Low Resolution Refinement

REFMAC5 / ProSMART / LIBG / COOT

DLS-CCP4 Data Collection and Analysis Workshop

December 10-17 2014

Diamond Light Source, Oxfordshire, UK

Rob Nicholls

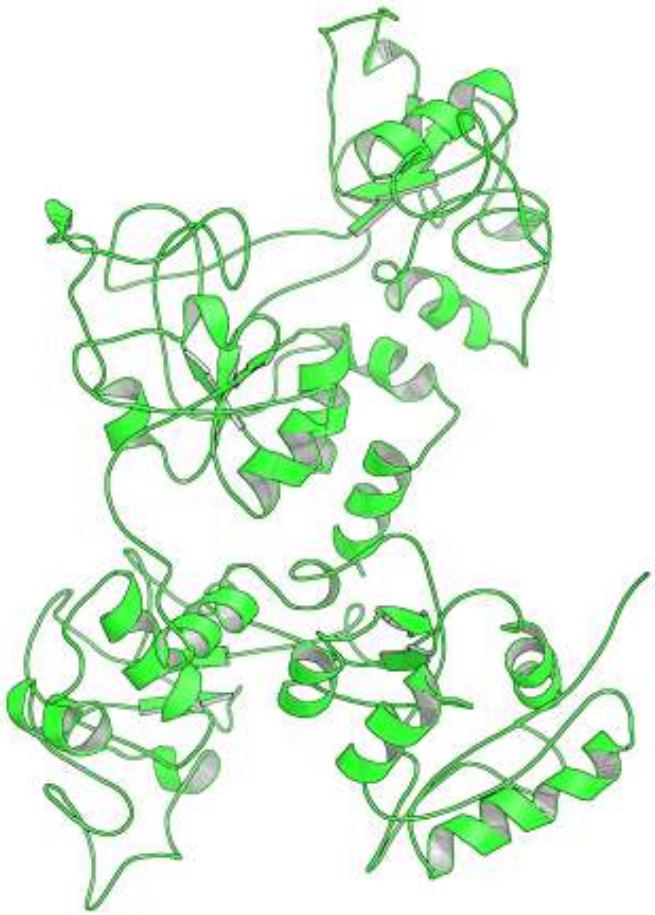
nicholls@mrc-lmb.cam.ac.uk

MRC Laboratory of Molecular Biology

CCP4

Motivational Example

Ovotransferrin



1ryx – 3.5Å

Low-resolution refinement:

Weak signal
Noisy data

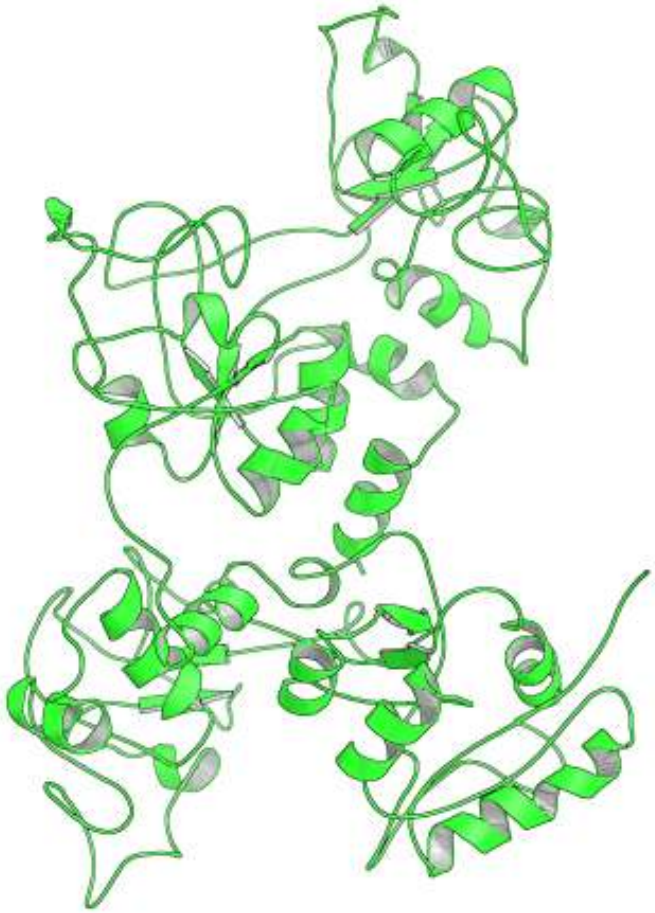
↓

Unstable refinement

Result:
Poor quality model

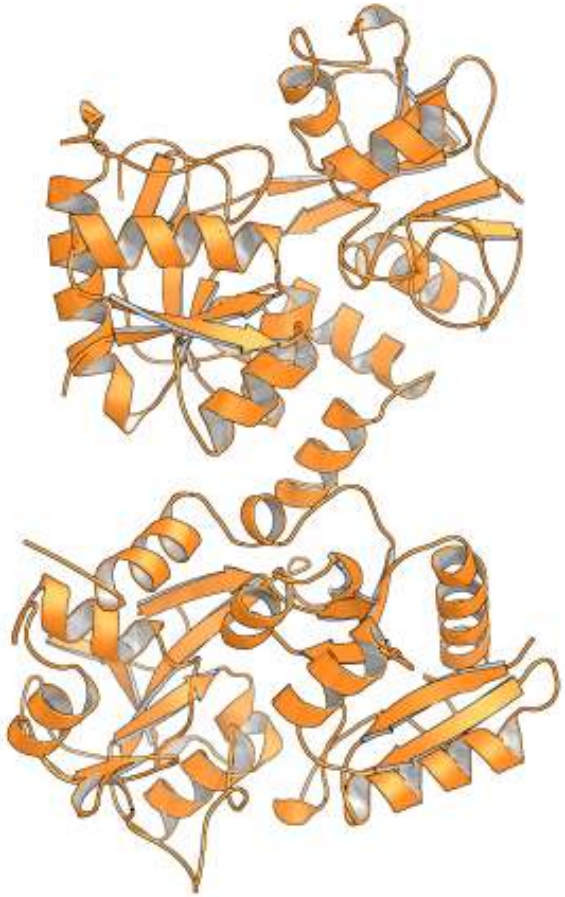
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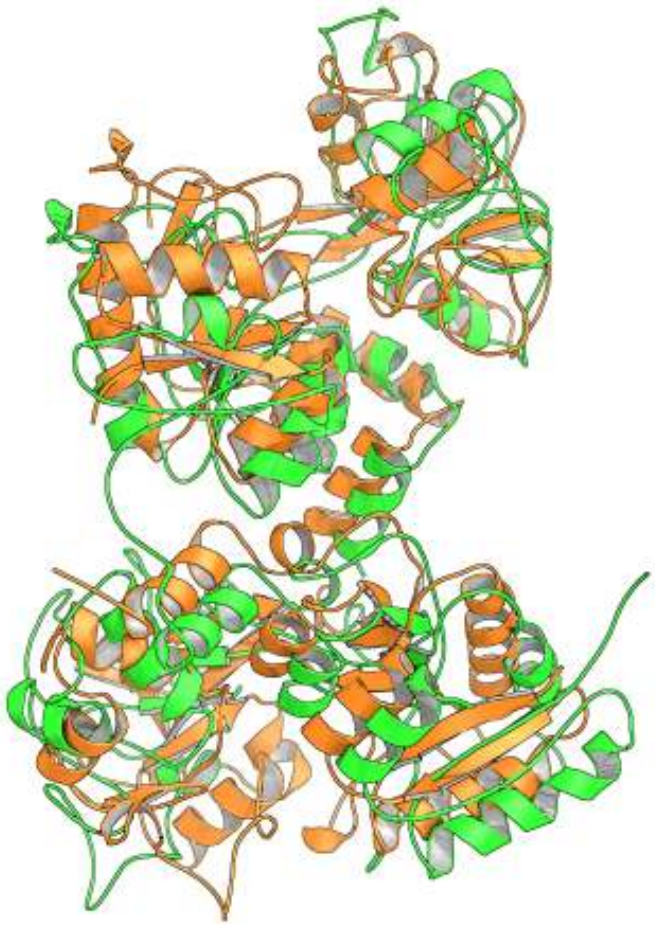
High-resolution homologue



2d3i – 2.15Å

Motivational Example

Ovotransferrin

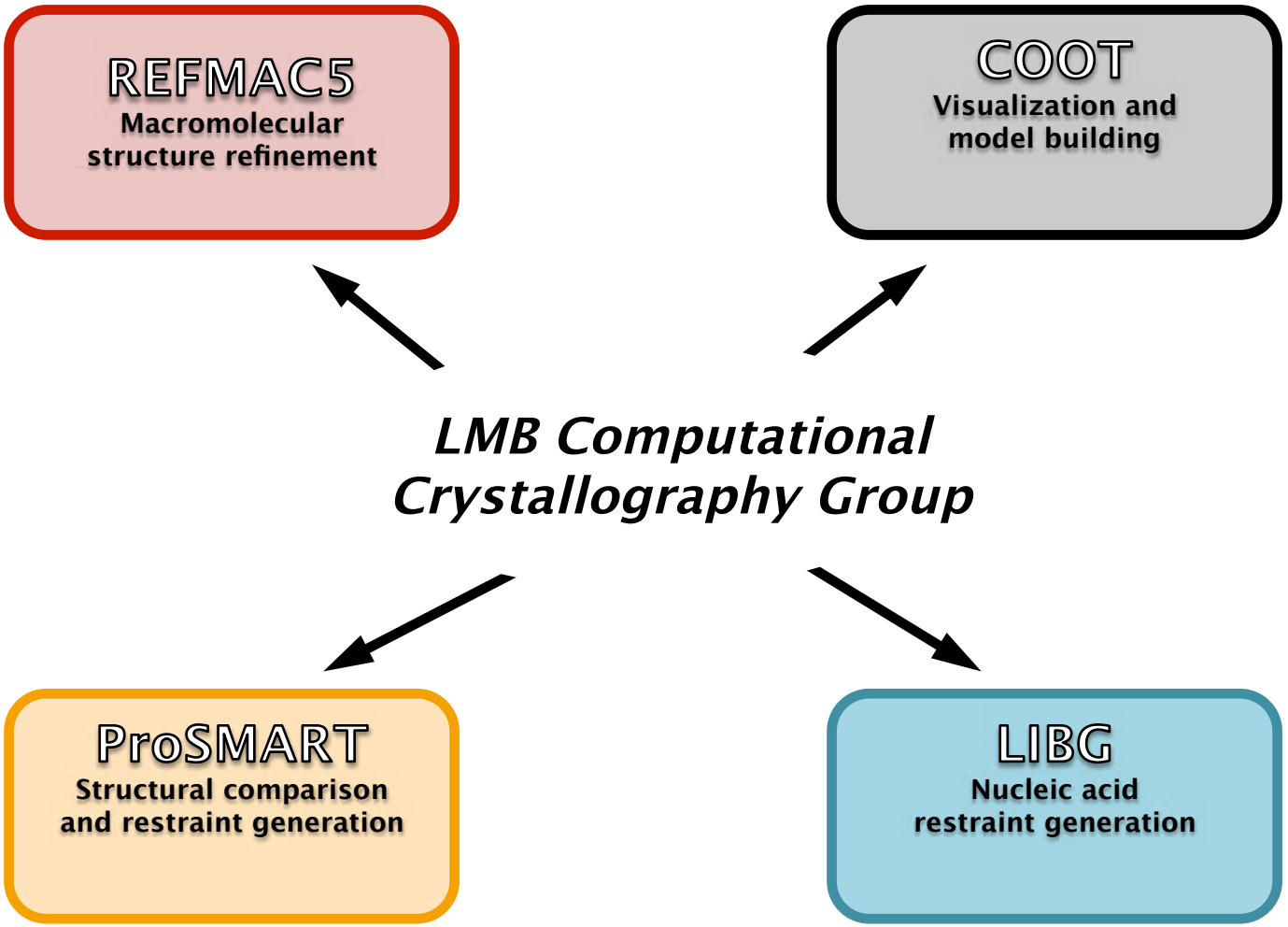


Models don’t superpose well

Contents

Tools to aid low-resolution refinement

- Regularisers in low-resolution crystallographic refinement
- Jelly body restraints
- External restraints
- Comparative structural analysis
- Map sharpening and blurring



Tools for low-resolution refinement

Low Resolution Refinement

- ~3Å or lower (tools are sometimes useful at slightly higher resolutions)
- Reflection intensities often noisy
- Limited data – poor observation:parameter ratio

At low resolution, refinement often becomes unstable

Overfitting – R-factors diverge

Low Resolution Refinement

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Overfitting – R-factors diverge

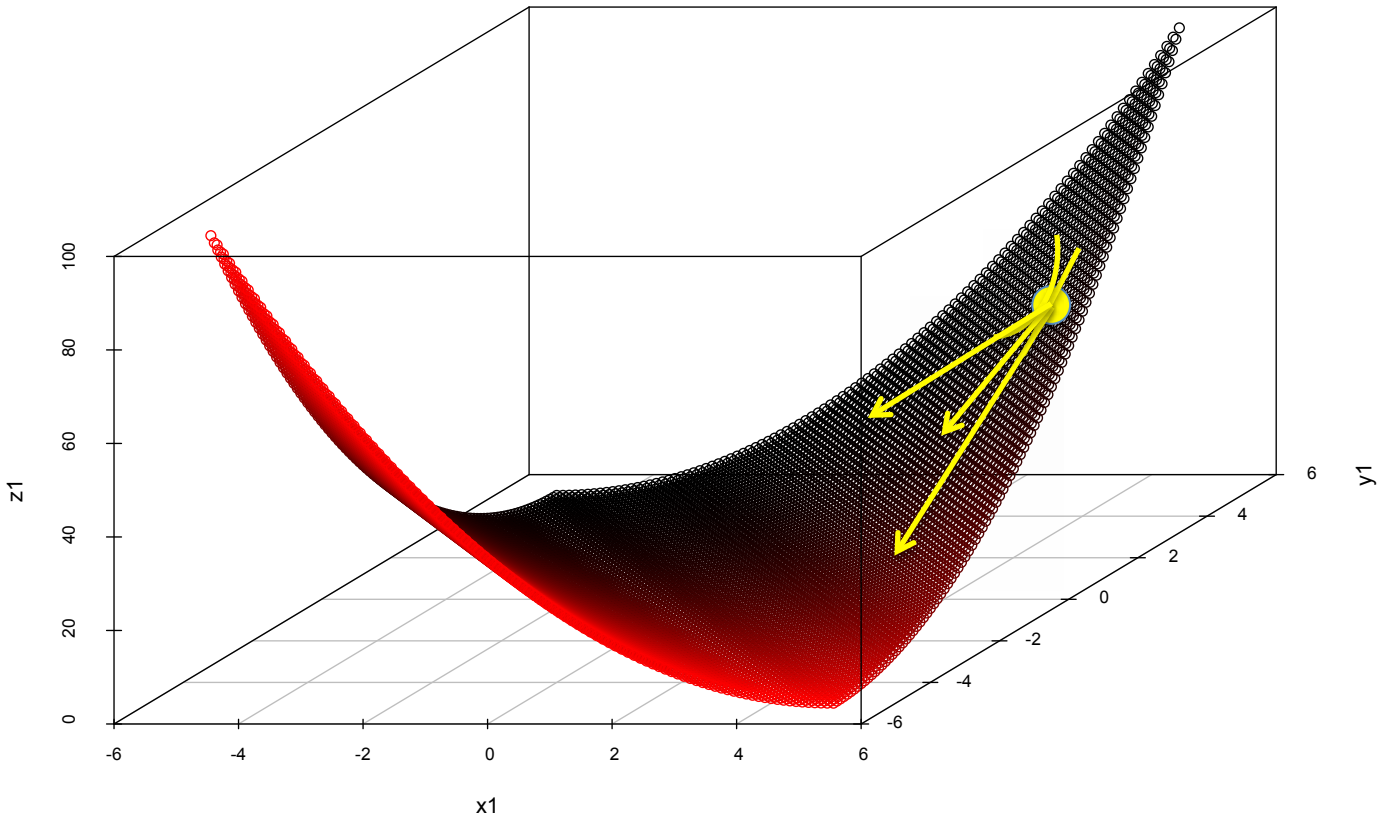
To improve models, we need to:

- **Ease** manual **model building**
- Use of **prior knowledge** to **stabilize refinement** – ***Regularisers***

Regularisation

Example:

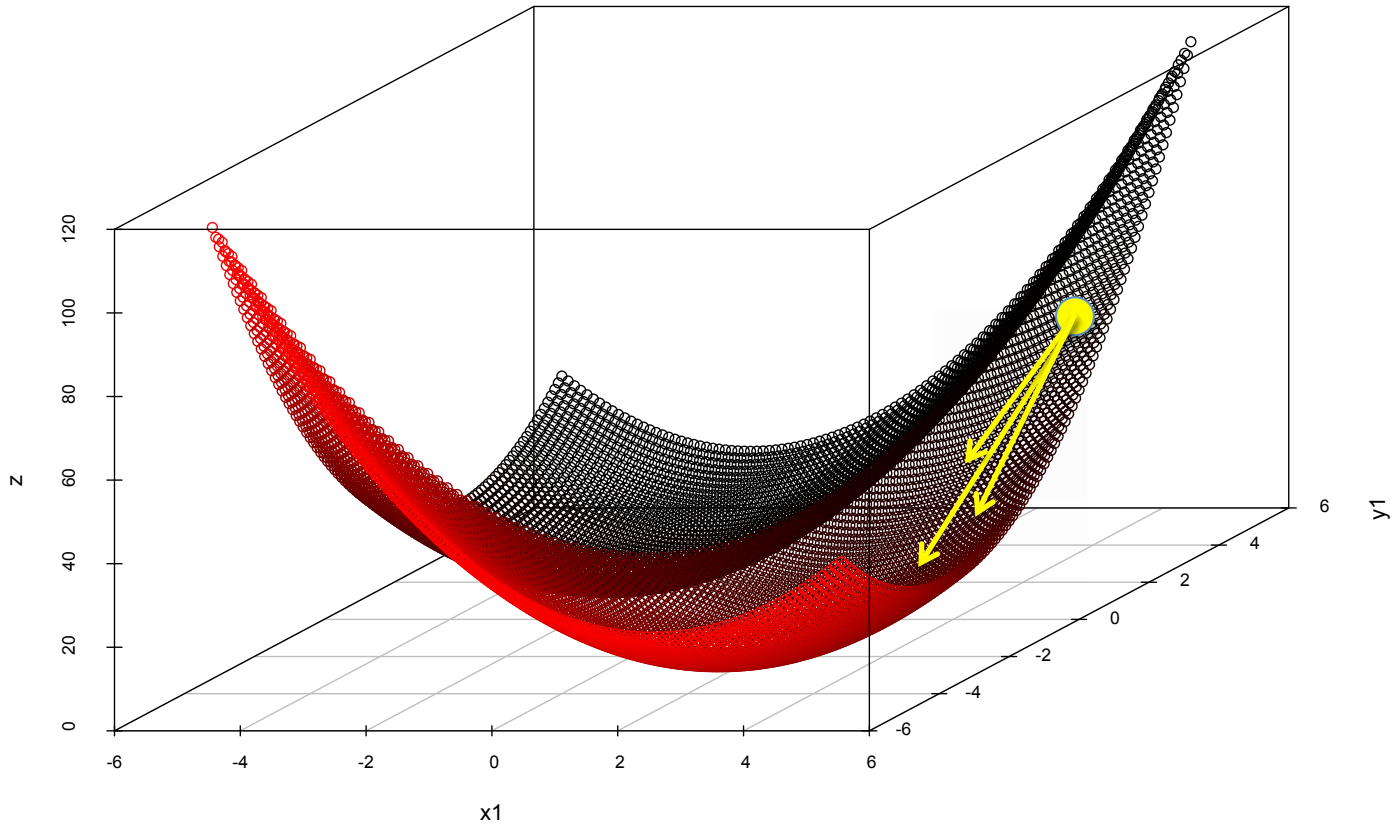
$$z = (x + y)^2$$



Regularisation

Example:

$$z = (x + y)^2 + (|x - y| - 4)^2$$



Regularise using prior information: $|x - y| = 4$

Regularisation

Use of available knowledge (prior information):

High-low resolution:

- Geometry restraints (chemical information)

Medium-low resolution:

- Local NCS restraints
- B-value restraints
- Jelly body restraints

Low resolution (and medium-low resolution model building):

- External restraints

Regularisation

Use of available knowledge (prior information):

High-low resolution:

- **Geometry restraints (chemical information)**

Medium-low resolution:

- **Local NCS restraints**
- **B-value restraints**
- Jelly body restraints

Low resolution (and medium-low resolution model building):

- **External restraints**

Regularisers with a target value

Regularisation

Use of available knowledge (prior information):

High-low resolution:

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Medium-low resolution:

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- B-value restraints
- **Jelly body restraints**

Low resolution (and medium-low resolution model building):

- External restraints

Regularisers without an external target value

Jelly Body Restraints

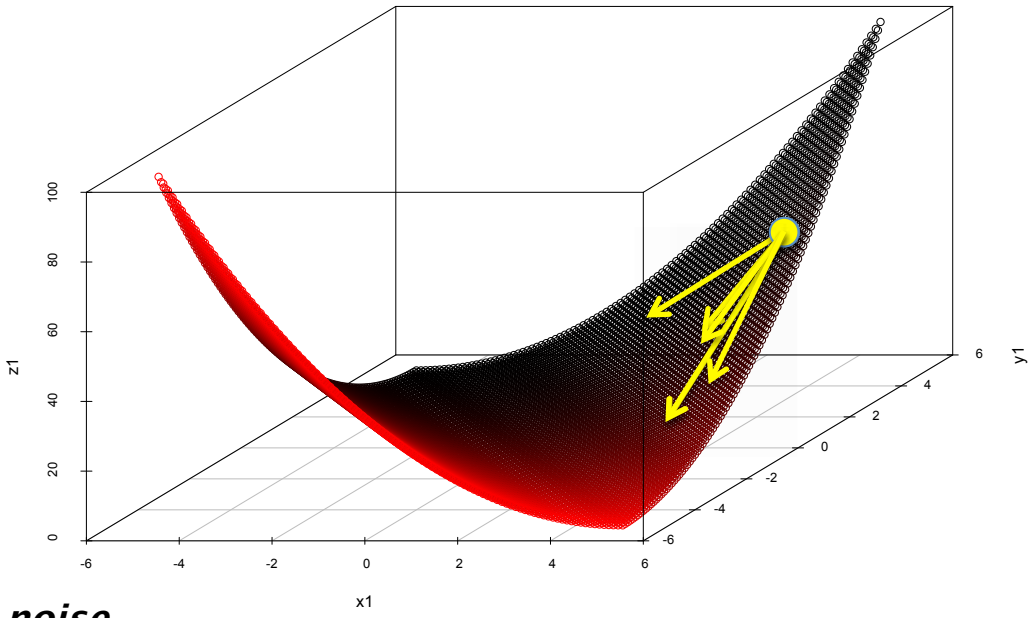
Regularisers without a target:

$$f = \sum_{\text{i : close atom pairs}} \frac{1}{\sigma^2}(|d_i| - |d_{i,\text{current}}|)^2$$

$|d_i|$: interatomic distance

$|d_{i,current}|$: current interatomic distance

σ : restraint standard deviation



Does not change likelihood function.

Does not change derivative.

Does change 2nd derivative.

Model should be less prone to fitting into noise

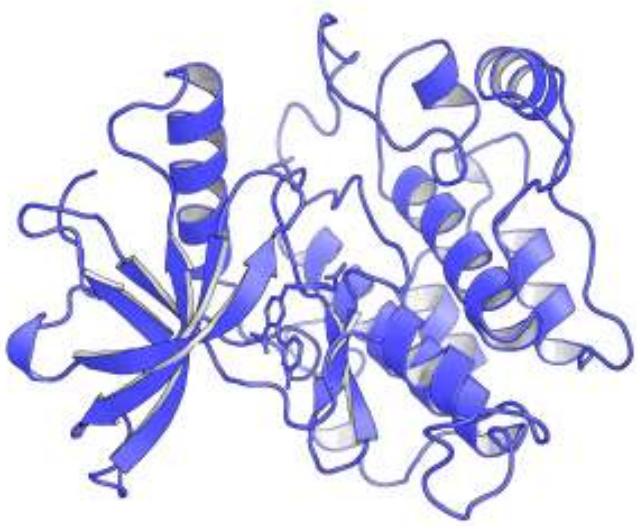
Should only work if parameters are near the minima (model is good)

Typical: σ = 0.01–0.02

Distance threshold: 4.2Å

Jelly Body Restraints

Tyrosine Kinase



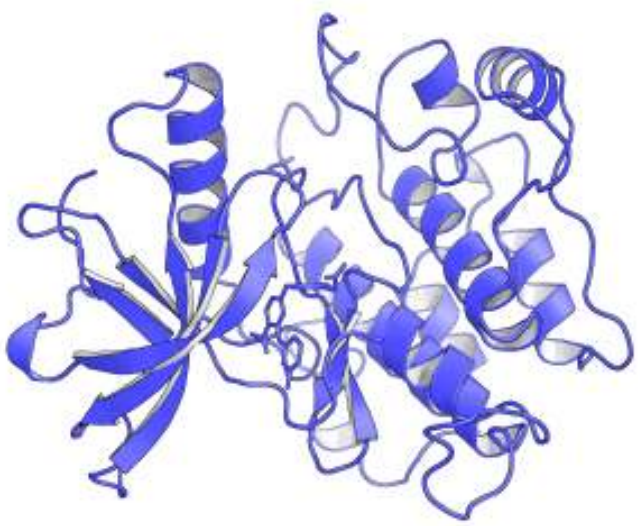
2z8c (2007) – 3.25Å

R_{work}/R_{free} : 21.7/29.2 %

Thanks to Oleg Kovalevskiy

Jelly Body Restraints

Tyrosine Kinase



2z8c (2007) – 3.25Å

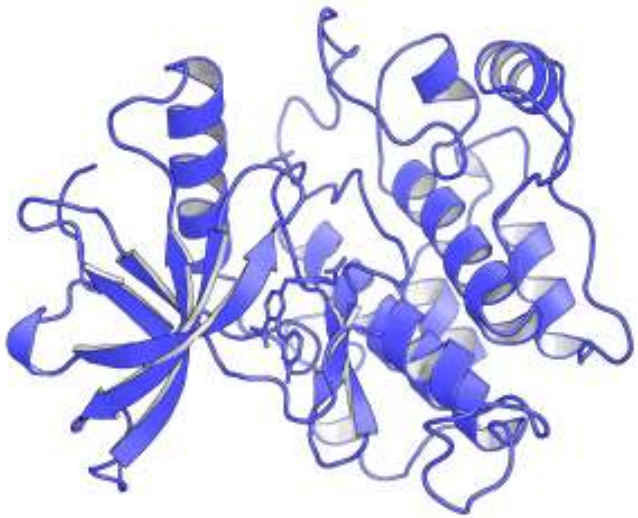
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Jelly-body: R_{work}/R_{free} : 19.7/27.3 %

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Jelly Body Restraints

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2auh (2005) – 3.2Å

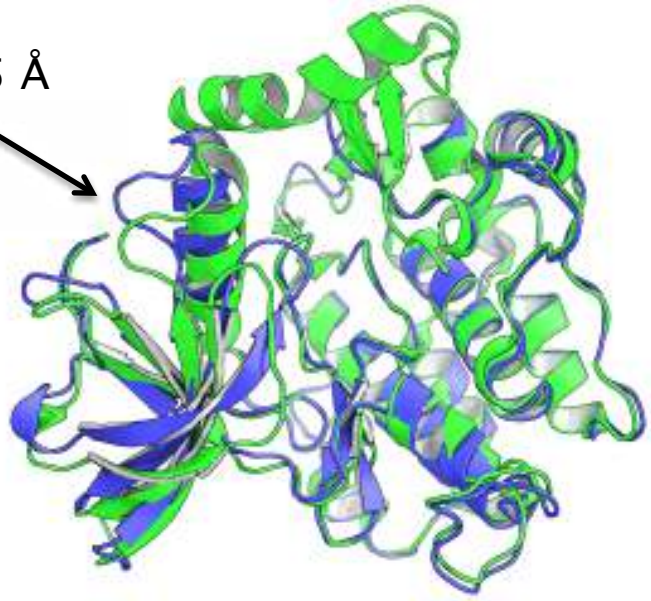
R_{work}/R_{free} : 22.3/25.4 %

Thanks to Oleg Kovalevskiy

Jelly Body Restraints

Tyrosine Kinase

Positional shift up to 4.5 Å



Molecular replacement
(Molrep, 10s)

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2z8c solved using 2auh,

re-refined using jelly-body

R_{work}/R_{free} : 20.2/25.8 %

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Jelly Body Restraints

Tyrosine Kinase



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R_{work}/R_{free} : 21.7/29.2 %

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Molprobity clashscore : 28.3

2z8c solved using 2auh,

re-refined using jelly-body

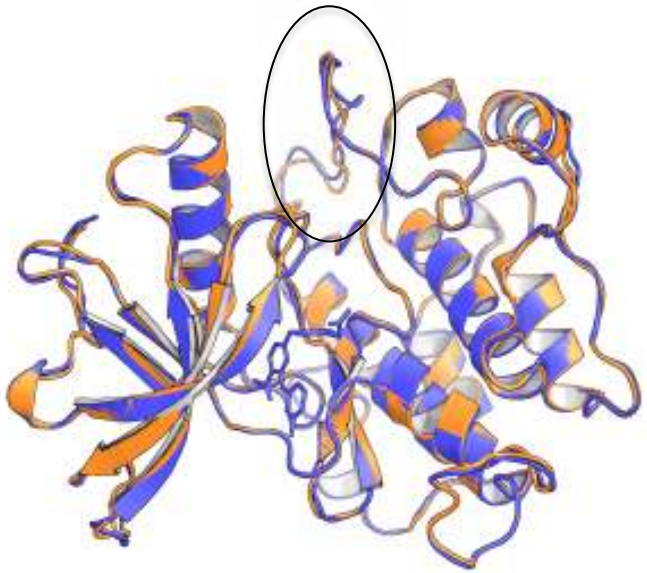
R_{work}/R_{free} : 20.2/25.8 %

Molprobity clashscore : 16.9

Thanks to Oleg Kovalevskiy

Jelly Body Restraints

Tyrosine Kinase



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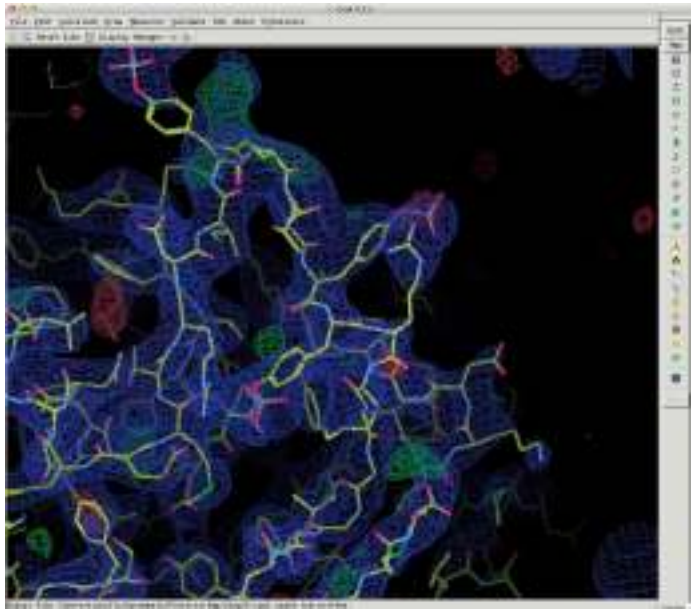
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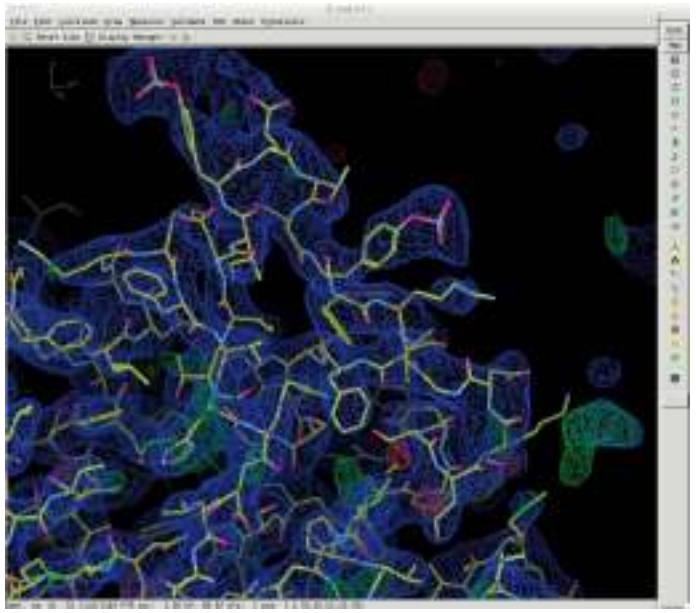
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Jelly Body Restraints

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Jelly Body Restraints

Conclusions:

(1) Jelly-body restraints are great at stabilising refinement, and can have quite a wide radius of convergence… but can't alone improve a model dramatically

(2) We can't rely on the reliability of models deposited in the PDB (applies for more moderate-resolution models also)

(3) Structural information in the PDB can be useful in the determination of new structures

ProSMART in MX Refinement

External restraint generation:

Injection of prior knowledge to aid new structure determination:

- External Restraints from homologous structures
- Hydrogen bond restraints

Restraints utilised by: REFMAC5, Coot

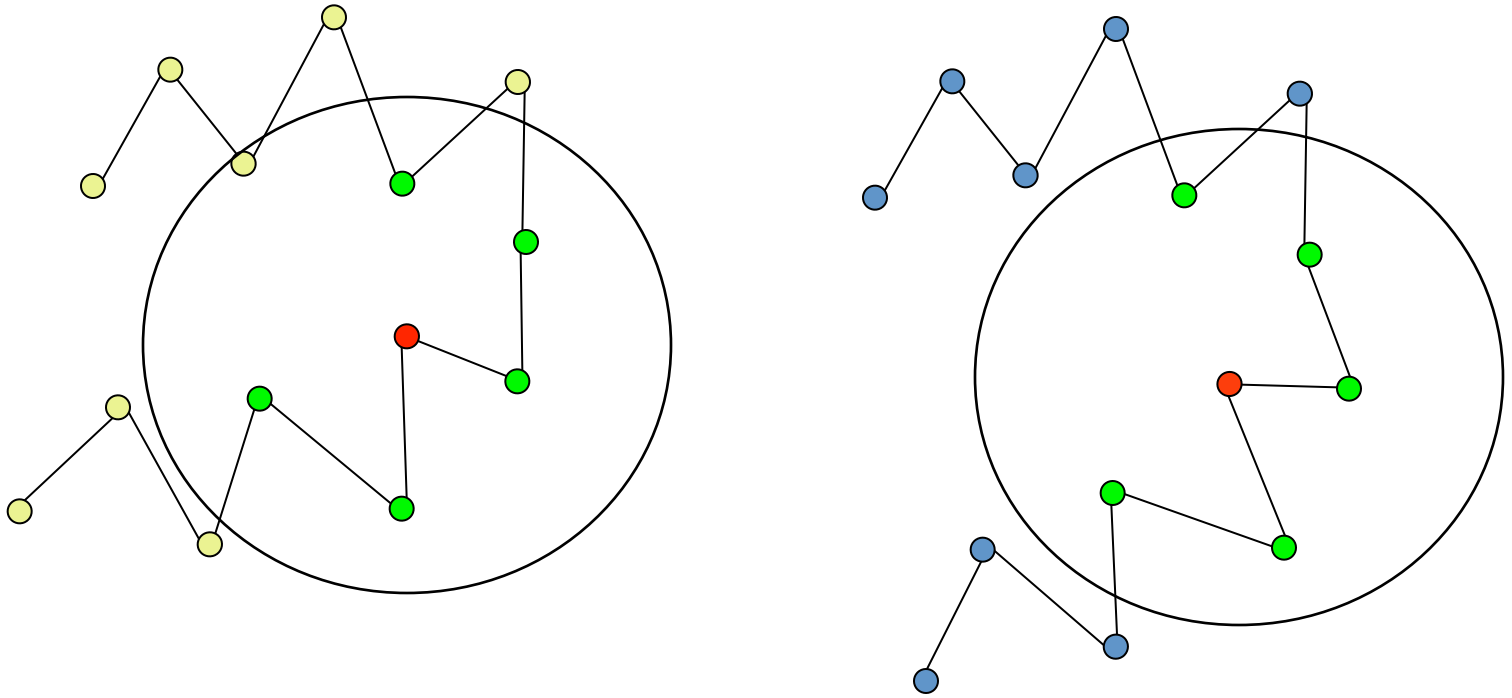
Executed from: CCP4i, CCP4mg, Coot, command line

Independent of global conformation

External Restraint Generation

structure to be refined

known similar structure (prior)

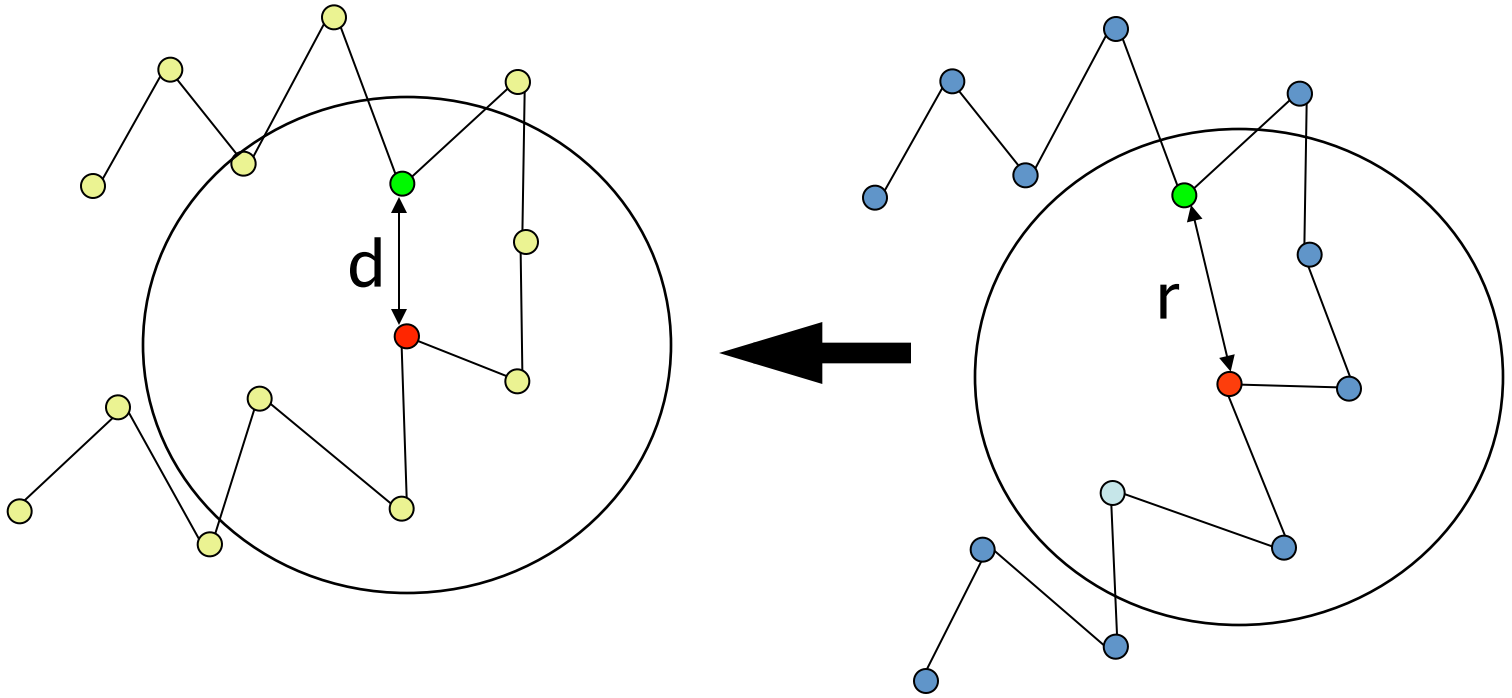


(abstract representation of an atomic model; circles = atoms)

External Restraint Generation

structure to be refined

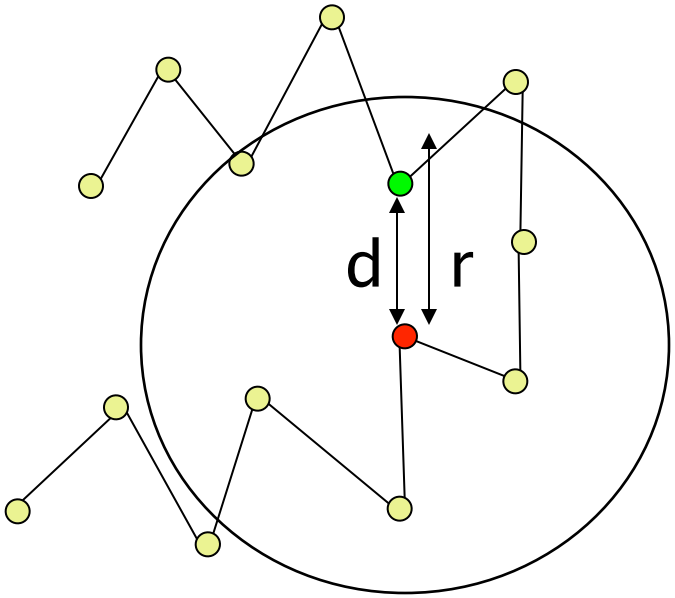
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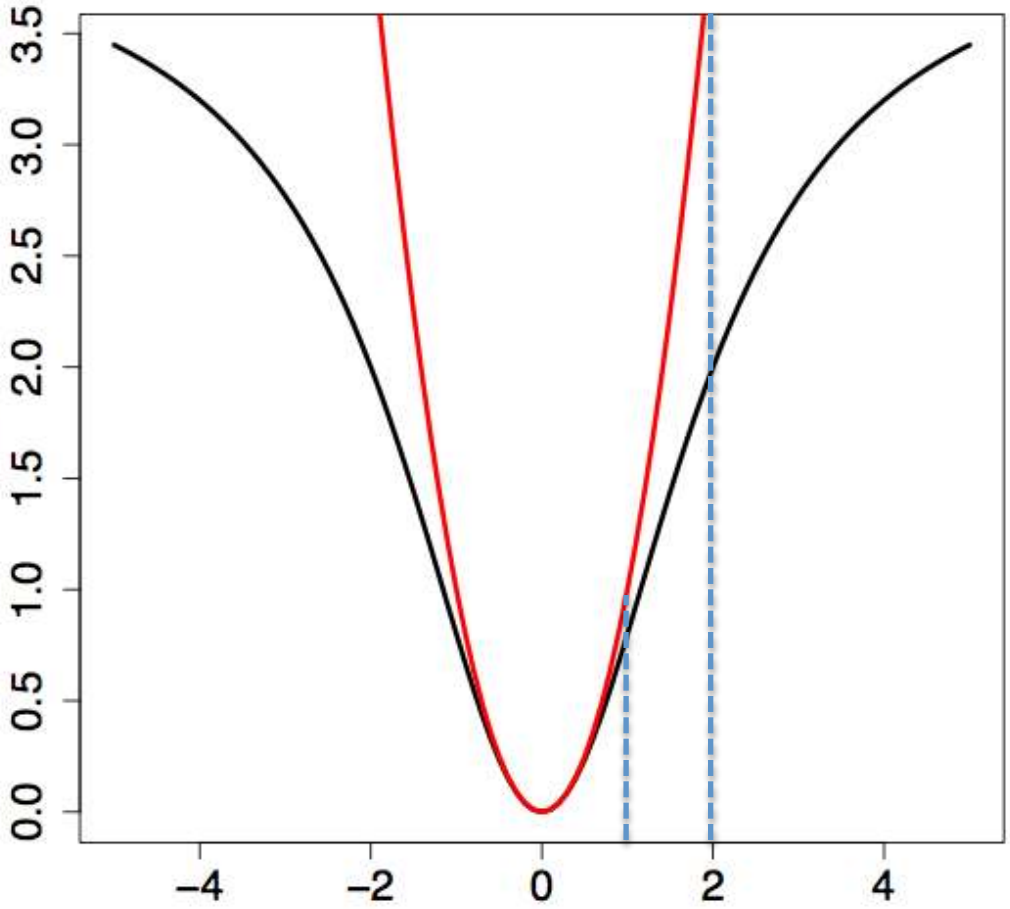
structure to be refined



$d \sim N(r,\sigma^2)$

(abstract representation of an atomic model; circles = atoms)

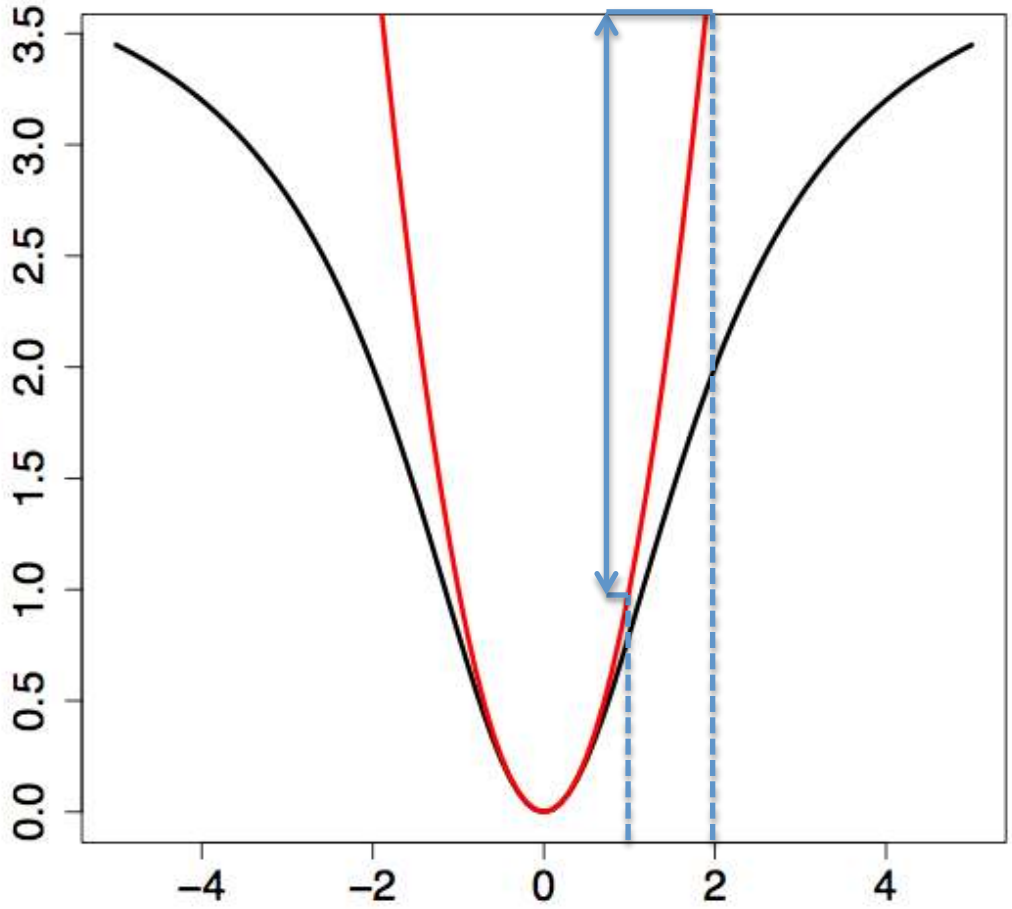
External Restraints



Least squares: x^2

Geman-McClure: $\frac{x^2}{1 + wx^2}$

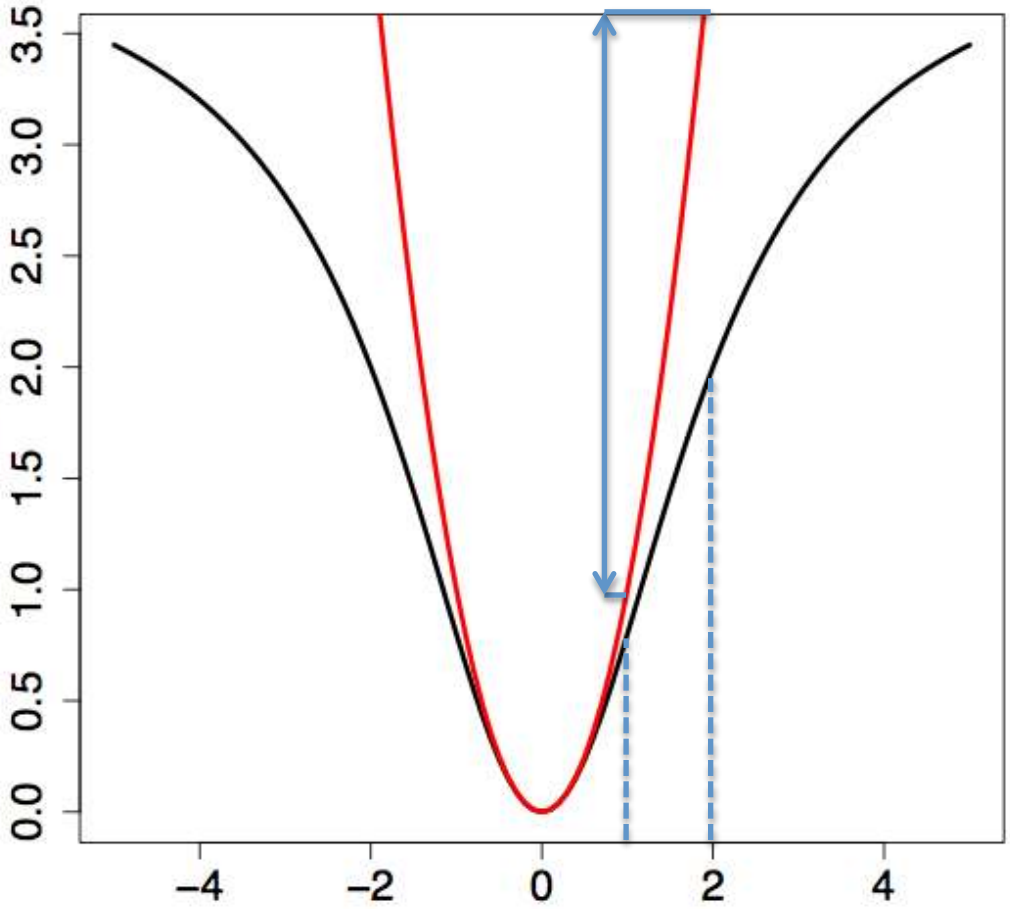
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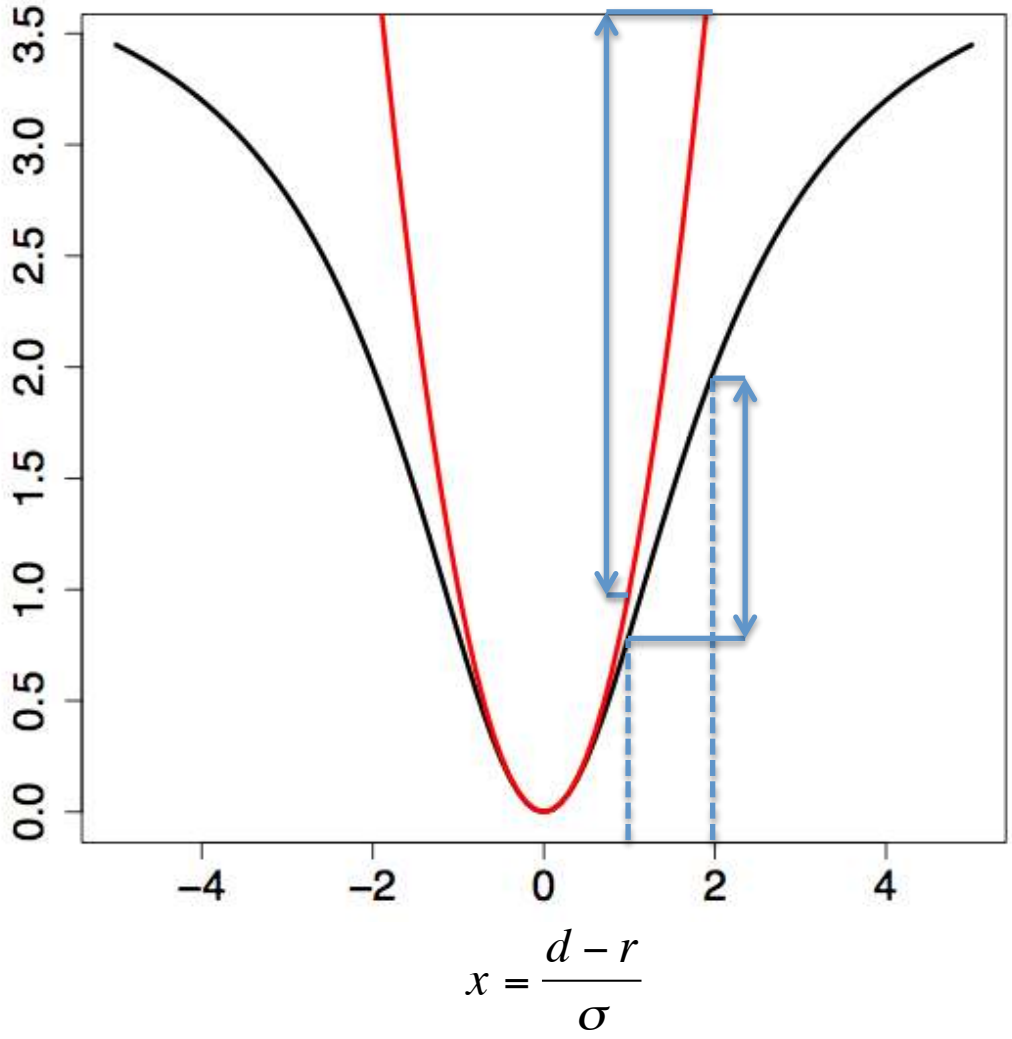
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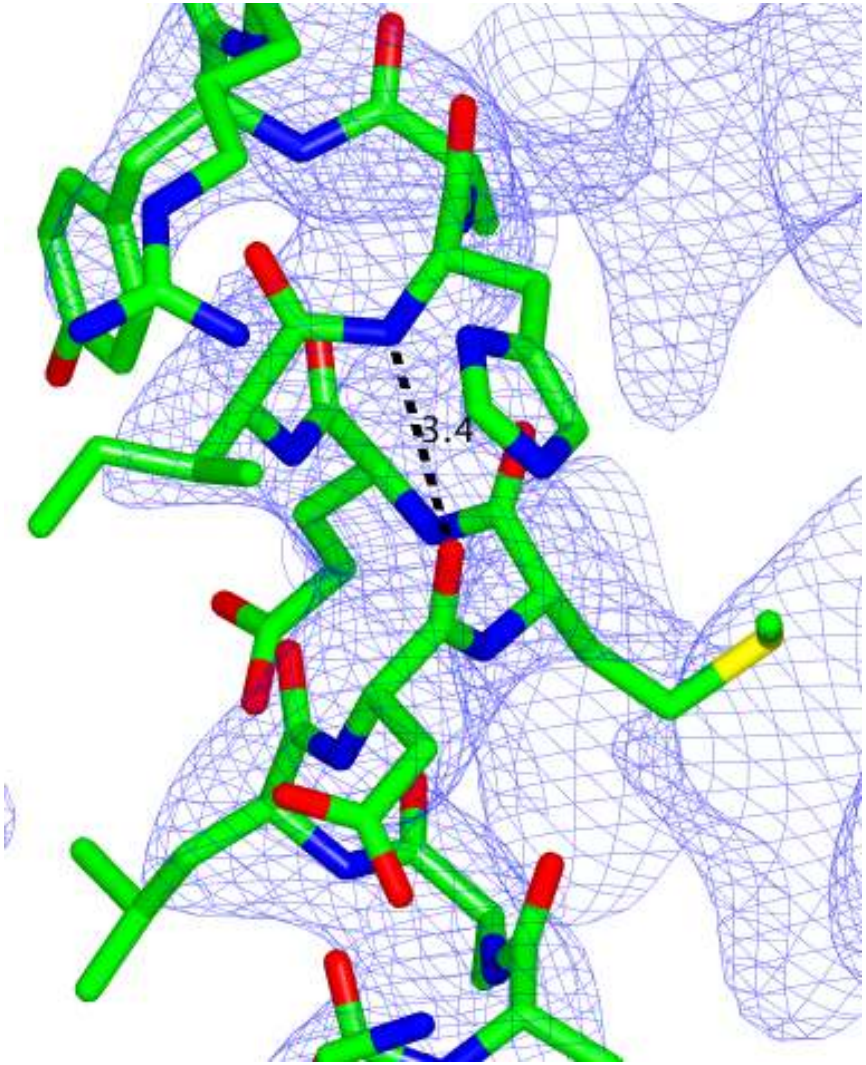
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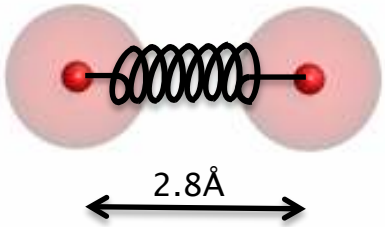
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Hydrogen Bond Restraints



3g4w – 3.7Å

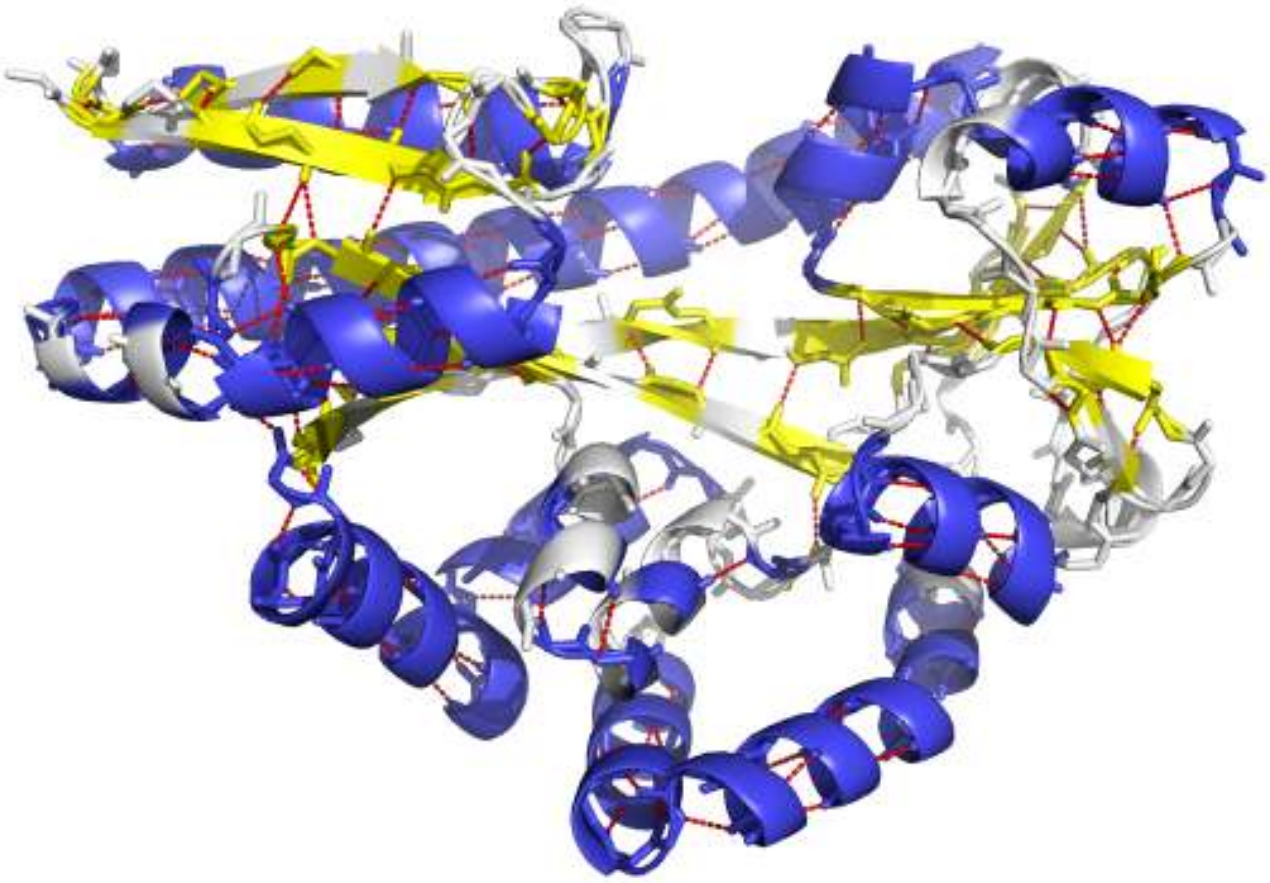
Prior information:



Stabilises structural features

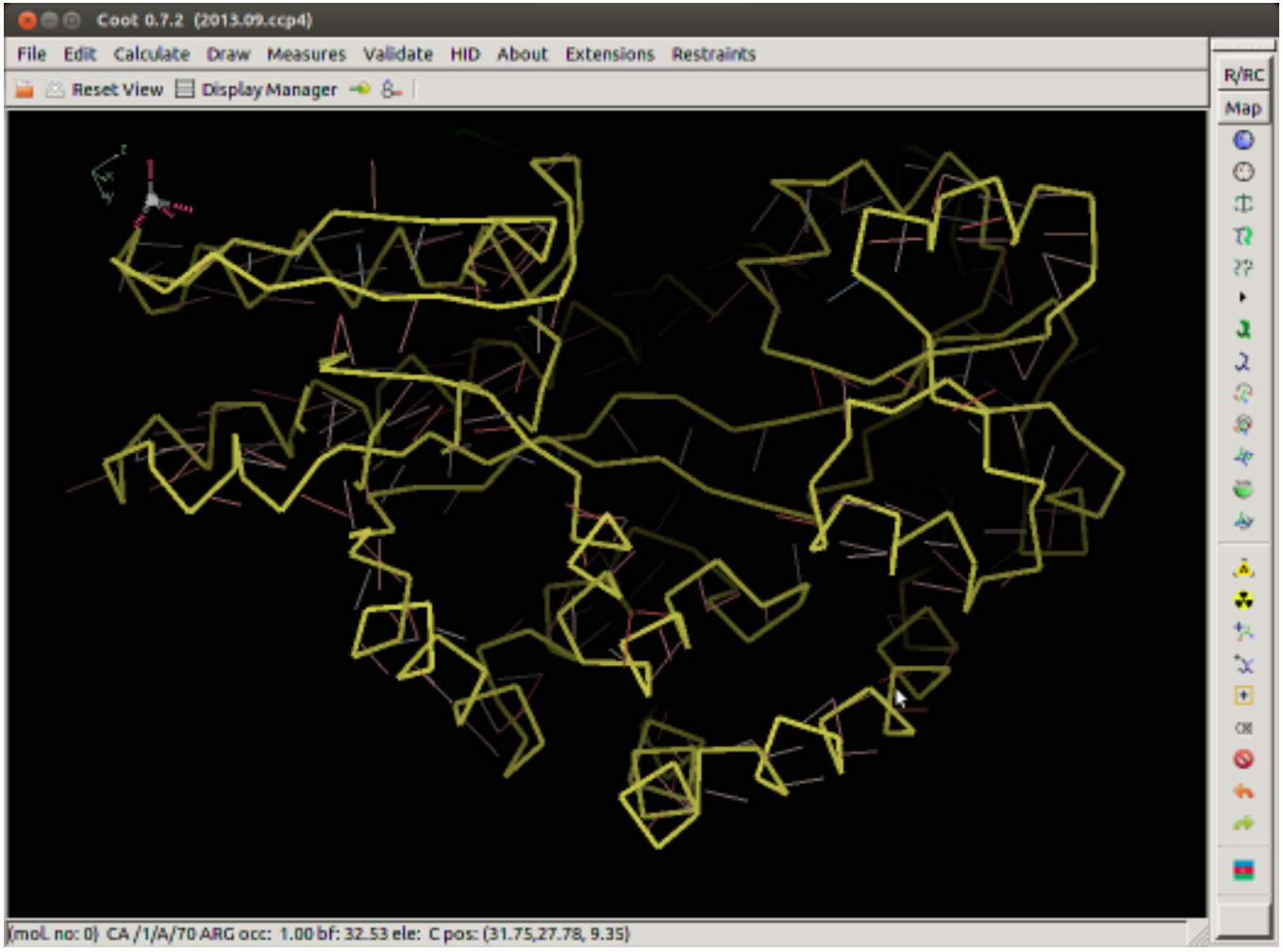
Hydrogen Bond Restraints

Visualisation of ProSMART hydrogen bond restraints using PyMOL:



Hydrogen Bond Restraints

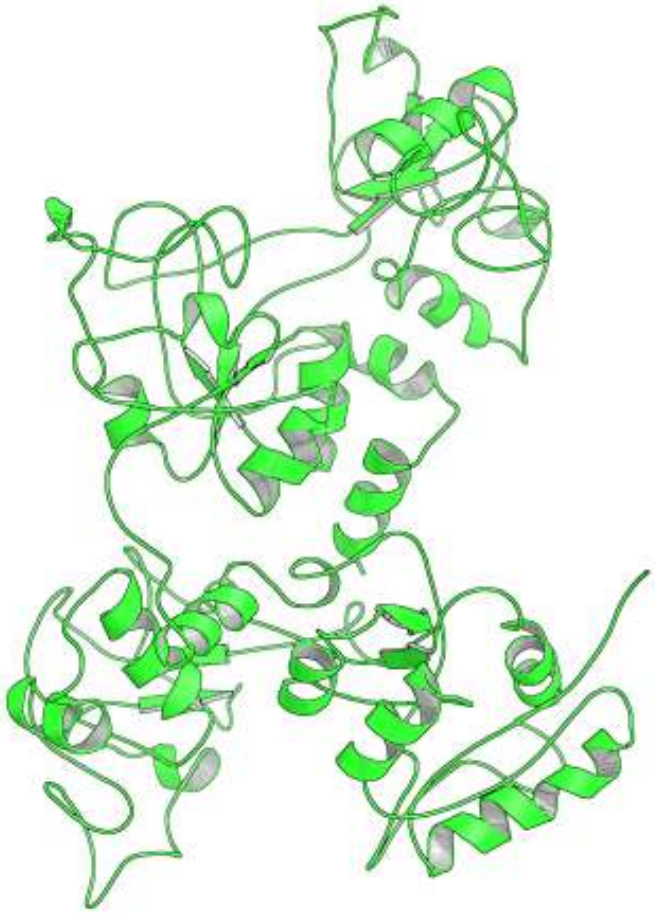
Visualisation of ProSMART hydrogen bond restraints using Coot:



Thanks to Paul Emsley

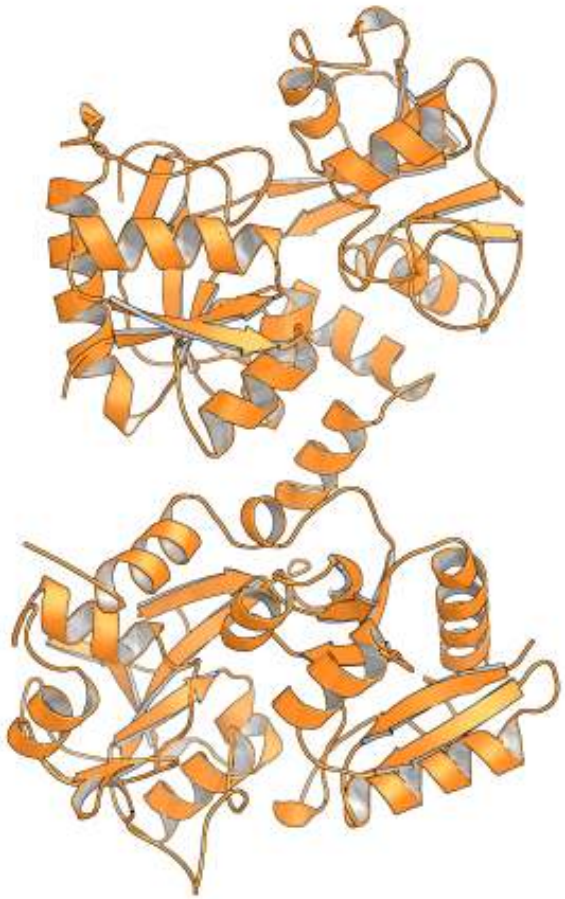
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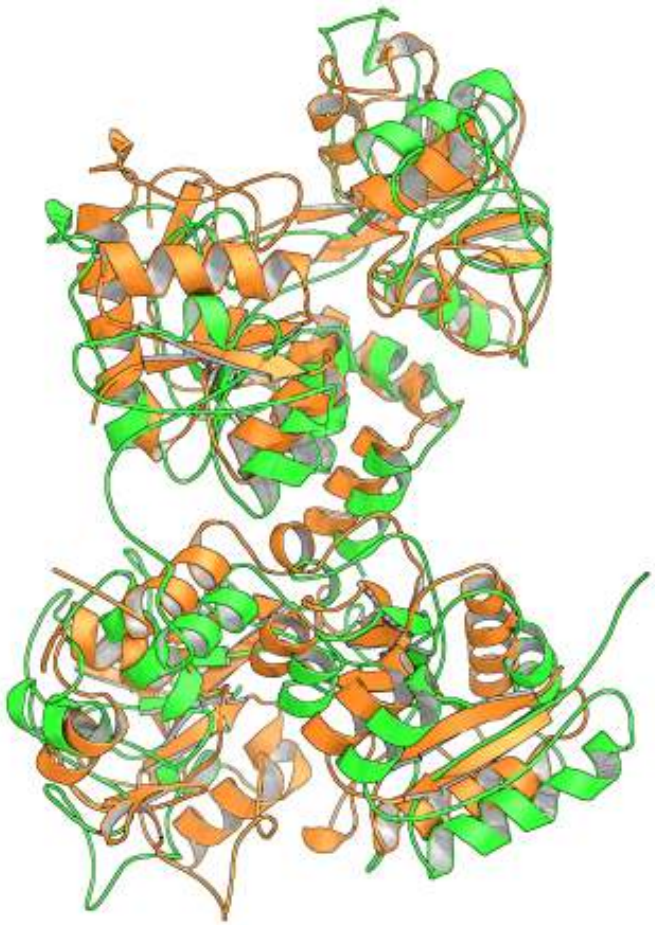
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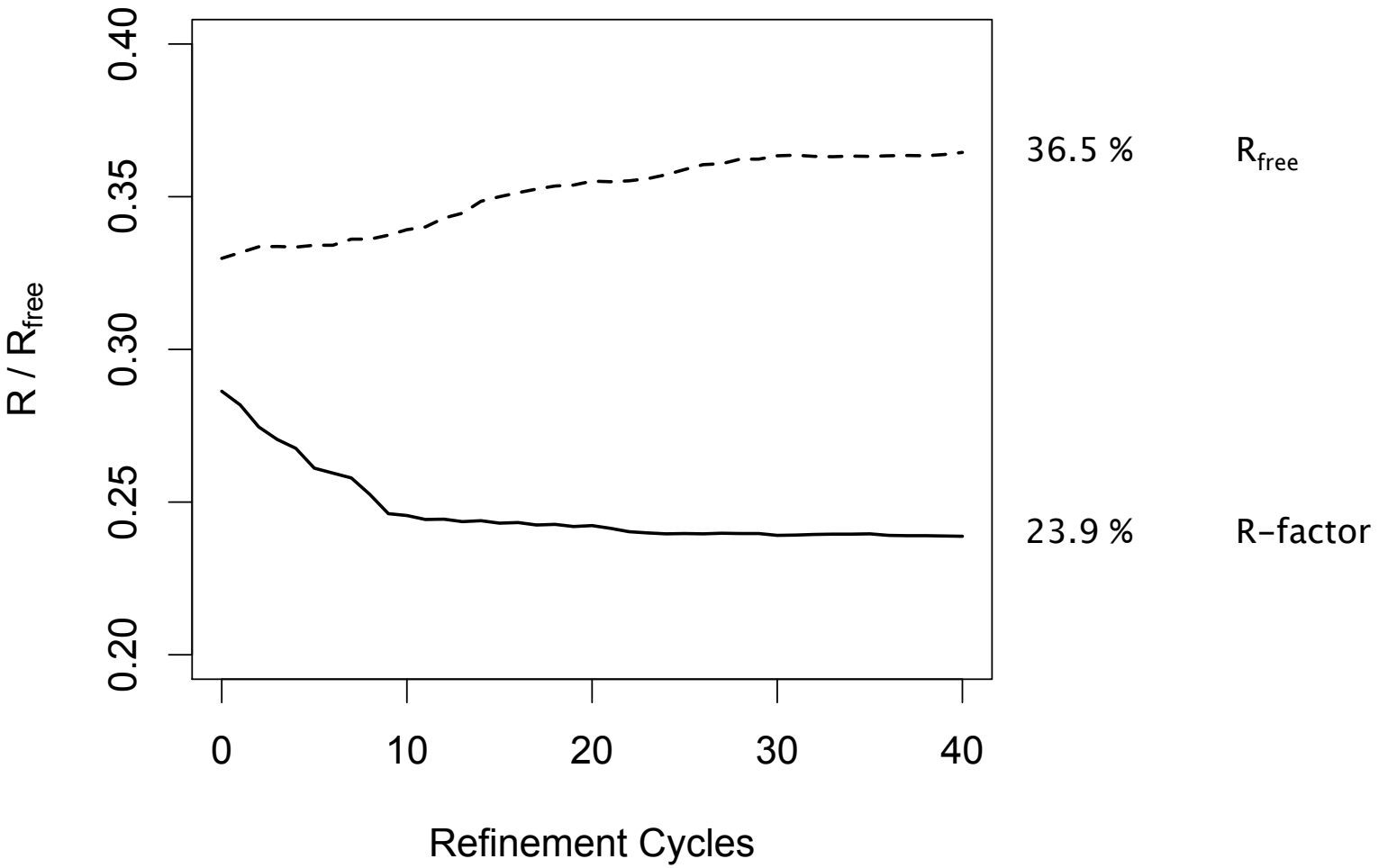
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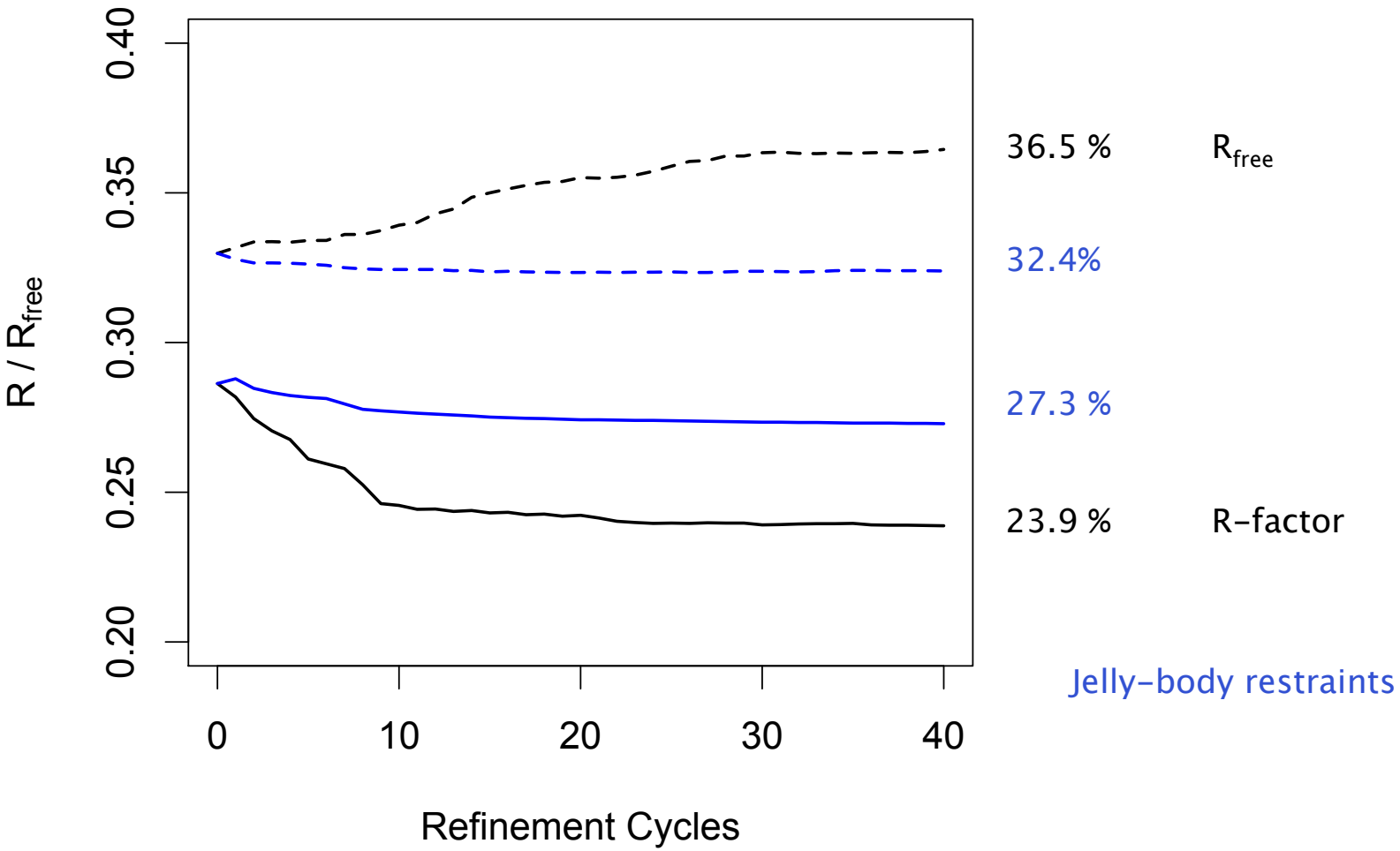
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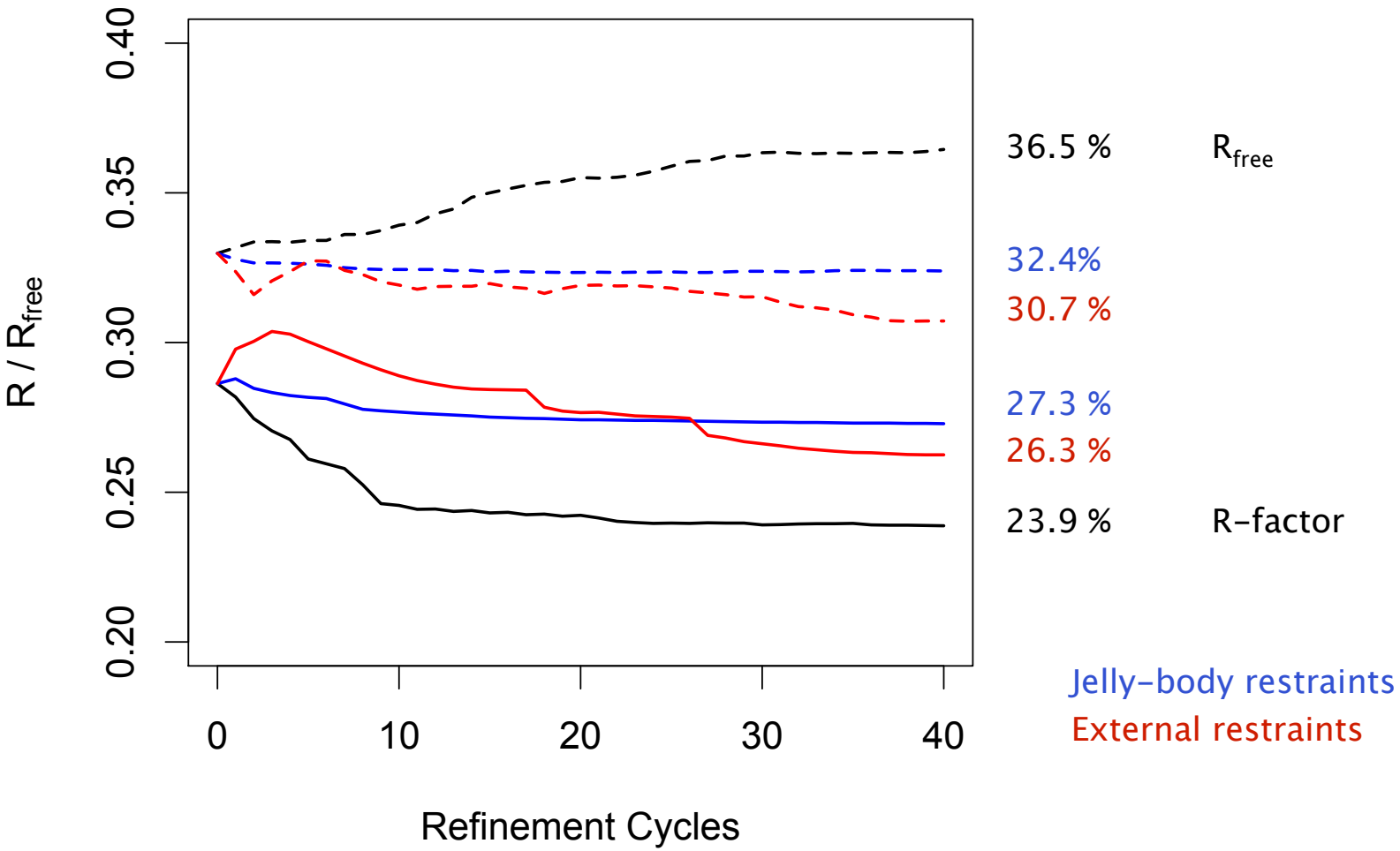
External Restraints

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External Restraints

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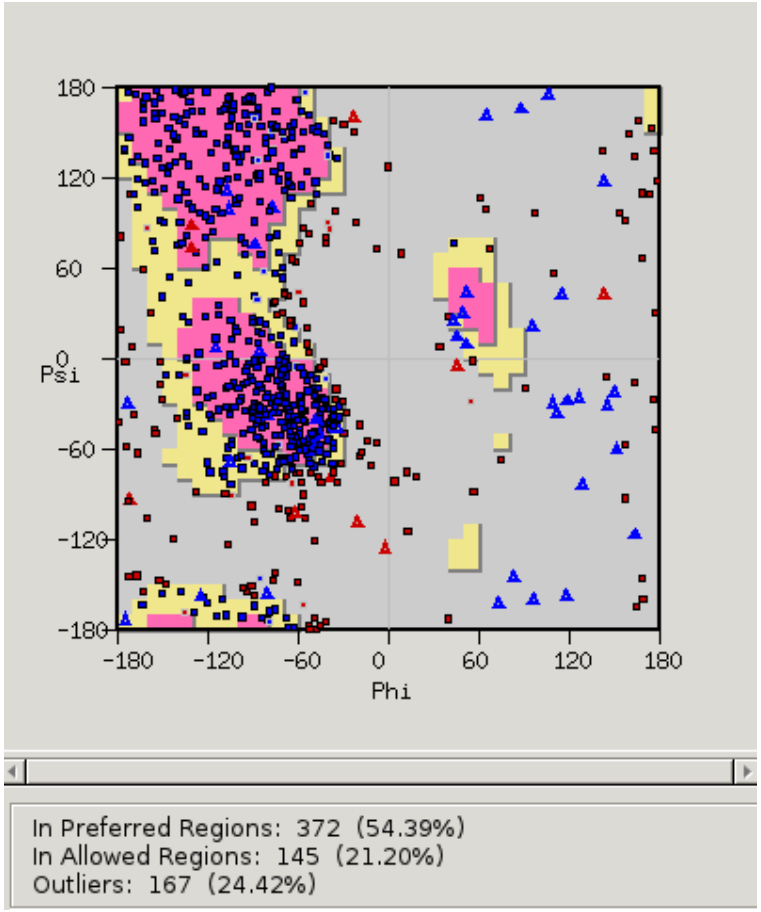


External Restraints

Ovotransferrin

Original Structure

R/R_{free} : 0.286/0.330

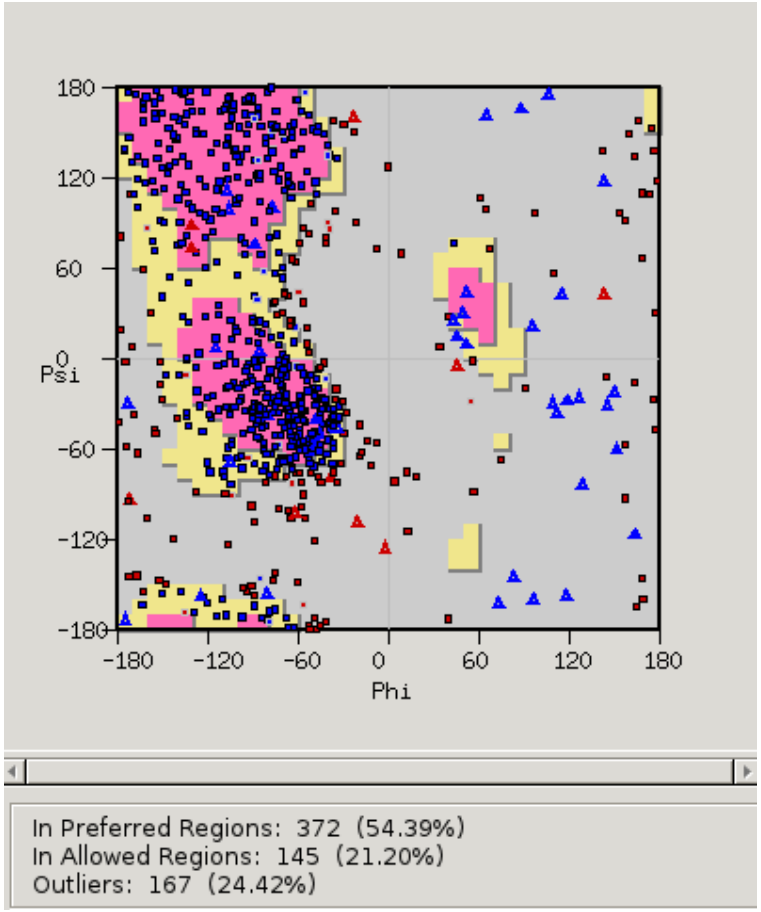


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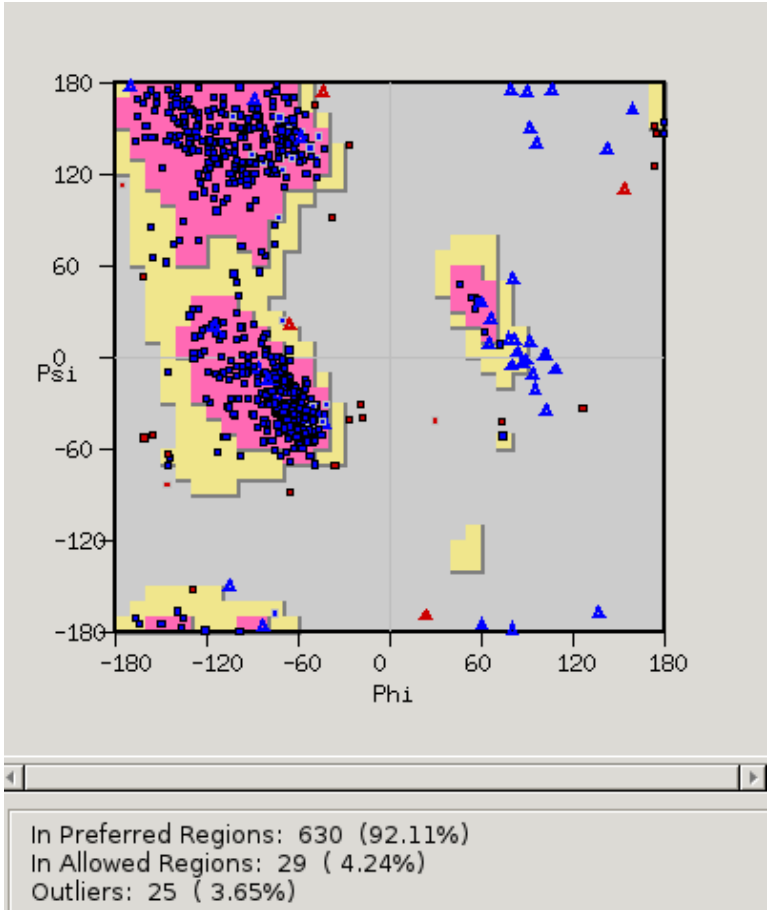
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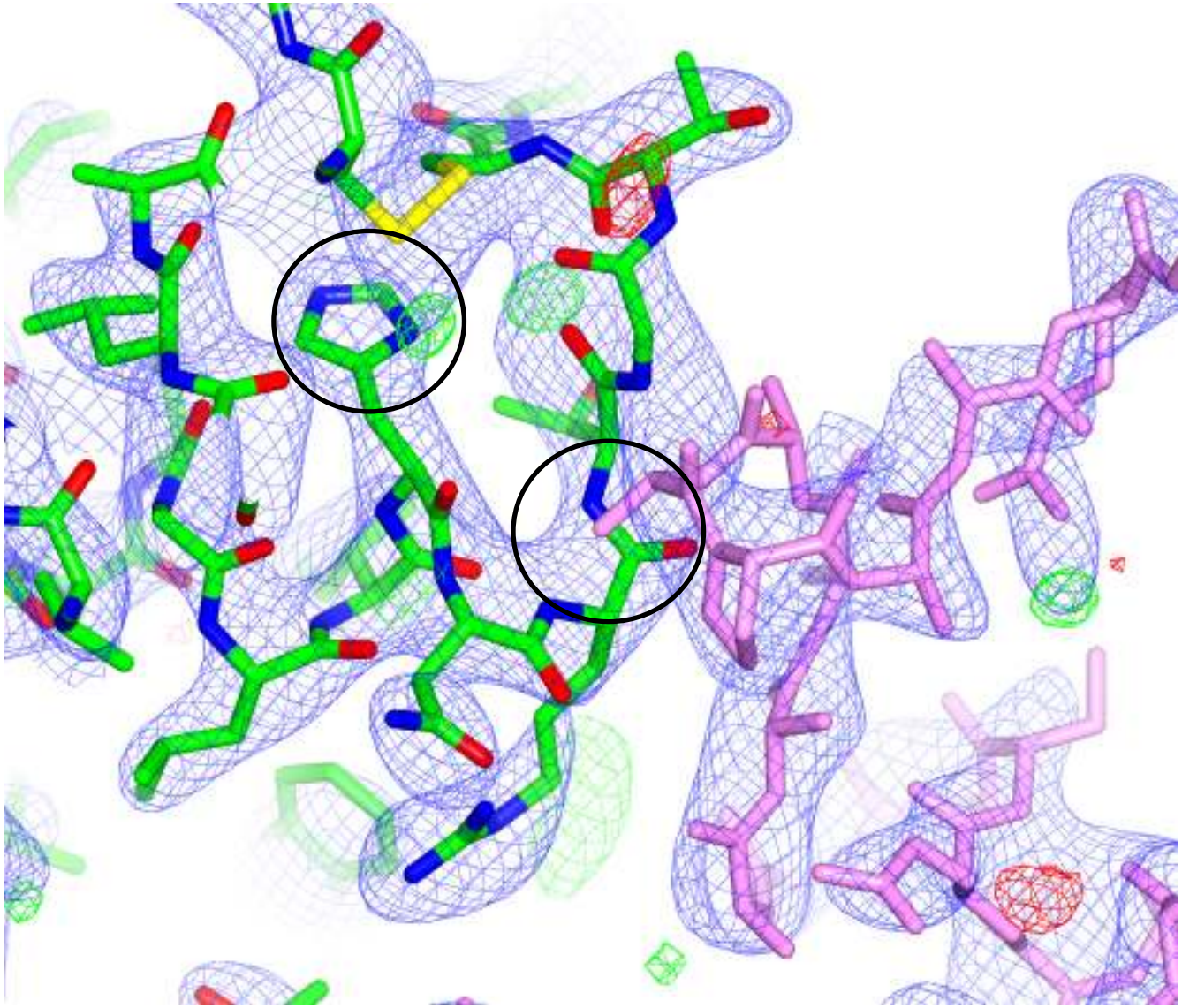


Re-refined with External Restraints

R/R_{free} : 0.263/0.307



External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

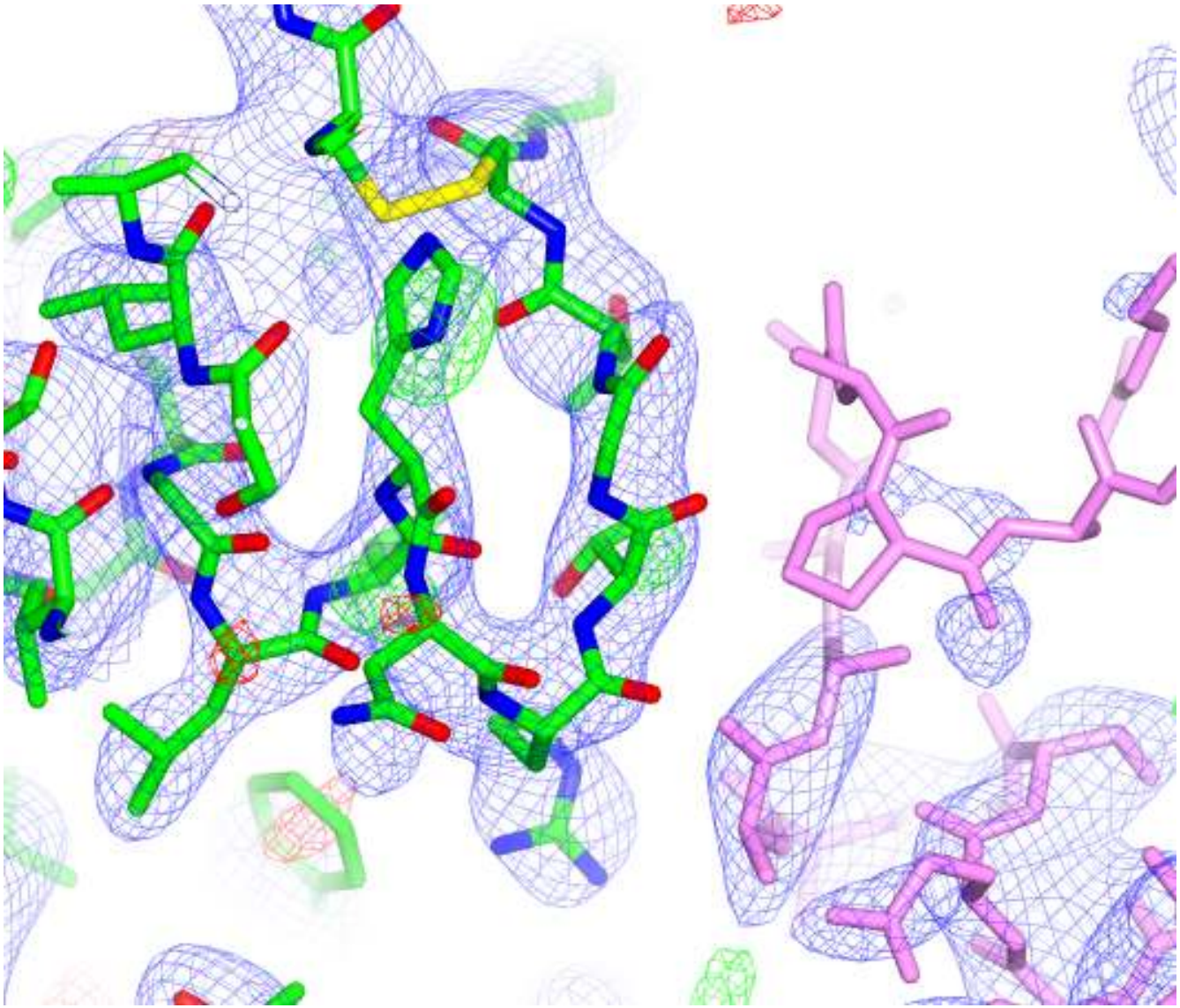
↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

↓

Modify

Real Space Refine

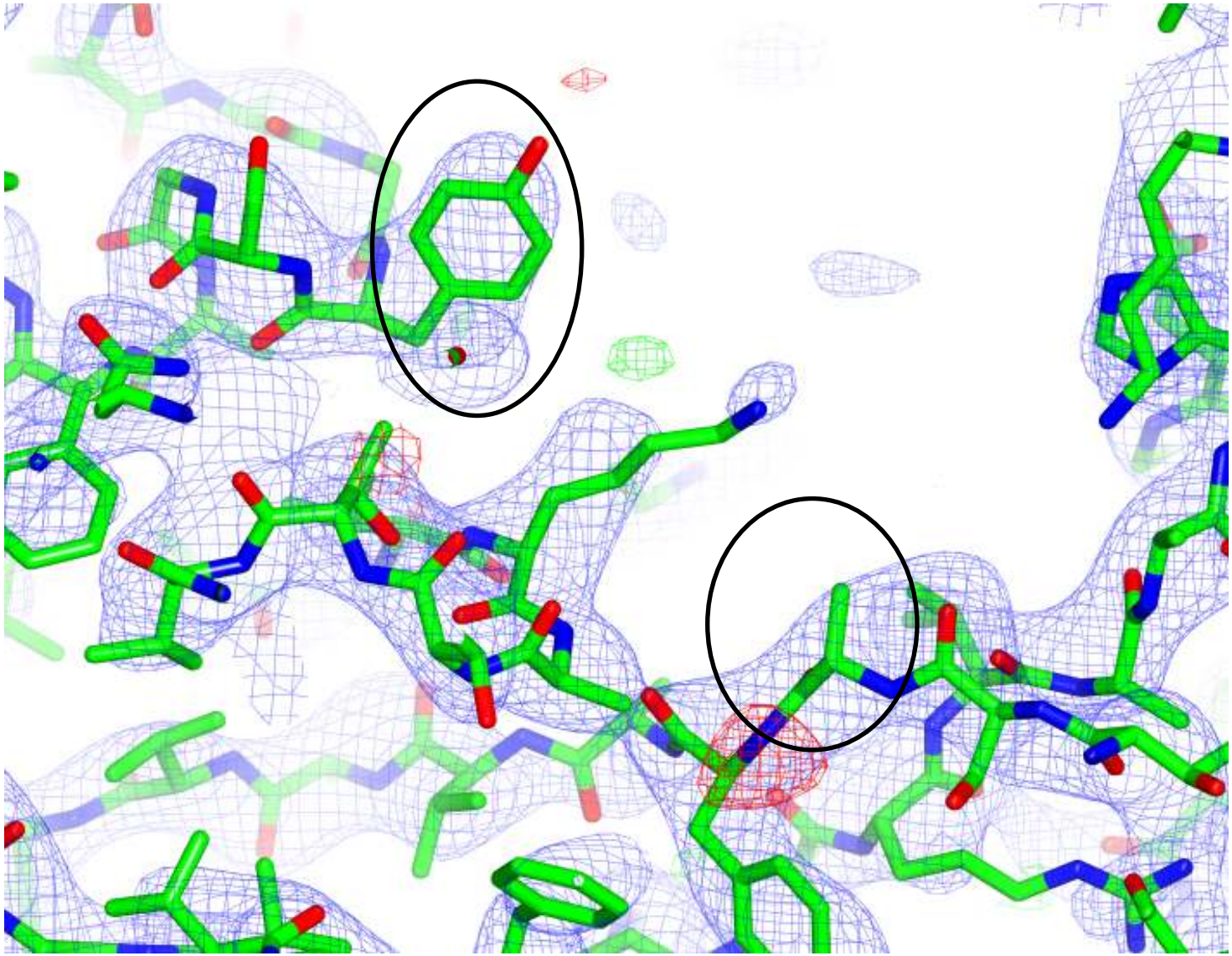
↓

Jelly body

(40 cycles)

R/R_{free} : 0.253/0.304

External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

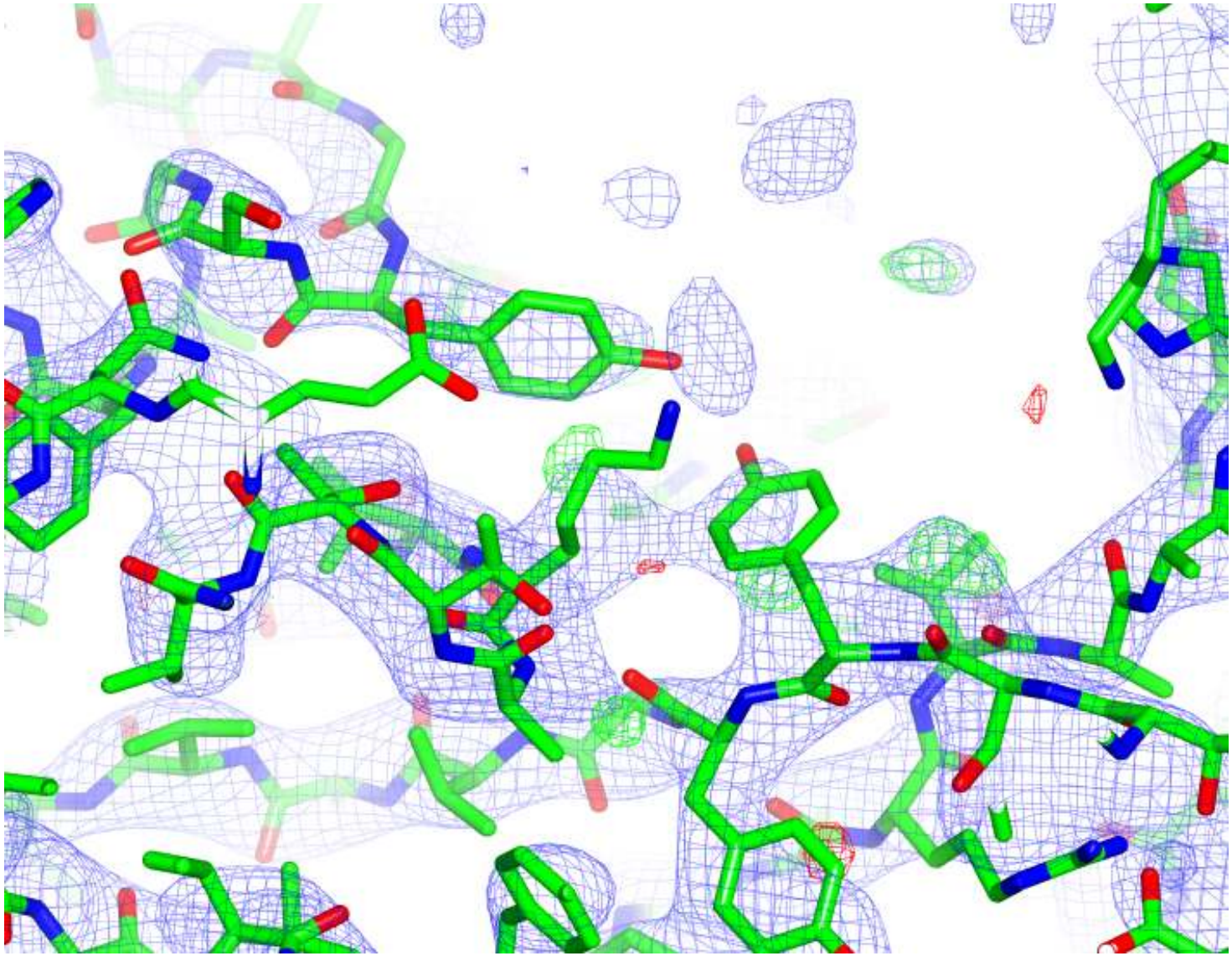
↓

External restraints

(40 cycles)

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External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

↓

Build TYR92

Modify LYS209

↓

Jelly body

(40 cycles)

R/R_{free} : 0.252/0.307

External Restraints

When refining at low resolution, check:

- Refinement statistics – ***Not always conclusive***
- Geometry – ***Not always conclusive***
- Electron density – ***Not always reliable***

Conclusion: **At low resolution, everything has to add up!**

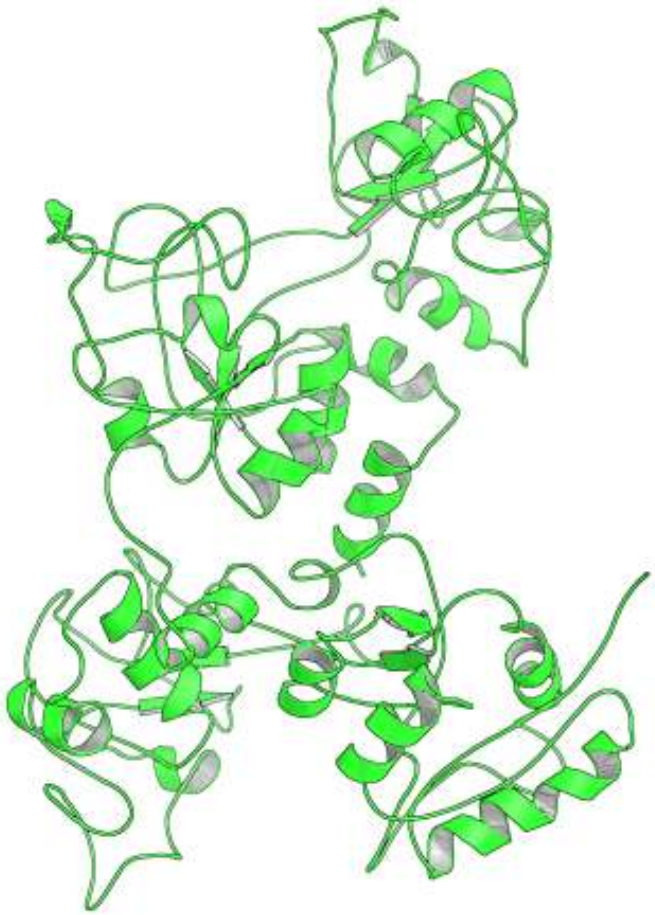
Take care; reflect

Quality of prior information is important **– consider manual re-refinement**

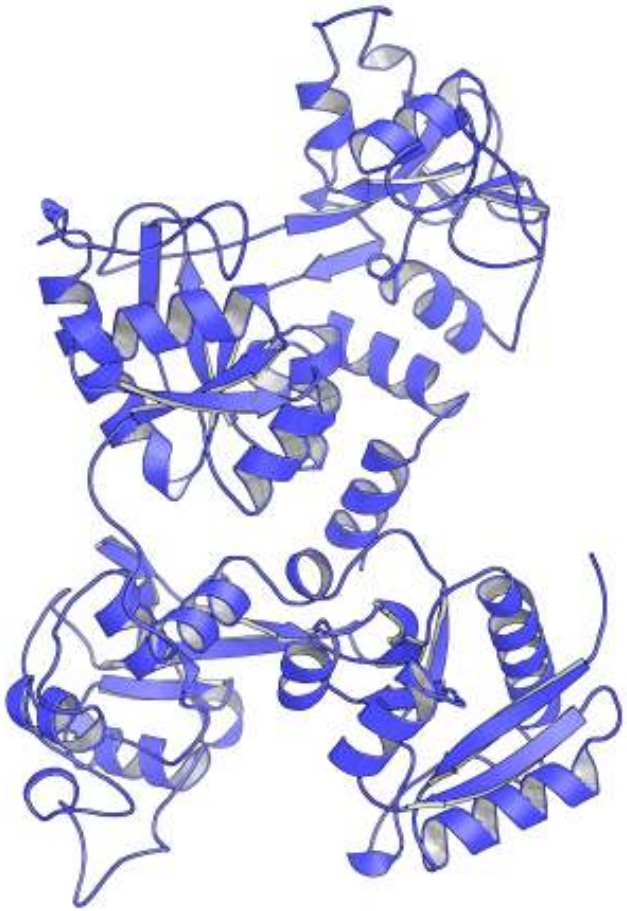
– PDB_REDO is useful

Changes During Refinement

Ovotransferrin



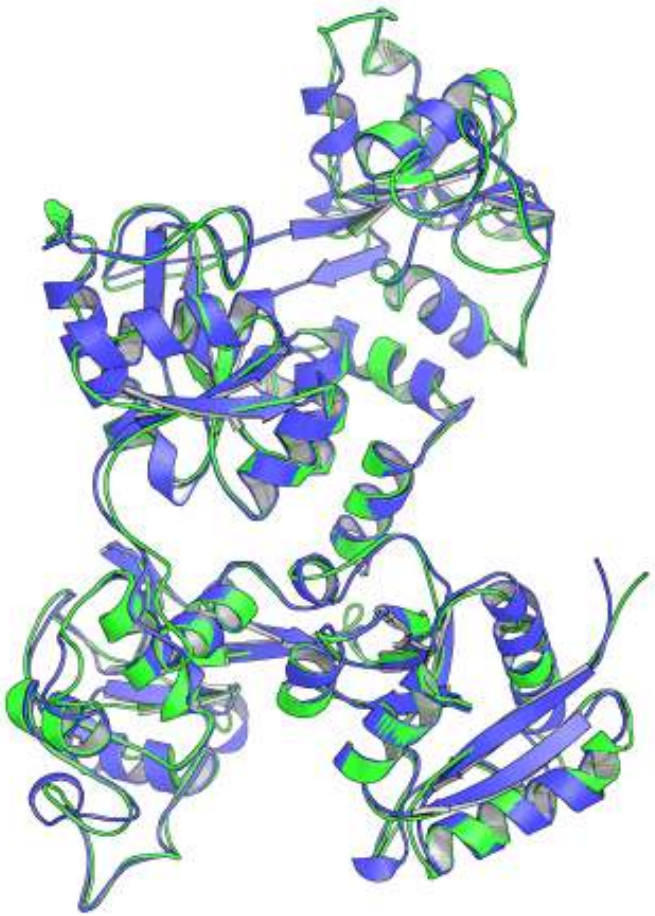
1ryx – 3.5Å



Re-refined with external restraints

Changes During Refinement

Ovotransferrin



original model vs. re-refined model

ProSMART – Local Structural Comparison

Detailed analysis of local structure, e.g. side chains in binding sites, conserved regions

Main features:

- Conformation-independent structural alignment
- Local structural comparison and visualisation
- Identification of rigidly conserved regions

Useful for comparing:

- Homologous structures
- Models at different stages of model-building/refinement

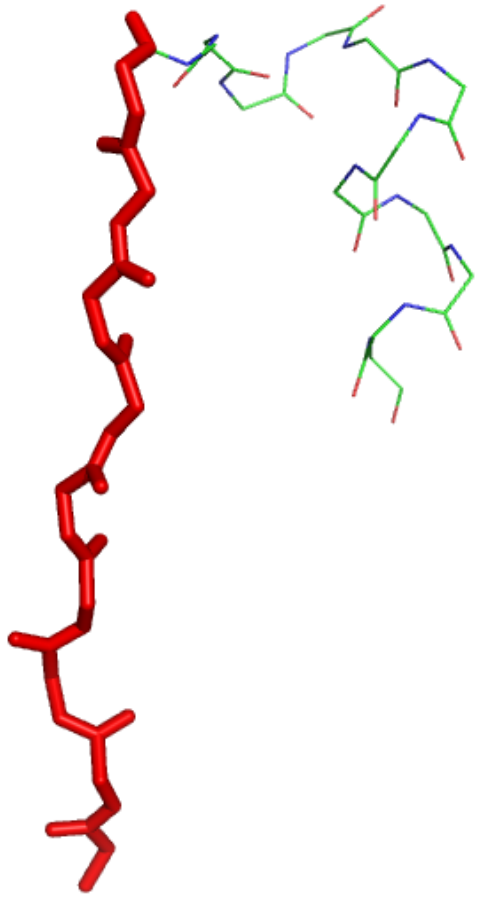
Results visualised in graphics software: CCP4mg, PyMOL, Chimera

Executed from: CCP4i, CCP4mg, Coot, command line

ProSMART – Local Structural Comparison

How it works:

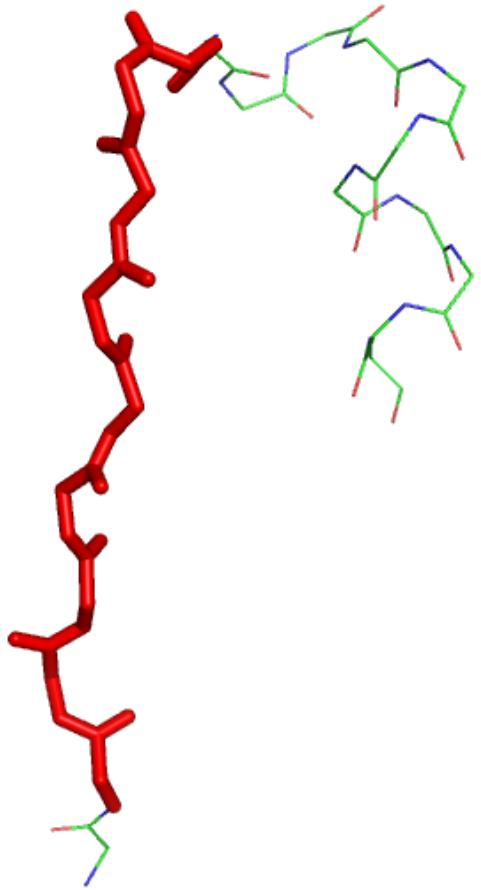
Backbone structural fragments:



ProSMART – Local Structural Comparison

How it works:

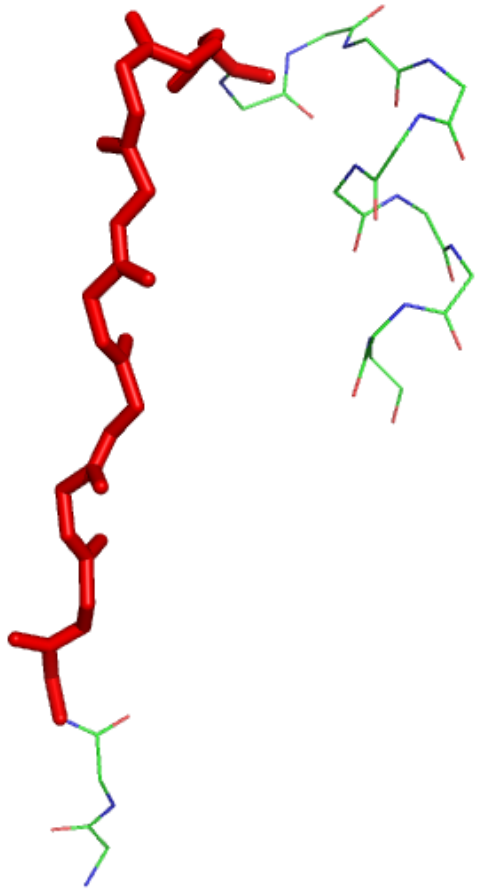
Backbone structural fragments:



ProSMART – Local Structural Comparison

How it works:

Backbone structural fragments:



ProSMART – Local Structural Comparison

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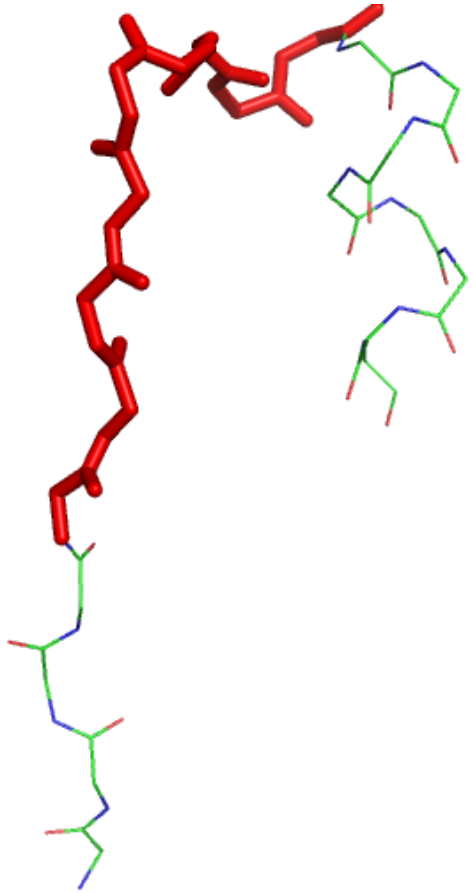
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ProSMART – Local Structural Comparison

How it works:

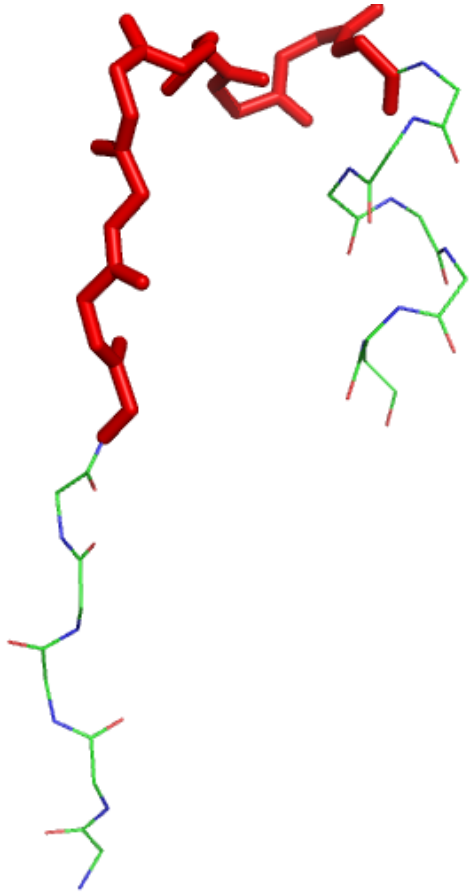
Backbone structural fragments:



ProSMART – Local Structural Comparison

How it works:

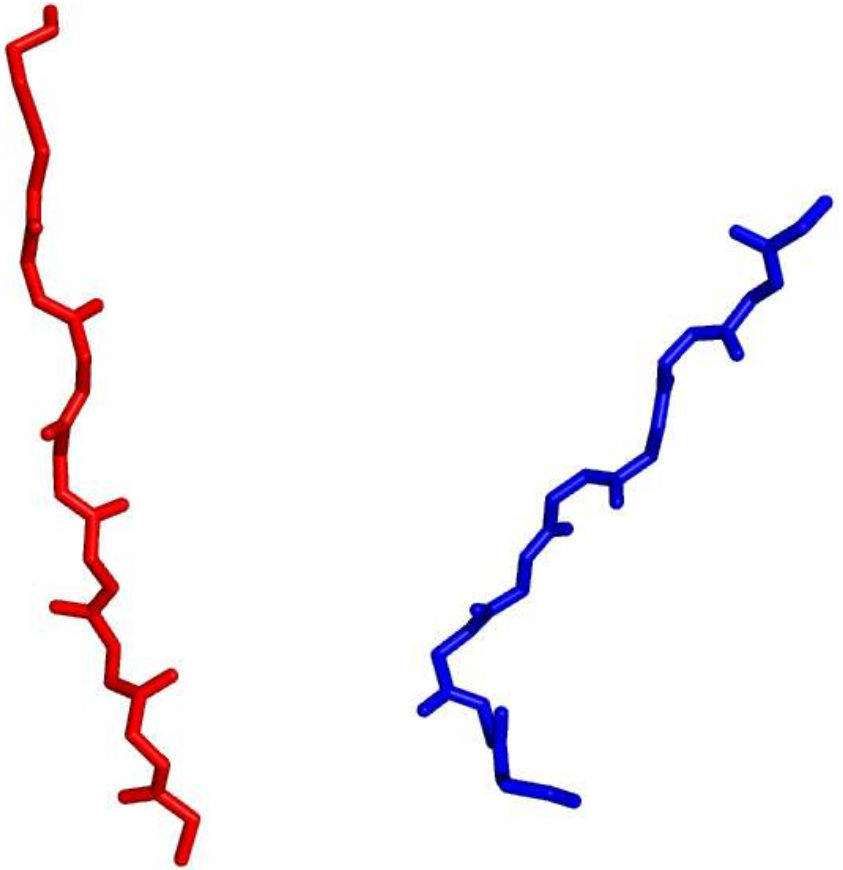
Backbone structural fragments:



ProSMART – Local Structural Comparison

How it works:

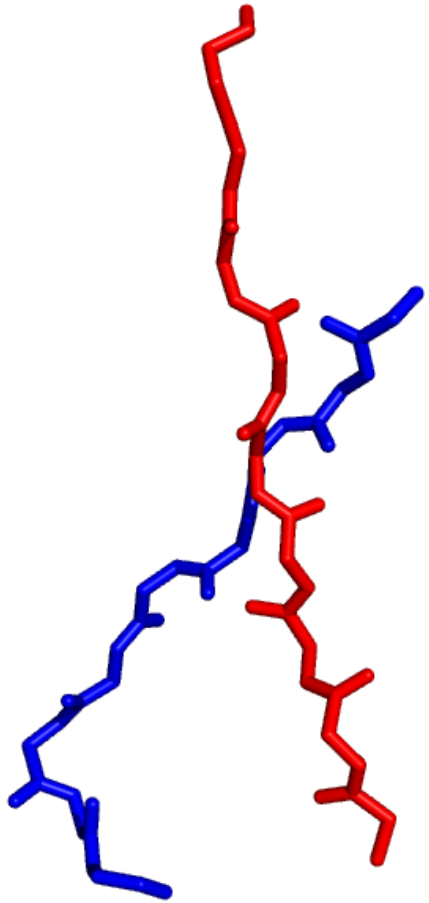
Fragment comparison:



ProSMART – Local Structural Comparison

How it works:

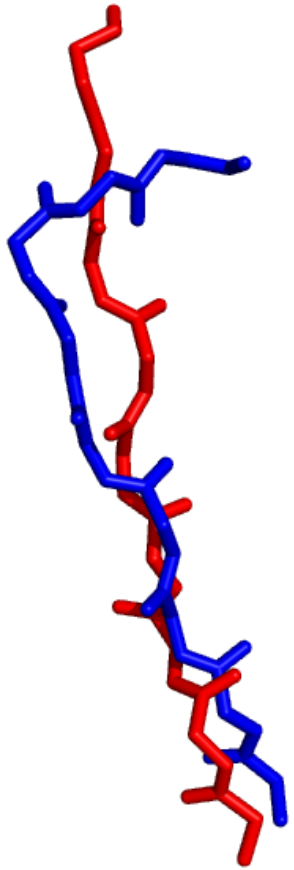
Fragment comparison:



ProSMART – Local Structural Comparison

How it works:

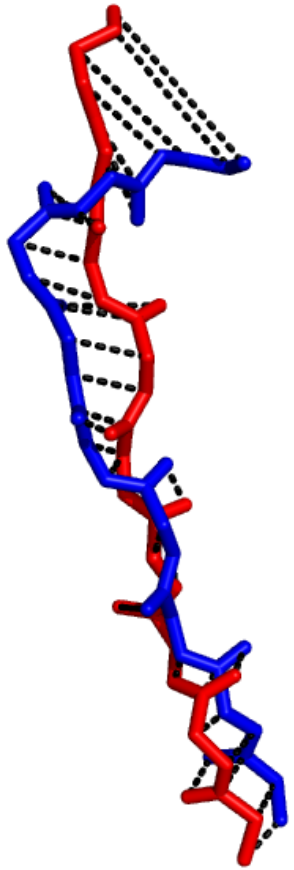
Fragment comparison:



ProSMART – Local Structural Comparison

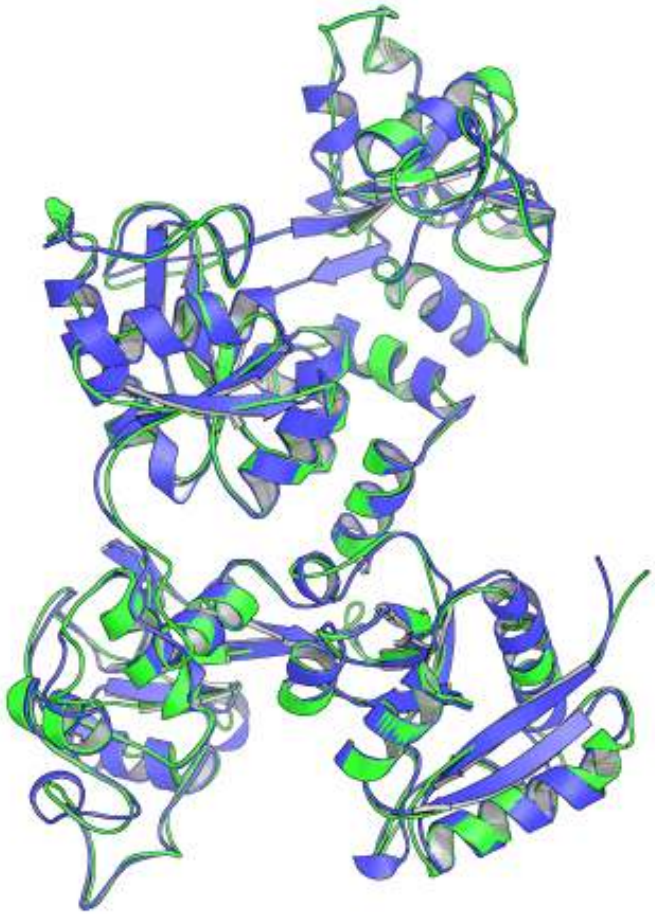
How it works:

Fragment comparison:



Changes During Refinement

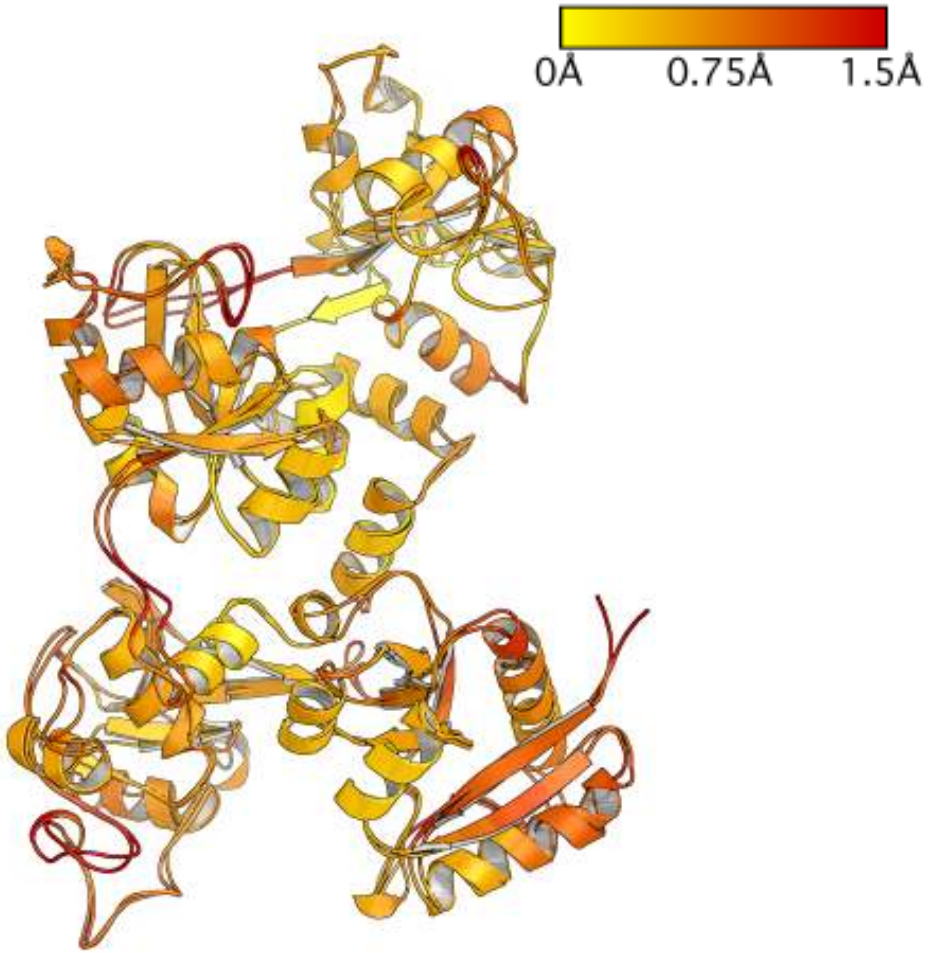
Example: Ovotransferrin



1ryx original versus re-refined

Changes During Refinement

Local Backbone RMSD



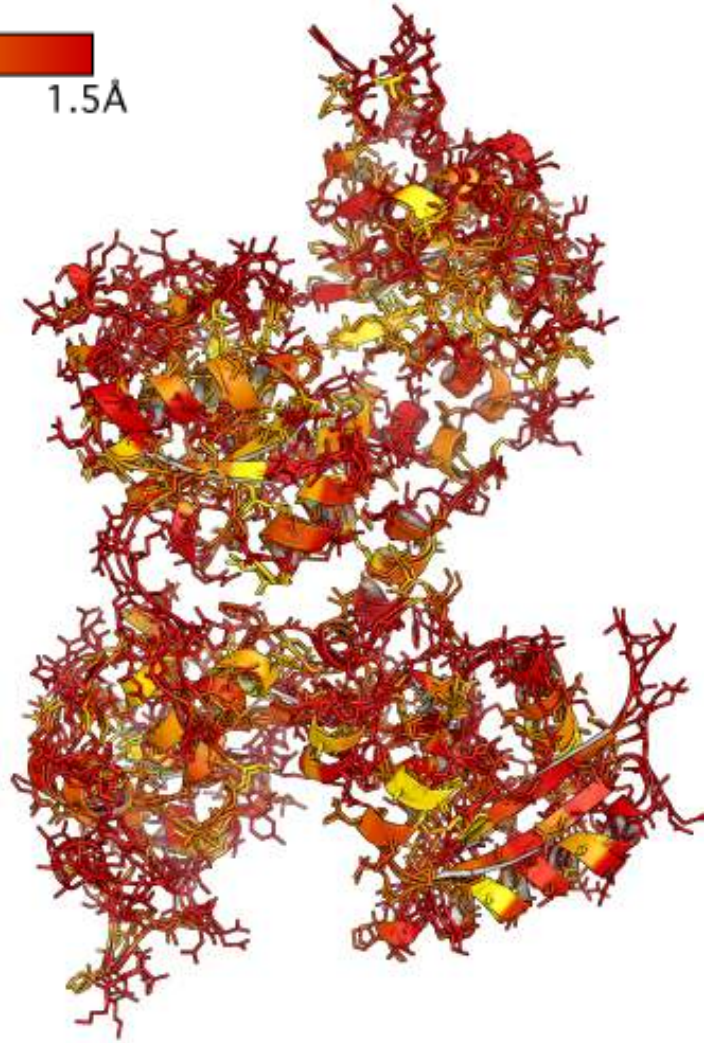
1ryx original versus re-refined

Changes During Refinement

Local Backbone RMSD



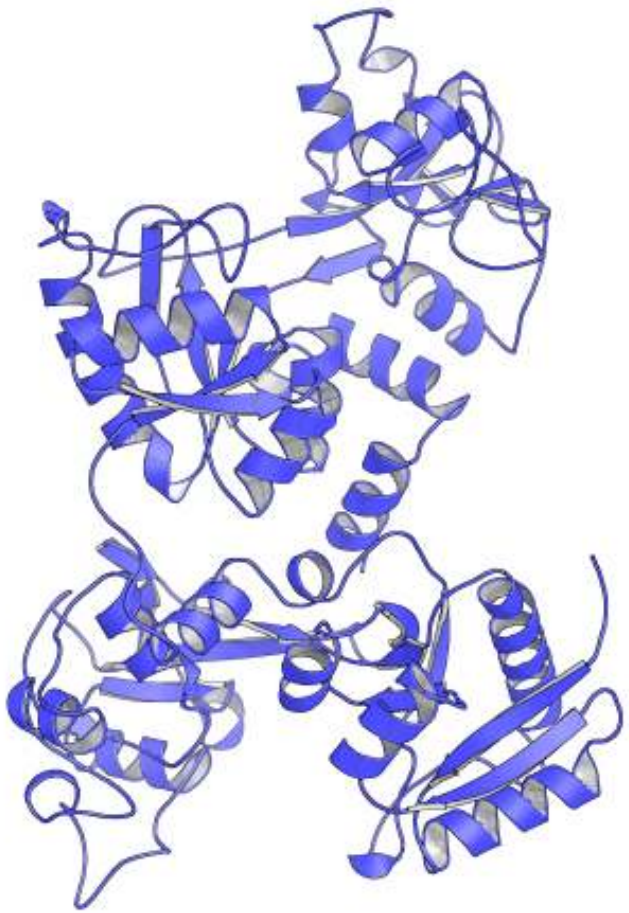
Local Side Chain RMSD



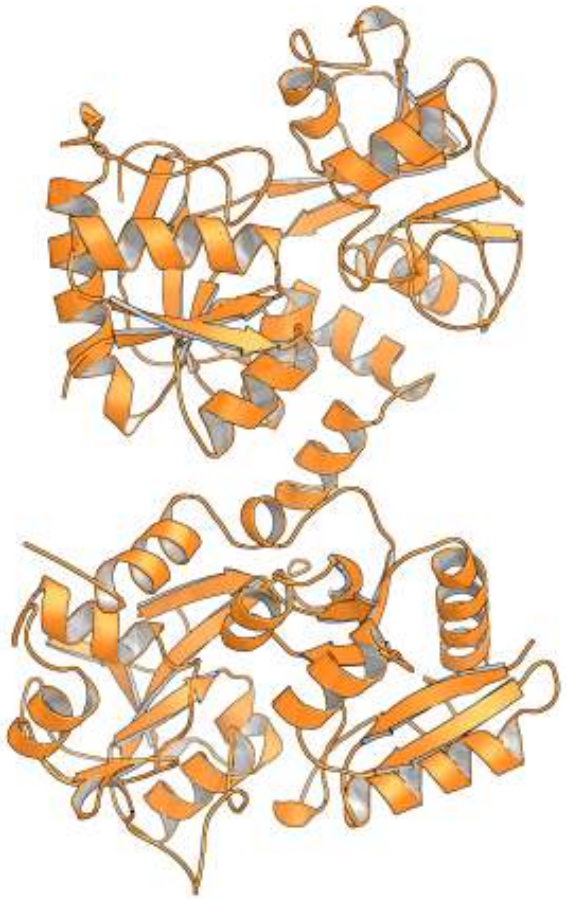
1ryx original versus re-refined

Example: Comparing Homologues

Comparing the re-refined model and the high-res homologue



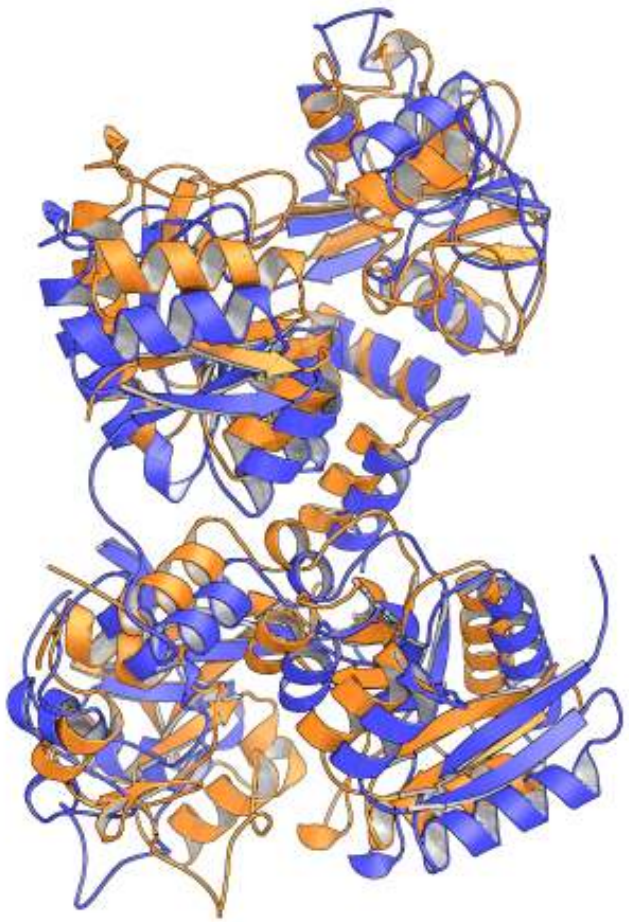
1ryx re-refined – 3.5Å



2d3i – 2.15Å

Example: Comparing Homologues

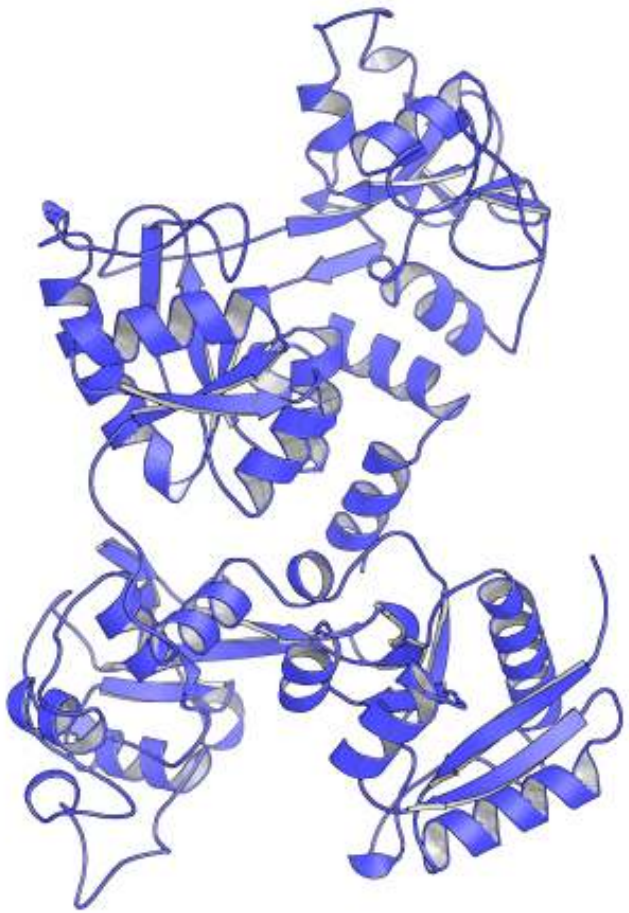
Comparing the re-refined model and the high-res homologue



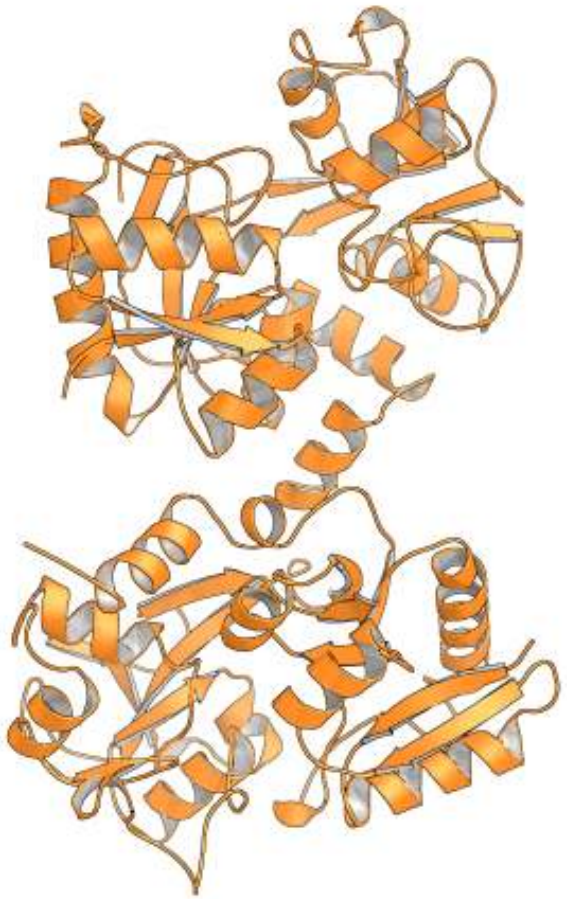
Models don’t superpose well

Example: Comparing Homologues

Comparing the re-refined model and the high-res homologue



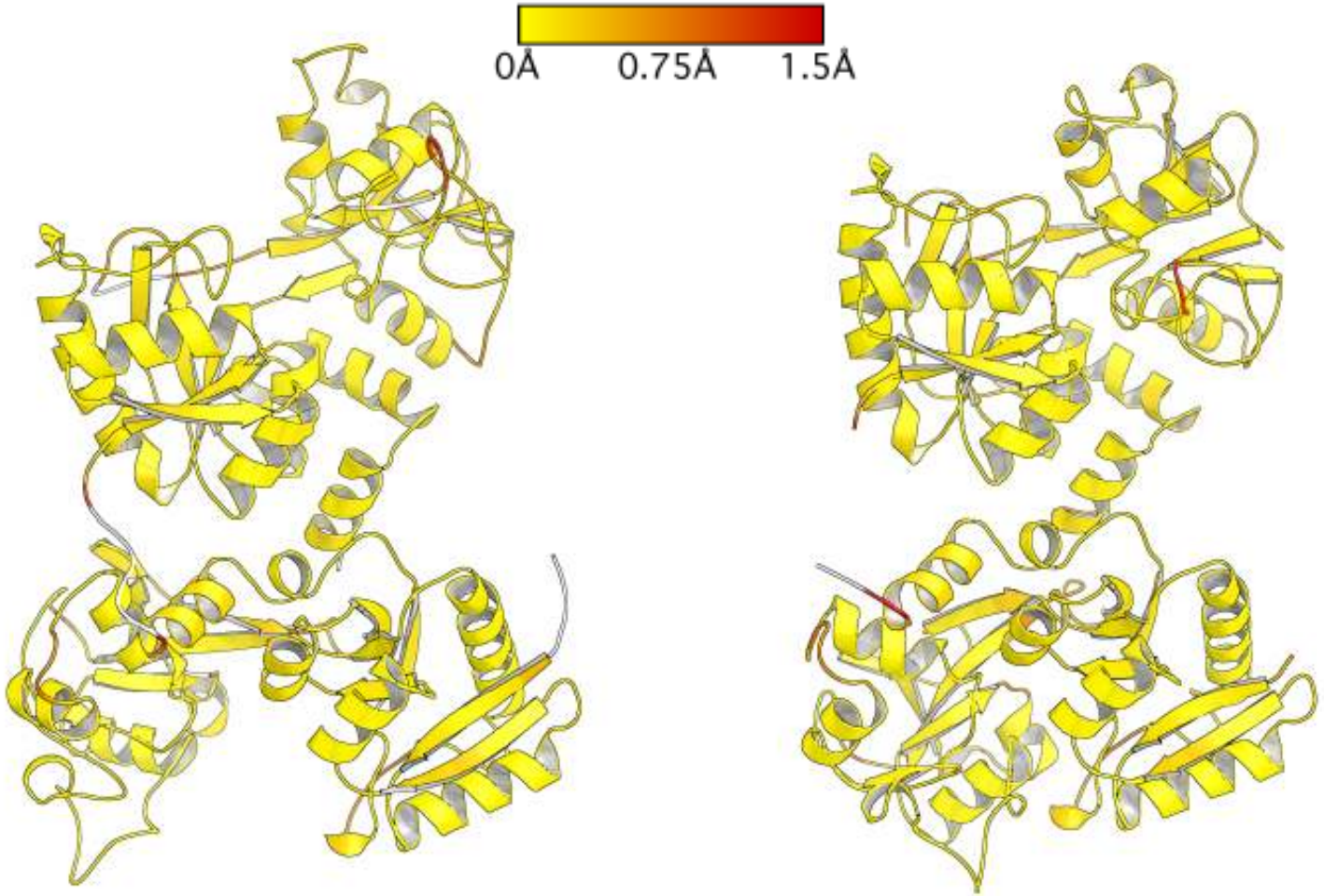
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Example: Comparing Homologues

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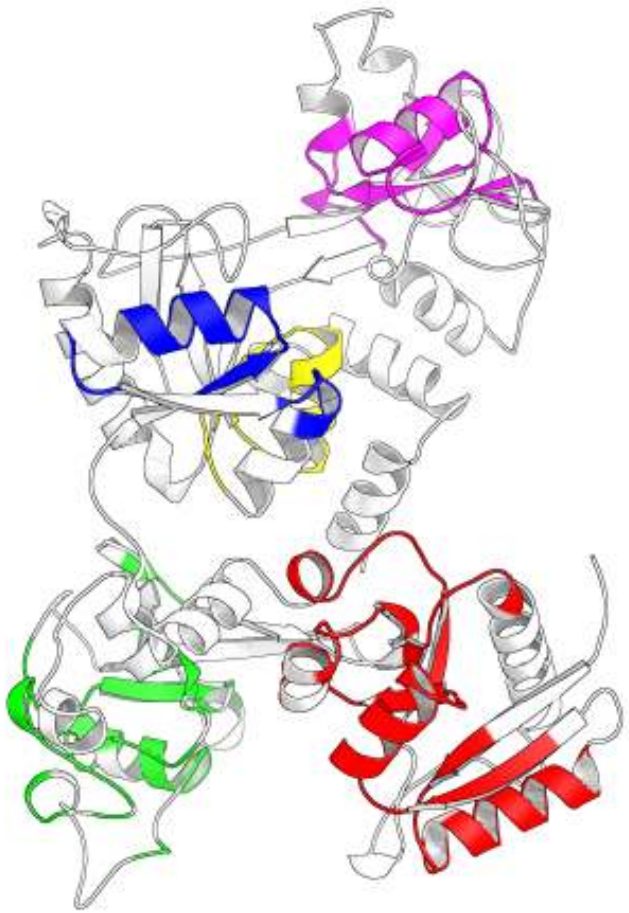


1ryx re-refined – 3.5Å

2d3i – 2.15Å

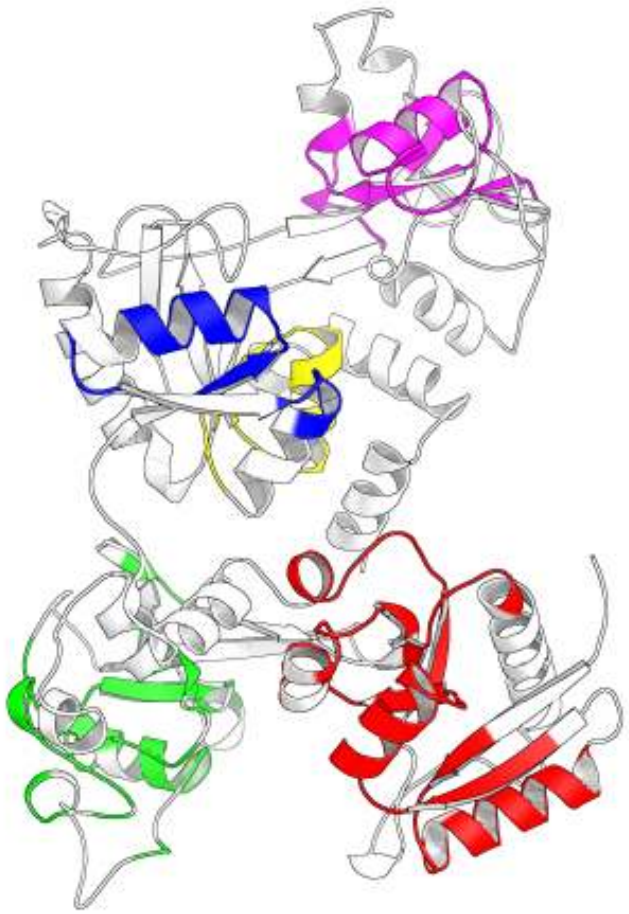
Example: Identifying Conserved Regions

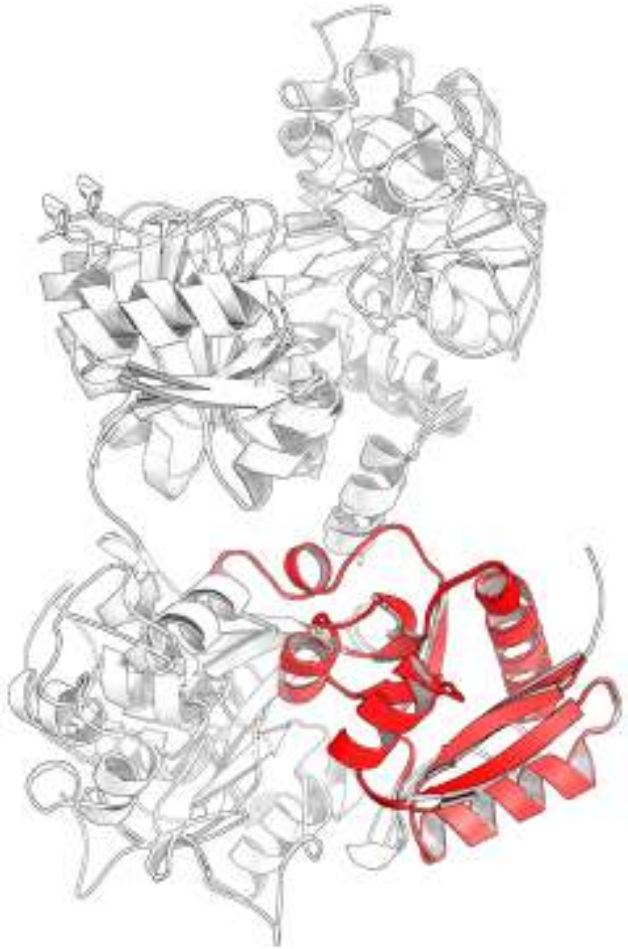
Identified 5 spatially-conserved regions between the two homologues



Example: Identifying Conserved Regions

Identified 5 spatially-conserved regions between the two homologues

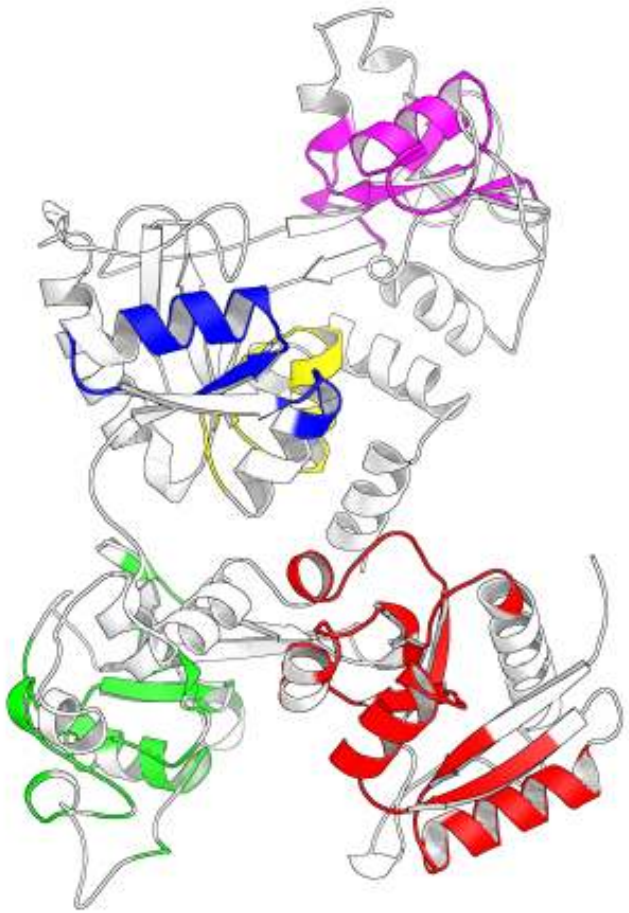




Superposition of conserved region

Example: Identifying Conserved Regions

Identified 5 spatially-conserved regions between the two homologues

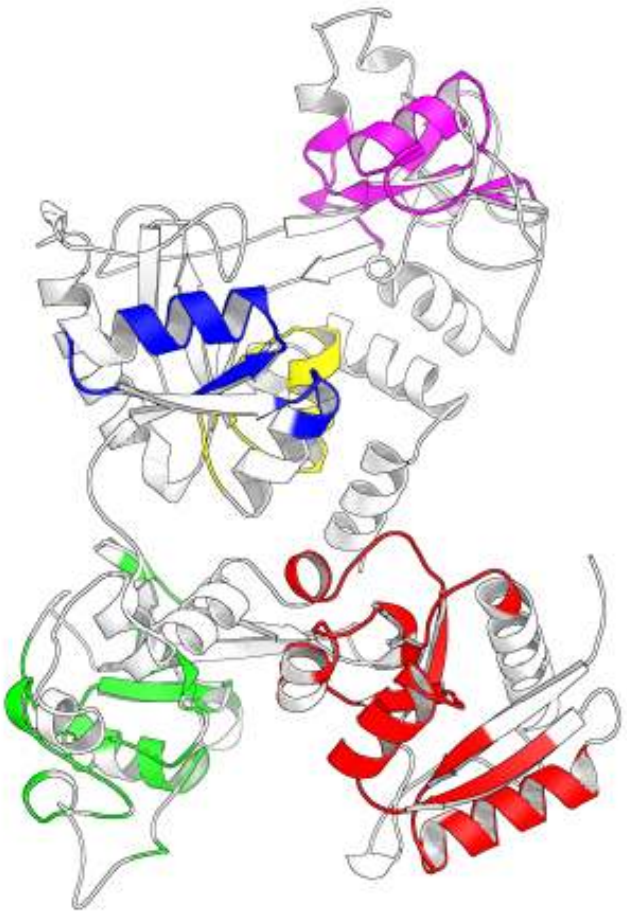


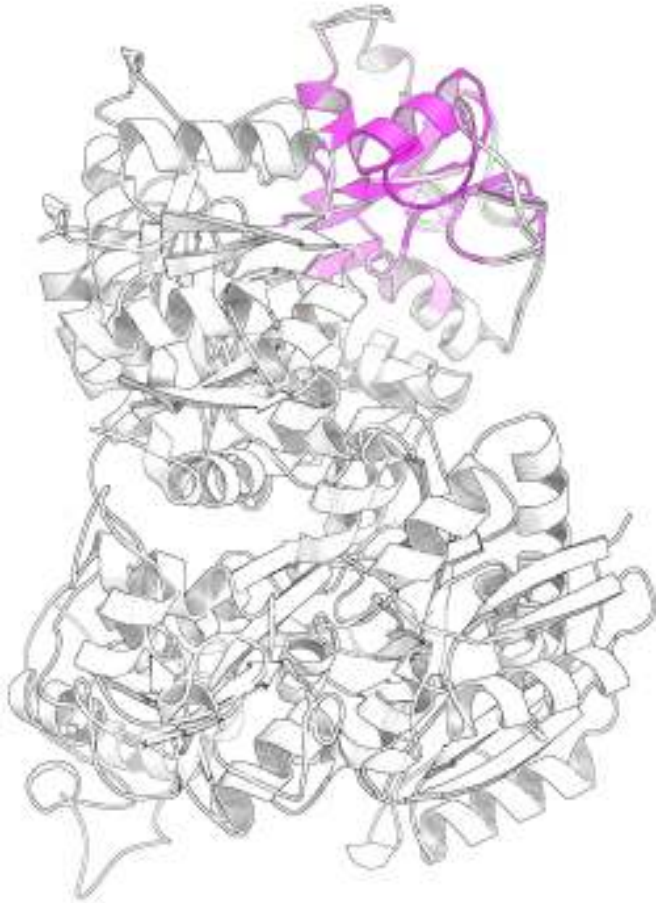


Superposition of conserved region

Example: Identifying Conserved Regions

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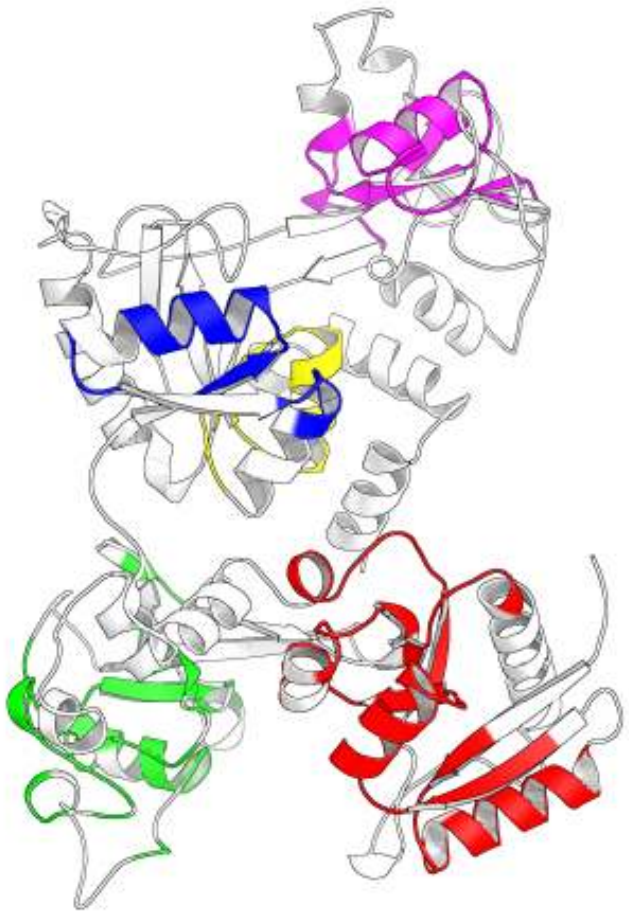


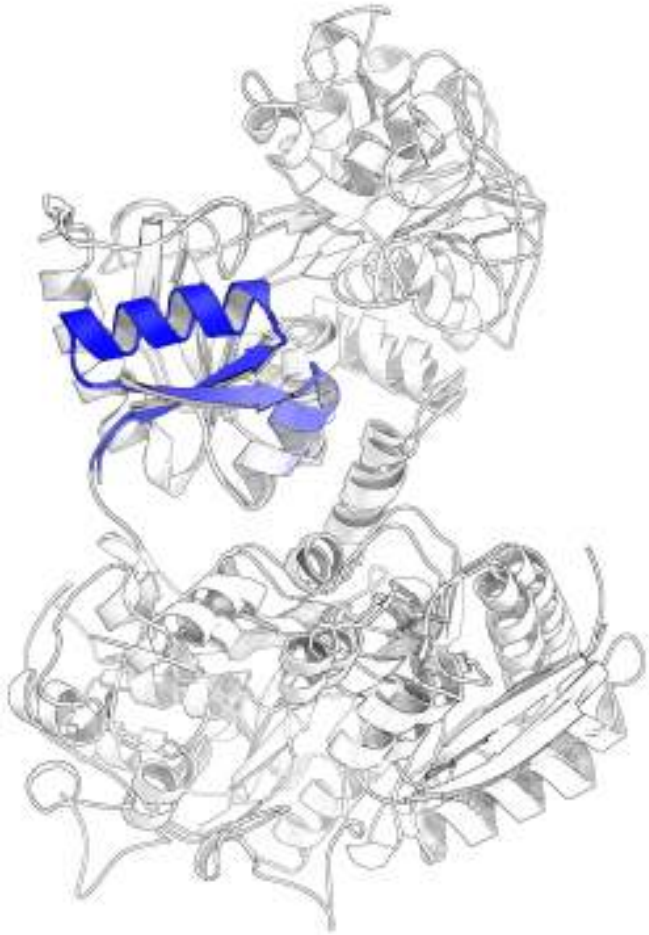


Superposition of conserved region

Example: Identifying Conserved Regions

Identified 5 spatially-conserved regions between the two homologues

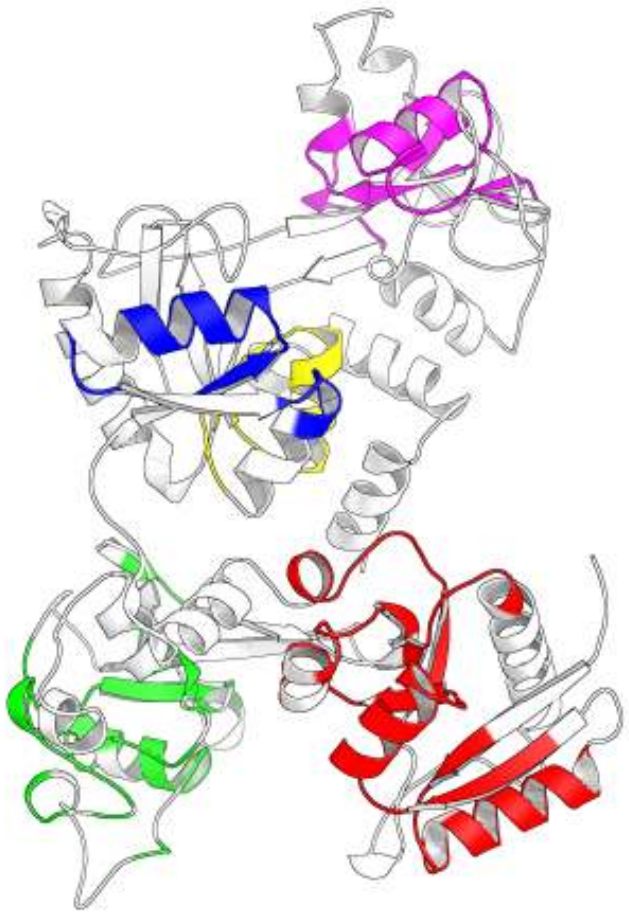




Superposition of conserved region

Example: Identifying Conserved Regions

Identified 5 spatially-conserved regions between the two homologues





Superposition of conserved region

Example: Tyrosine Kinase



Molecular replacement
(Molrep, 10s)

2z8c (2007) – 3.25Å

R_{work}/R_{free} : 21.7/29.2 %

Jelly-body: R_{work}/R_{free} : 19.7/27.3 %

Molprobity clashscore : 28.3

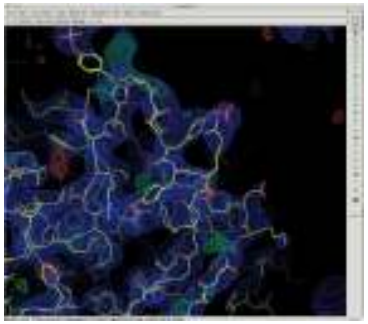
2z8c solved using 2auh,

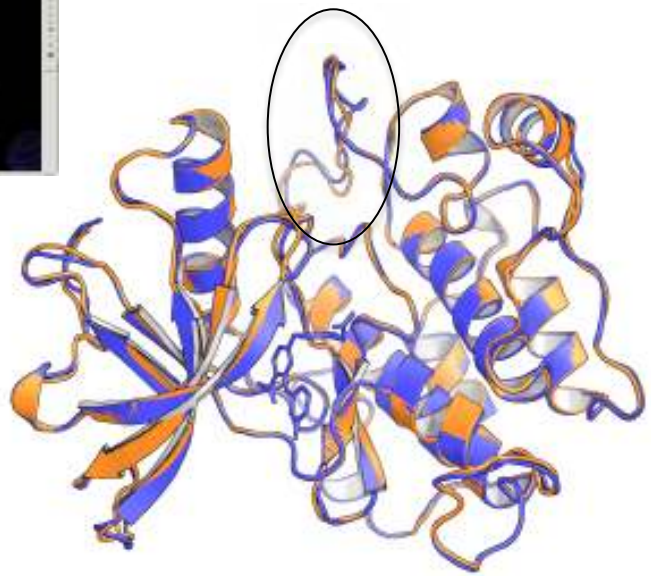
re-refined using jelly-body

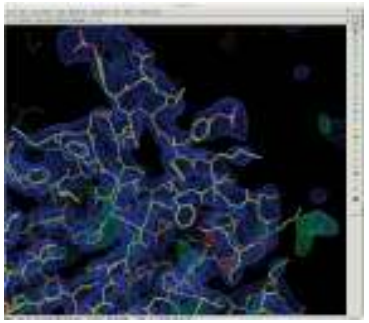
R_{work}/R_{free} : 20.2/25.8 %

Molprobity clashscore : 16.9

Example: Tyrosine Kinase







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(Molrep, 10s)

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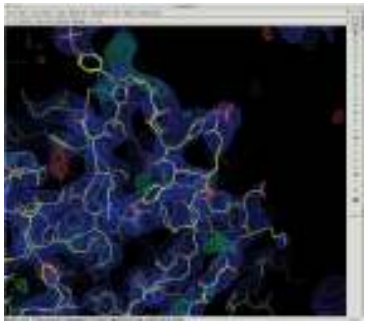
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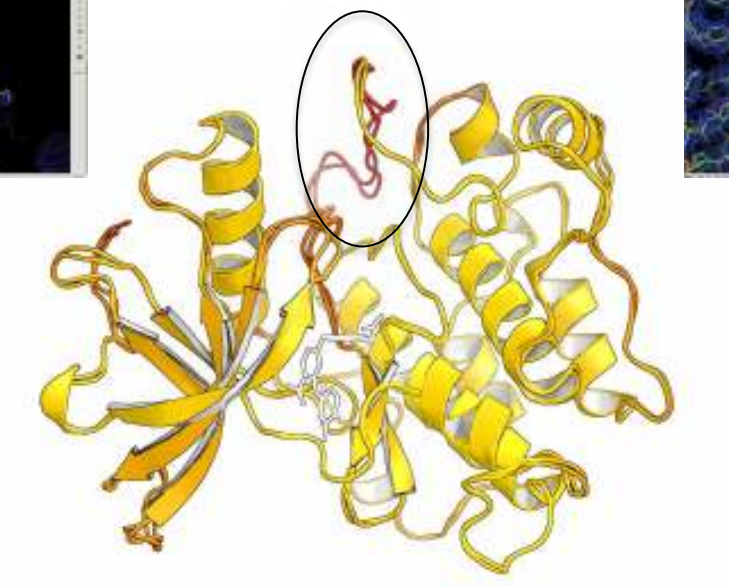
re-refined using jelly-body

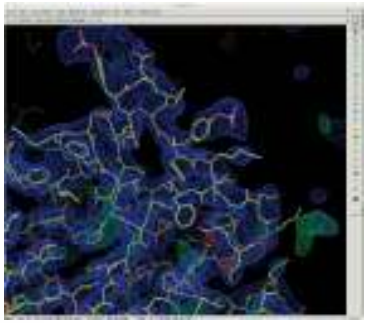
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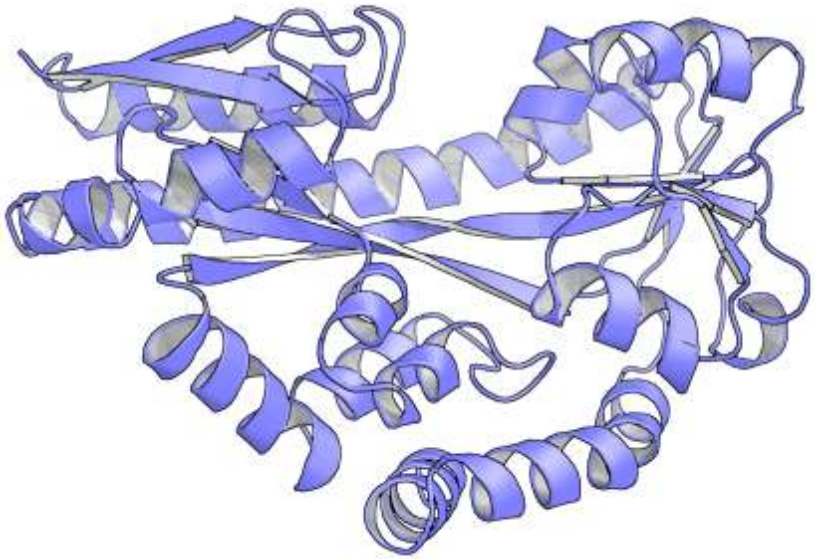
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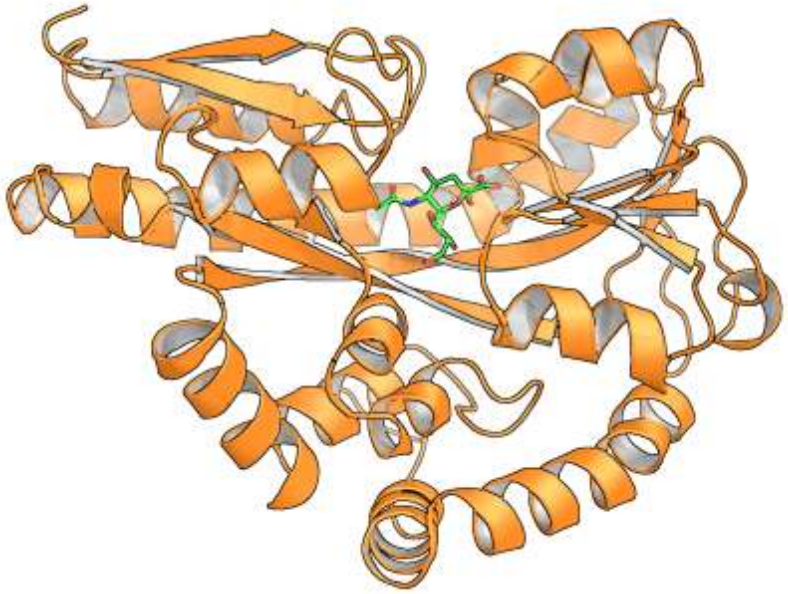
Molprobity clashscore : 16.9

Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states



2cex (2.2Å) – open

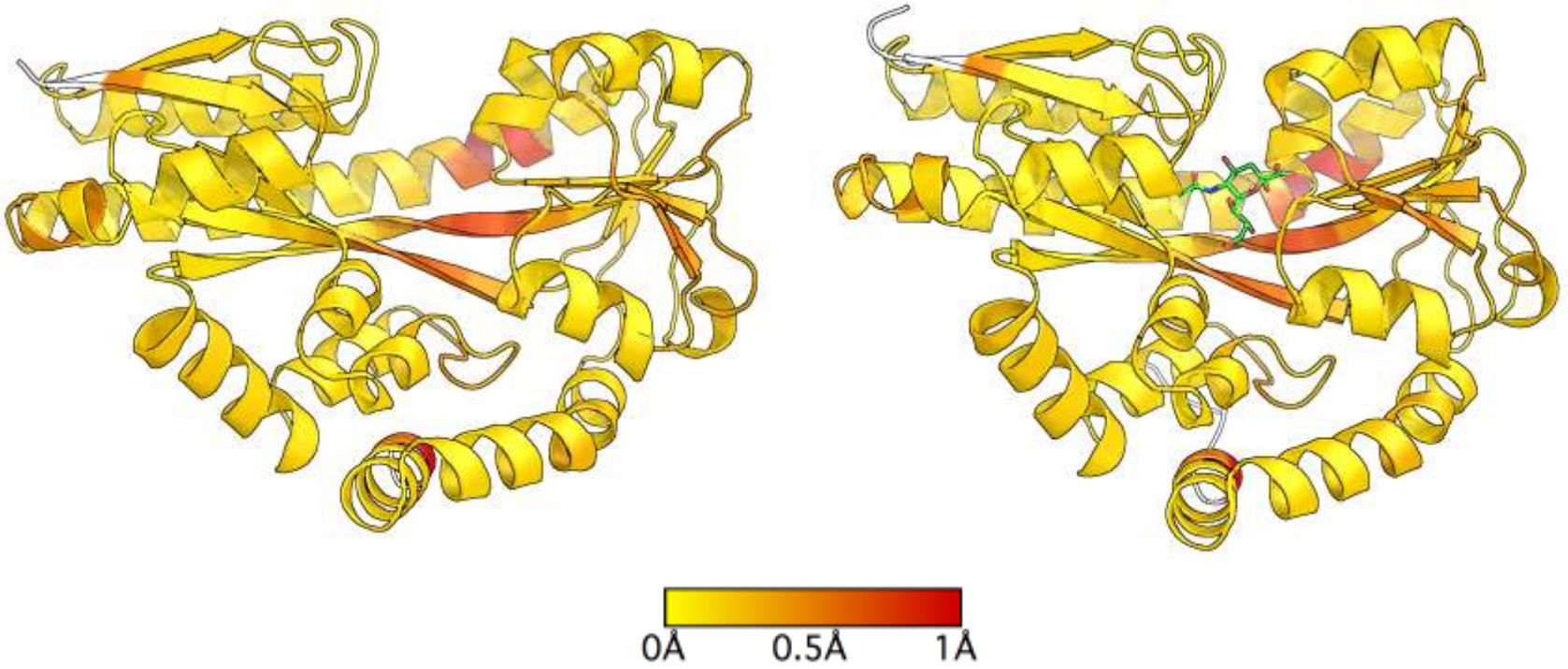


3b50 (1.4Å) – bound

Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states

Coloured by local backbone RMSD



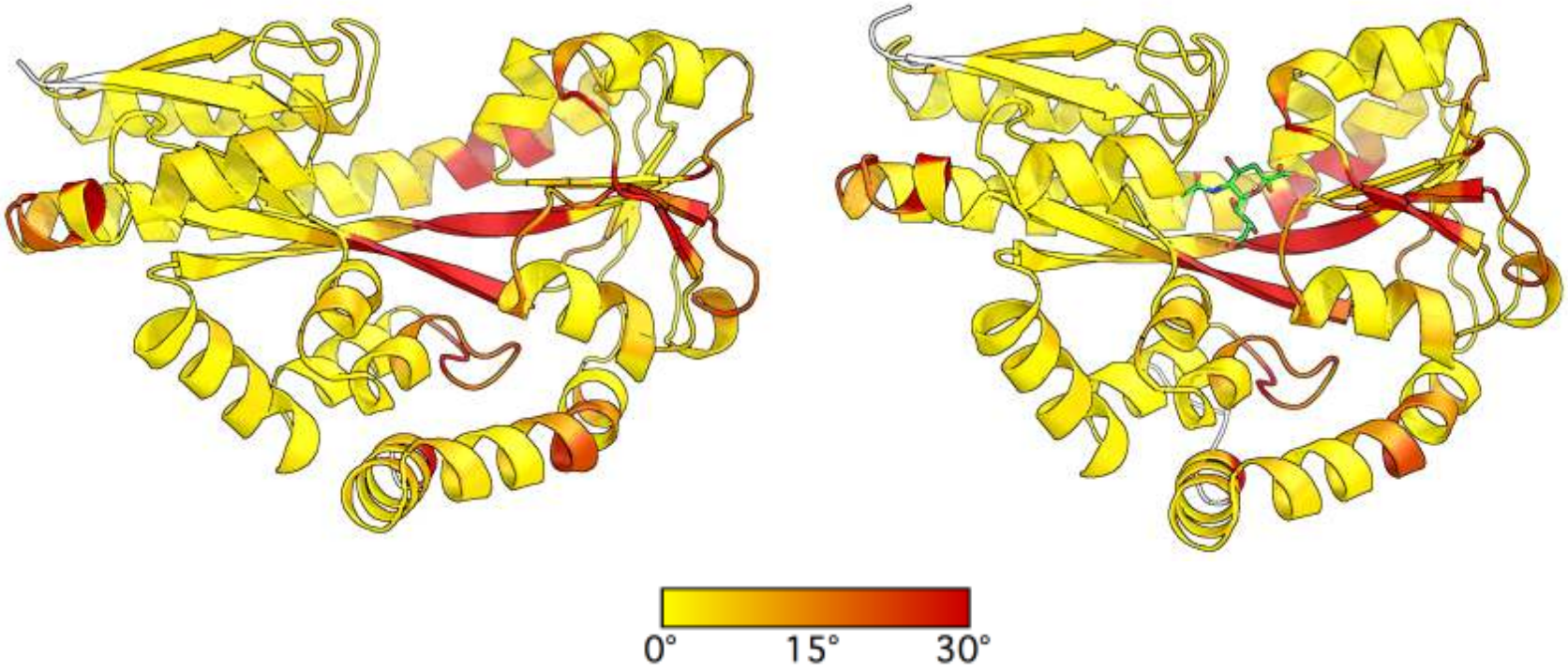
2cex (2.2Å) – open

3b50 (1.4Å) – bound

Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states

Identifying hinge residues



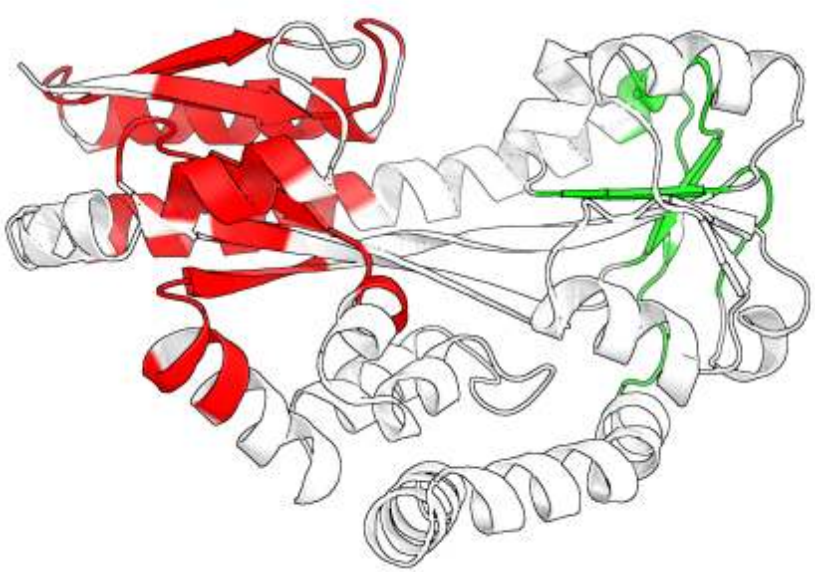
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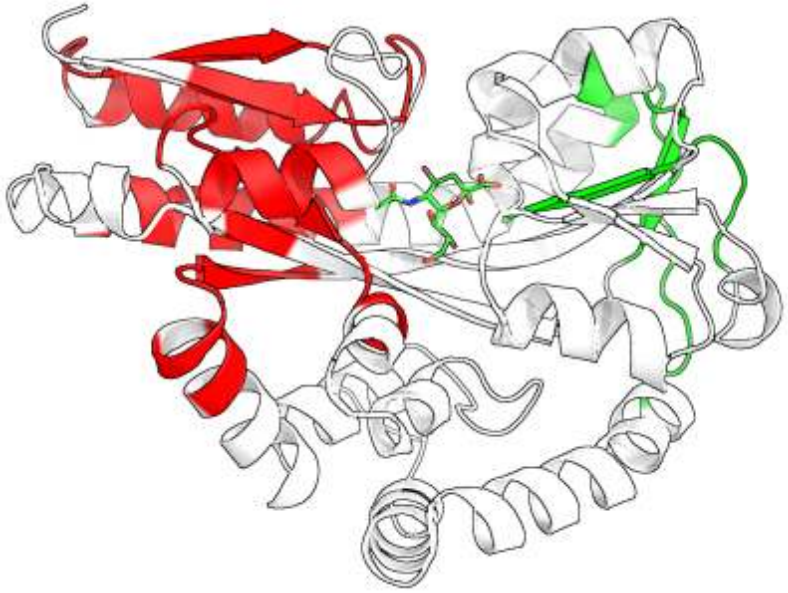
Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states

Identifying spatially-conserved regions



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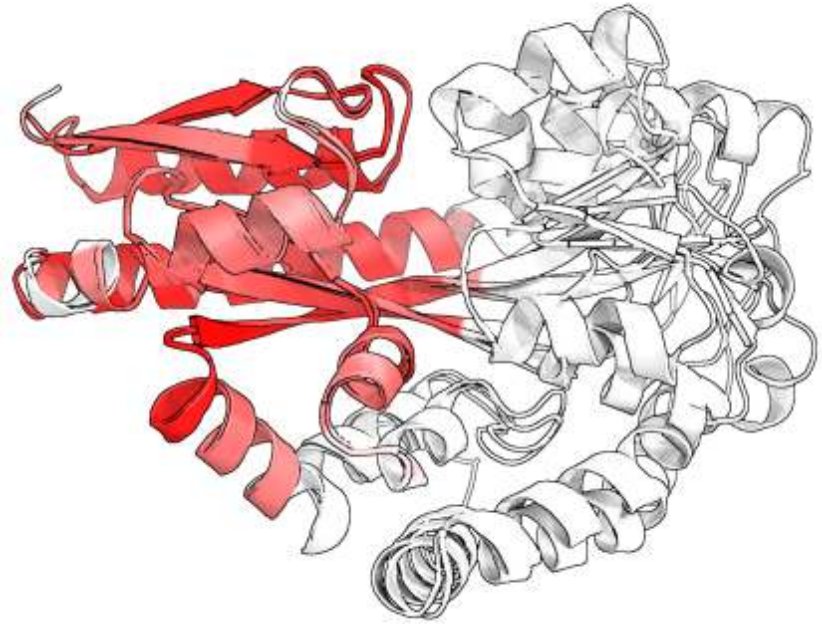


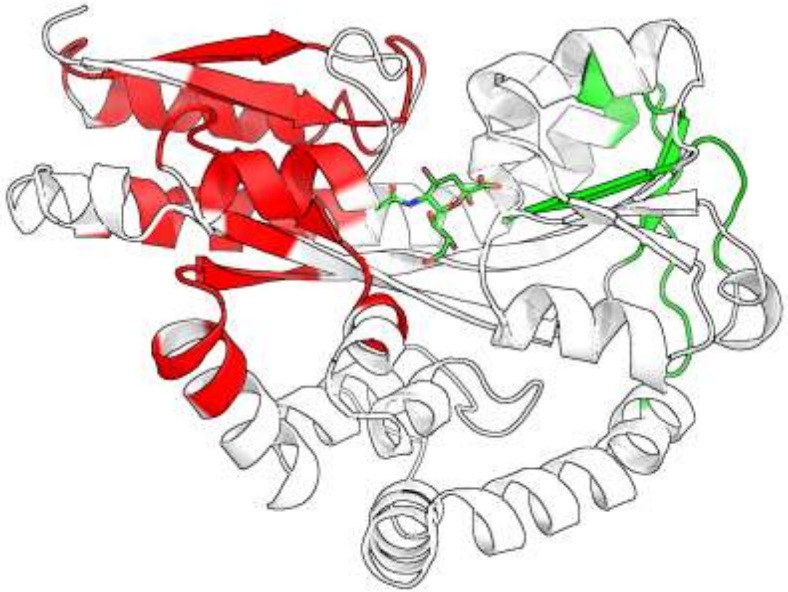
3b50 (1.4Å) – bound

Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states

Identifying spatially-conserved regions





Coloured by conservation of coordinate frame (superposition quality)

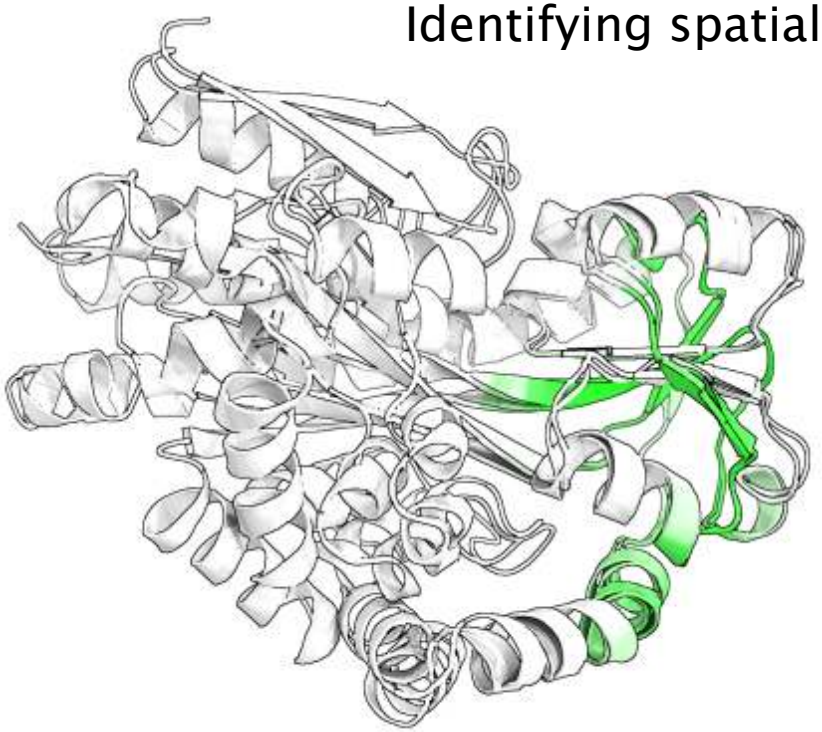
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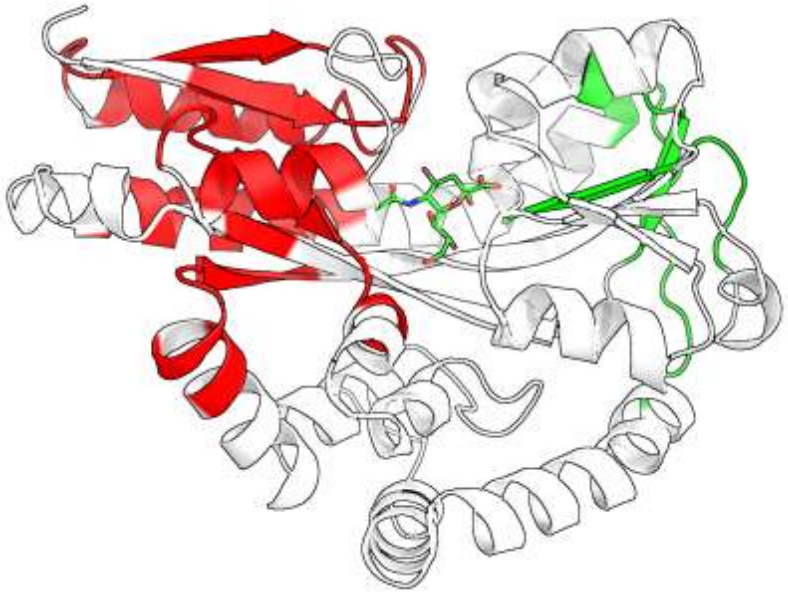
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Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states

Identifying spatially-conserved regions





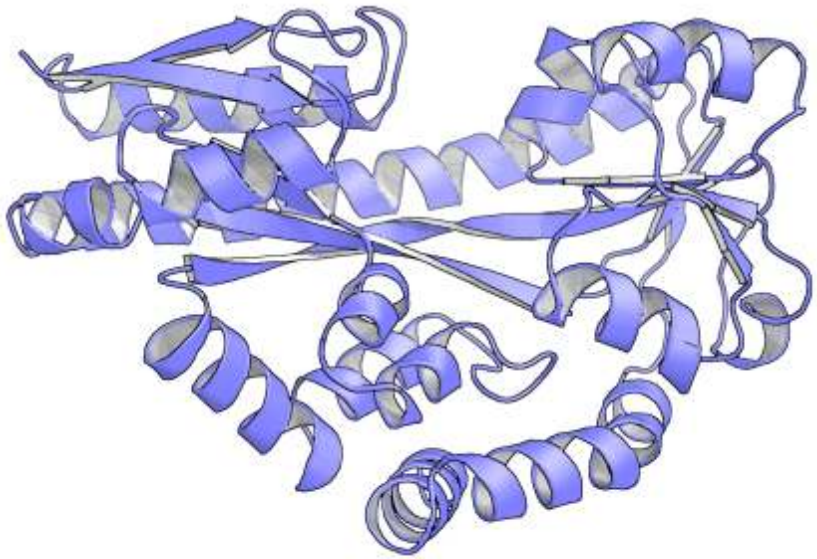
Coloured by conservation of coordinate frame (superposition quality)

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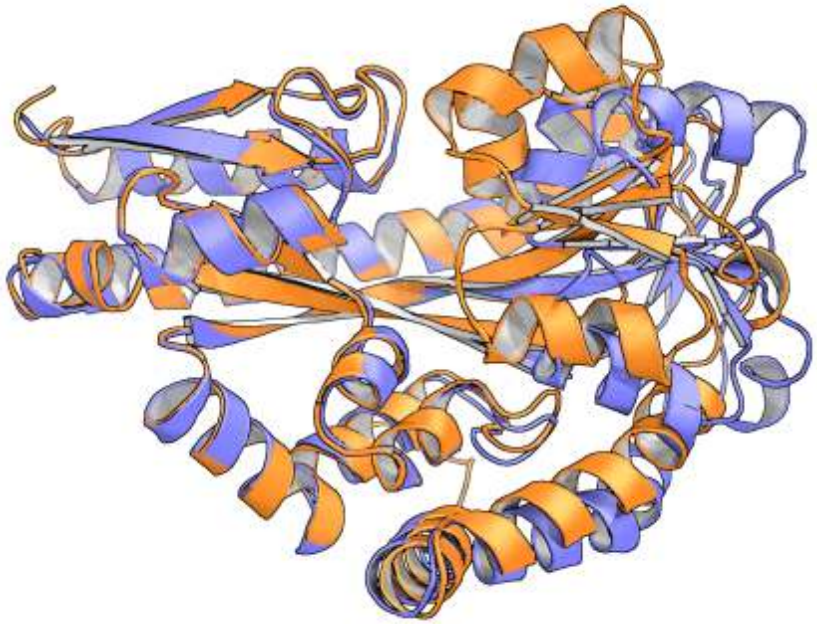
Sialic acid binding protein in different bound states



Describing domain motion – screw axis and angle

Example: Ligand-Induced Changes

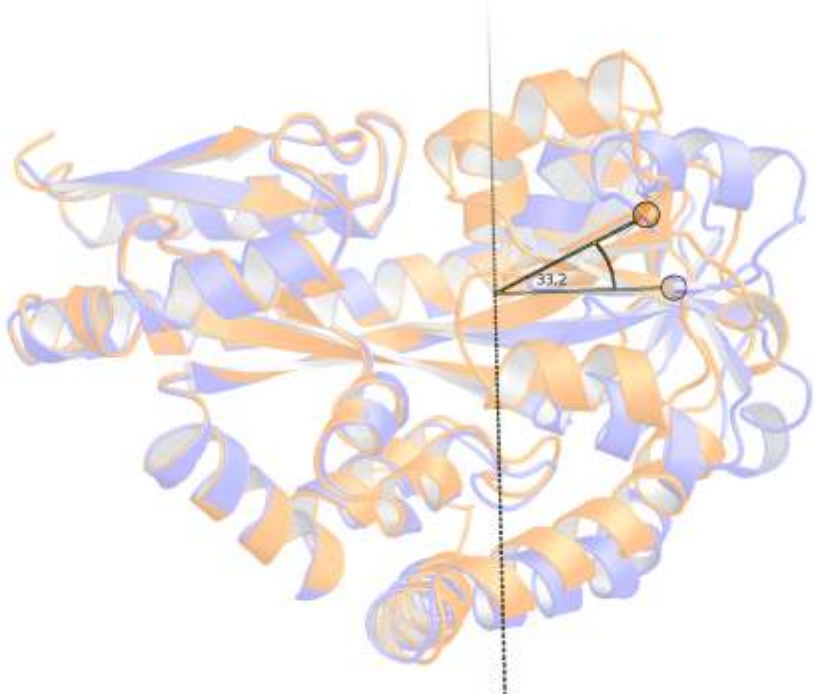
Sialic acid binding protein in different bound states



Describing domain motion – screw axis and angle

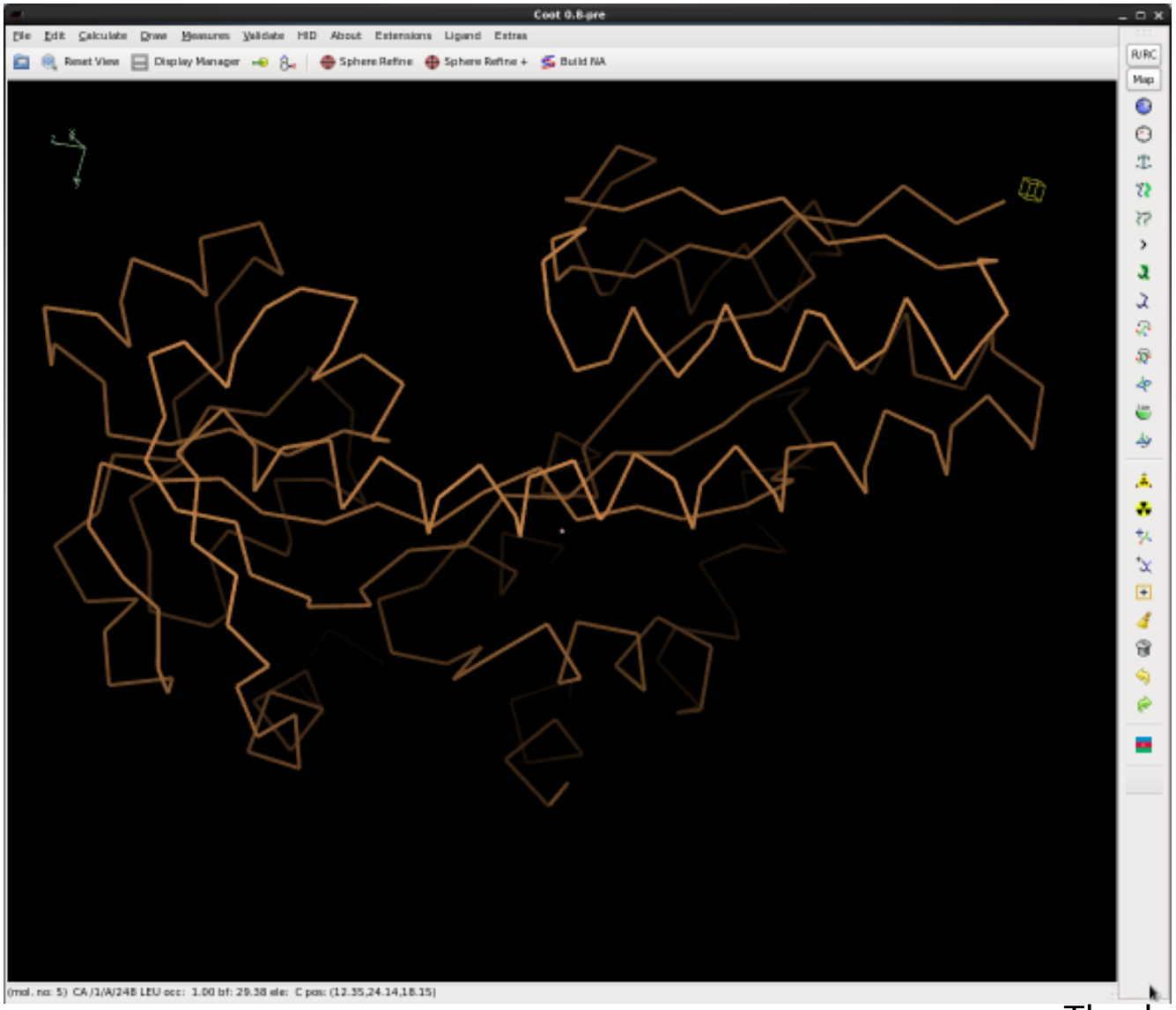
Example: Ligand-Induced Changes

Sialic acid binding protein in different bound states



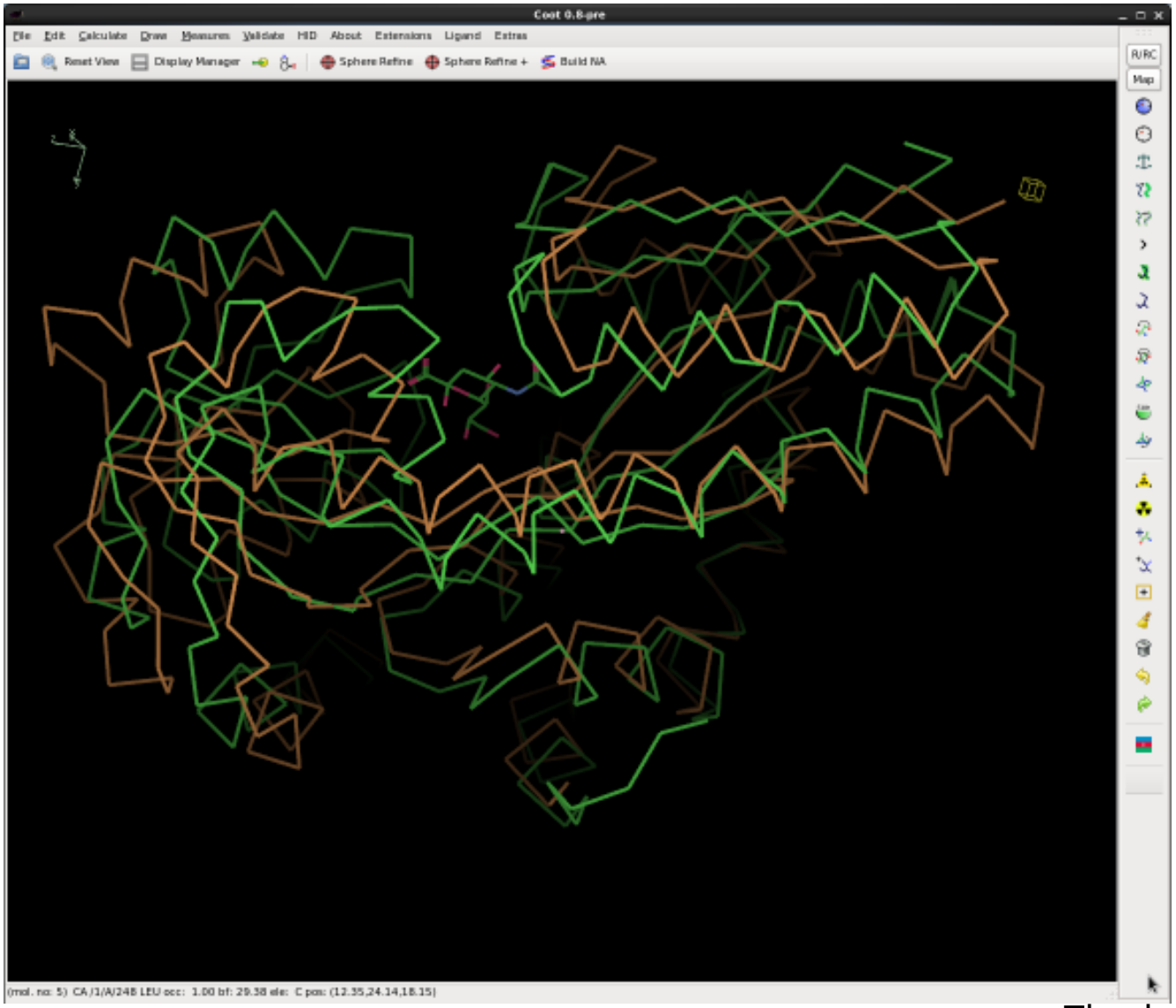
Describing domain motion – screw axis and angle

ProSMART Restraint Visualisation in Coot



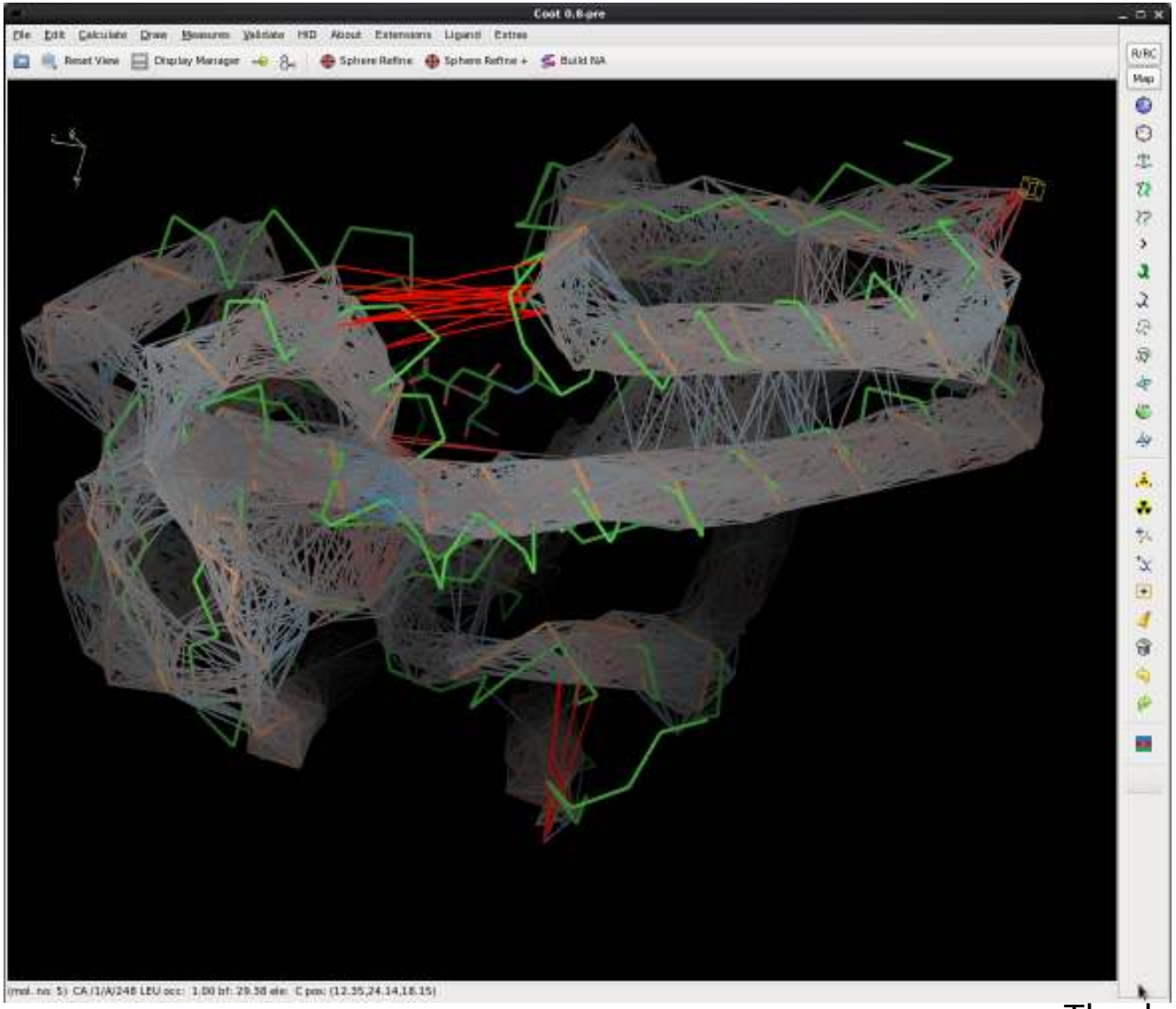
Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot



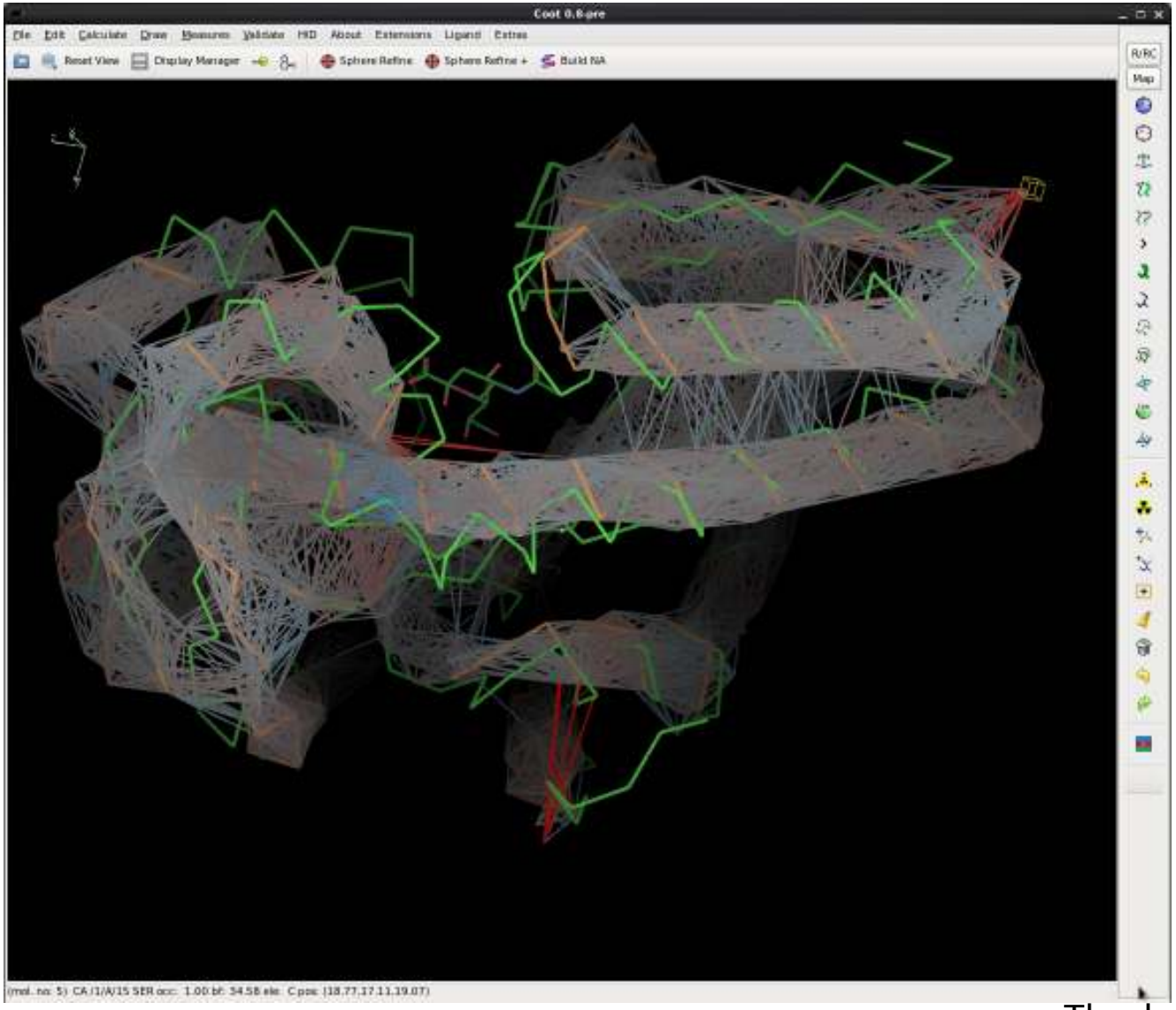
Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot



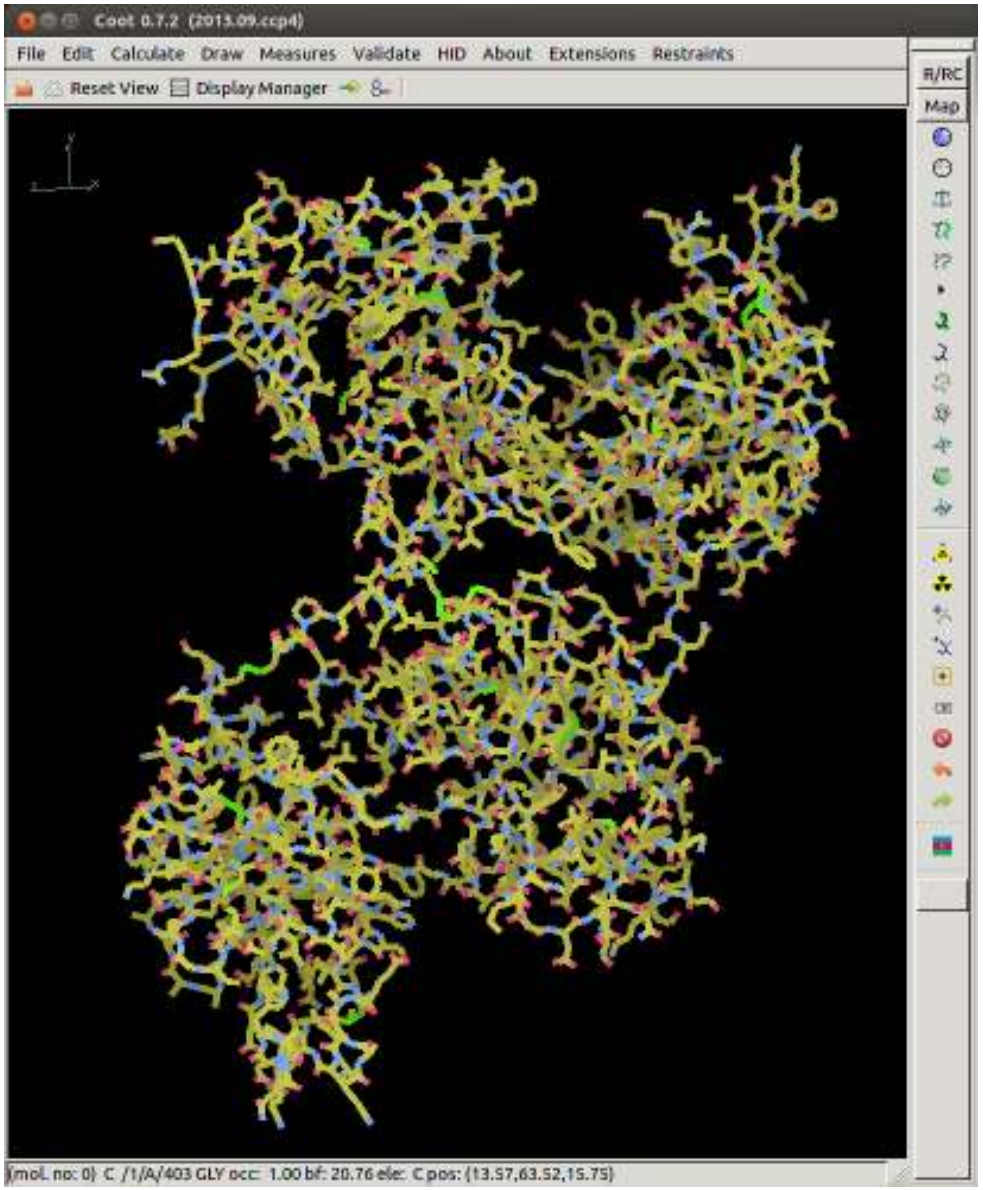
Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot



Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot



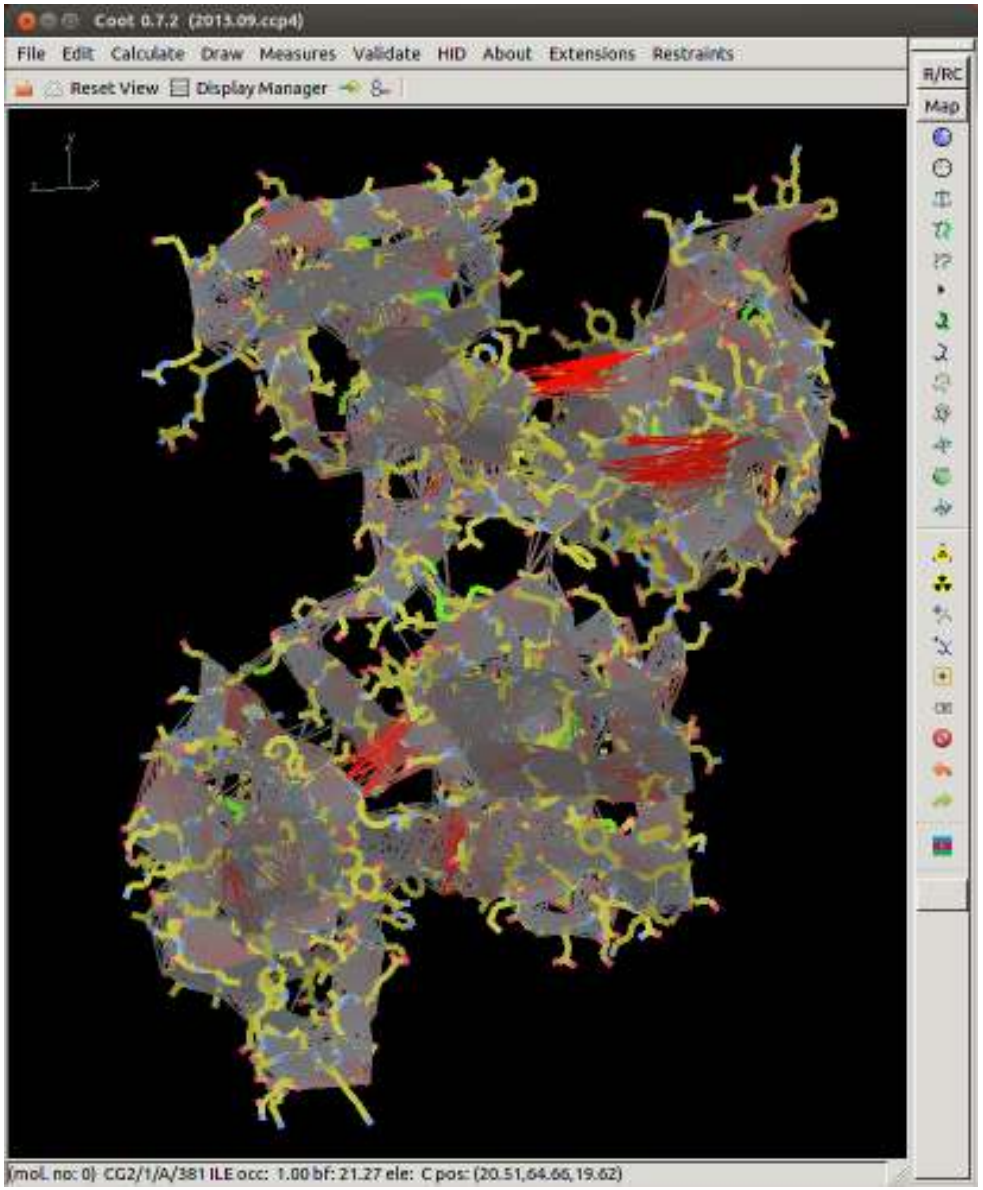
Ovotransferrin

1ryx (3.5Å)

Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot

Backbone Restraints



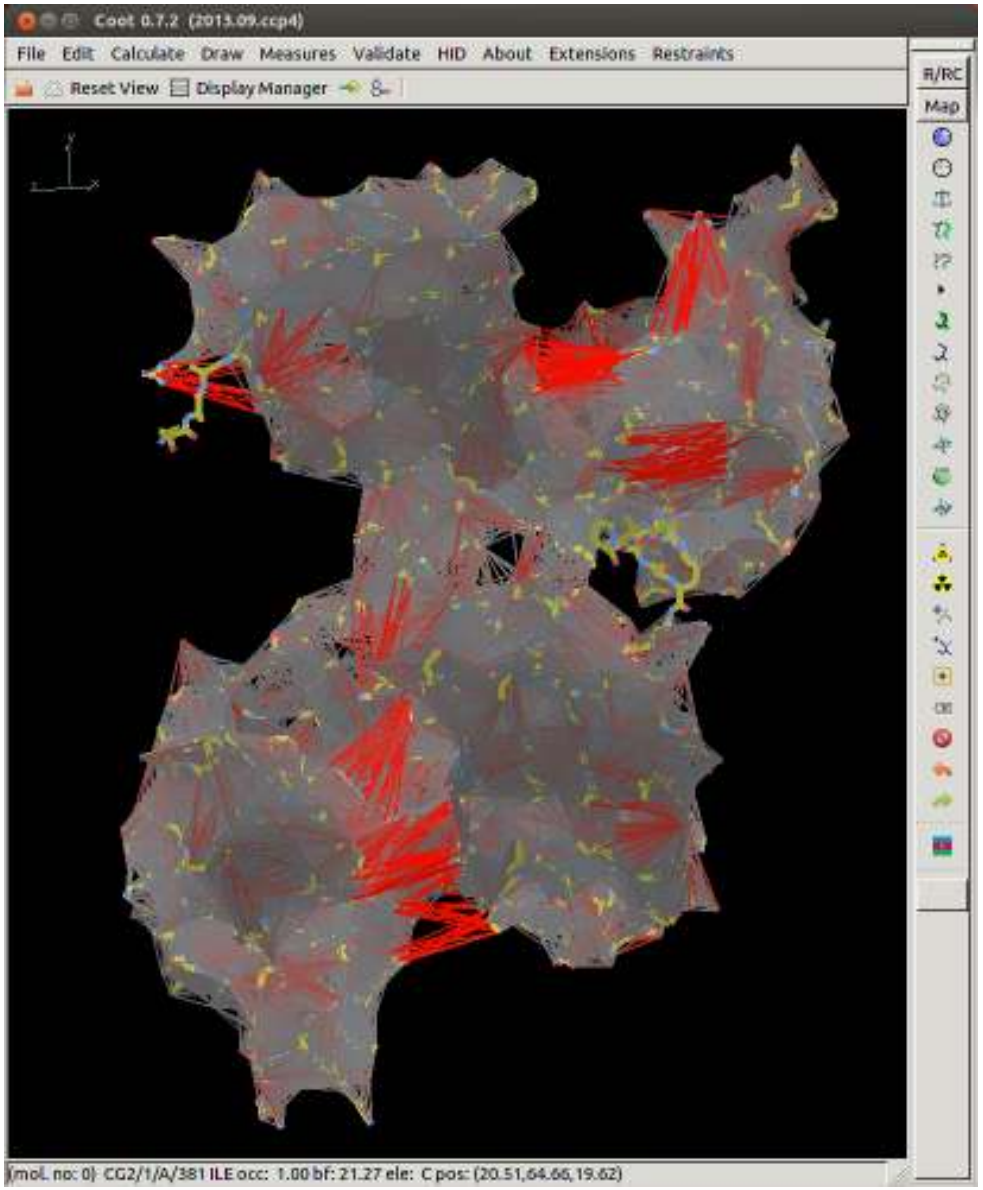
Ovotransferrin

1ryx (3.5Å)
restrained to
2d3i (2.15Å)

Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot

Backbone
& Side Chain
Restraints



Ovotransferrin

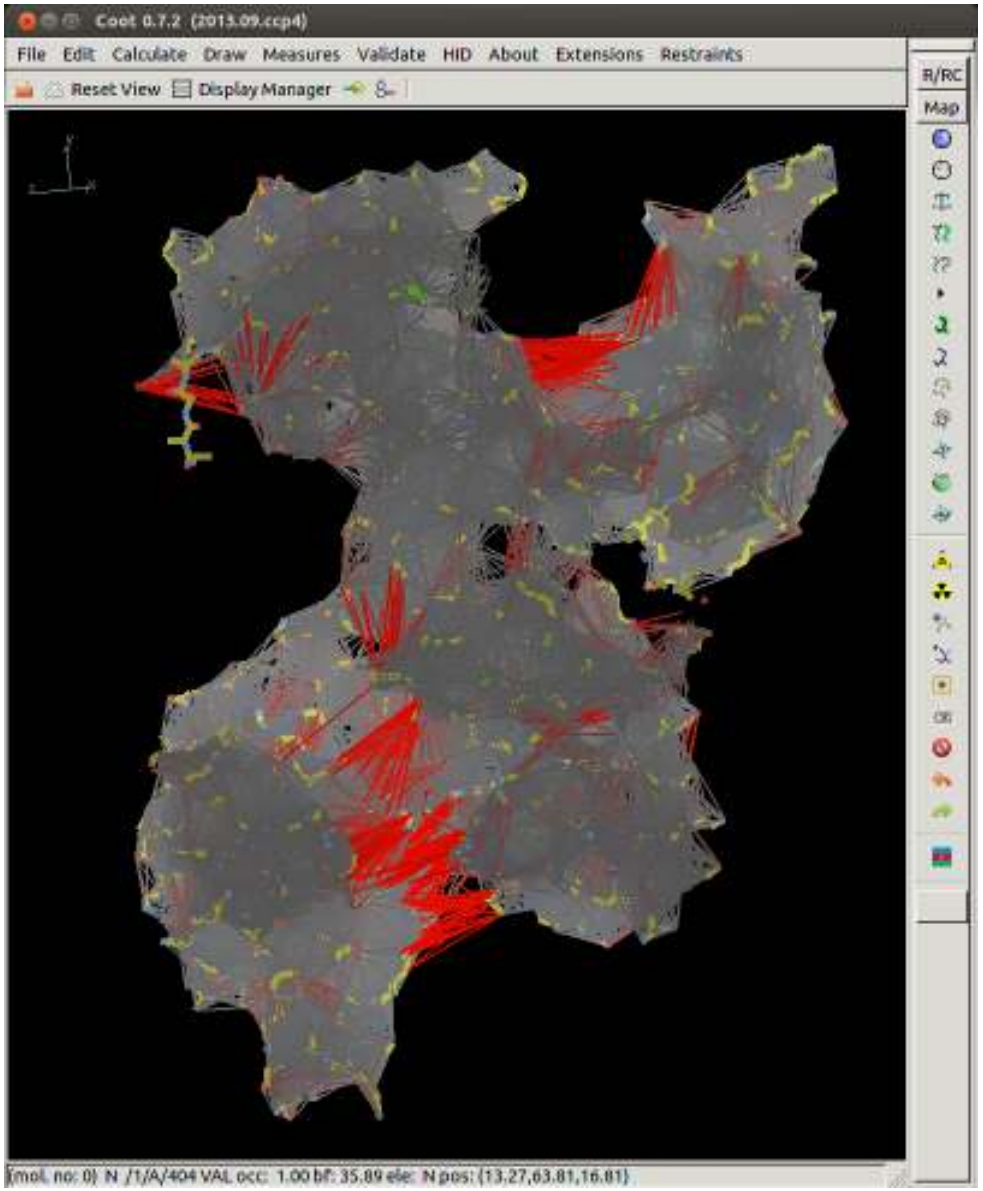
1ryx (3.5Å)
restrained to
2d3i (2.15Å)

Thanks to Paul Emsley

ProSMART Restraint Visualisation in Coot

Backbone
& Side Chain
Restraints

Regenerated after
re-refinement



Ovotransferrin

1ryx re-refined
restrained to
2d3i (2.15Å)

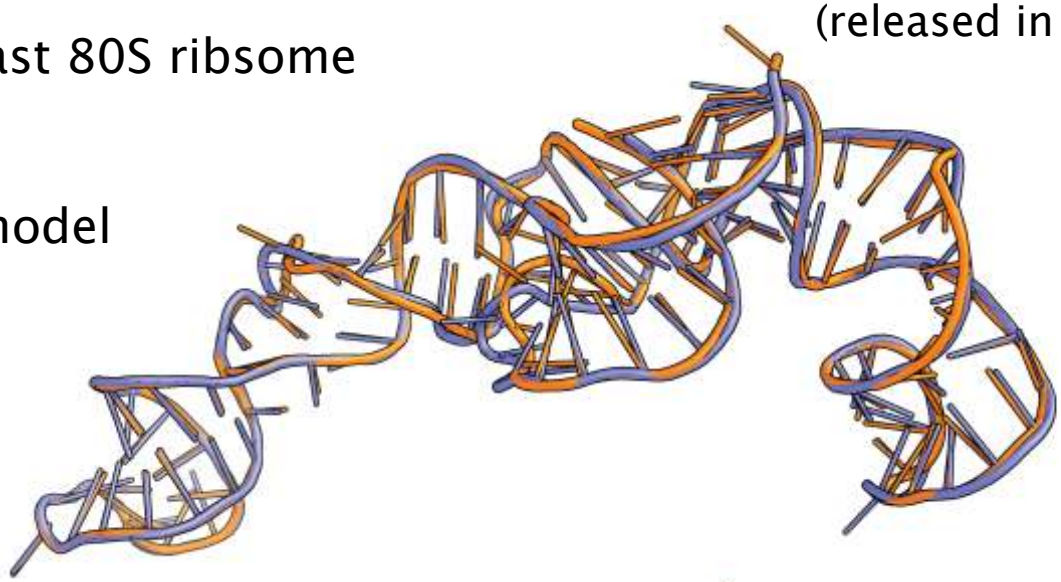
Thanks to Paul Emsley

Support for DNA/RNA – ProSMART

(released in CCP4 6.5)

5S rRNA from yeast 80S ribsome

Blue - EM model
Orange – X-ray model

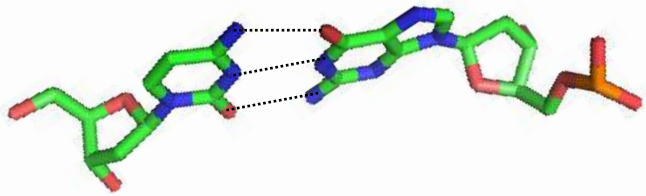


Support for DNA/RNA – LIBG

(released in CCP4 6.5)

LIBG (Fei Long) – for the generation of nucleic acid restraints

Base-pair restraints:

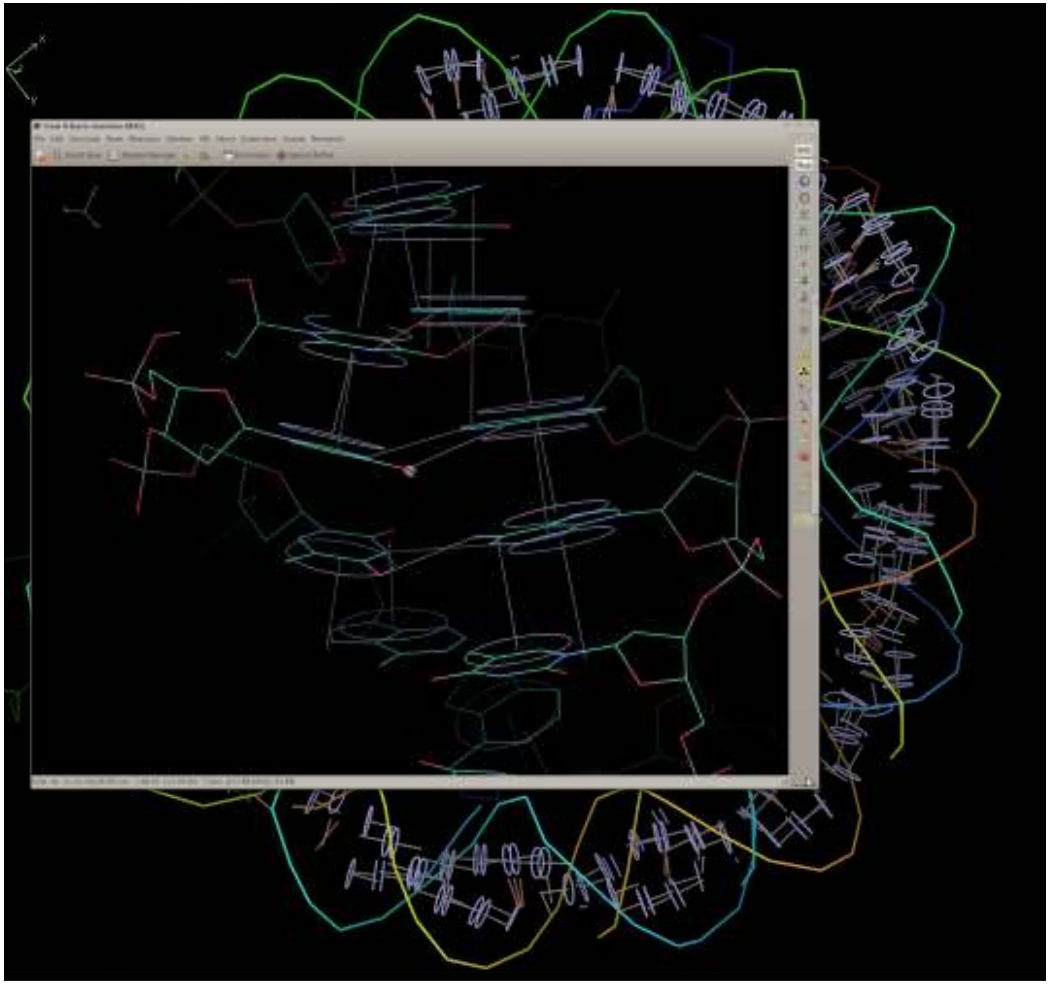


Support for DNA/RNA – LIBG

(released in CCP4 6.5)

LIBG (Fei Long) – for the generation of nucleic acid restraints

Base-stacking restraints:
(parallel plane restraints)



Thanks to Fei Long, Paul Emsley

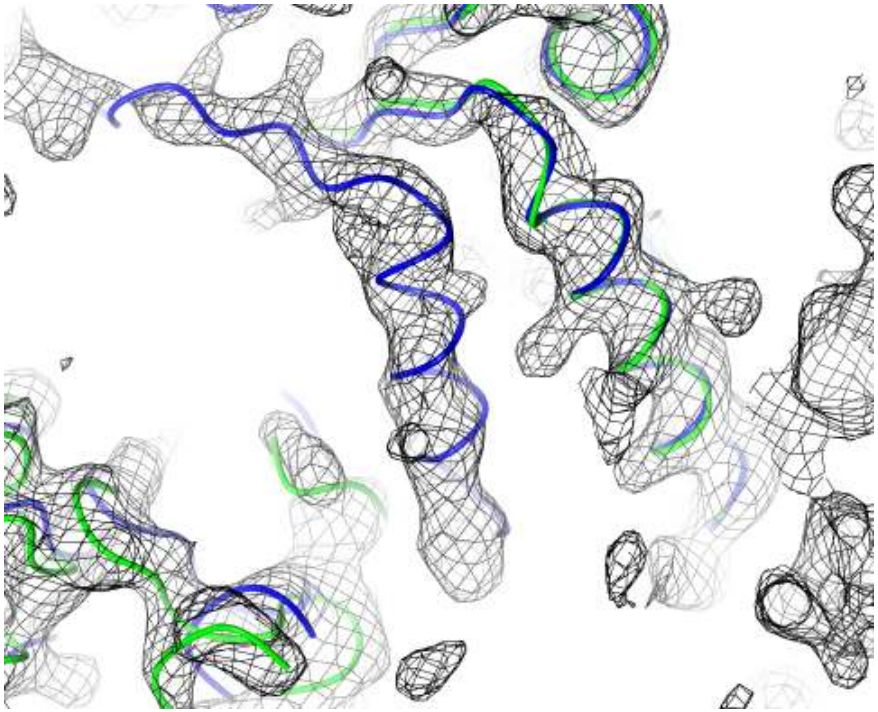
REFMAC Anisotropic Map Sharpening

Idea – remove an overall B value

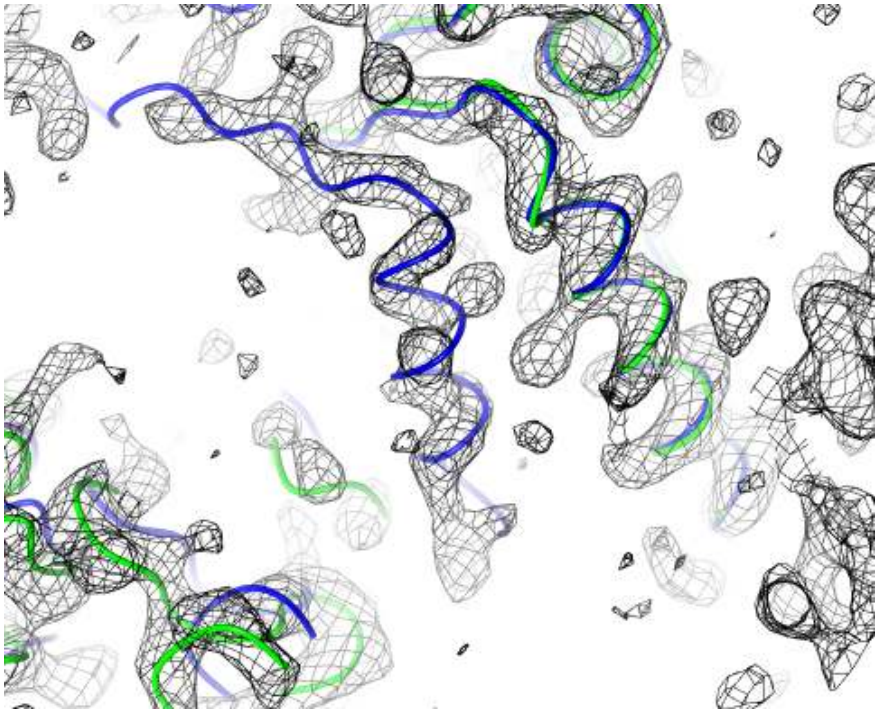
REFMAC Anisotropic Map Sharpening

Idea – remove an overall B value

Original Map



Sharpened map from REFMAC



Green: original structure 2r6c (4.0Å) – helix unmodelled

Blue: homologous structure 2r6a (2.9Å)

Thanks to Garib Murshudov

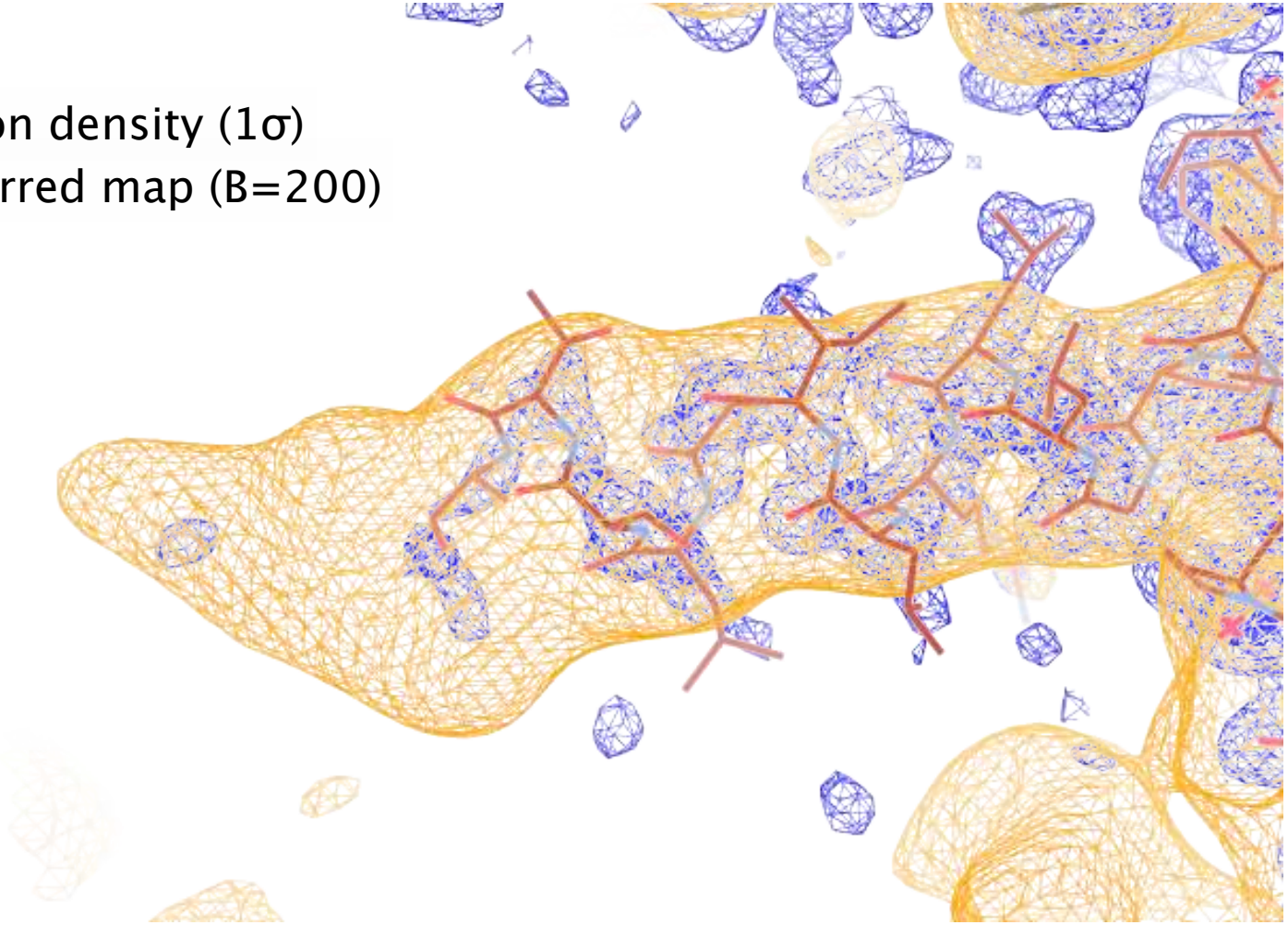
Map Blurring

Idea – apply an overall B value

Map Blurring

Idea – apply an overall B value

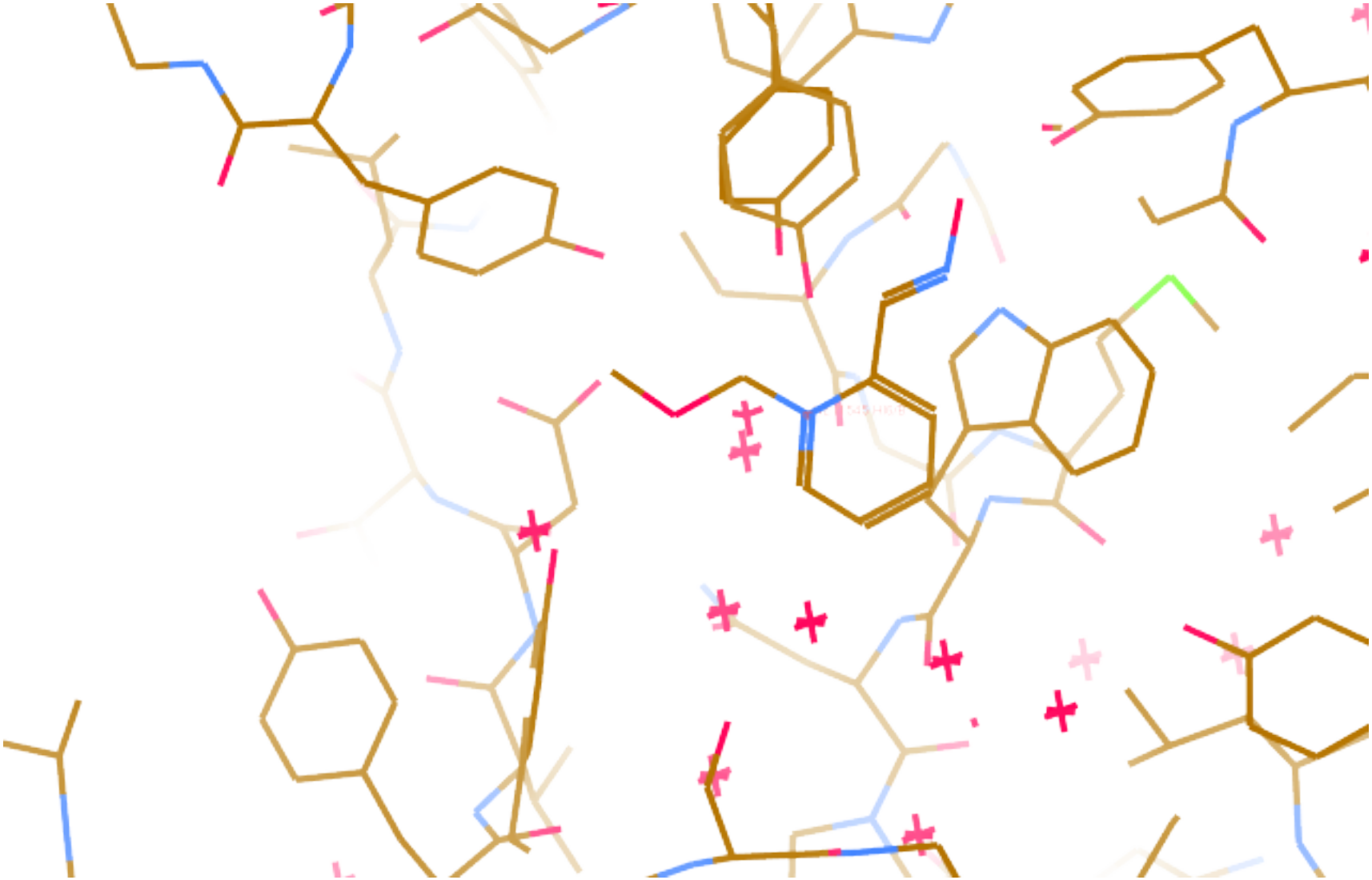
Blue – electron density (1σ)
Orange – blurred map (B=200)



1s3e

Thanks to Andrea Thorn

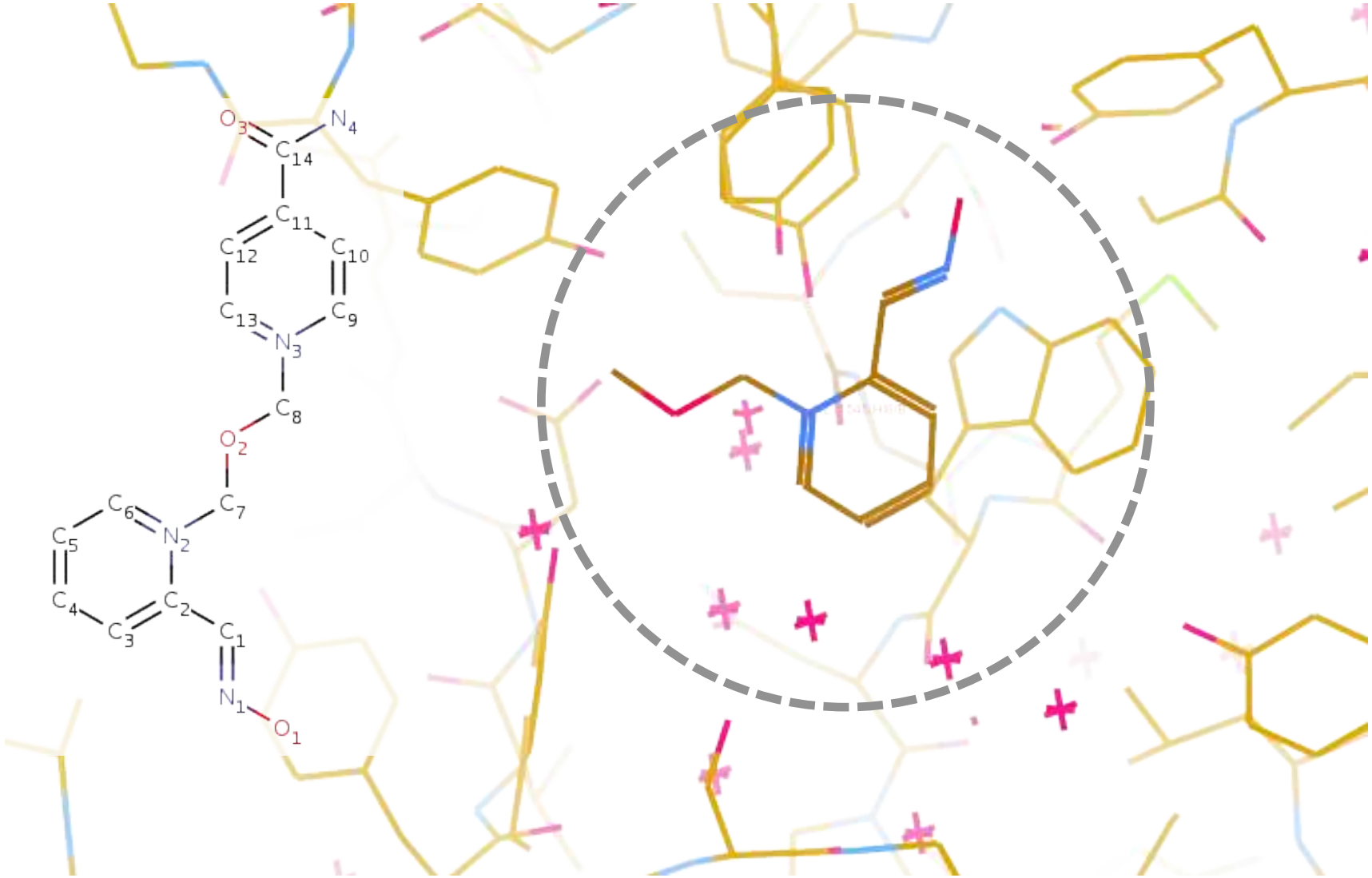
Map Blurring



2whq

Thanks to Andrea Thorn

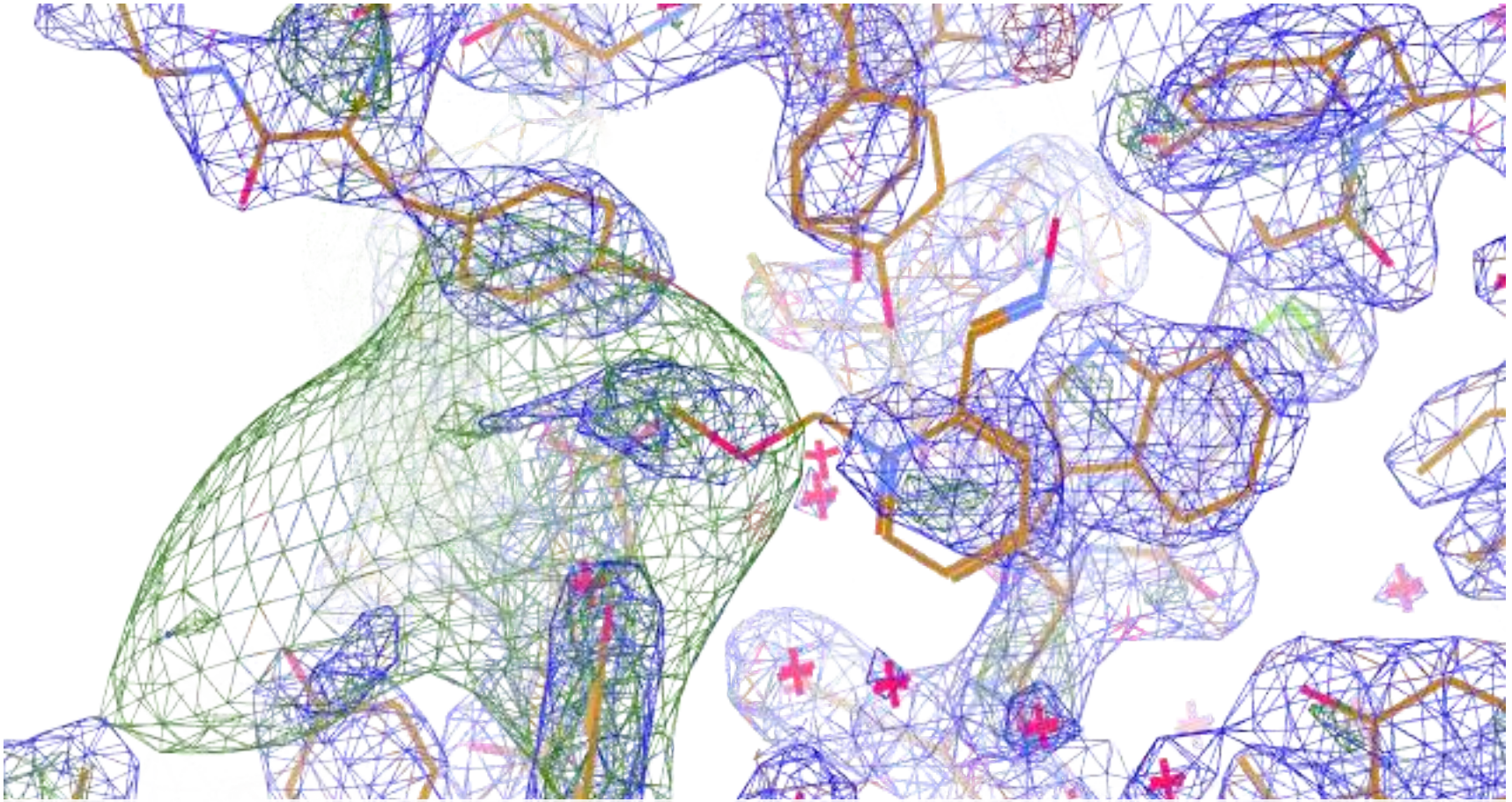
Map Blurring



2whq

Thanks to Andrea Thorn

Map Blurring



Electron density at 1.7σ; Residual density at 3.5σ
Blurred residual map, B = 200

2whq

Thanks to Andrea Thorn

Summary

Tools to help in low-resolution model building and refinement:

REFMAC: Jelly body refinement, map sharpening

ProSMART: External restraints, comparative analysis

LIBG: Nucleic acid restraints

COOT: Visualisation & manipulation of restraints, map blurring…
…also morphing, backrub rotamer fitting…

Tools are applicable to cryo-EM as well as MX

Acknowledgements

Contact: **nicholls@mrc-lmb.cam.ac.uk**

Computational Crystallography Group:

Garib Murshudov
Paul Emsley
Fei Long
Andrea Thorn
Oleg Kovalevskiy
Michal Tykac

Many Thanks:

Marcus Fischer
Stuart McNicholas
CCP4 core team
Colleagues from MRC-LMB, YSBL and CCP4

Software available for Mac/Linux/Windows via CCP4

Computational Crystallography Group website:
www2.mrc-lmb.cam.ac.uk/groups/murshudov/

ProSMART tutorials: **www2.mrc-lmb.cam.ac.uk/groups/murshudov/content/tutorials/prosmart_tutorial/**

REFMAC tutorials: **www2.mrc-lmb.cam.ac.uk/groups/murshudov/content/tutorials/refmac_tutorial/**

ProSMART:

Nicholls *et al.* (*2014*) Conformation-Independent Structural Comparison of Macromolecules with ProSMART. *Acta Cryst.* D70, 2487-99.
Nicholls *et al.* (2012) An Overview of ProSMART. *CCP4 Newsletter on Protein Crystallography*.
Nicholls (2011) Conformation-Independent Comparison of Macromolecules. University of York.

Low-resolution refinement with REFMAC5 and ProSMART:

Nicholls *et al.* (2013) Recent Advances in Low Resolution Refinement Tools in REFMAC5. Adv. Methods for Bio. Xtallography, 231–58.
Nicholls *et al.* (2012) Low Resolution Refinement Tools in REFMAC5. *Acta Cryst.* D68, 404-17.

Refmac:

Murshudov *et al.* (2011) REFMAC5 for the refinement of macromolecular crystal structures. *Acta Cryst.* D67, 355-67.

Tools for cryo-EM fitting:

Brown *et al.* (2014) Tools for macromolecular model building and refinement into electron cryo-microscopy reconstructions. *Acta Cryst.* D70.







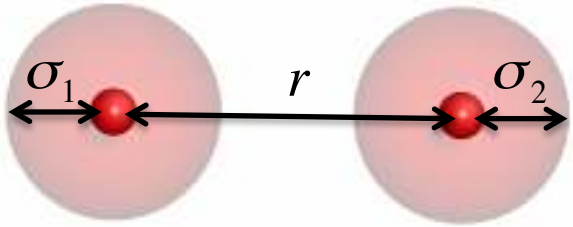


Extra Slides

External Restraints

Restraint Parameter Estimation:

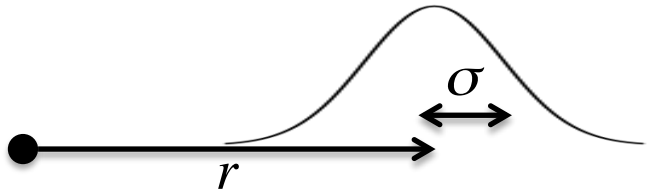
Atoms in homologous structure:



σ_i estimated as by Murshudov & Dodson (CCP4 Newsletter 1997)

Dependencies:

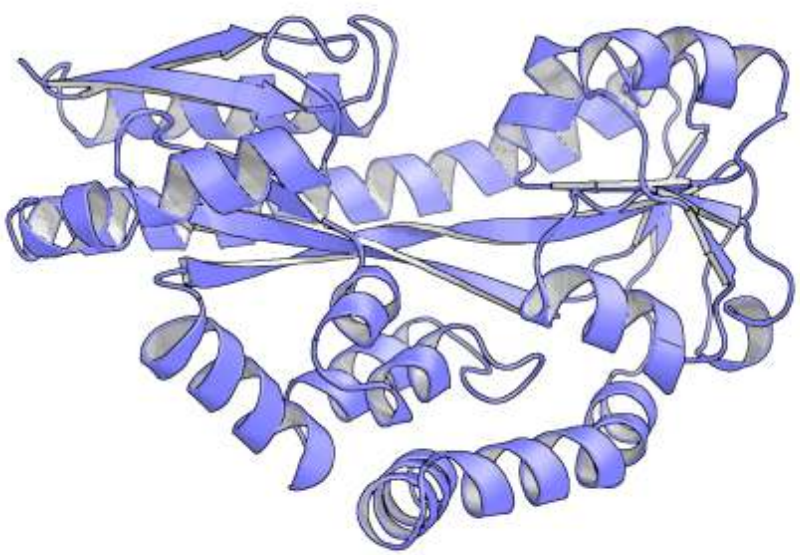
- B-value
- Resolution
- Model/data completeness
- Data quality



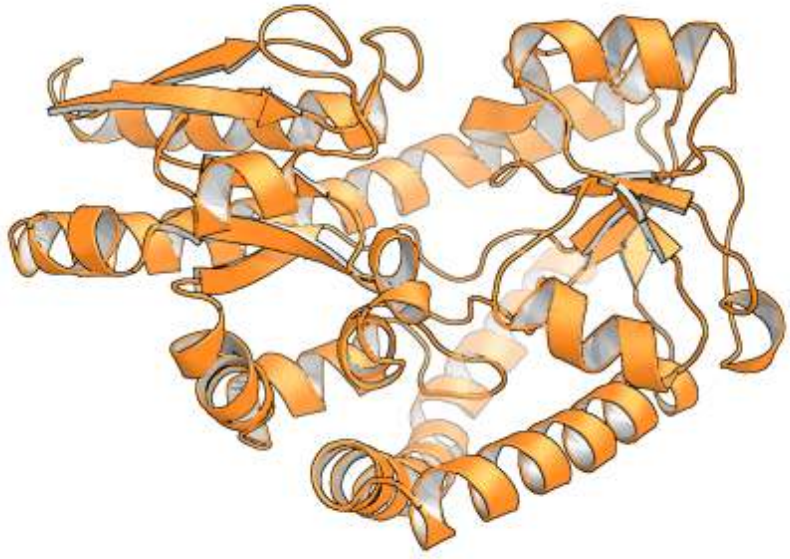
Plus a modification that increases robustness to outliers (use of Geman-McClure function)

Example: Comparing Distant Structures

Comparison of open forms of binding proteins – **14% sequence identity**



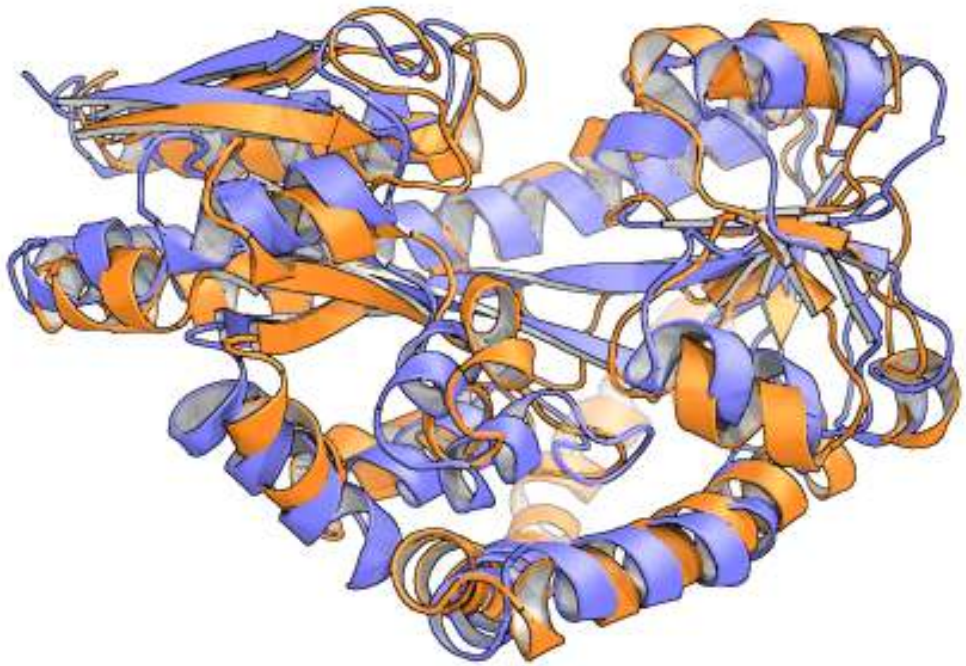
2cex – Sialic acid binding protein



2hzk – sodium-alpha-keto binding protein

Example: Comparing Distant Structures

Comparison of open forms of binding proteins – **14% sequence identity**

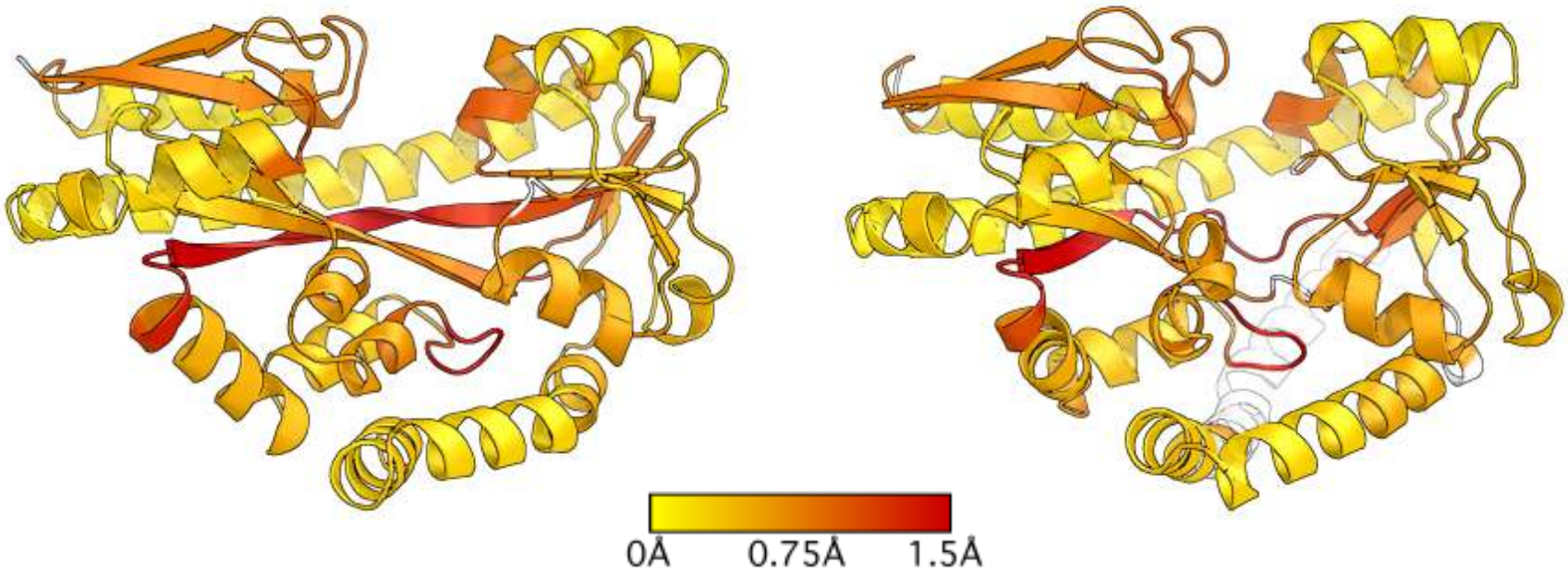


2cex and 2hzk, superposed

Example: Comparing Distant Structures

Comparison of open forms of binding proteins – **14% sequence identity**

Coloured by local backbone RMSD



2cex – Sialic acid binding protein

2hzk – sodium-alpha-keto binding protein

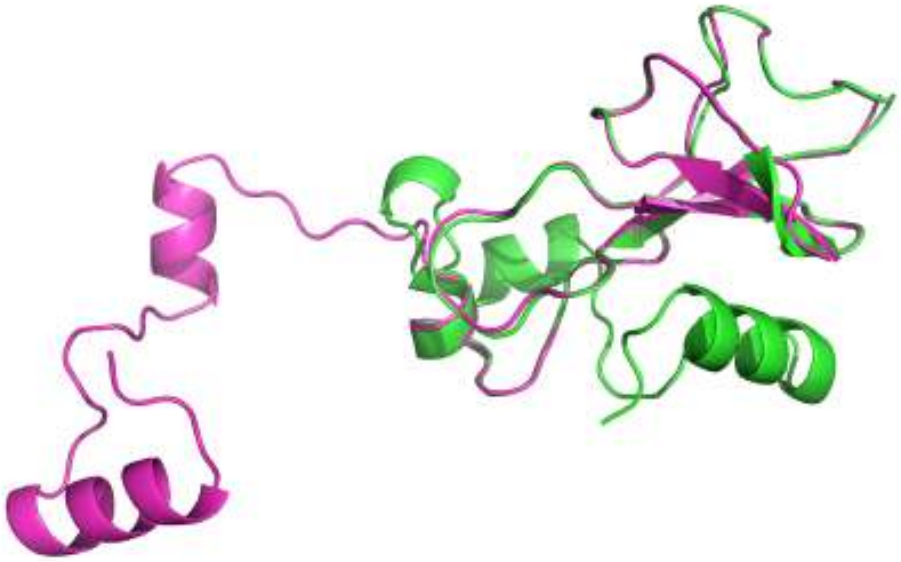
Example

Example: structural analysis of domain swaps (Barnase – 2za4, 1yvs)



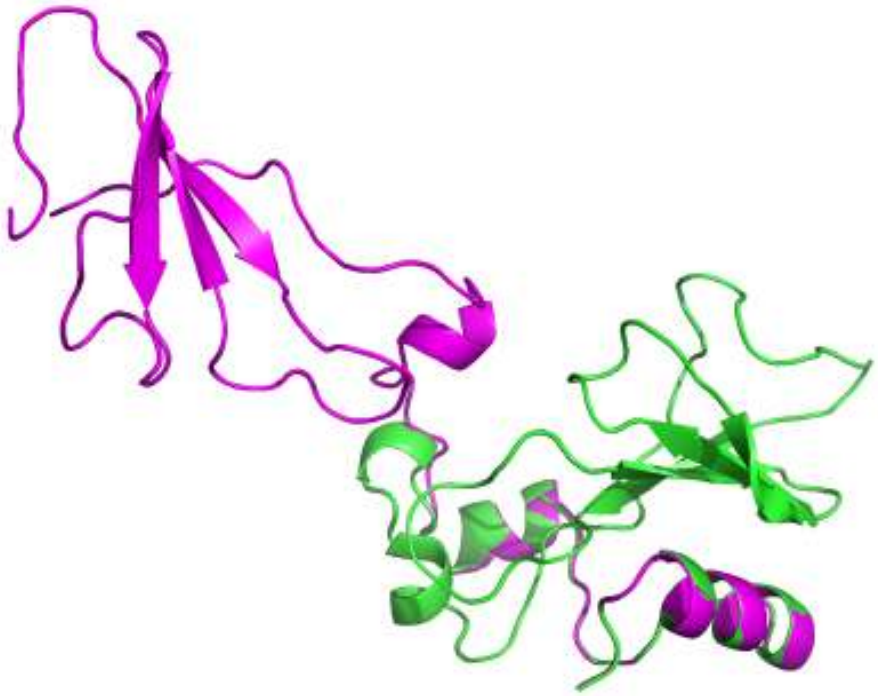
Example

Example: structural analysis of domain swaps (Barnase – 2za4, 1yvs)



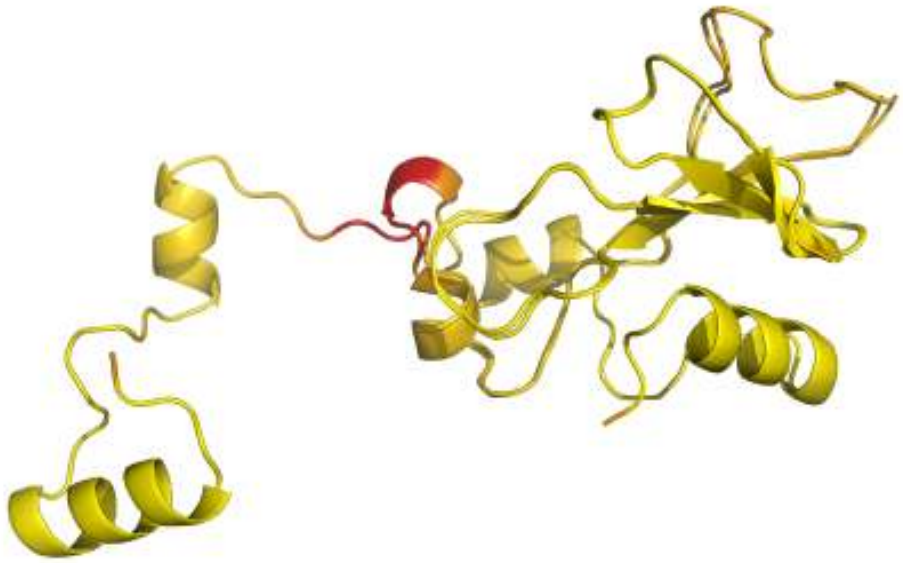
Example

Example: structural analysis of domain swaps (Barnase – 2za4, 1yvs)



Example

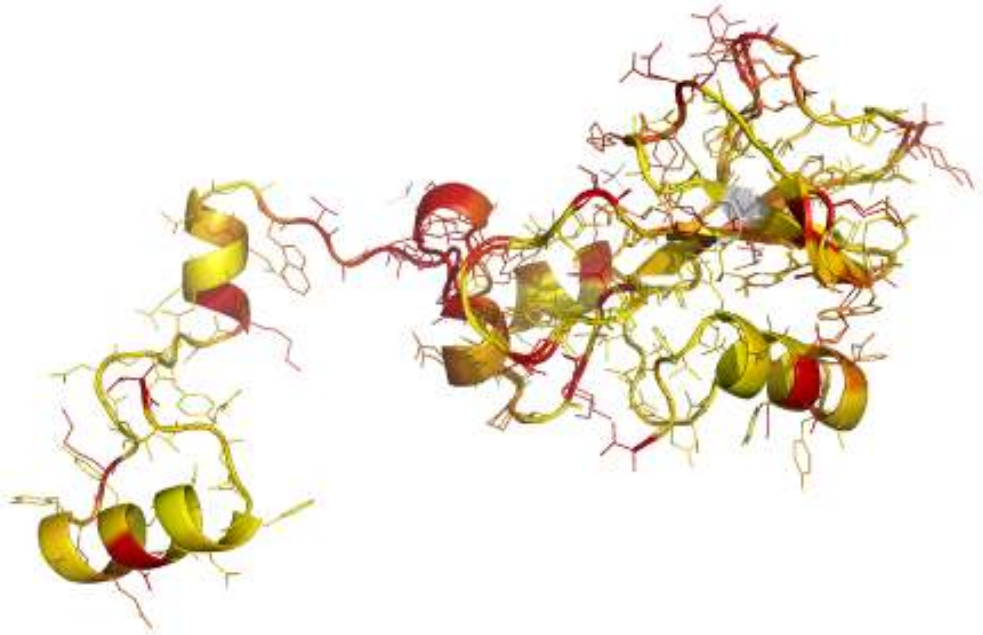
Example: structural analysis of domain swaps (Barnase – 2za4, 1yvs)



Coloured by local backbone RMSD

Example

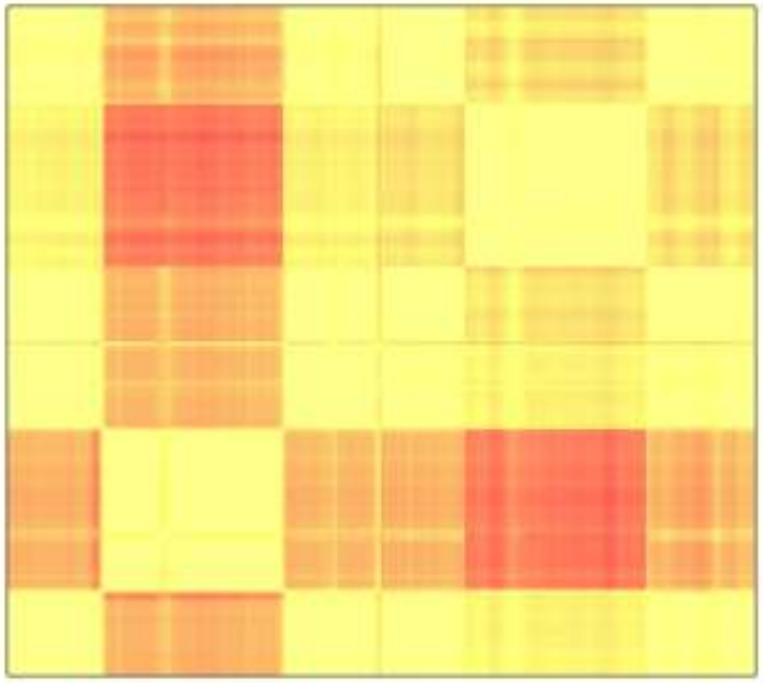
Example: structural analysis of domain swaps (Barnase – 2za4, 1yvs)



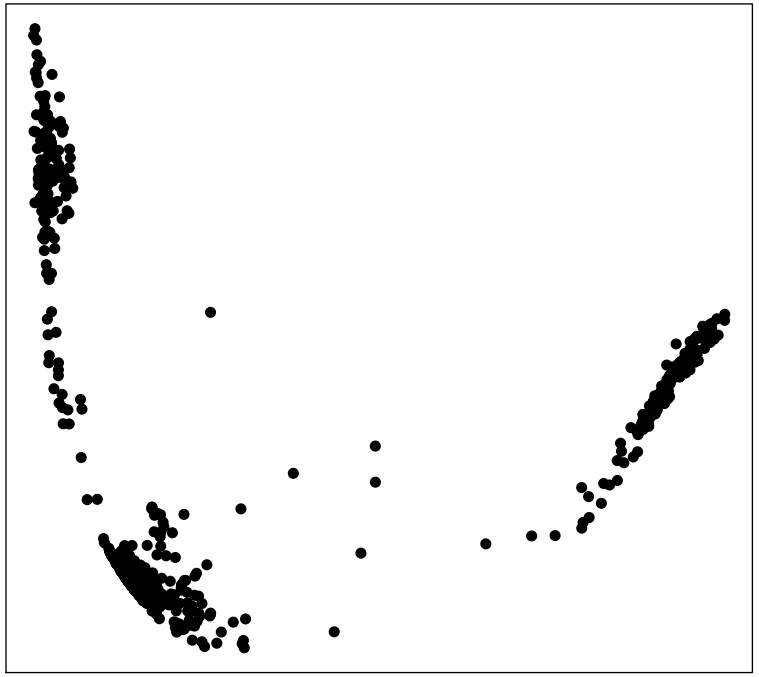
Coloured by local side chain conservation

Identifying Conserved Regions

Cluster aligned fragment-pairs by comparing their coordinate frames



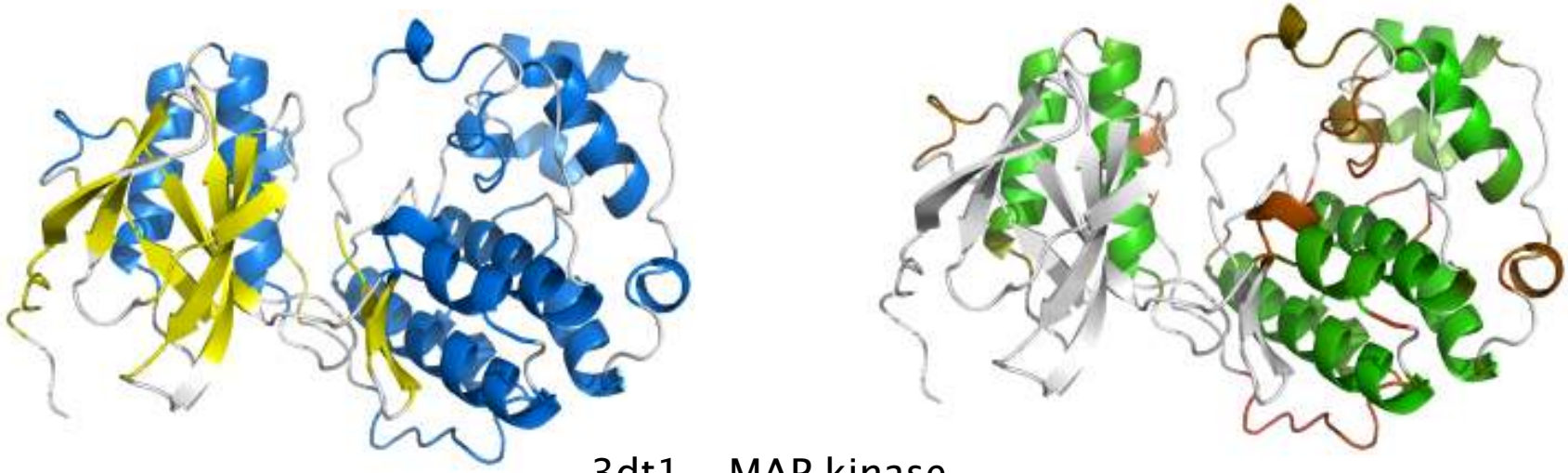
yellow – similar
red – dissimilar



Motif Identification and Scoring

Identifying an ideal α-helix
and a representative β-strand

Similarity to the ideal helix



3dt1 - MAP kinase

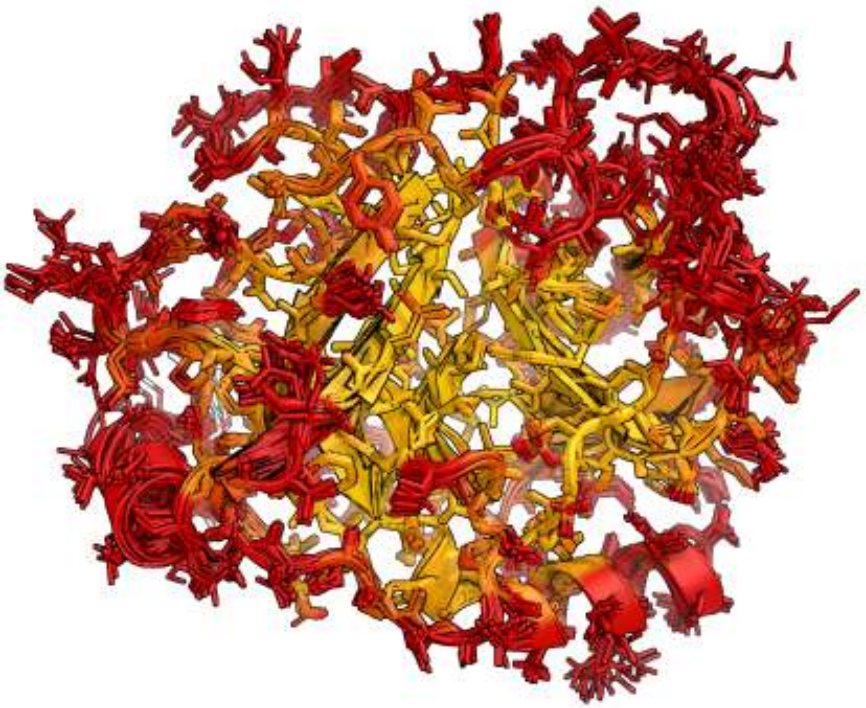
This is not equivalent to traditional secondary structure identification.

May be used to generate external restraints for use in refinement.

Multi-Chain Comparison

Comparison of 19 models of trypsin

Same sequence, different space group, 0.8–2.0Å

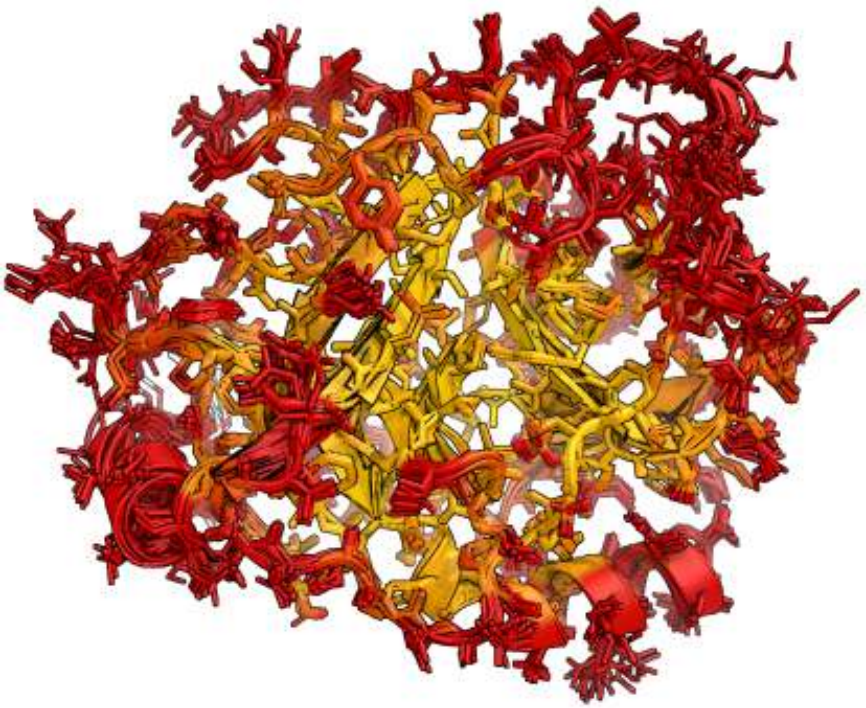


Max atomic deviation

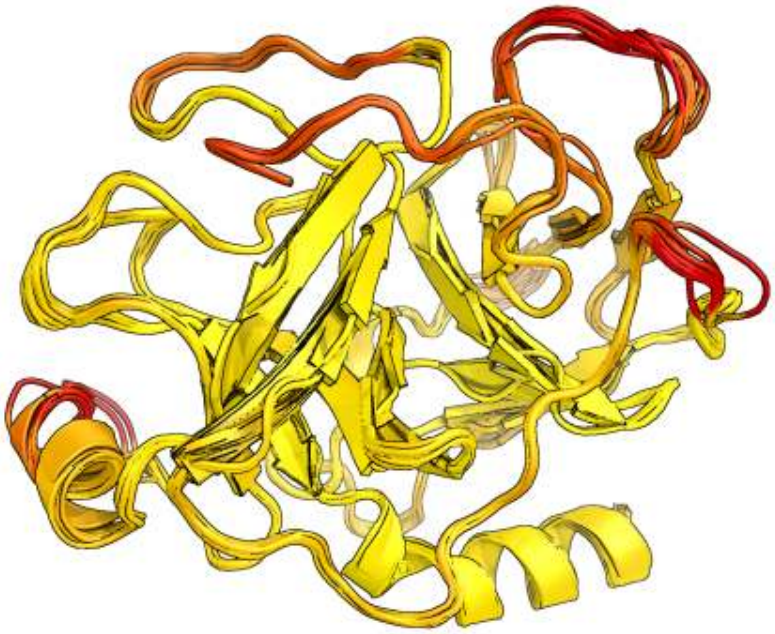
Multi-Chain Comparison

Comparison of 19 models of trypsin

Same sequence, different space group, 0.8–2.0Å



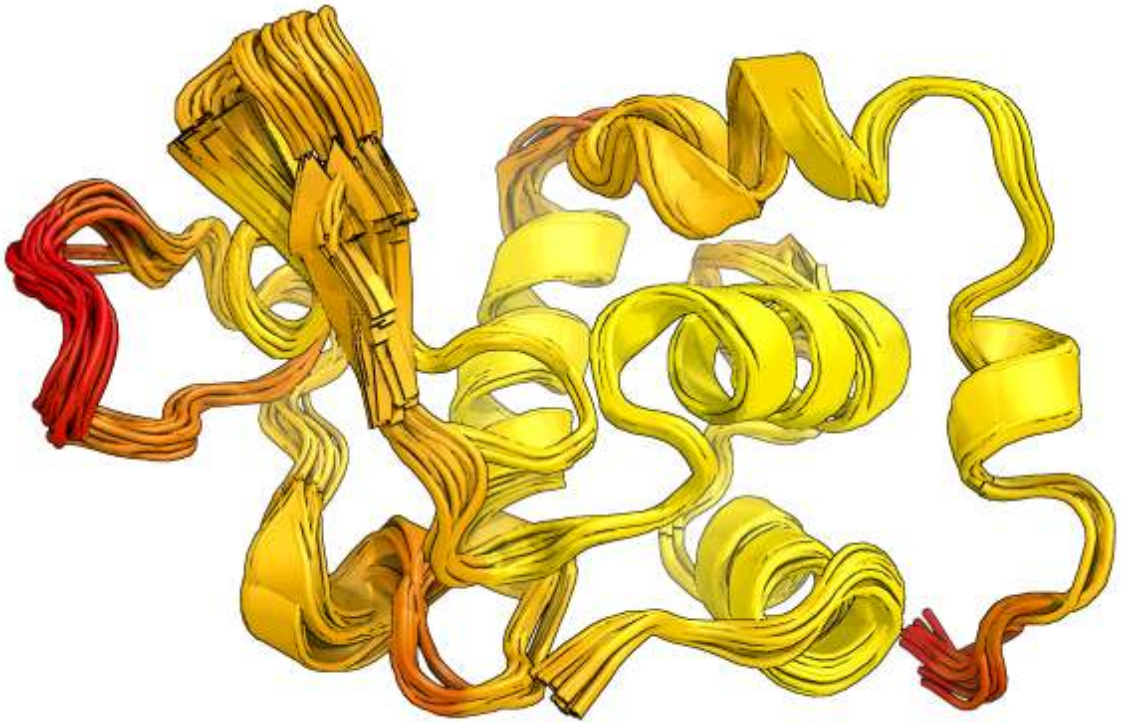
Max atomic deviation



Max local backbone RMSD

Multidisciplinary Usage

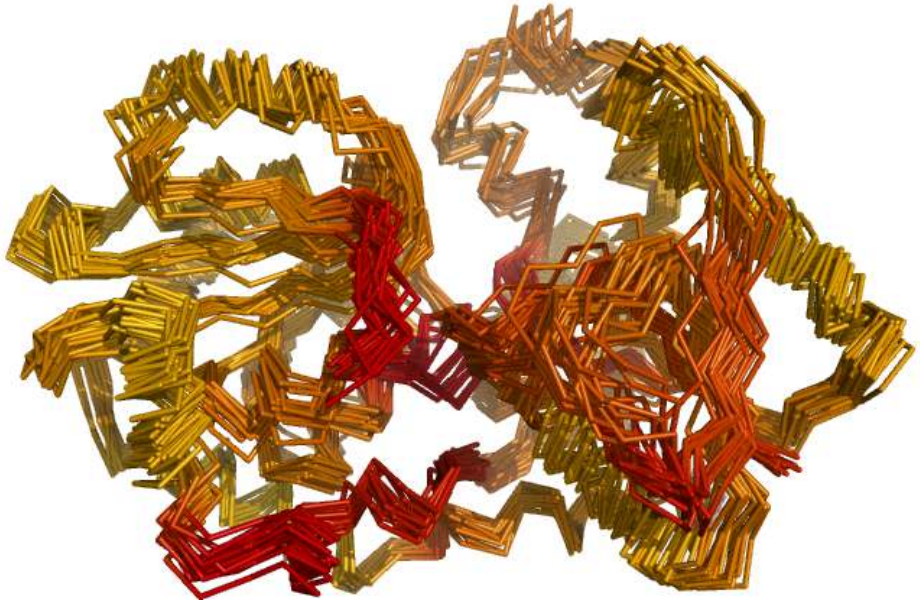
NMR solution ensemble of hen egg white lysozyme (50 models)



Max local backbone RMSD

Multidisciplinary Usage

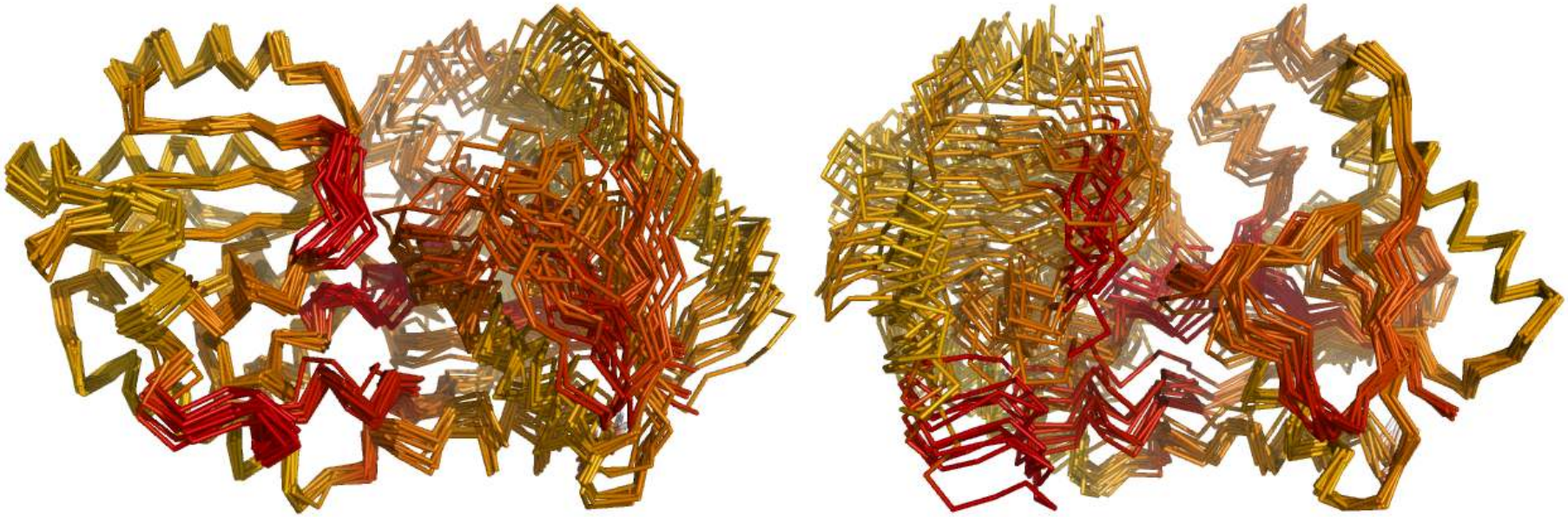
Ensemble from a 55ns MD simulation of WaaG



Images thanks to Jens Landström

Multidisciplinary Usage

Ensemble from a 55ns MD simulation of WaaG



Images thanks to Jens Landström

Anisotropic Regularised Map Sharpening

Map sharpening attempts to remove overall B-value.

Can be considered as an inverse de-blurring problem:

$$\int k(x,y)\rho_0(y)\,dy = \rho(x)$$

$k(x,y)$ is unknown

Problem is ill-posed

Assume blurring is homogeneous: $k(x,y) = k(x-y,0)$

Use regularisation: $\| \int k(x,y)\rho_0(y)\,dy - \rho(x) \|^2 + f(\rho_0(x)) \rightarrow \min$

Solution: $F_{0hkl} = \frac{K(s)}{K(s)^2 + \alpha(s)} F_{hkl}$ $\qquad K(s) = \exp\left(\frac{-s^T U_{deblur} s}{4}\right)$