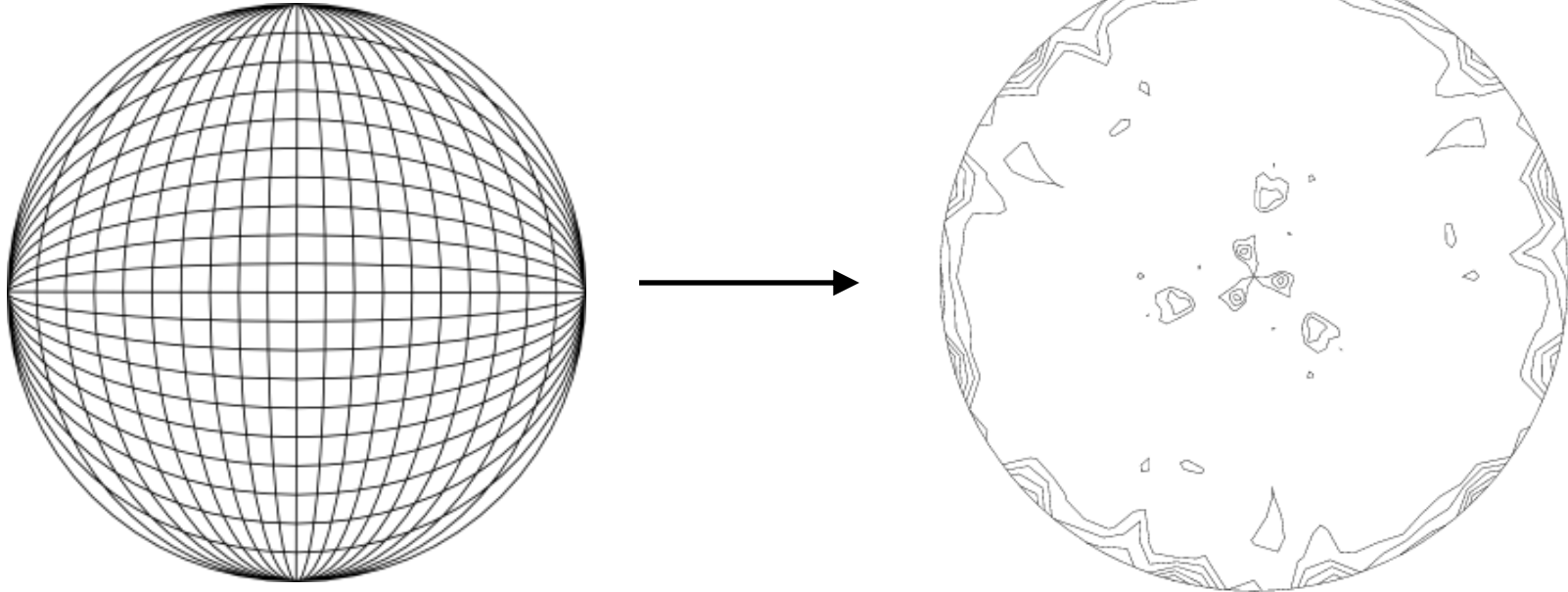
# Fast Search



“Rescore” with likelihood



# Rotation Search



⇒ Typically small signal

⇒ Dependent only on the point group

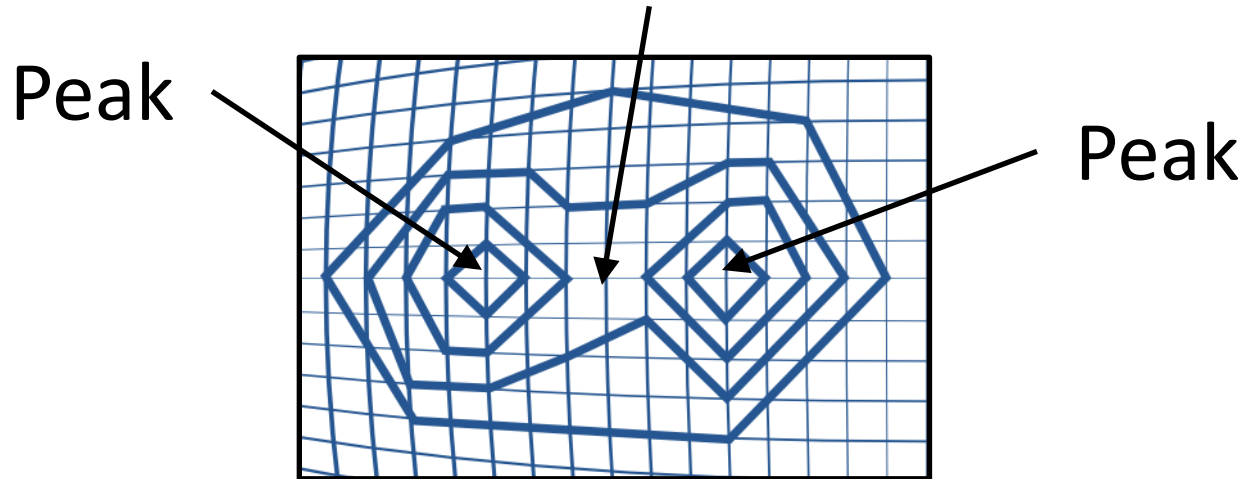






# Rotation peaks

Centre of coalesced peak



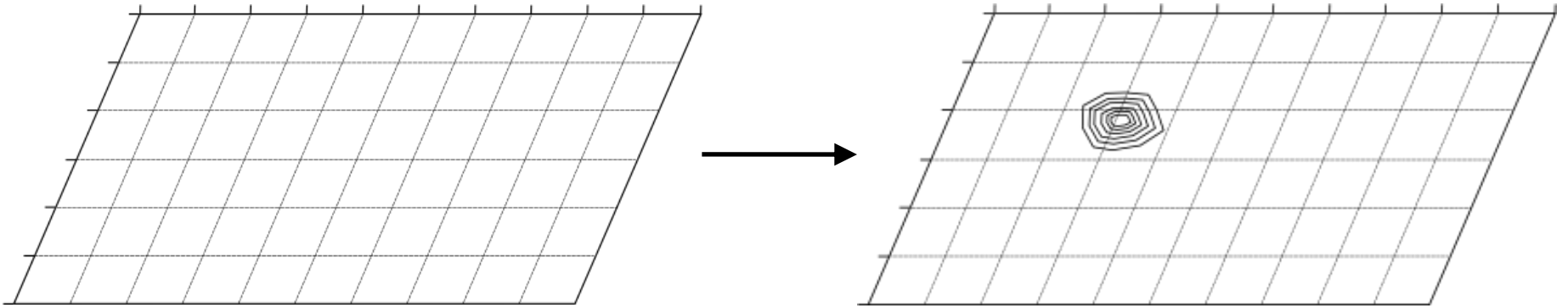
Translation function is very sensitive to orientation and may not find a solution in such a case!





# Translation Search

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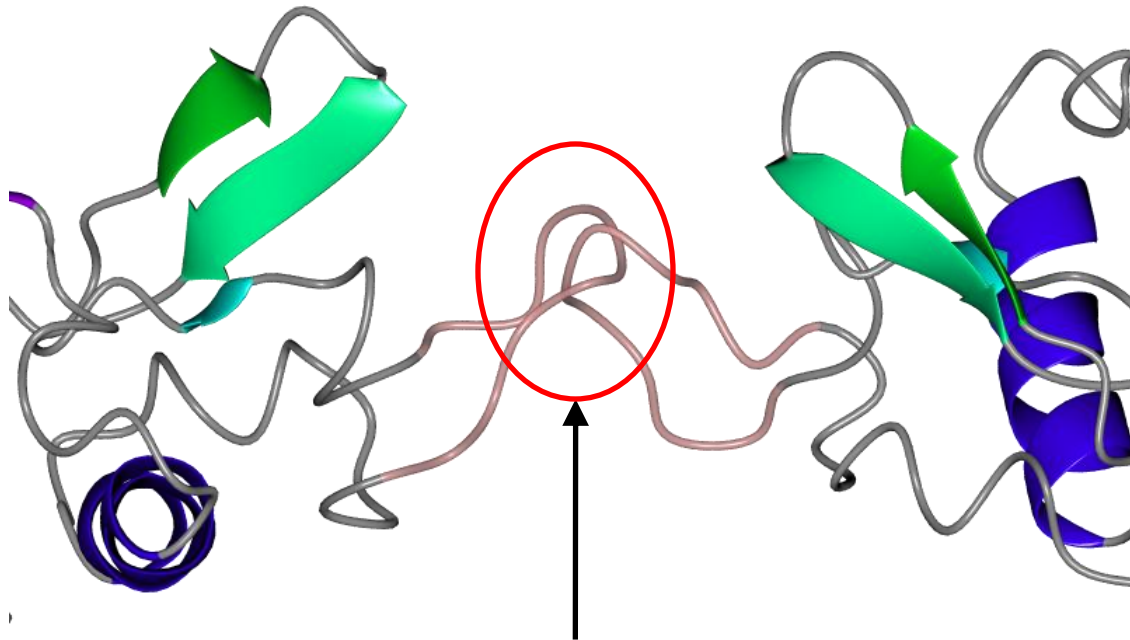
Solutions are recognizable at this stage  
(high Z-score)





# Packing

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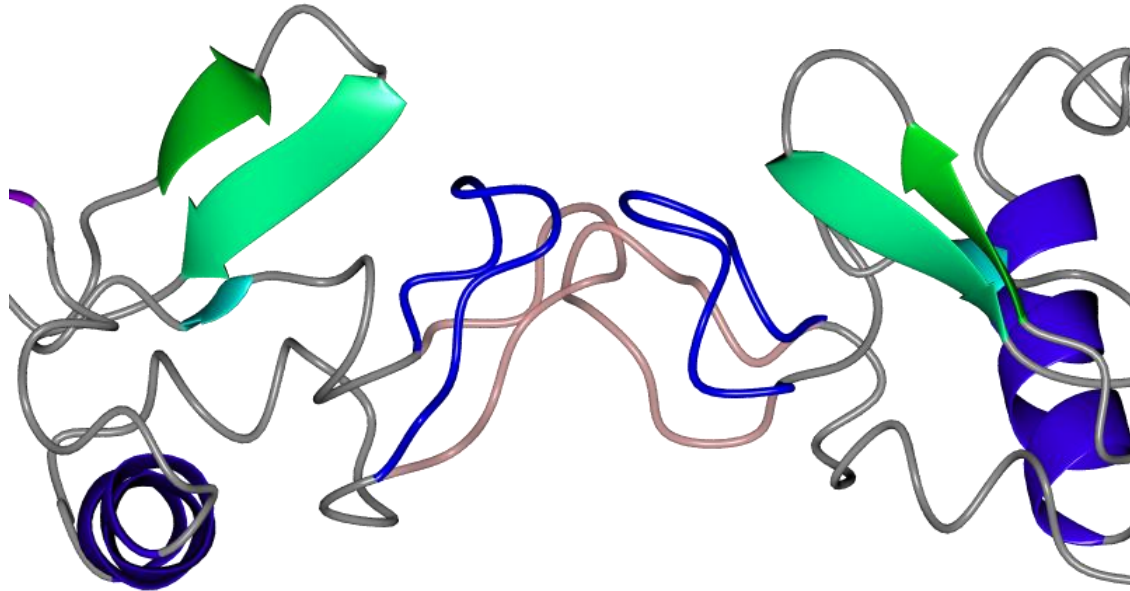


Clashes between models  
decrease credibility in  
solution



# Packing

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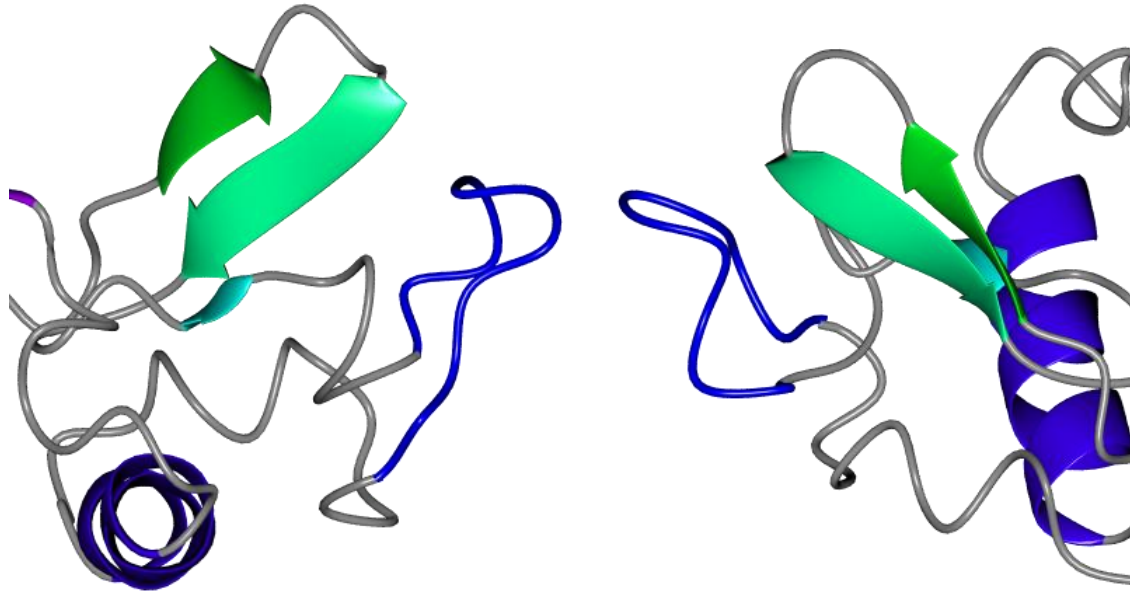


Surface loops may change  
conformation



# Packing

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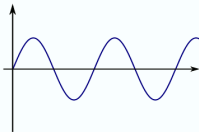
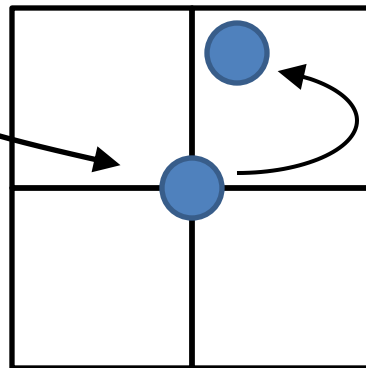
A small number of clashes  
is acceptable



# Refinement

The rotation and translation functions were performed on a (not very fine) grid

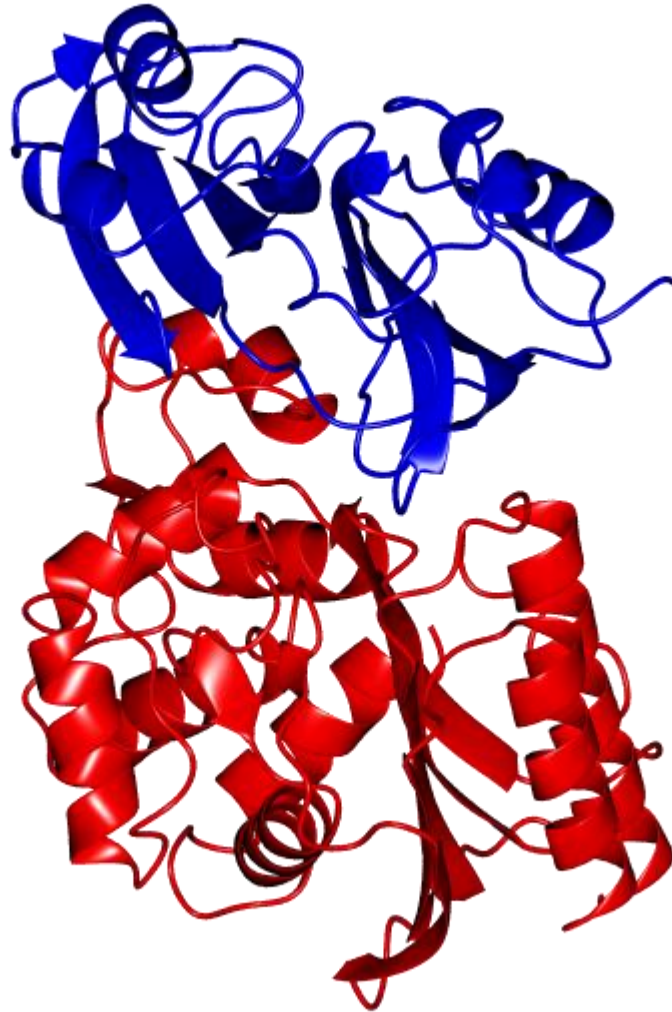
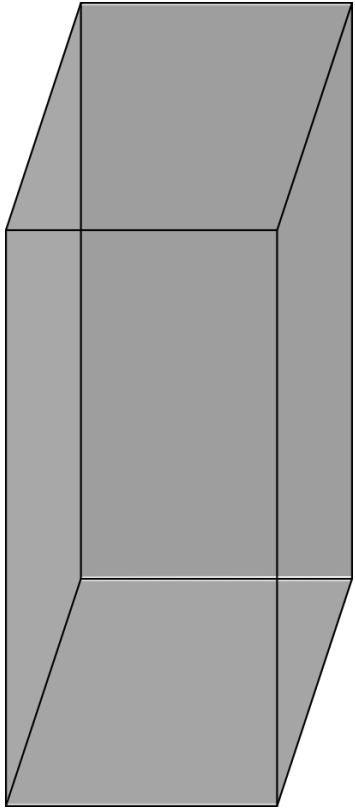
The solution can be improved if the grid is taken away and the rotational and translational parameters optimized





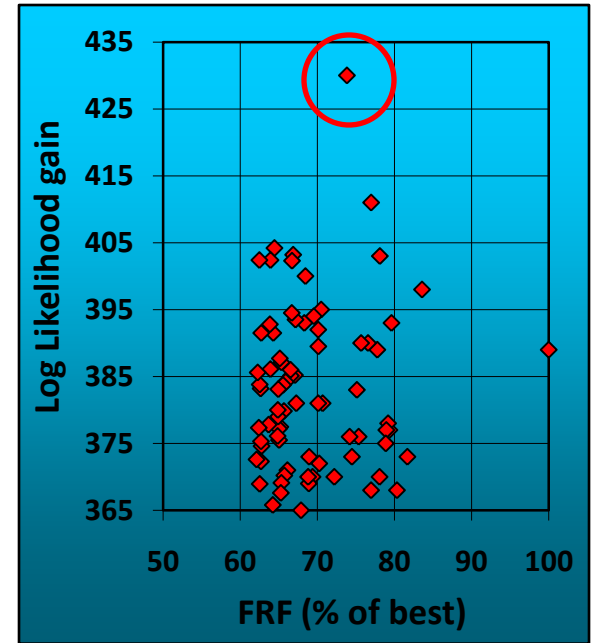
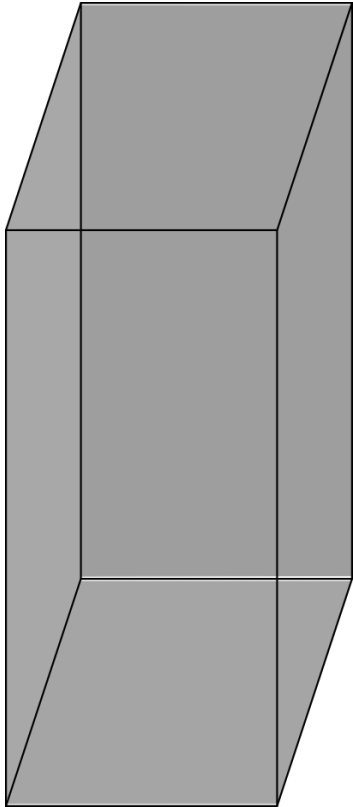
# Partial Structure

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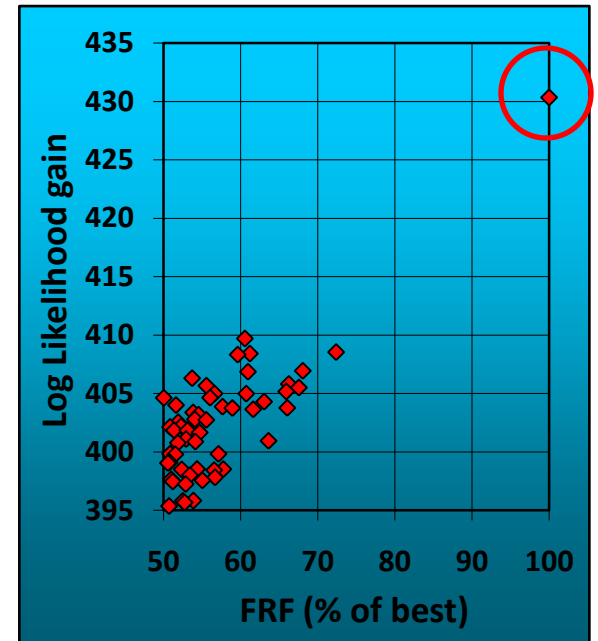
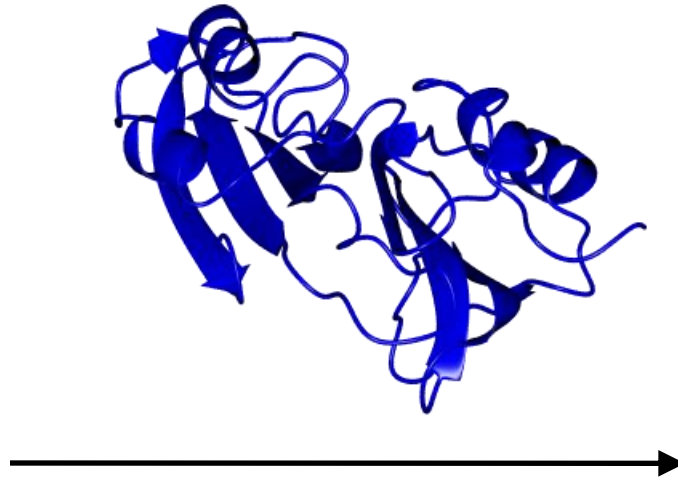
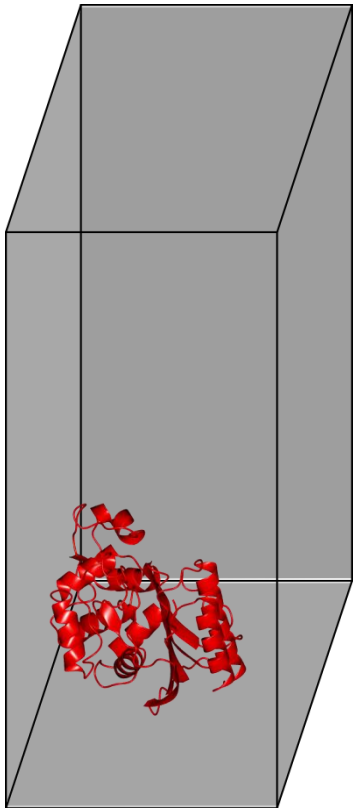
# Partial Structure







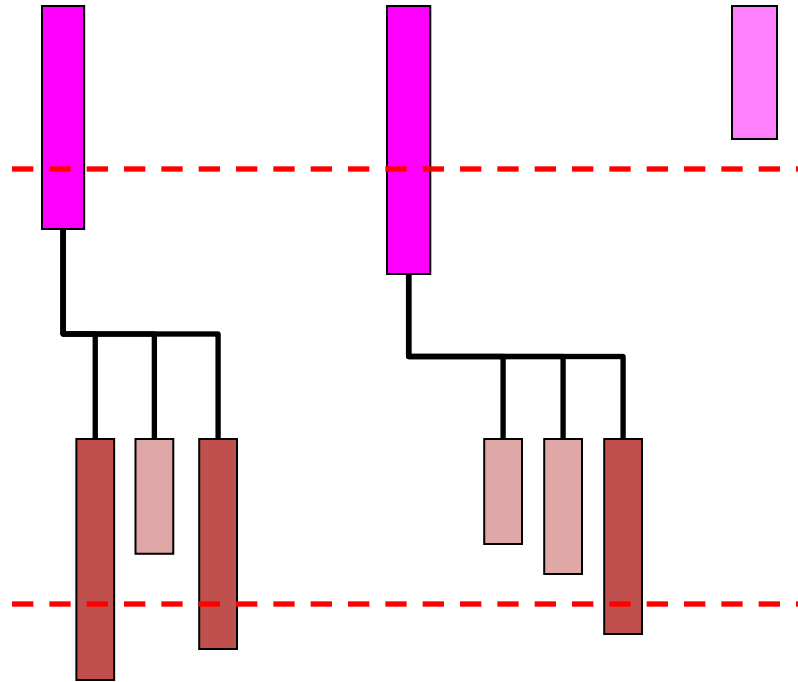
# Partial Structure





# Tree search with pruning

1<sup>st</sup> component

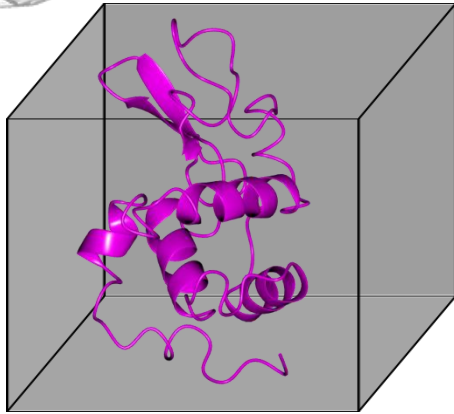


2<sup>nd</sup> component

Search order set up automatically based on resolution and expected error!



# “Manual” molecular replacement



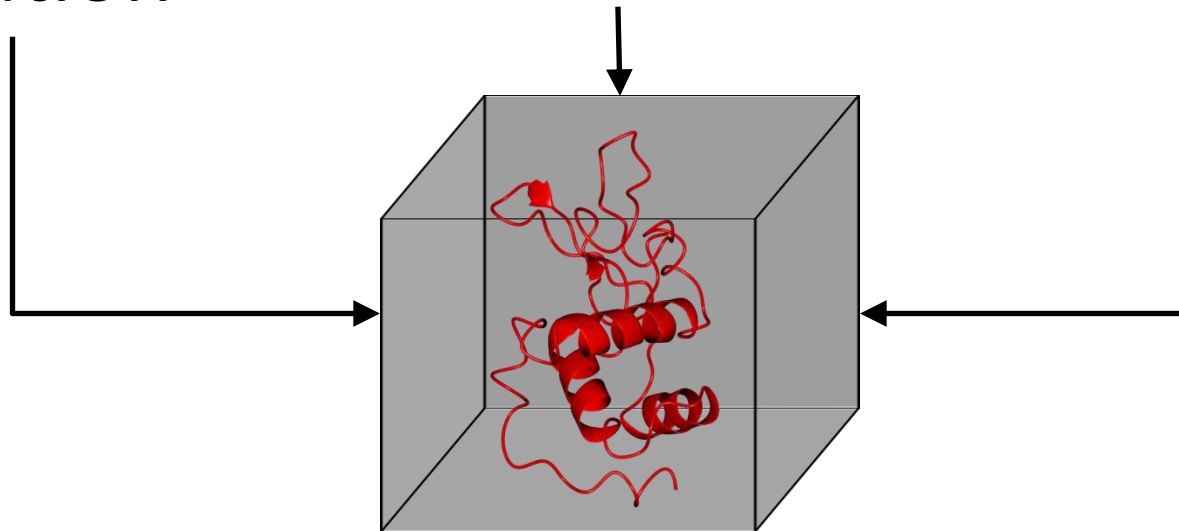
Solution



Model



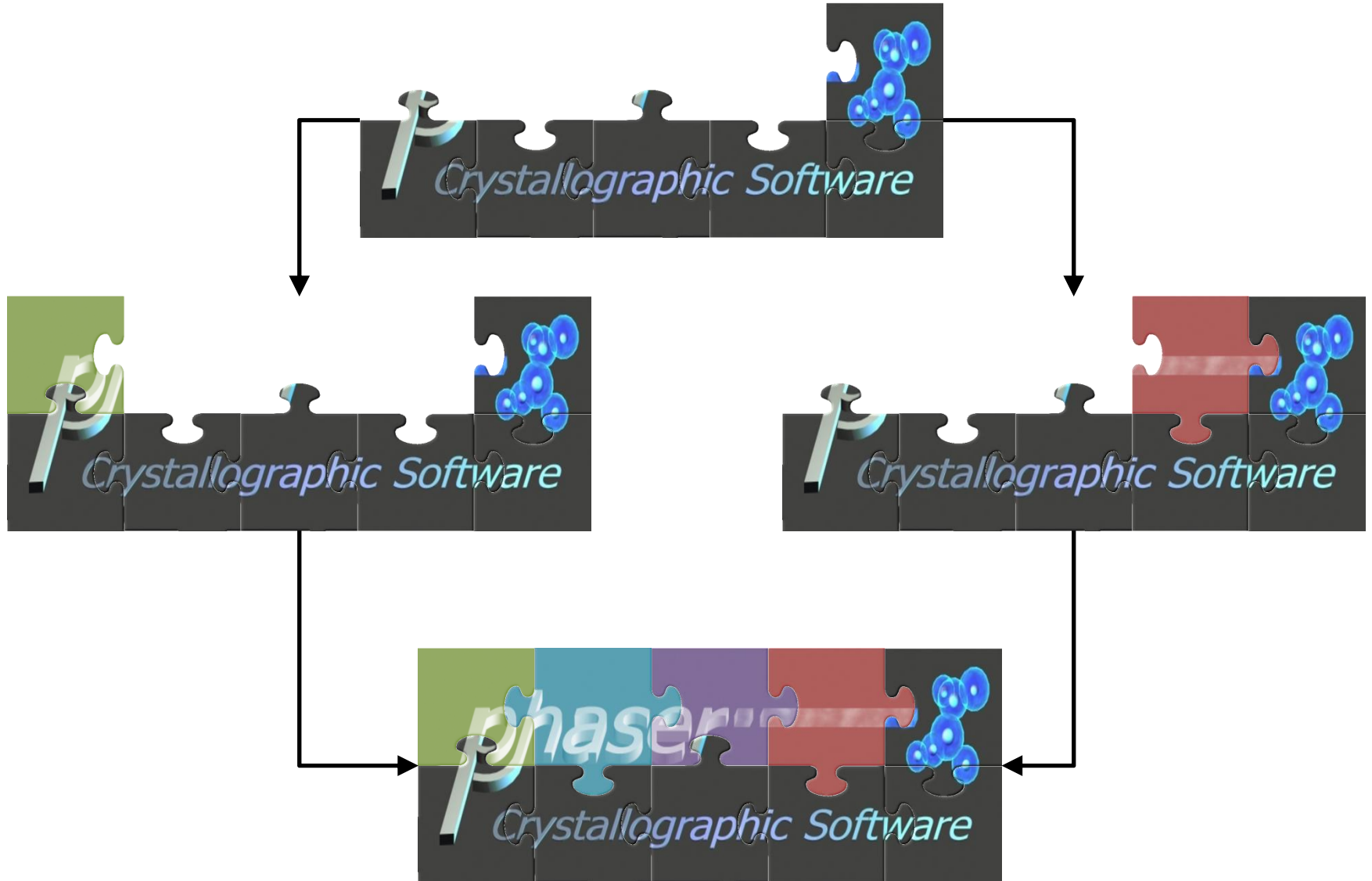
Superposition



New solution

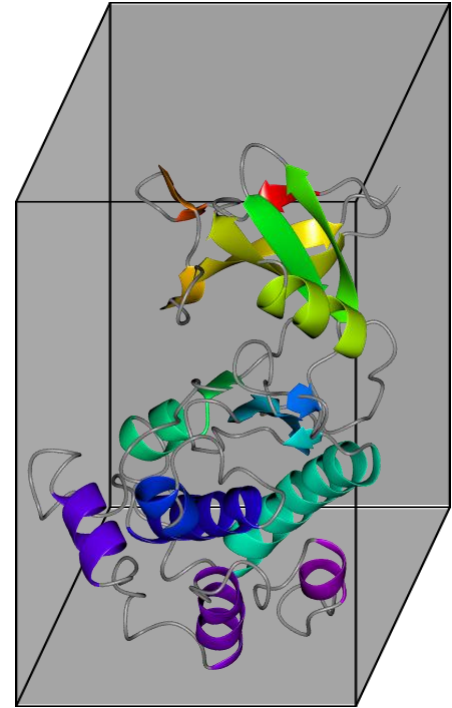
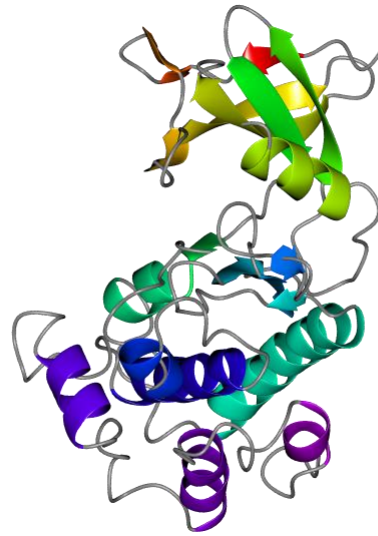
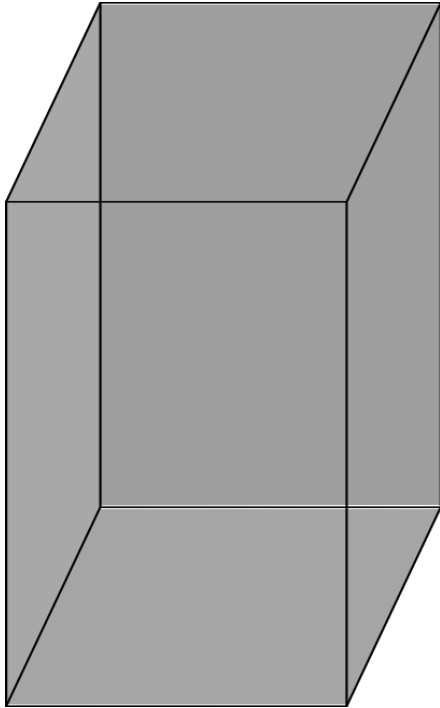


# “Jigsaw puzzle” strategy



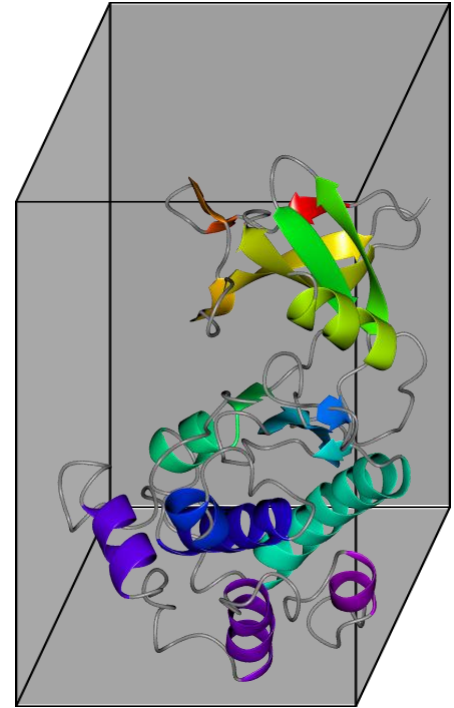
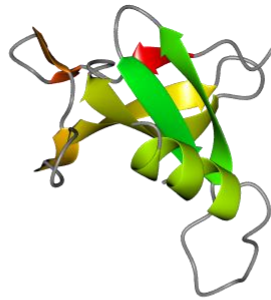
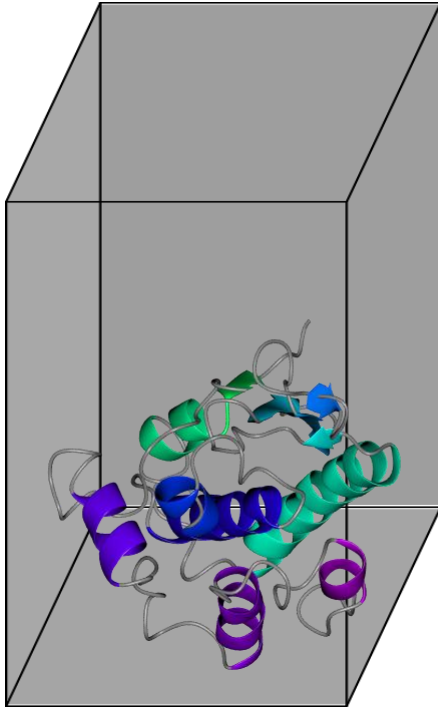


# Restricted search



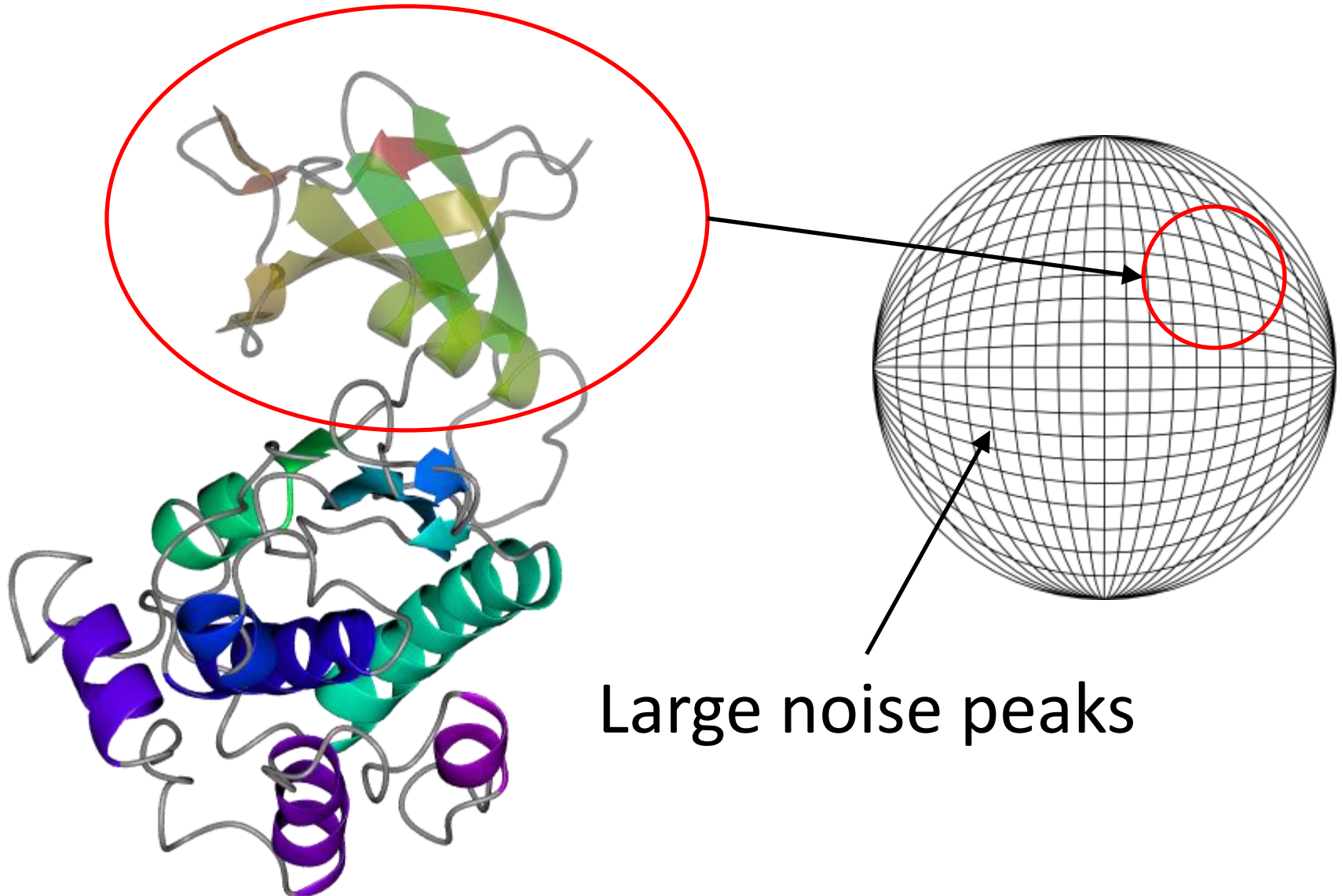


# Restricted search





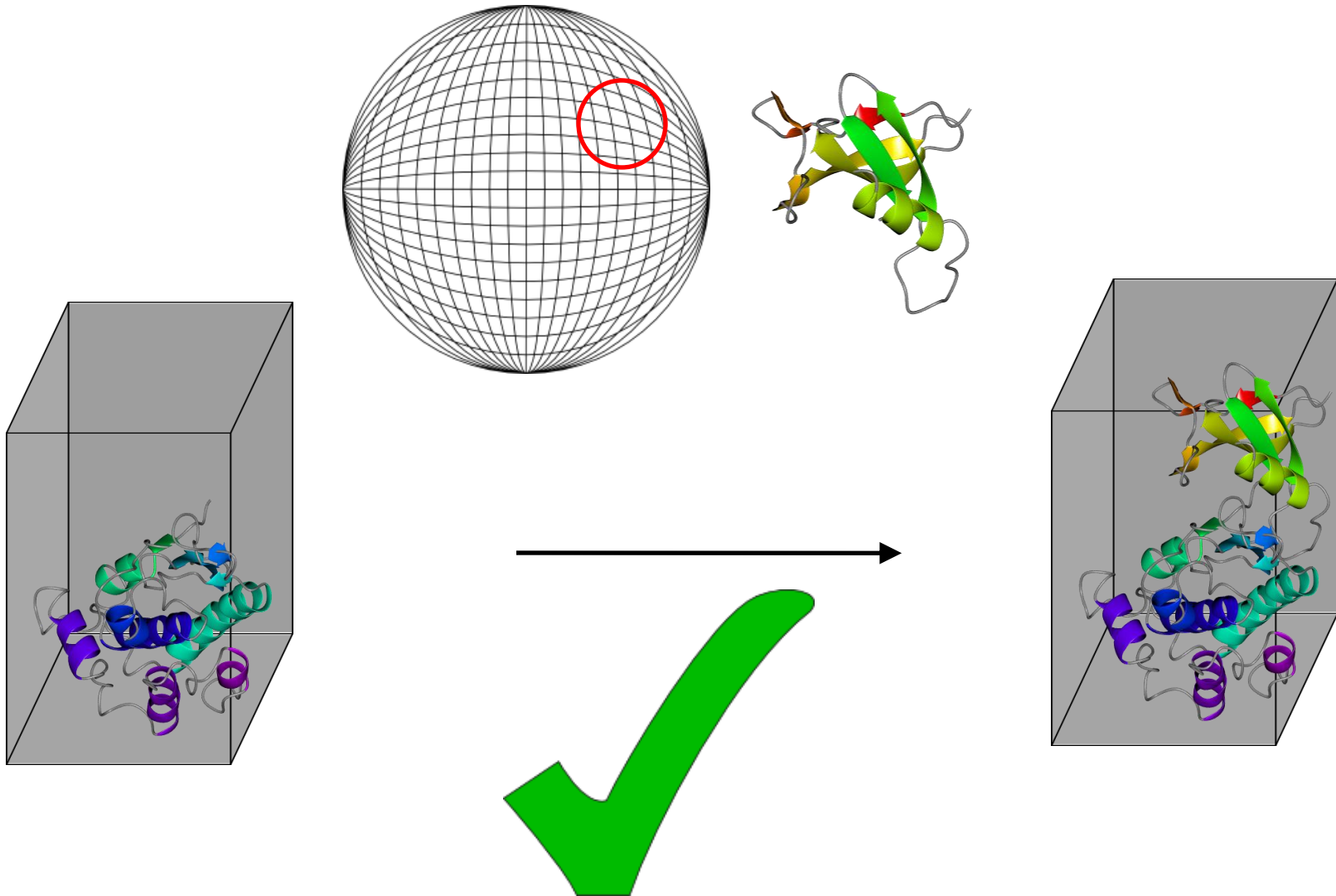
# Restricted search



Large noise peaks



# Restricted search







# Ensembles

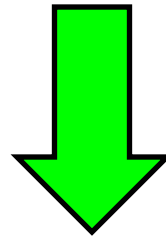
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Ensembles may be more suitable models for certain flexible proteins families



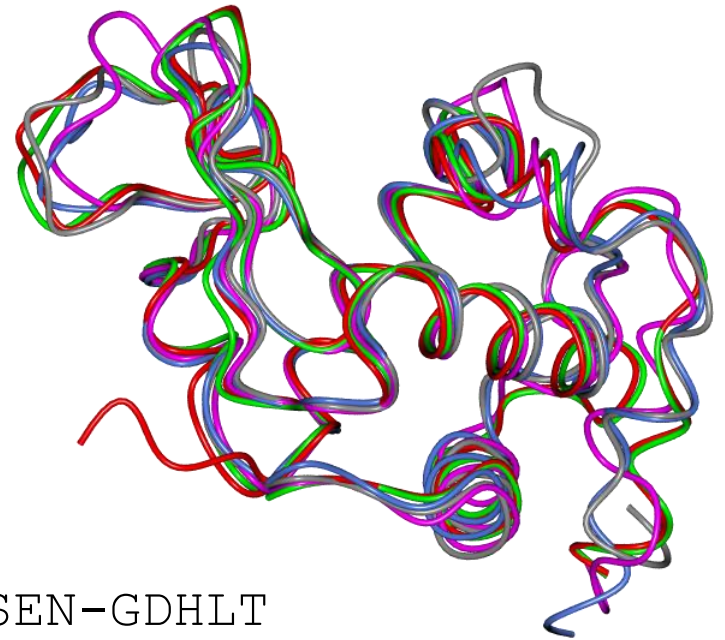
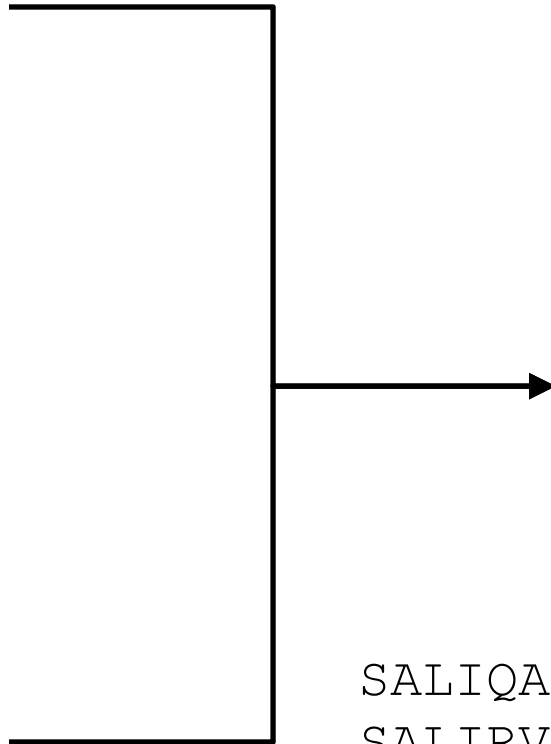
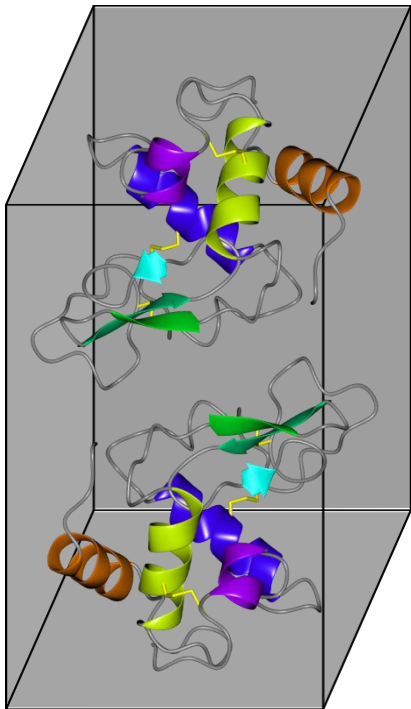
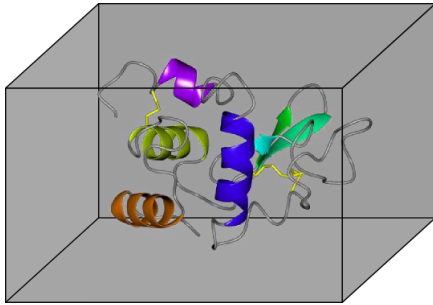
However, it is usually time-consuming to assemble the models



Automatic ensemble model generation



# Usage

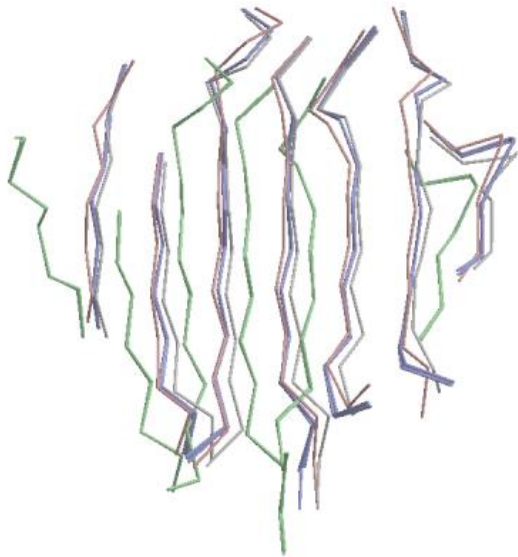


```
SALIQA-SEN-GDHLT  
SALIRV-QDDDDGRLS  
GAVVAEY--GDD--AT  
SKLCTE-QVKPG-NID
```

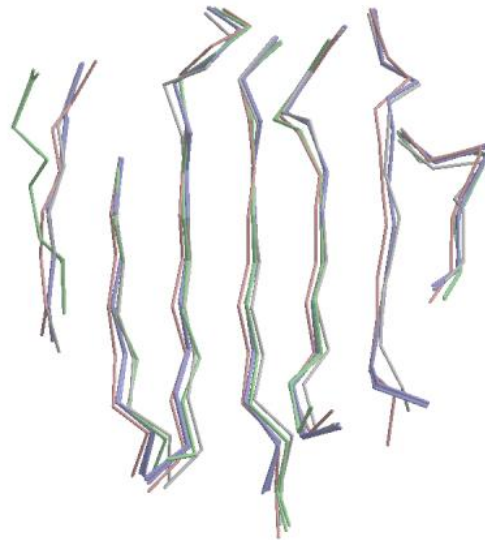


# Weighting

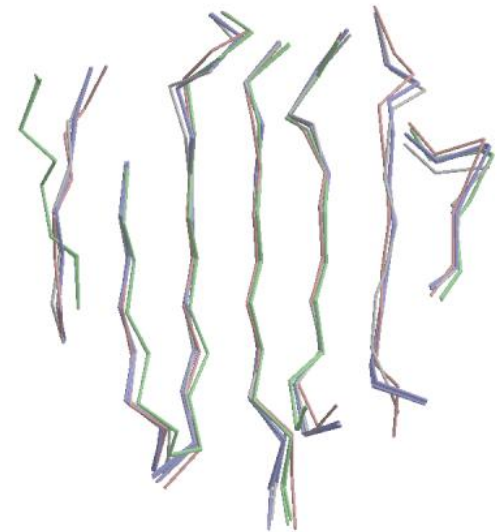
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**Sequence  
alignment  
unweighted**



**Structural  
alignment  
unweighted**

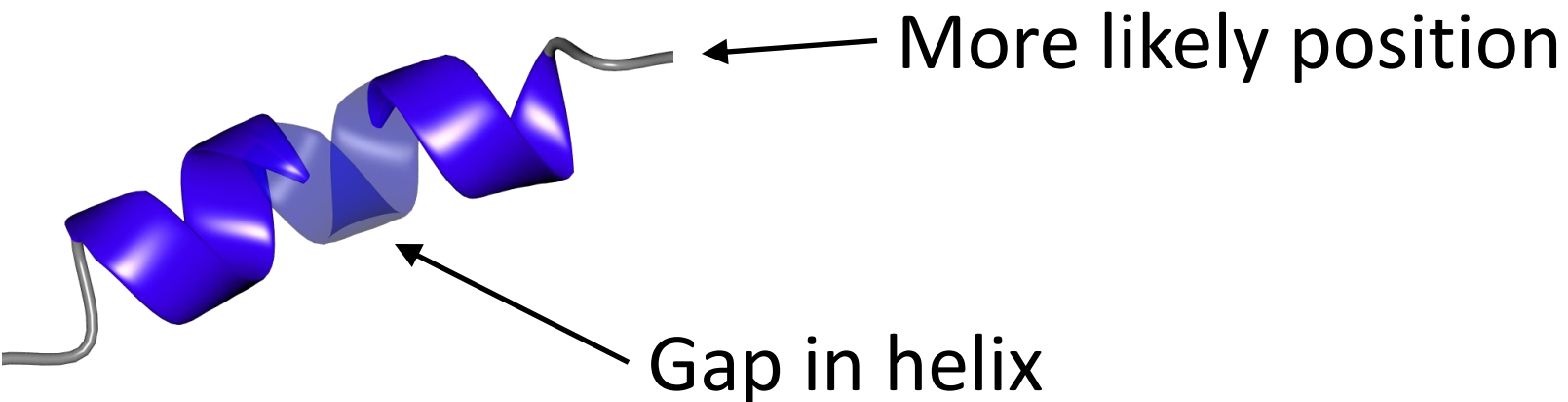
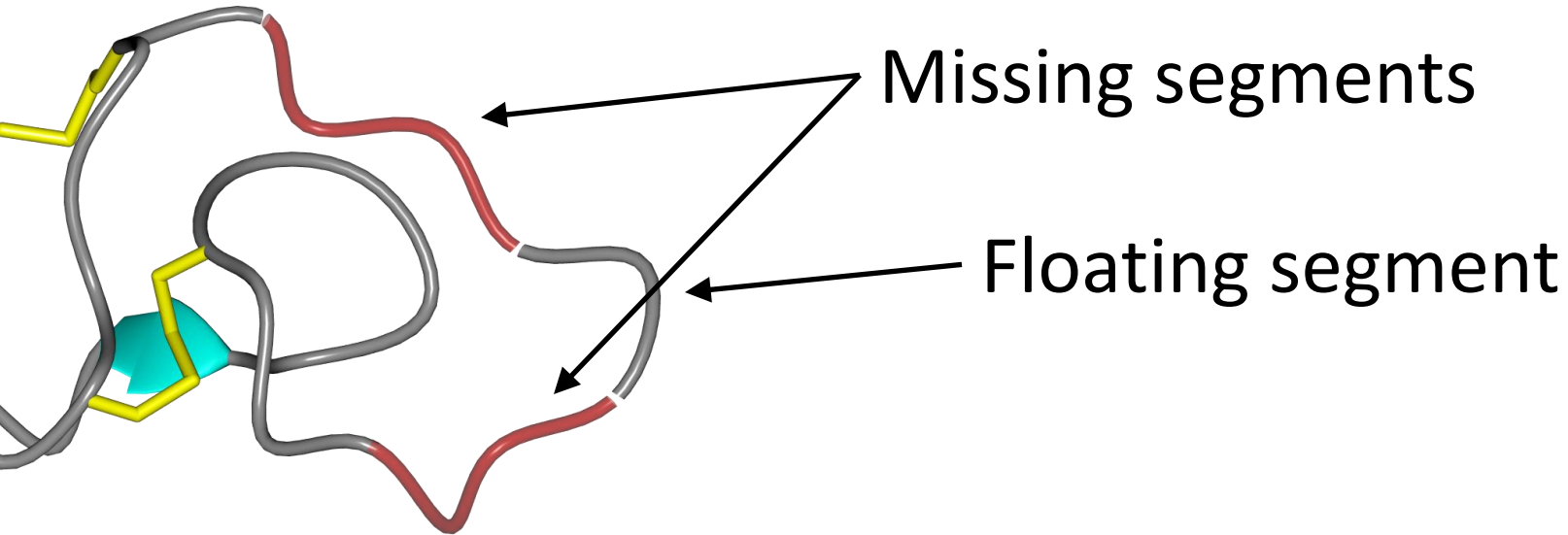


**Sequence  
alignment  
weighted**



# Editing models

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# Interactive model editing

Alignment

Model

Chain

A screenshot of the Coot software interface. The main window displays a 3D molecular model of a protein structure, colored in yellow, blue, and pink, with several red 'x' marks indicating specific atoms or residues. Overlaid on the top left is a dialog box titled "Sculptor GUI" with fields for "Alignment:", "Model:", and "Chain:", and buttons for "Preview", "Toggle", "Create", and "Cancel". The background shows a desktop environment with a blue sidebar containing "gaborb's Home" and "Trash" icons. The bottom of the screen shows a taskbar with various application icons and a system tray displaying the date and time: "Fri Jan 1, 5:54 PM" and the name "Gabor Bunkoczi".



# Interactive model editing

Preview

A screenshot of the Coot software interface. The main window is titled "Coot" and has a menu bar with "Draw", "Measures", "Validate", "HID", "About", and "Extensions". Below the menu bar is a "Display Manager" section. The "Sculptor GUI" is open as a dialog box, showing "Alignment: ui/1vkk\_1ahq\_trunc", "Model: 1ahq\_trunc.pdb", and "Chain: A". It has buttons for "Preview", "Toggle", "Create", and "Cancel". An arrow points from the word "Preview" on the left to the "Preview" button in the dialog. The main 3D view shows a protein structure with blue and pink outlines. The left sidebar shows "gaborb's Home" and "Trash". The bottom taskbar shows "Applications", "Places", "System", and the system tray with the date "Fri Jan 1, 5:56 PM" and the name "Gabor Bunkoczi".



# Interactive model editing

Preview

Toggle

Create

A screenshot of the Coot software interface. The main window is titled "Coot" and contains a menu bar with "Draw", "Measures", "Validate", "HID", "About", and "Extensions". Below the menu bar is a "Display Manager" section. The central area shows a 3D model of a protein structure, represented by blue and pink lines. On the left side, there is a sidebar with "gabor's Home" and a "Trash" icon. Overlaid on the top left of the Coot window is a smaller window titled "Sculptor GUI". This window has a menu bar with "Preview", "Toggle", "Create", and "Cancel". The "Sculptor GUI" window contains the following fields: "Alignment: ui/1vkk\_1ahq\_trunc", "Model: 1ahq\_trunc.pdb", and "Chain: A". Three black arrows point from the text labels on the left to the "Preview", "Toggle", and "Create" buttons in the "Sculptor GUI" window. The system tray at the bottom shows the date and time as "Fri Jan 1, 5:57 PM" and the name "Gabor Bunkoczi".



# Interactive model editing

A screenshot of the Coot software interface. The window title is "Coot". The menu bar includes "File", "Edit", "Calculate", "Draw", "Measures", "Validate", "HID", "About", and "Extensions". Below the menu bar are buttons for "Reset View" and "Display Manager". The main workspace displays a 3D molecular model of a protein structure, rendered in blue and red sticks. A small 3D coordinate system (x, y, z) is visible in the top-left corner of the workspace. On the right side of the window, there is a vertical toolbar with various icons for navigation and editing, including "R/RC" and "Map" buttons. The Windows taskbar at the bottom shows the system tray with icons for Applications, Places, System, and the date/time: "Fri Jan 1, 5:58 PM" and the user name "Gabor Bunkoczi".





# Acknowledgements

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## Python-based Hierarchical Environment for Integrated Xtallography



### Computational Crystallography Initiative / LBNL

Paul Adams, Ralf Grosse-Kunstleve, Pavel Afonine, Nathaniel Echols, Nigel Moriarty, Nicholas Sauter, Peter Zwart



### Los Alamos National Lab

Tom Terwilliger, Li-Wei Hung



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Jane and Dave Richardson, Vincent Chen, Jeffrey Headd