



UNIVERSITY OF
LIVERPOOL

Ronan Keegan CCP₄ Group

MR model selection, preparation and assessing the solution

CCP₄-DLS-Workshop 2020

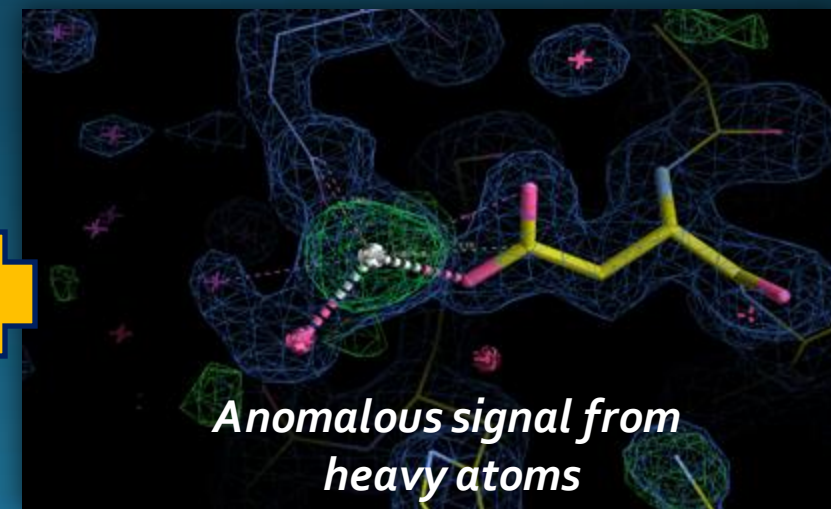
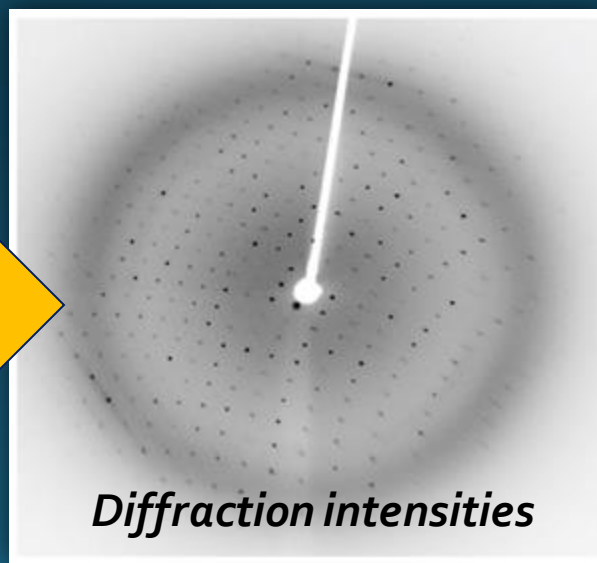
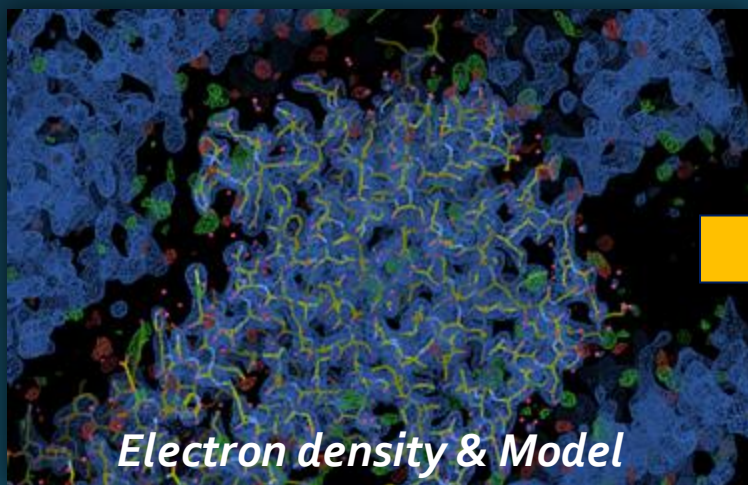
Introduction: *Basics of Molecular Replacement*

$$\rho(x, y, z) = \frac{1}{V} \sum_{hkl} |F_o(hkl)| \cos[-2\pi(hx + ky + lz) + \varphi_{hkl}]$$

Electron density
at (x,y,z)

Amplitudes

Phases



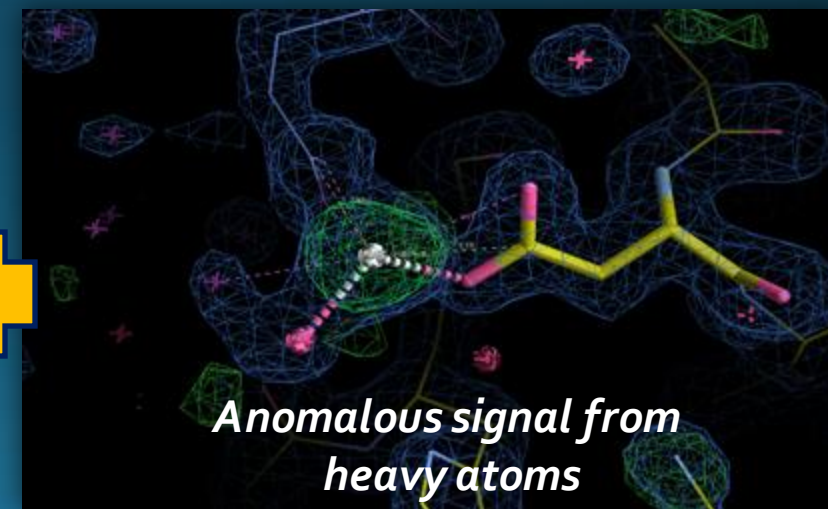
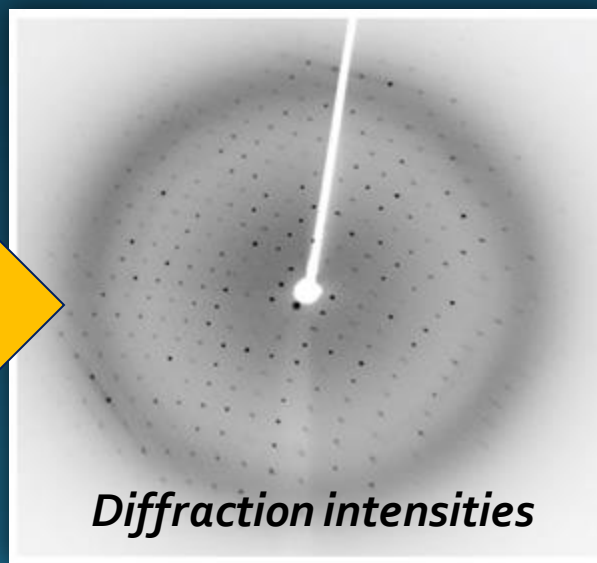
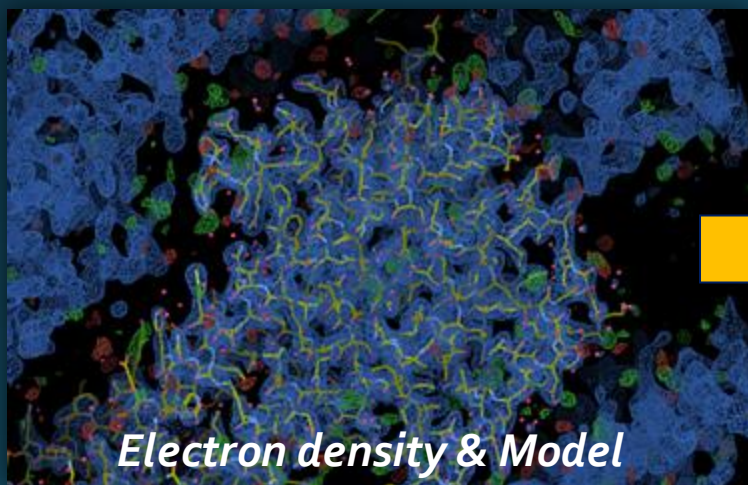
Introduction: *Basics of Molecular Replacement*

$$\rho(x, y, z) = \frac{1}{V} \sum_{hkl} |F_o(hkl)| \cos[-2\pi(hx + ky + lz) + \varphi_{hkl}]$$

Electron density
at (x,y,z)

Amplitudes

Phases

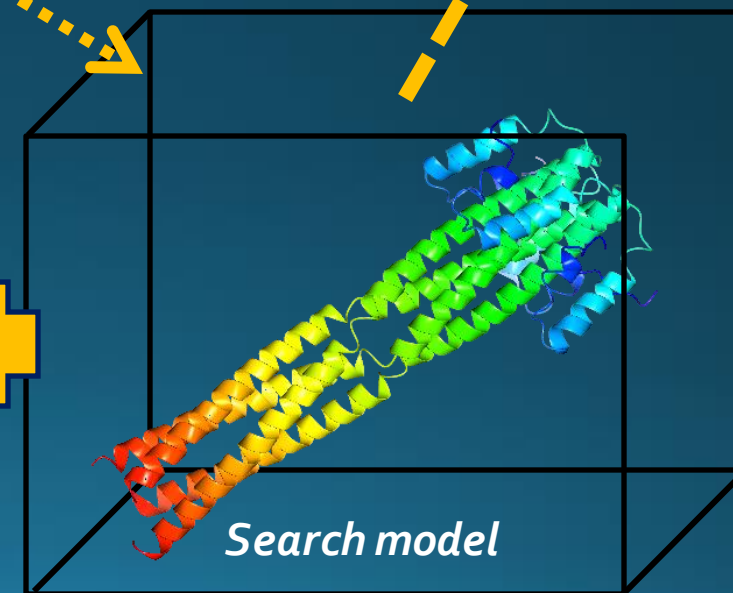
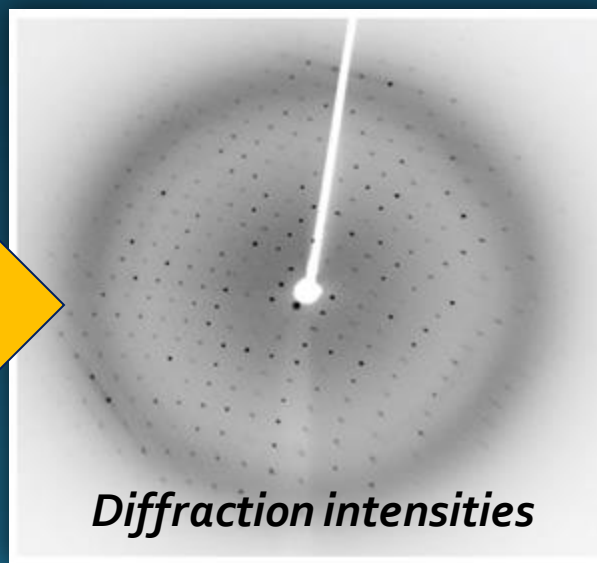
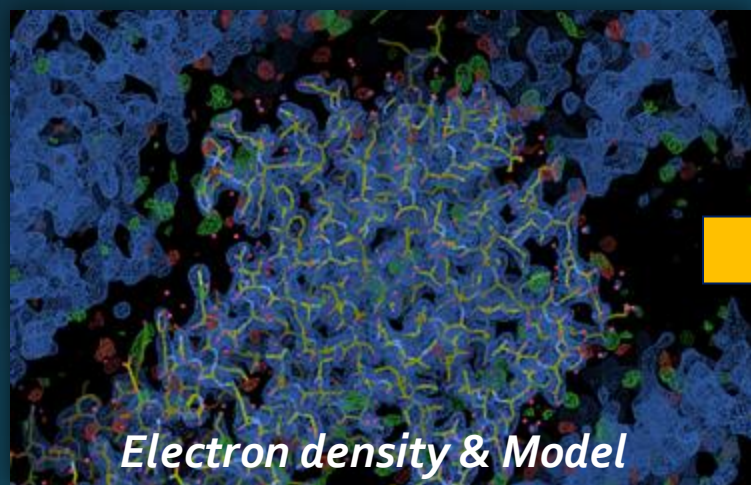


Introduction: *Basics of Molecular Replacement*

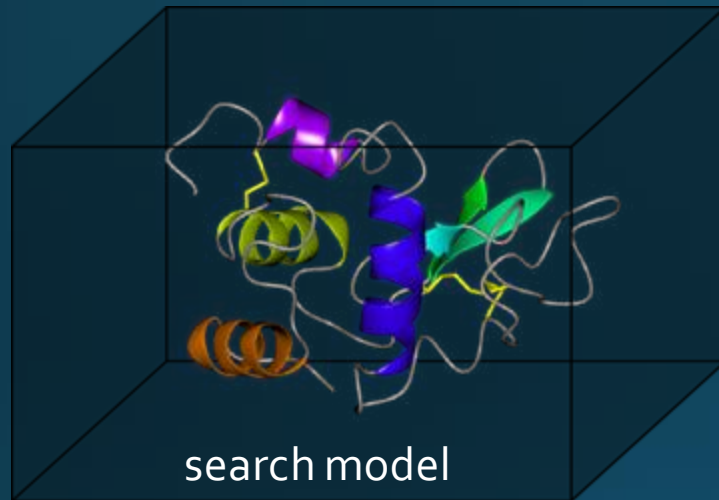
$$\rho(x, y, z) = \frac{1}{V} \sum_{hkl} |F_o(hkl)| \cos[-2\pi(hx + ky + lz) + \varphi_{hkl}]$$

Electron density
at (x,y,z)

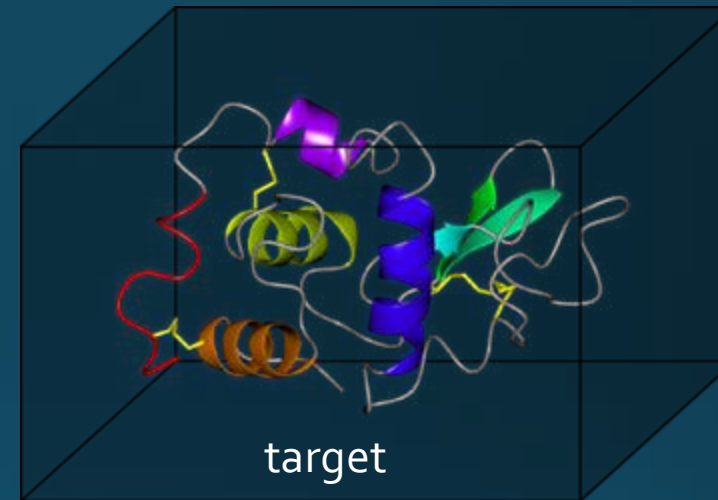
Amplitudes



Introduction: *Basics of Molecular Replacement*



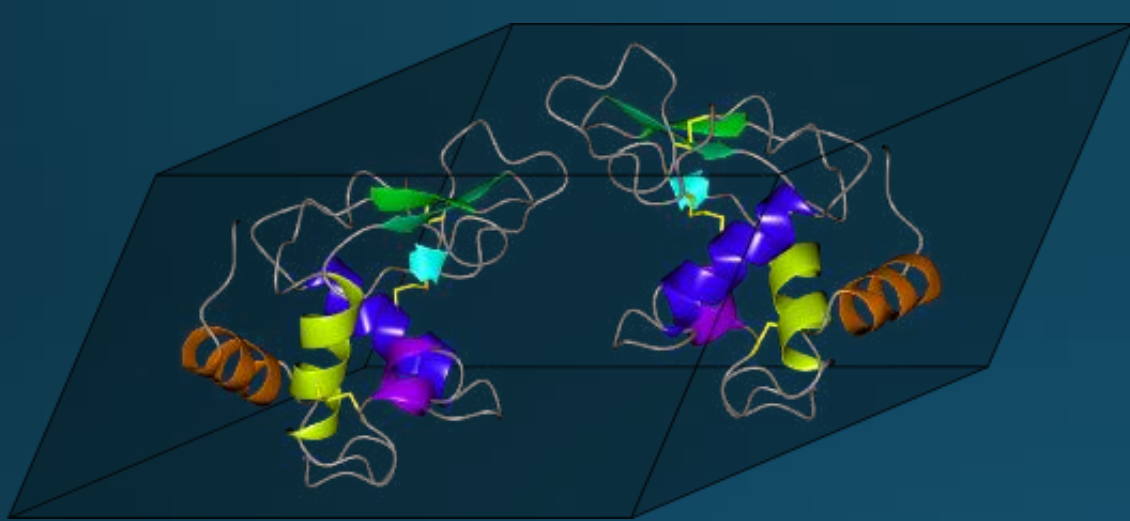
h	k	l	F	ϕ
0	0	1	12.6	123
0	0	2	2.1	12
0	0	3	69.9	287



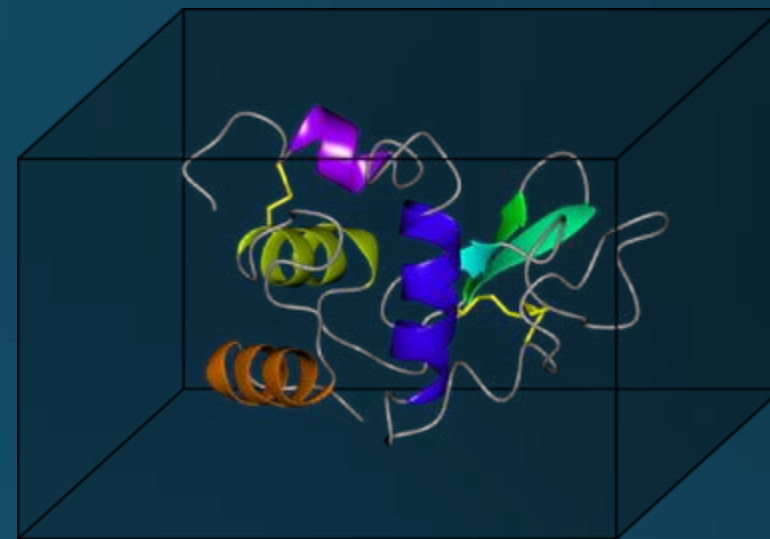
h	k	l	F	ϕ
0	0	1	10.4	113
0	0	2	3.5	18
0	0	3	57.2	265

(slide from Gabor Bunkoczi)

Introduction: *Basics of Molecular Replacement*



Search model crystal form

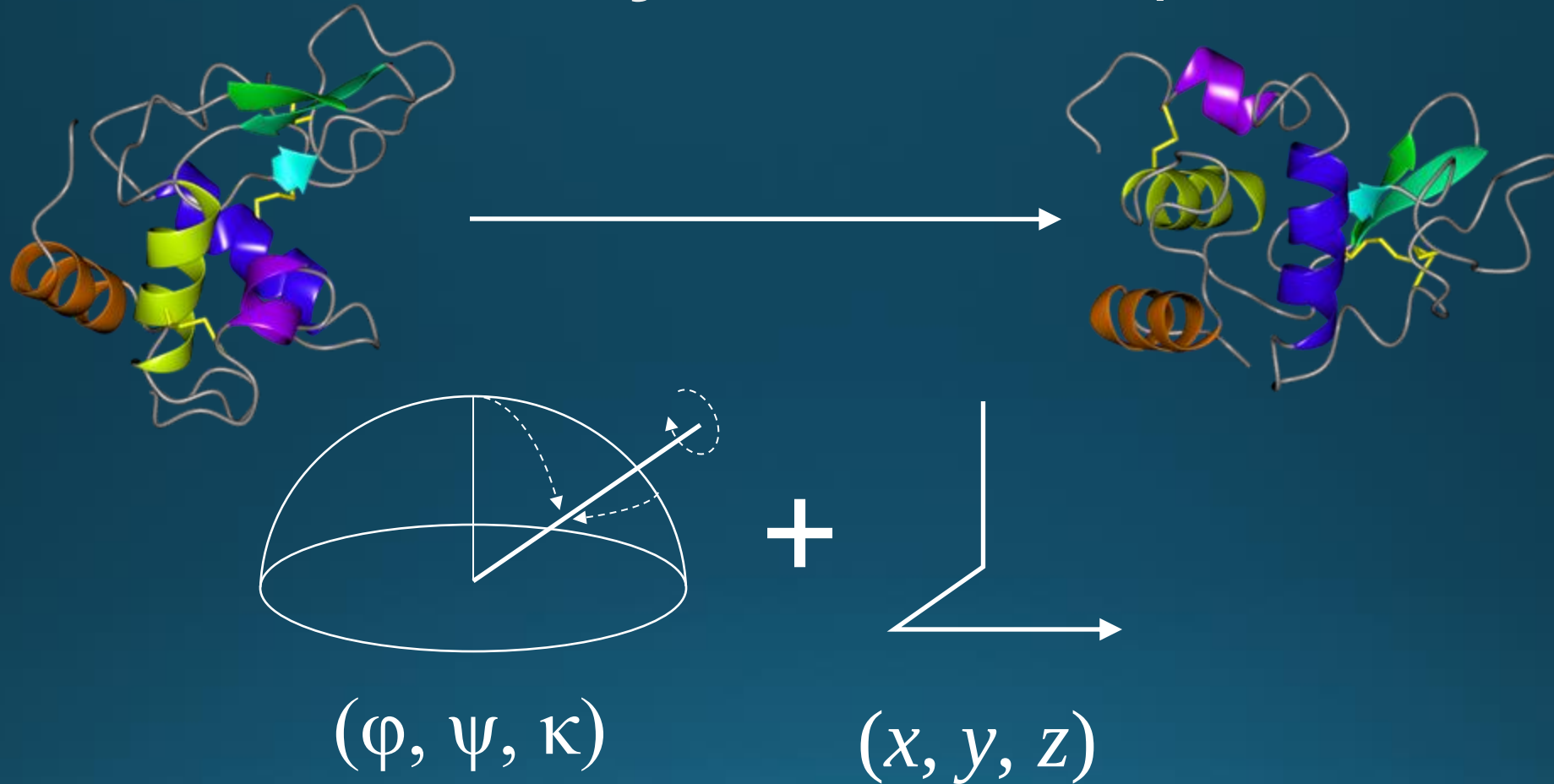


Target crystal form

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

(slide from Gabor Bunkoczi)

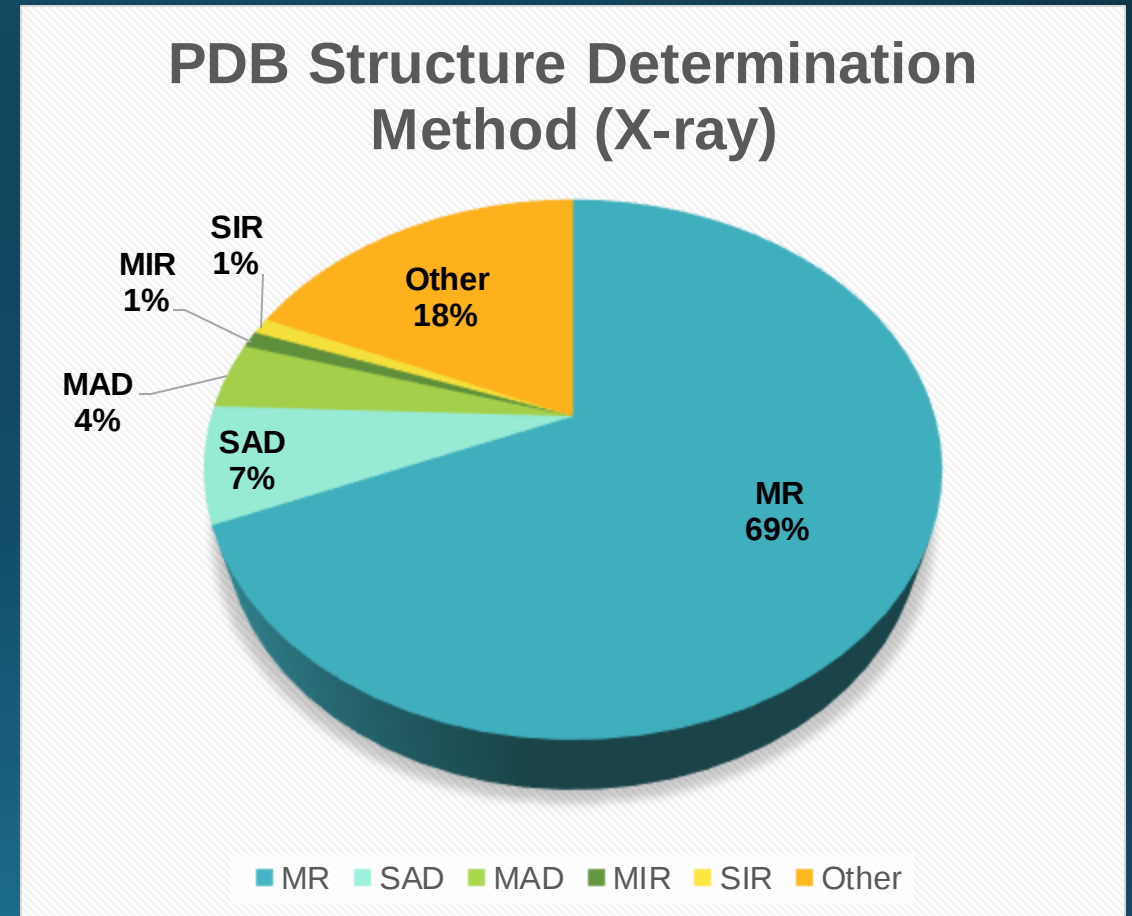
Introduction: *Basics of Molecular Replacement*



(slide from Gabor Bunkoczi)

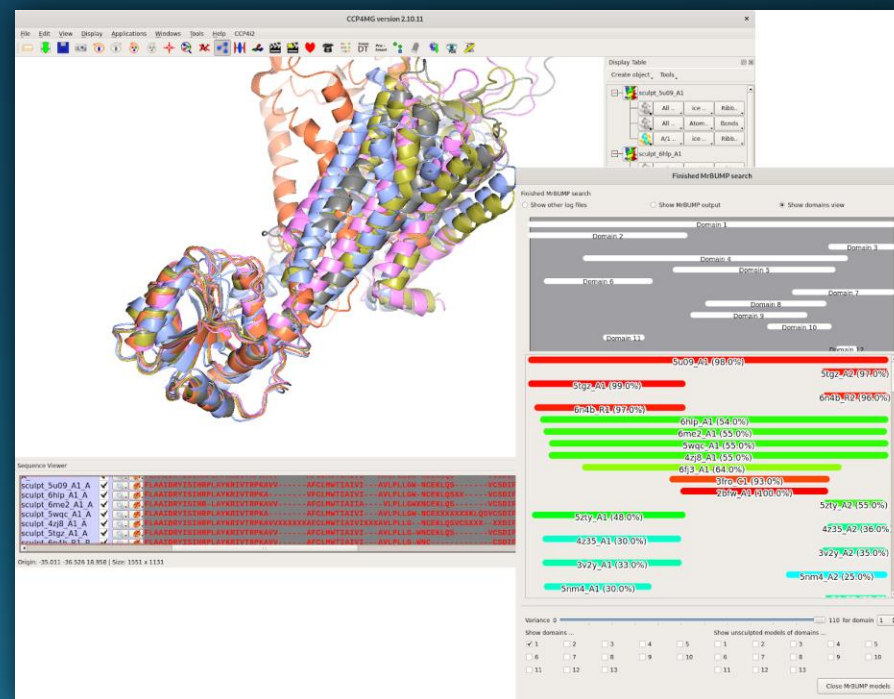
Introduction: *Molecular Replacement and the PDB*

- Today, the vast majority of X-ray entries in the PDB have been phased using MR
- Increasing success rate of MR has been due to:
 - Improving methods in software e.g. maximum likelihood in *Phaser*
 - Increasing availability of suitable search models



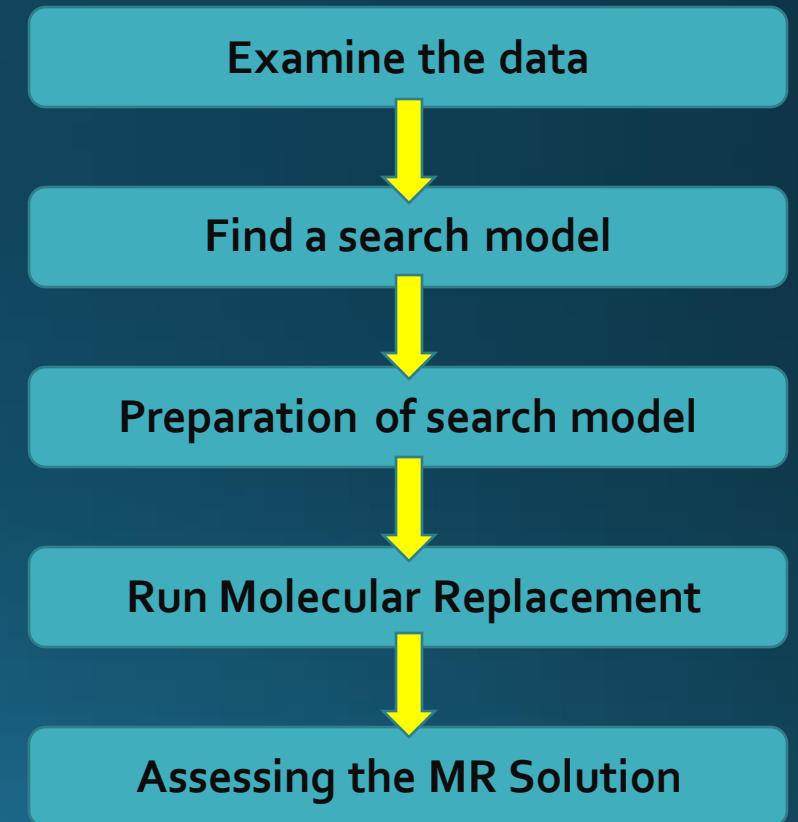
Introduction: *Automated Molecular Replacement in CCP4*

- Very amenable to automation
- Many applications in CCP4 that automate MR
 - Conventional pipelines:
 - Balbes
 - Dimple
 - MoRDa
 - MrBUMP
 - CCP4mg
 - Unconventional MR automation:
 - AMPLE
 - ARCIMBOLDO
 - Fragon
 - SIMBAD



Introduction: *Step-by-step Molecular Replacement*

- Despite this, successfully performing MR depends on attention to detail both before and after running the Molecular Replacement program



Step-by-Step MR: *Examining the data*

- Data issues can have an impact on how well MR will work

Examine the data

Step-by-Step MR: *Examining the data*

- Data issues can have an impact on how well MR will work
- Things to think about:
 - How many copies in the asymmetric unit?
 - Estimate through Matthews Coefficient
 - What's the resolution of the data?
 - Self-rotation function – signs of NCS?

Examine the data

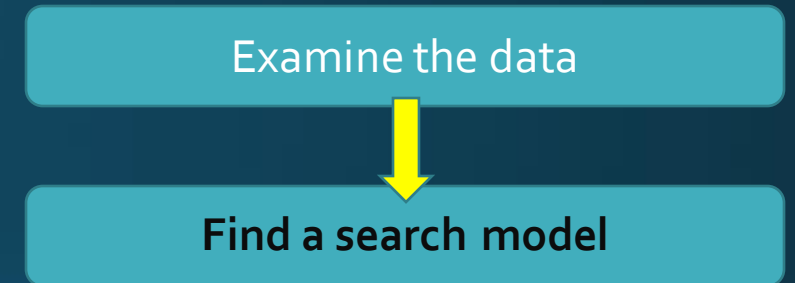
Step-by-Step MR: *Examining the data*

- Data issues can have an impact on how well MR will work
- Things to think about:
 - How many copies in the asymmetric unit?
 - Estimate through Matthews Coefficient
 - What's the resolution of the data?
 - Self-rotation function – signs of NCS?
- Potential problems:
 - Pseudo translation?
 - Twinned data?
 - Space group assignment correct?

Examine the data

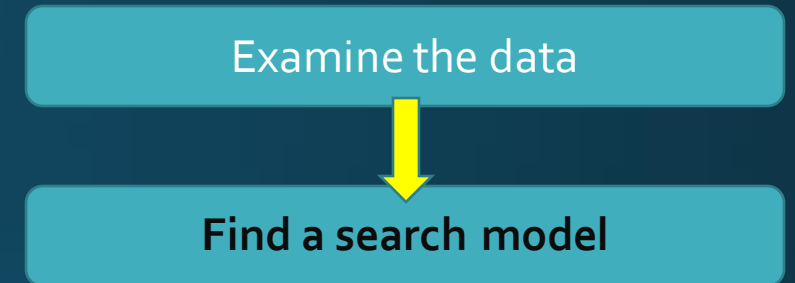
Step-by-Step MR: *Finding a search model*

- A well-chosen and optimized search model has two key advantages



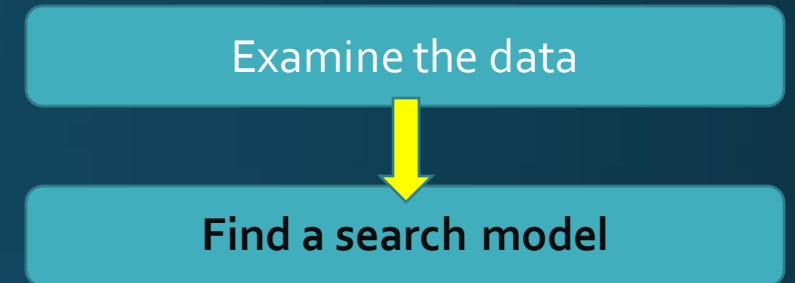
Step-by-Step MR: *Finding a search model*

- A well-chosen and optimized search model has two key advantages
 1. Makes it easier for the MR program to find the correct position for that search model and hence sensible starting phases



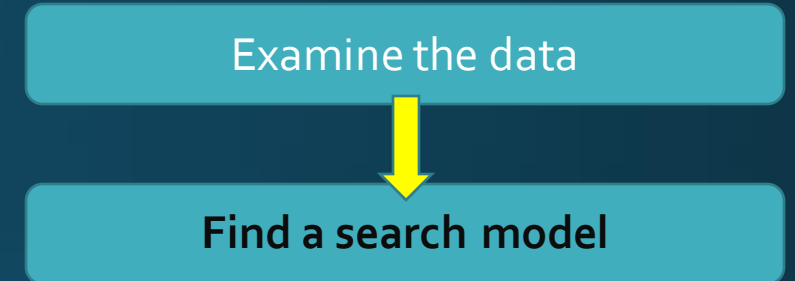
Step-by-Step MR: *Finding a search model*

- A well-chosen and optimized search model has two key advantages
 1. Makes it easier for the MR program to find the correct position for that search model and hence sensible starting phases
 2. Facilitates quicker and easier model building and refinement post MR



Step-by-Step MR: *Finding a search model*

- Considerations
 - Target Size:
 - The bigger the structure the more likely there will be sizeable conformational changes
 - Homologue Sequence identity $< 30\%$
 - Suitable search models may exist – sequence identity calculation is difficult
 - Data Resolution
 - eLLG from Phaser is the best measure of suitability of a search model for MR



Step-by-Step MR: *Resolution and MR*

- How does data resolution impact on molecular replacement?

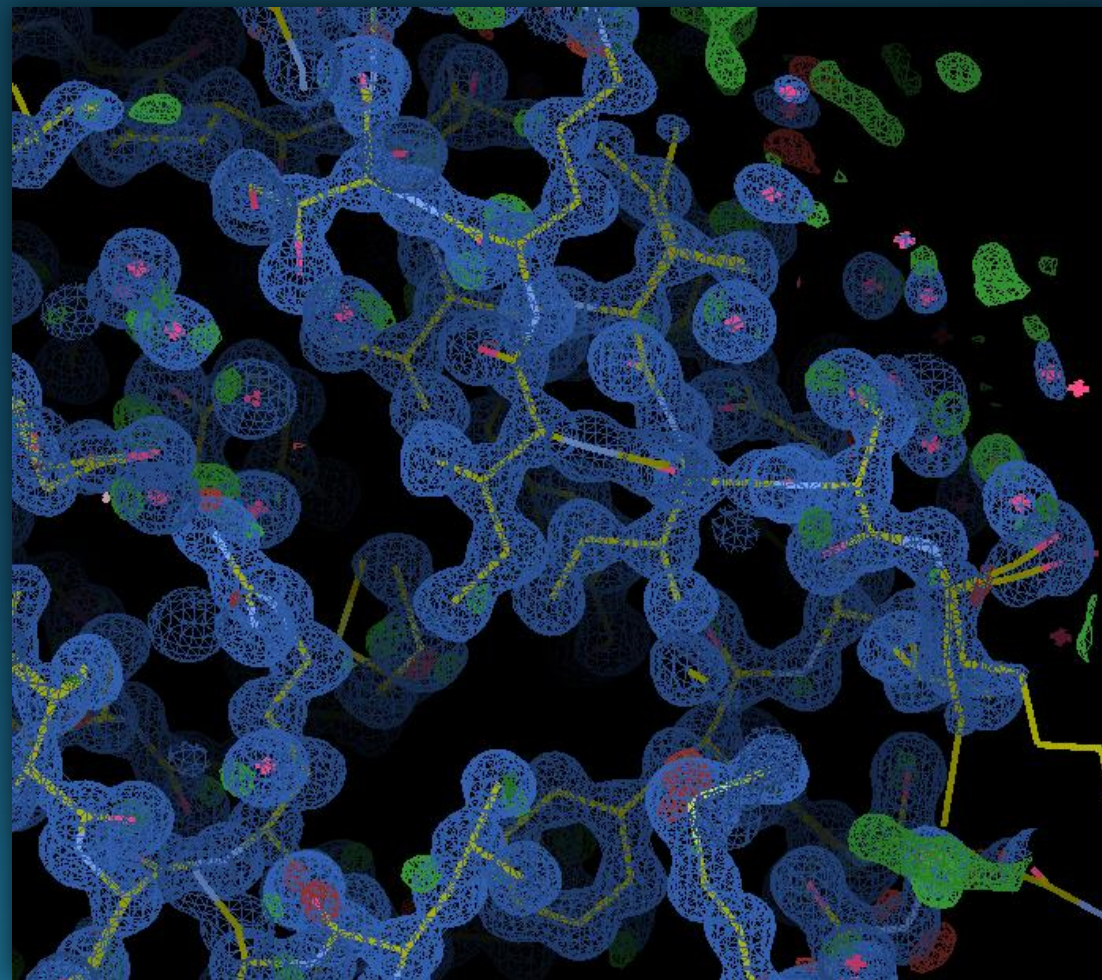
Examine the data



Find a search model

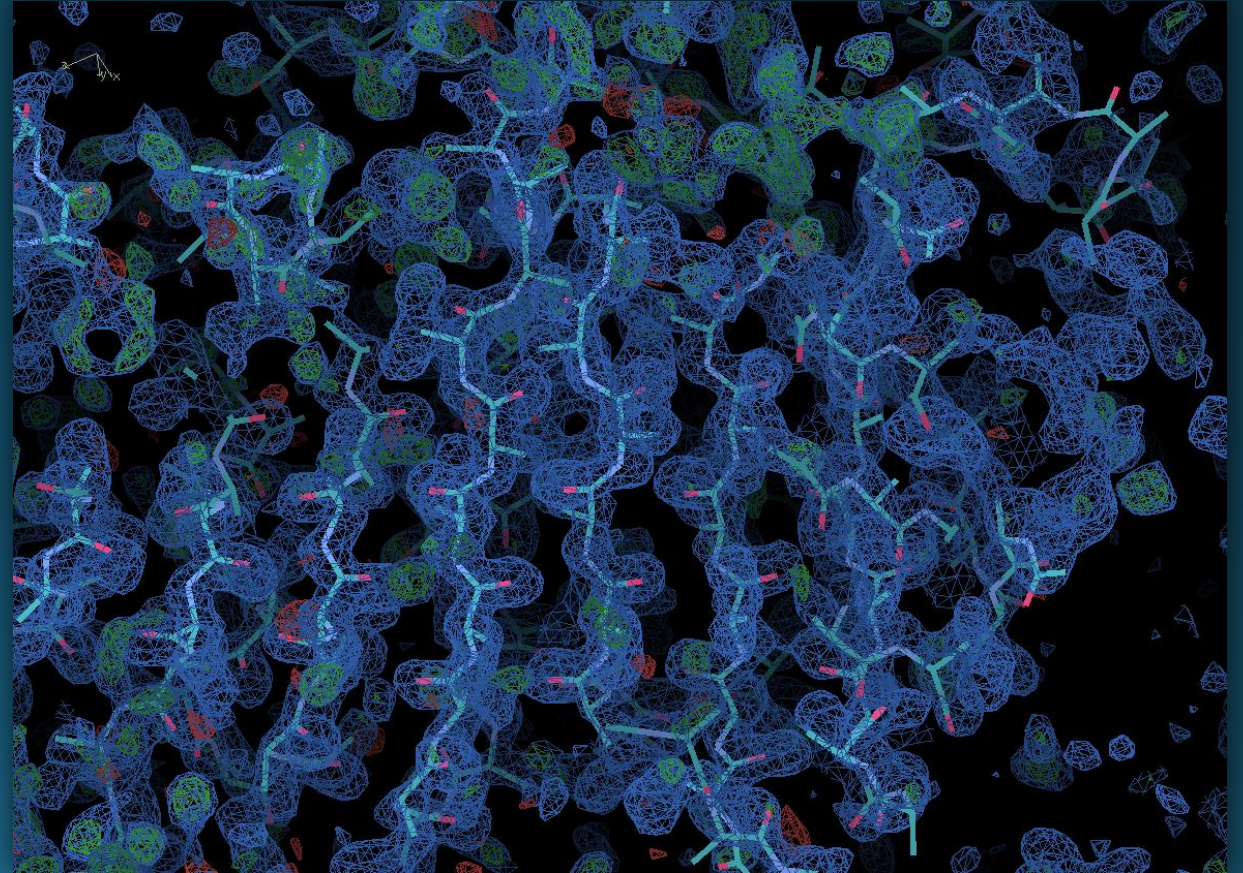
Step-by-Step MR: *Resolution and MR*

- Better than 1.0 Å – search model can be a small fragment or even a single atom in *Phaser*
(McCoy et al. 2017, PNAS)



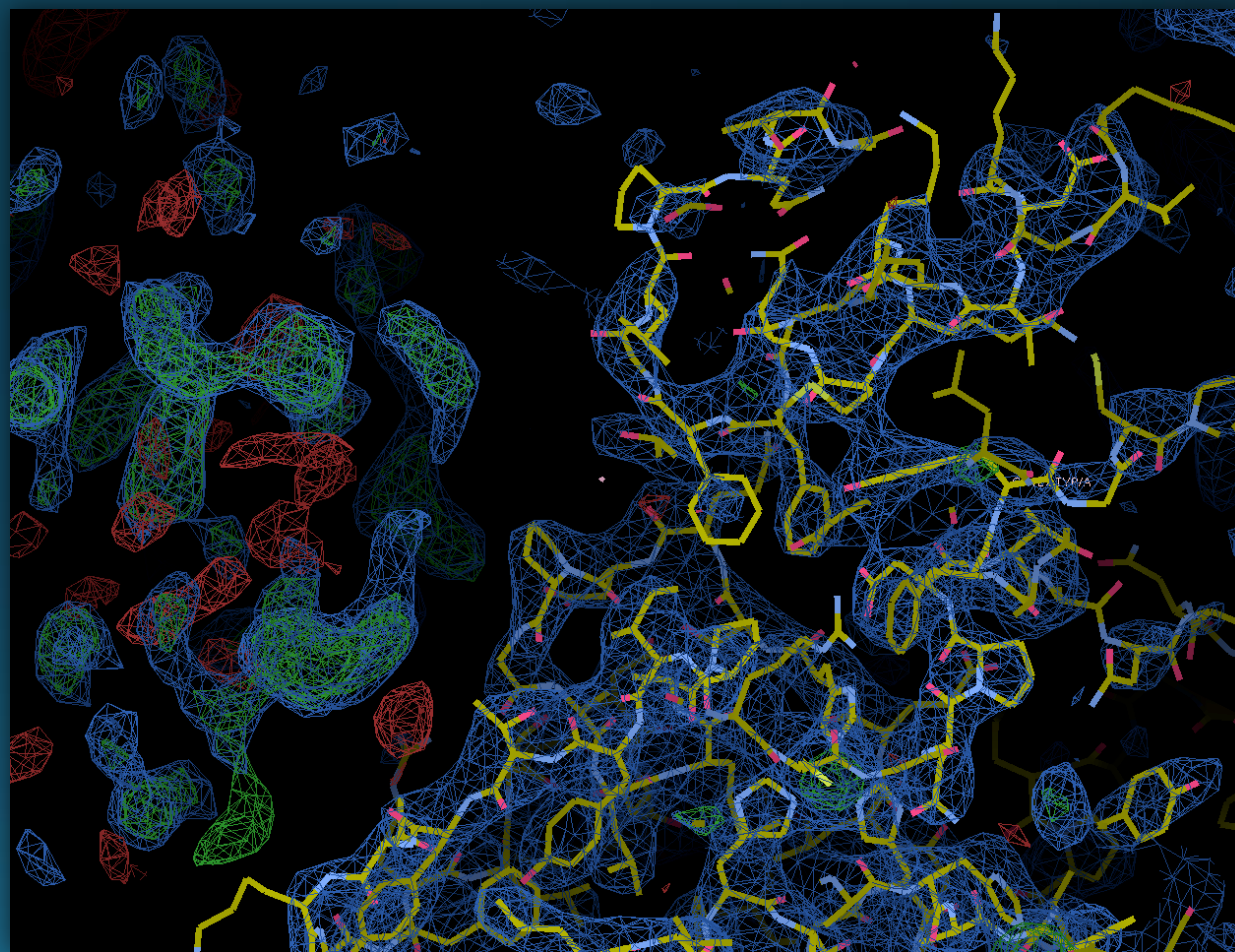
Step-by-Step MR: *Resolution and MR*

- At resolutions above $\sim 2.5 \text{ \AA}$
 - *Phaser* can place small fragments of total scattering
 - Applications like *SHELXE* and *Acorn* can improve through density modification (DM) and model building to a correct solution



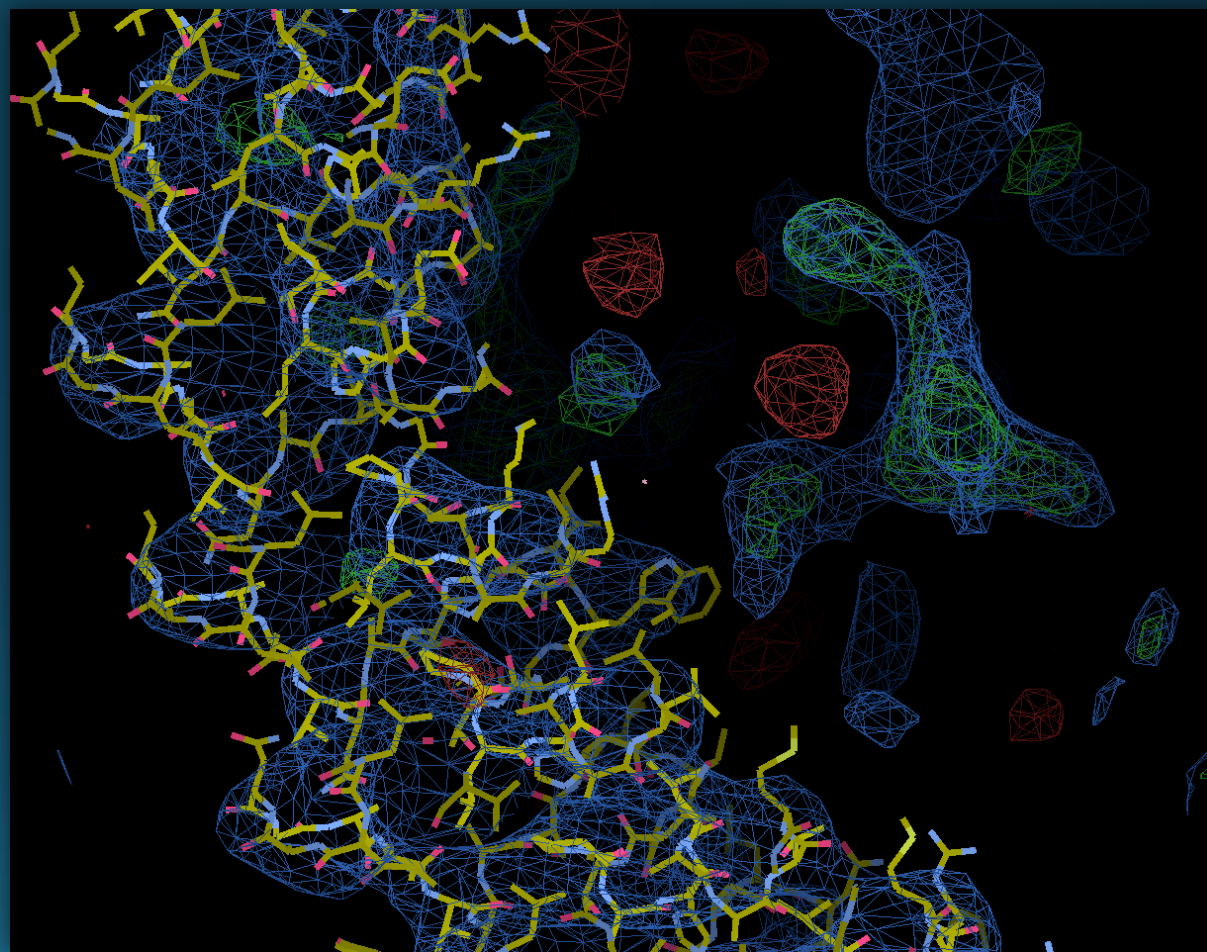
Step-by-Step MR: *Resolution and MR*

- 2.5Å or worse
 - DM techniques and fragment placing is less effective
 - Larger search models are required



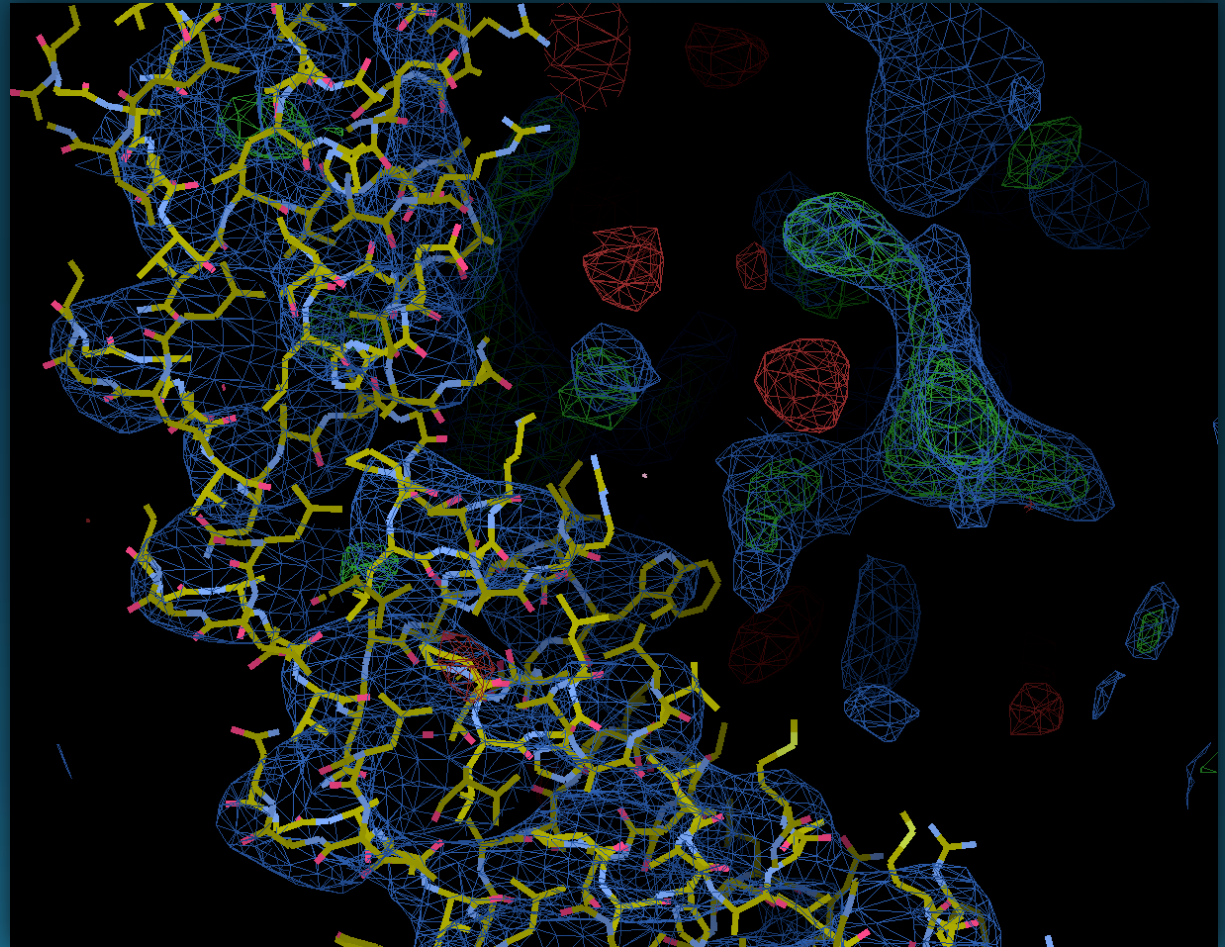
Step-by-Step MR: *Resolution and MR*

- Below 4Å automated model building becomes difficult



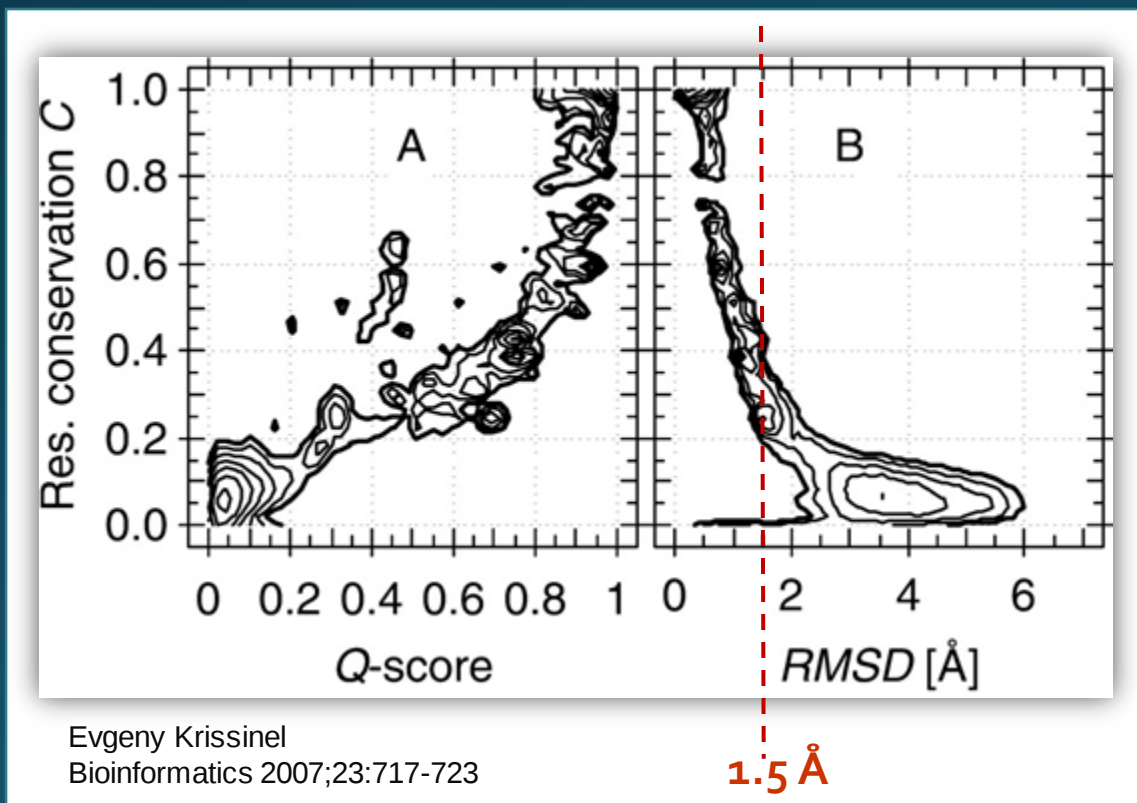
Step-by-Step MR: *Resolution and MR*

- Below 4Å automated model building becomes difficult
- However, given suitable search models, MR can be used to build up a model structure



Step-by-Step MR: *Finding a search model*

- How to find a search model?
 - Amino acid sequence similarity often correlates well with structural similarity



Examine the data

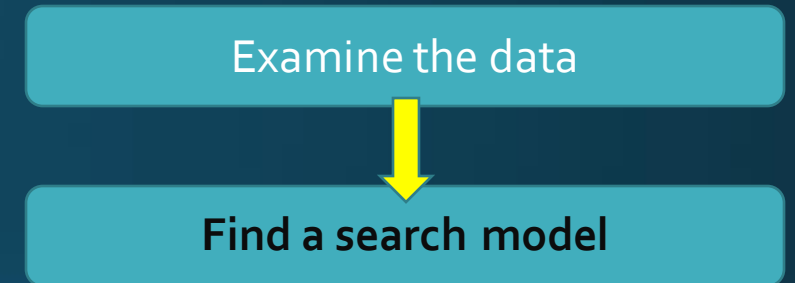


Find a search model

Krissinel et al. looked at structure-sequence relationship across entire PDB

Step-by-Step MR: *Finding a search model*

- Bioinformatics gives us sensitive sequence-based search tools for finding potential homologues from which we can derive search models for MR



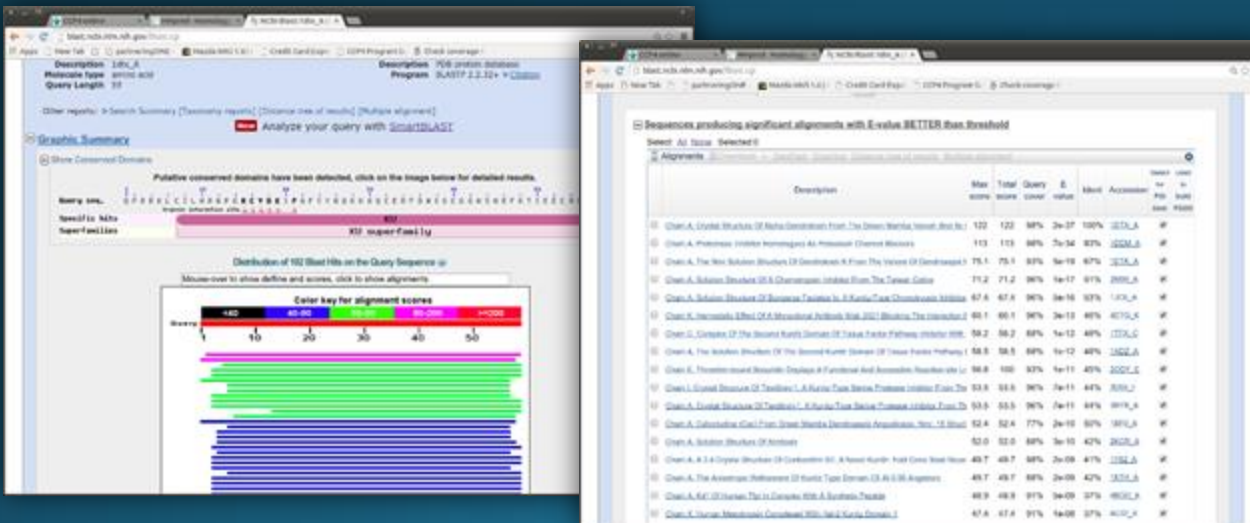
Step-by-Step MR: *Finding a search model*

- PSI-Blast
 - Profile-based searching
 - Online server, fast
 - Works well at finding suitable homologues down to sequence identities of 30%

Examine the data



Find a search model



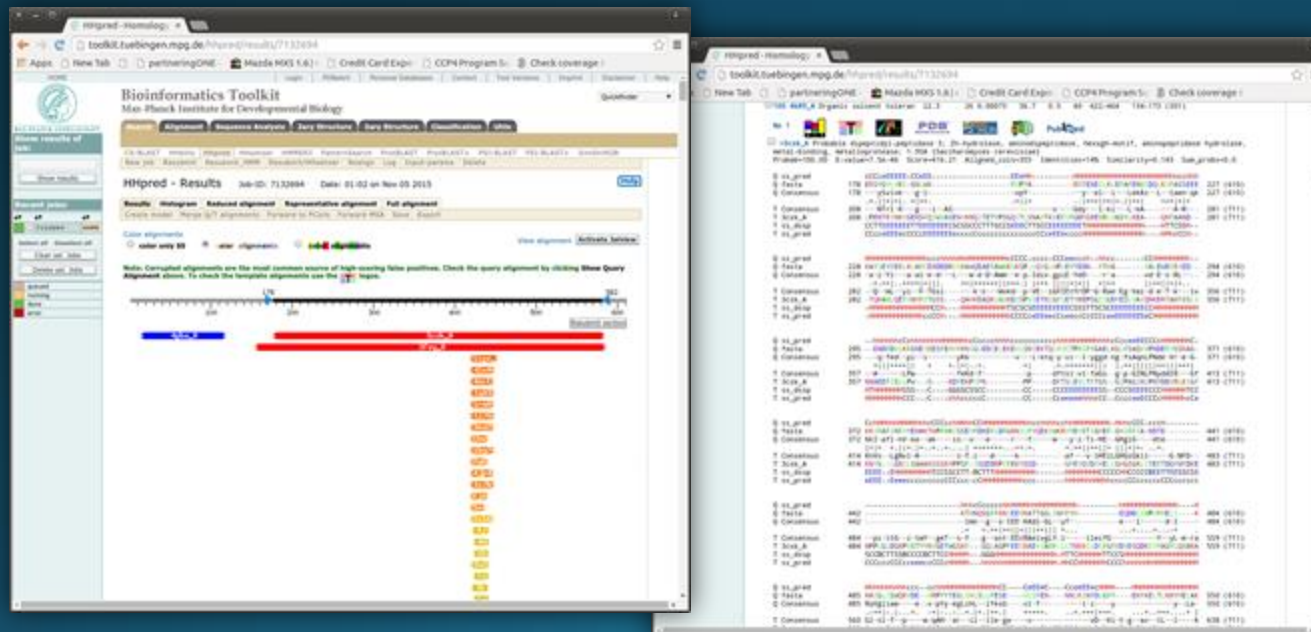
Step-by-Step MR: *Finding a search model*

- HHpred
 - Hidden Markov Model approach
 - Suitable for more difficult cases where no obvious homologue is available

Examine the data

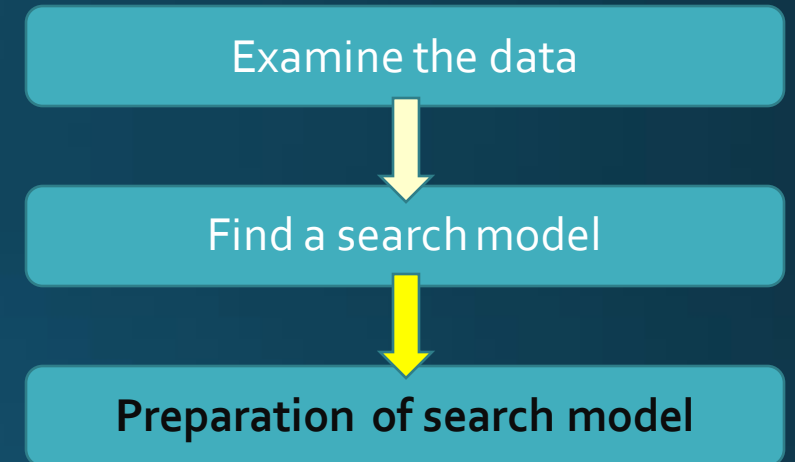


Find a search model



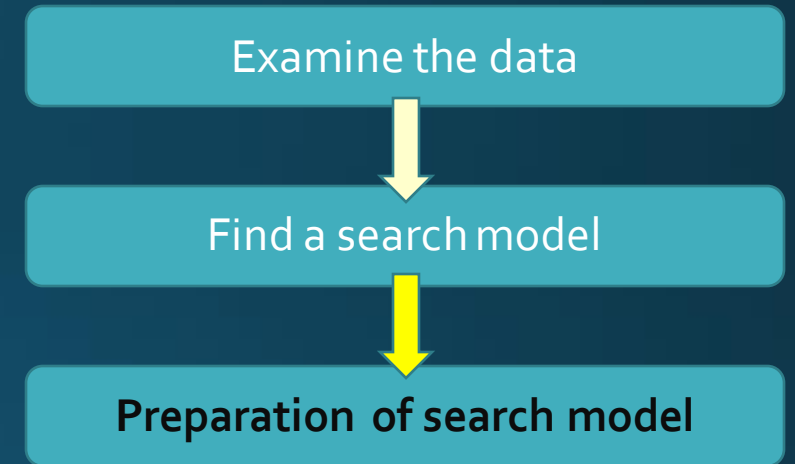
Step-by-Step MR: *Preparation of search model*

- Once a suitable homologue or set of homologues have been found they need to be prepared for use as search models



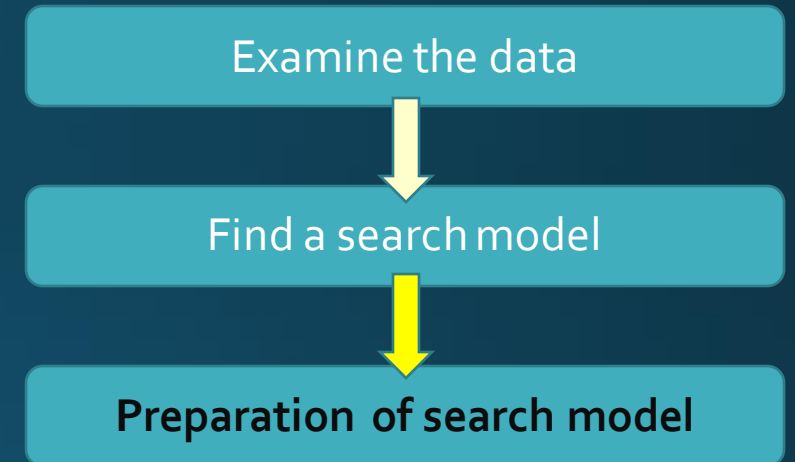
Step-by-Step MR: *Preparation of search model*

- Once a suitable homologue or set of homologues have been found they need to be prepared for use as search models
- The closer we can make the homologue to the target in terms of structural similarity the more likely it is to be successfully positioned in Molecular Replacement



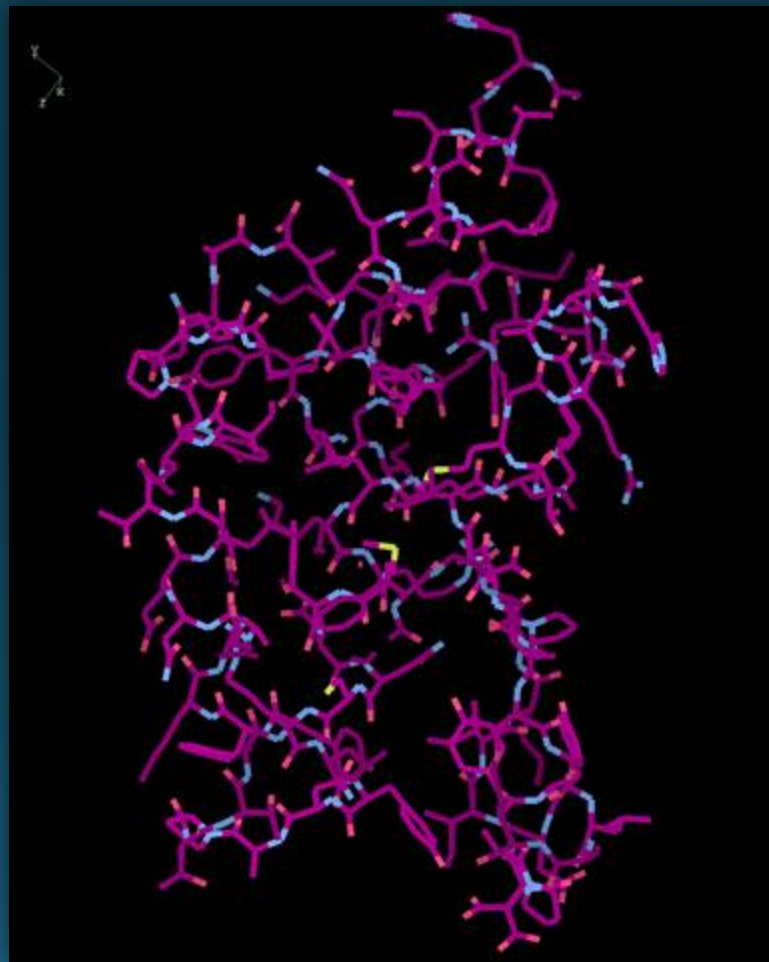
Step-by-Step MR: *Preparation of search model*

- Once a suitable homologue or set of homologues have been found they need to be prepared for use as search models
- The closer we can make the homologue to the target in terms of structural similarity the more likely it is to be successfully positioned in Molecular Replacement
- The sequence alignment generated in the search step can be used as a guide for the pruning of the homologue



Step-by-Step MR: *Preparation of search model*

- Example target:
 - 5TPX - Bromodomain from Plasmodium Faciparum Gcn5



Examine the data



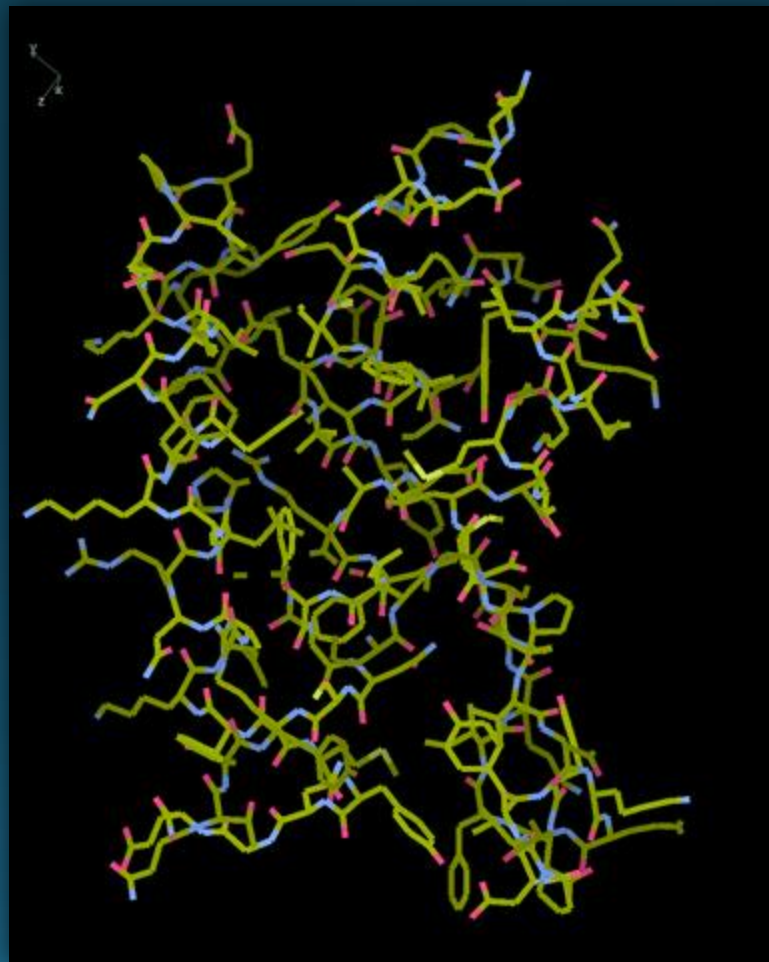
Find a search model



Preparation of search model

Step-by-Step MR: *Preparation of search model*

- Example target:
 - 5TPX - Bromodomain from Plasmodium Faciparum Gcn5
- Homologue:
 - 1E6I – chain A
 - 45% sequence identity over 82% coverage of target



Examine the data



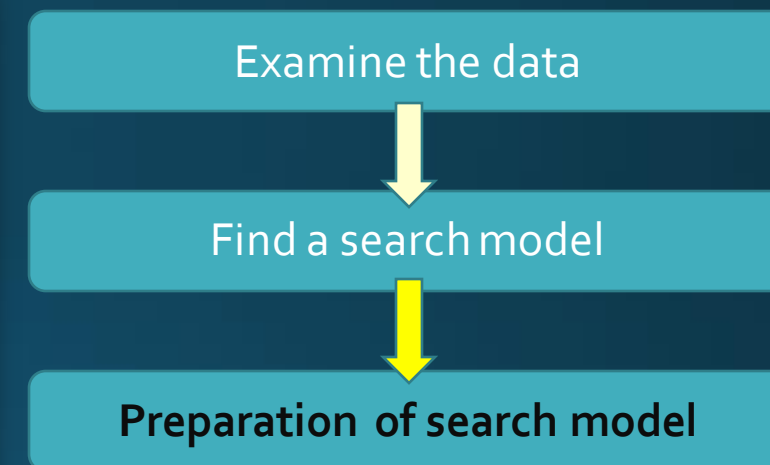
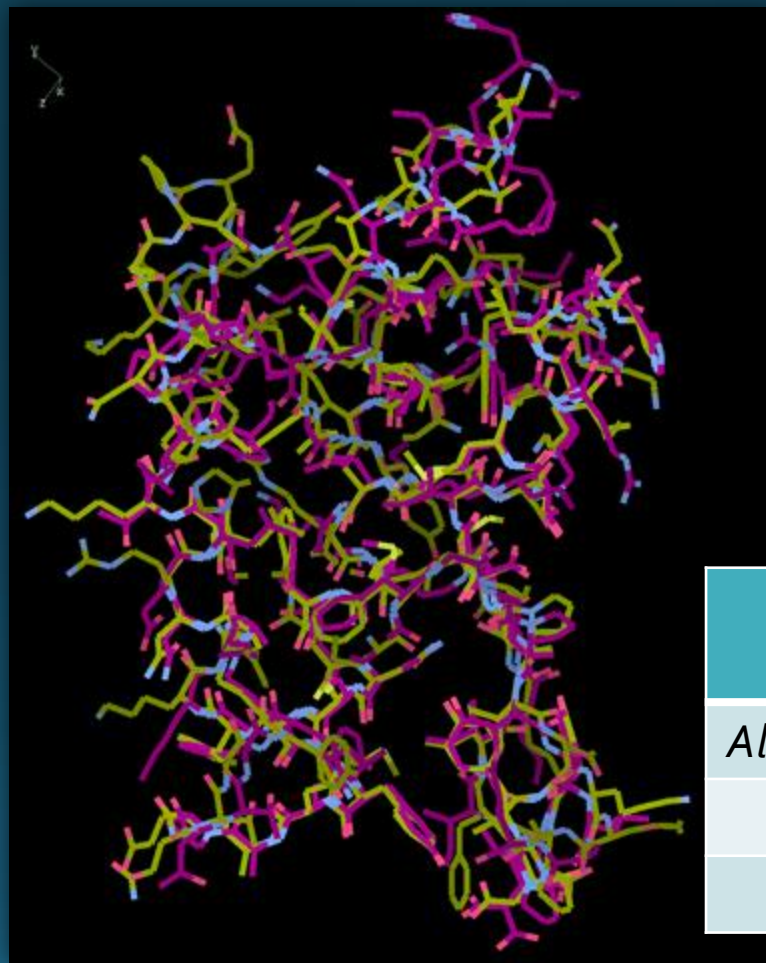
Find a search model



Preparation of search model

Step-by-Step MR: *Preparation of search model*

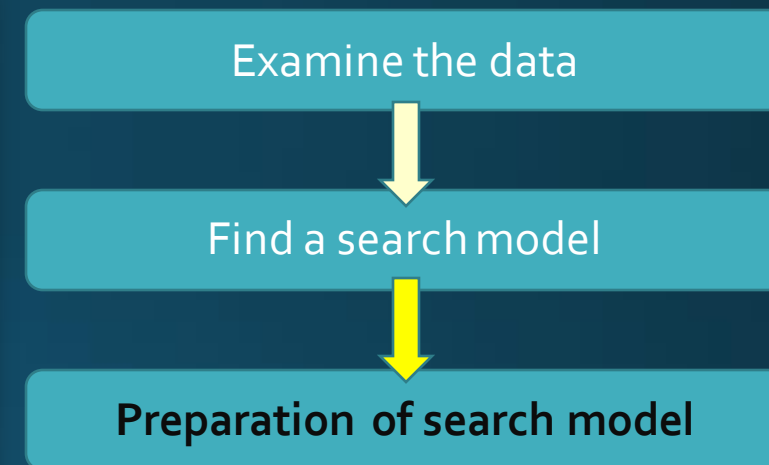
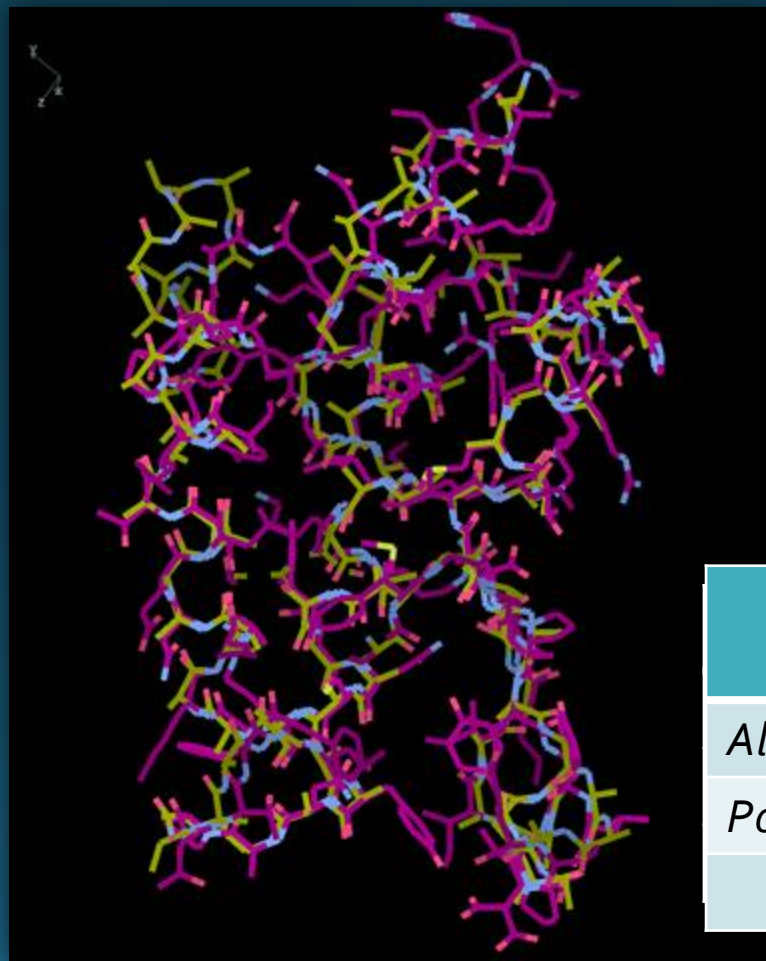
- Search Model preparation and MR:
 - All atoms?



	<i>Molrep</i> TF/sigma	<i>Refmac</i> Rfactor	<i>Refmac</i> Rfree
<i>All atoms</i>	6.82	0.418	0.481

Step-by-Step MR: *Preparation of search model*

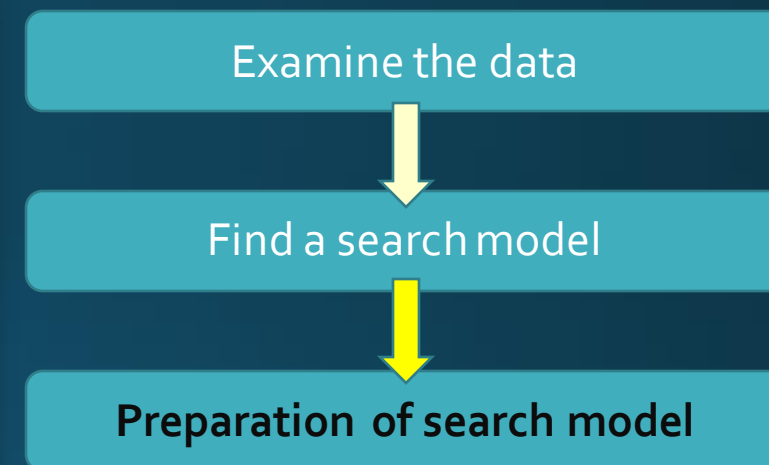
- Search Model preparation and MR:
 - All atoms?
 - Polyalanine?



	<i>Molrep</i> TF/sigma	<i>Refmac</i> Rfactor	<i>Refmac</i> Rfree
<i>All atoms</i>	6.82	0.418	0.481
<i>Poly</i>	4.98	0.46	0.482

Step-by-Step MR: *Preparation of search model*

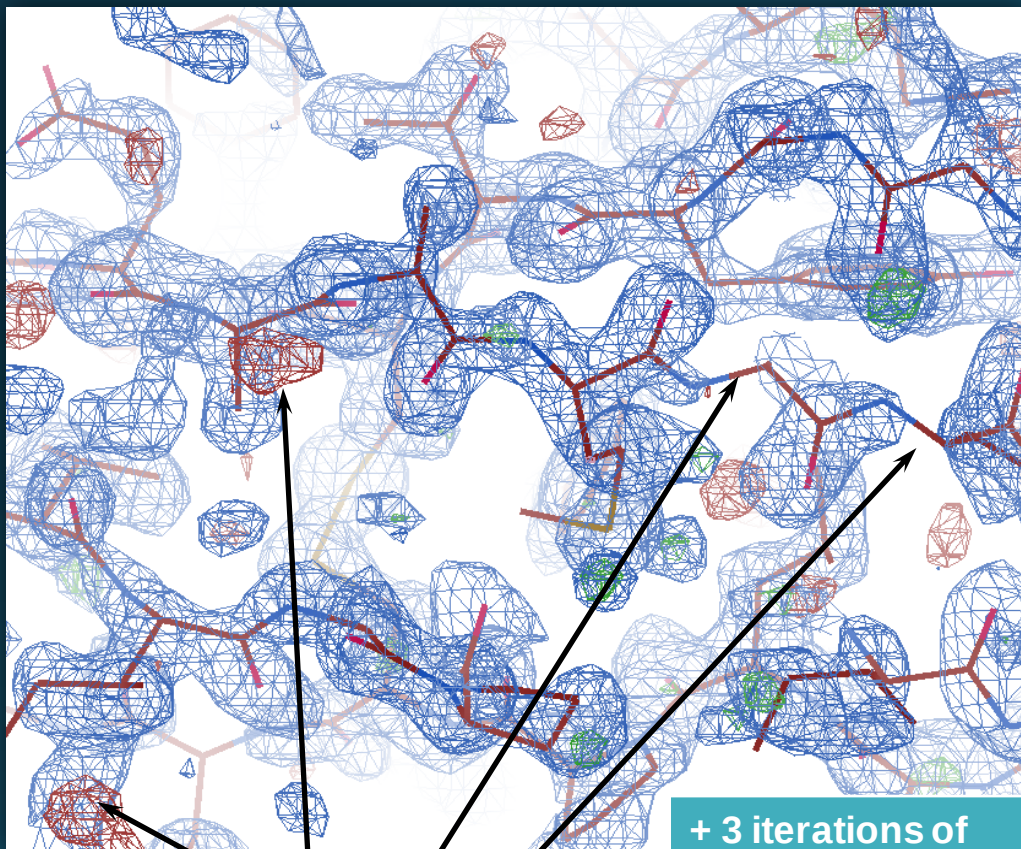
- Search Model preparation and MR:
 - All atoms?
 - Polyalanine?
 - Mixed model based on sequence alignment
 - Keep common residues
 - Truncate to common atoms on aligned residues



	<i>Molrep</i> TF/sigma	<i>Refmac</i> Rfactor	<i>Refmac</i> Rfree
<i>All atoms</i>	6.82	0.418	0.481
<i>Poly</i>	4.98	0.46	0.482
<i>Mixed</i>	10.27	0.379	0.418

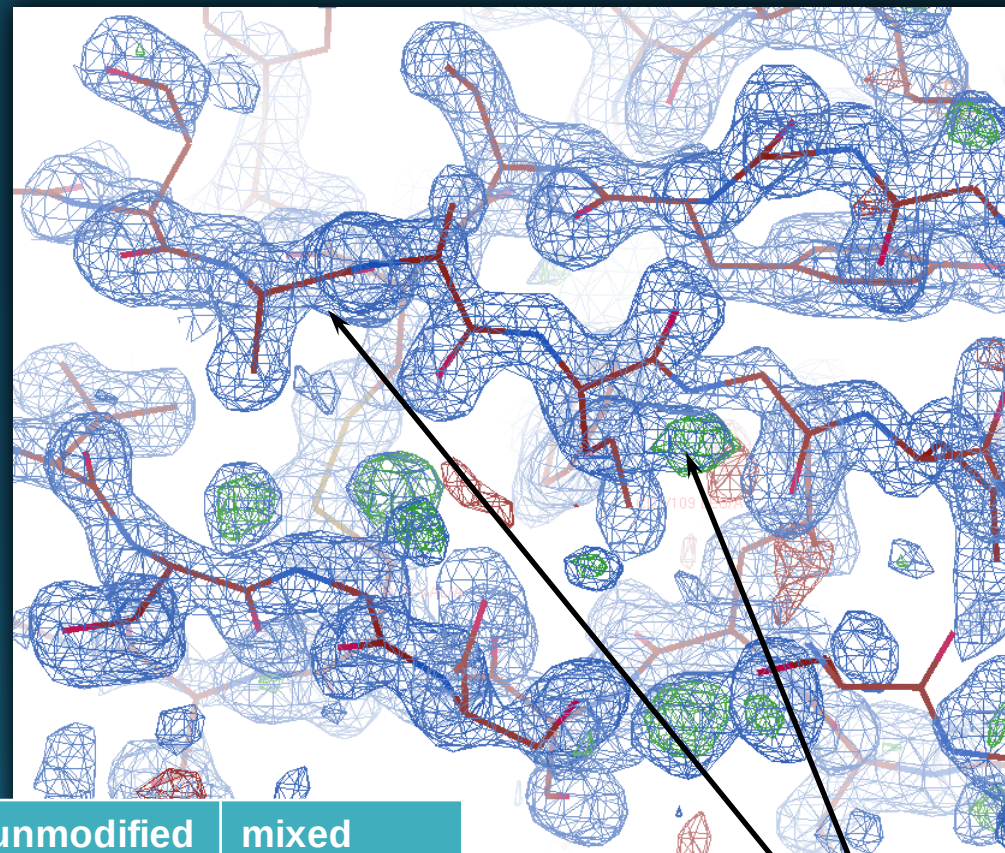
Step-by-Step MR: *Preparation of search model*

Unmodified model:



bad density features

Mixed model:

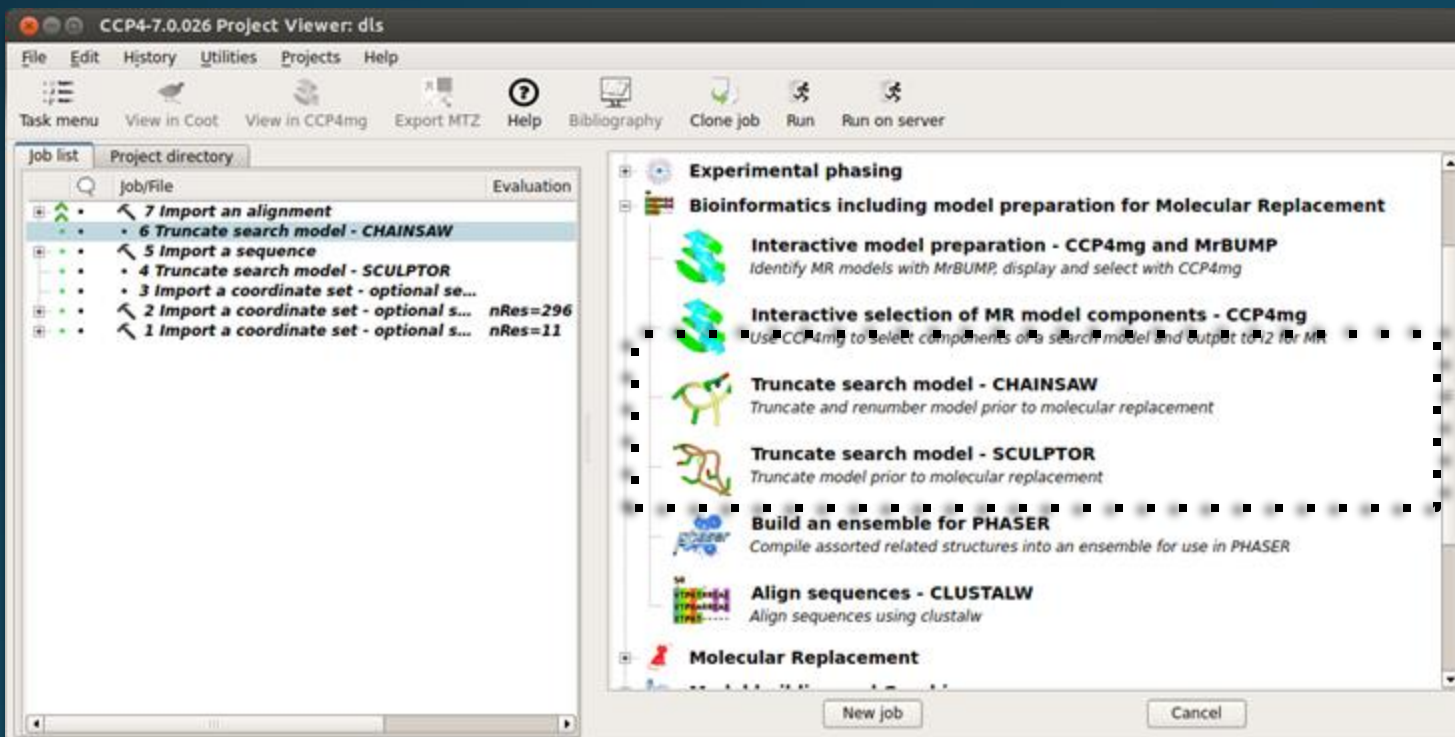


good density features

+ 3 iterations of buccaneer & refmac	unmodified	mixed
R	32	30
R-free	36	33

Step-by-Step MR: *Preparation of search model*

- Mixed-model generation in CCP4
 - Chainsaw & Sculptor



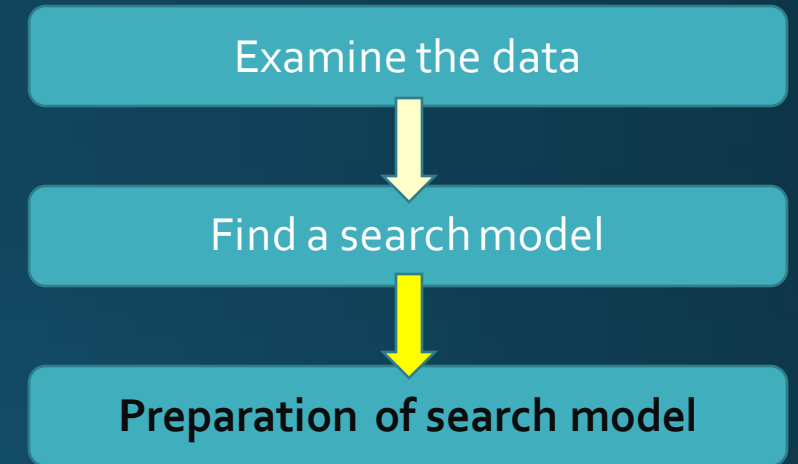
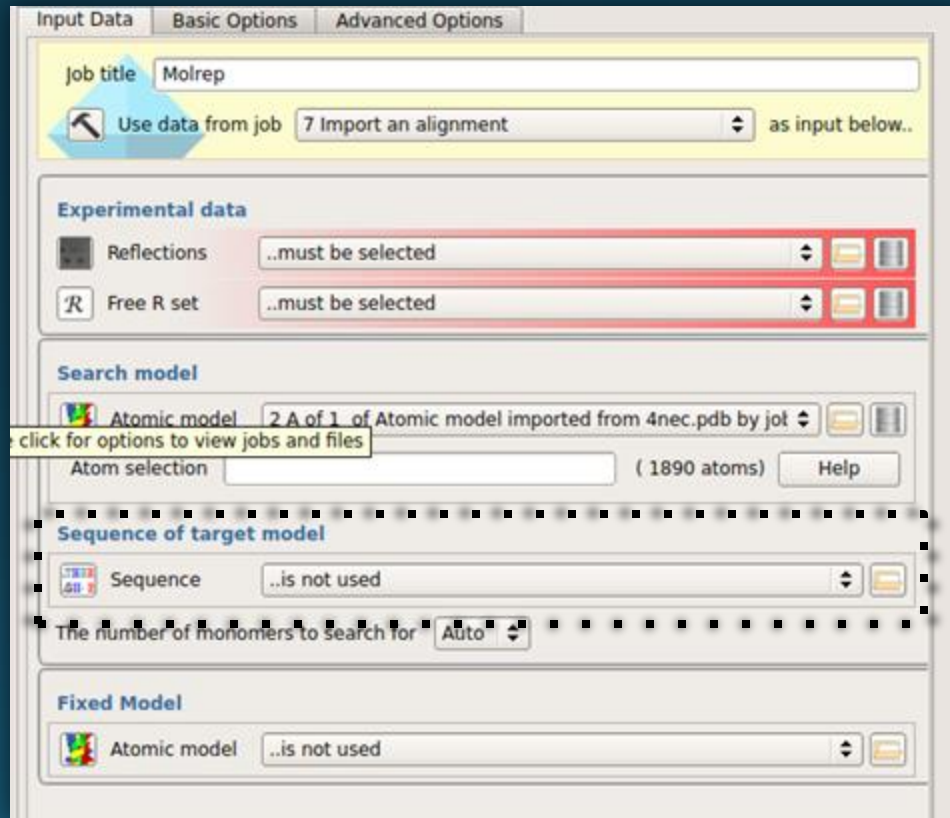
Examine the data

Find a search model

Preparation of search model

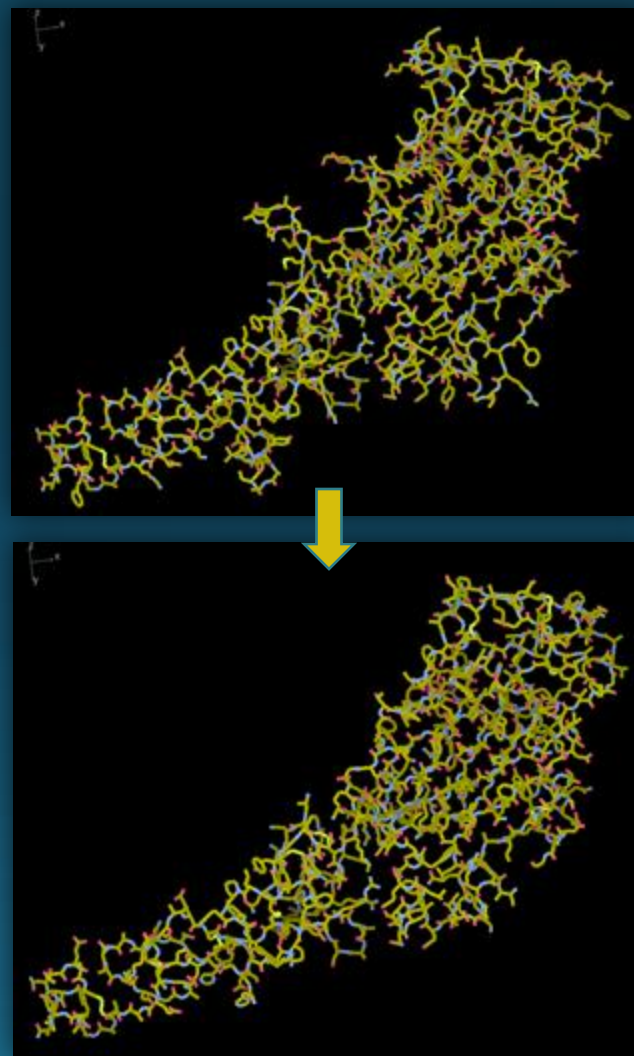
Step-by-Step MR: *Preparation of search model*

- Mixed-model generation in CCP4
 - Molrep



Step-by-Step MR: *Preparation of search model*

- Manual pruning in Coot
- Remove anything that is likely to be flexible:
 - External loops
 - Longer side chains on the surface of the molecule (e.g. LYS)
 - Domains if there is evidence of “hinge motion”



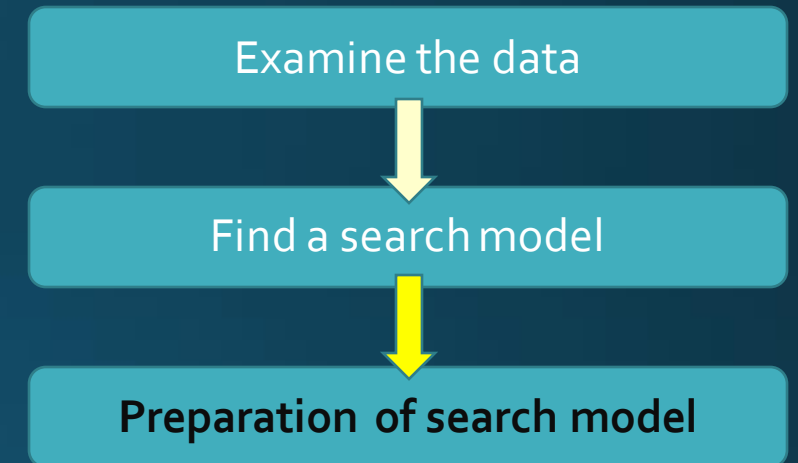
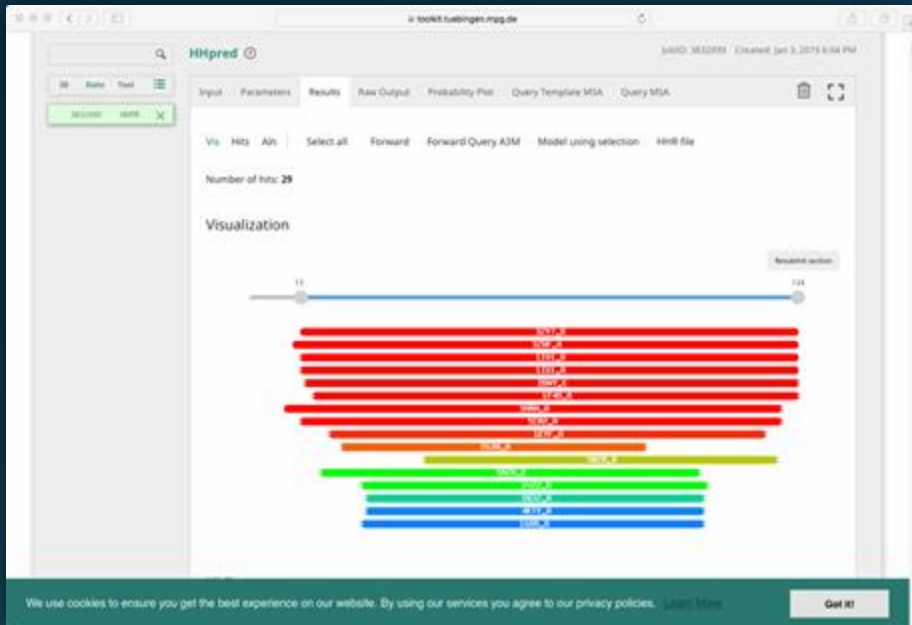
Examine the data

Find a search model

Preparation of search model

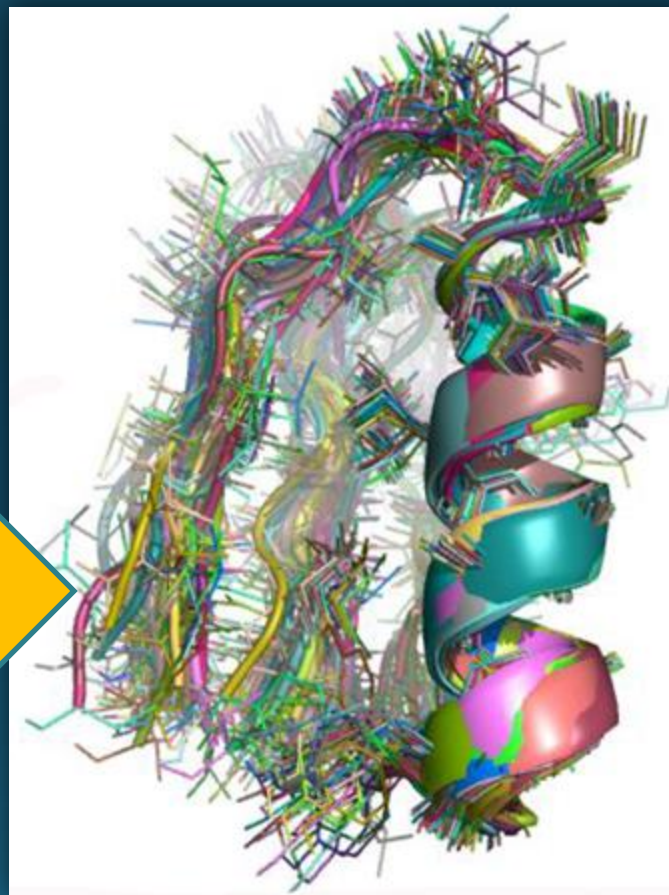
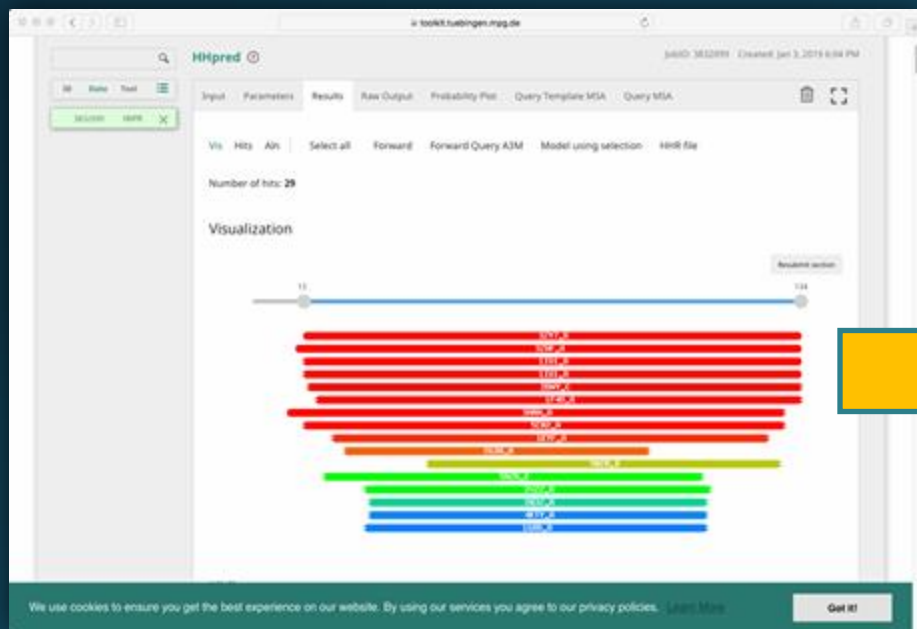
Step-by-Step MR: *Preparation of search model*

- Ensembles: alignment of search models : several homologues available



Step-by-Step MR: *Preparation of search model*

- Ensembles: alignment of search models



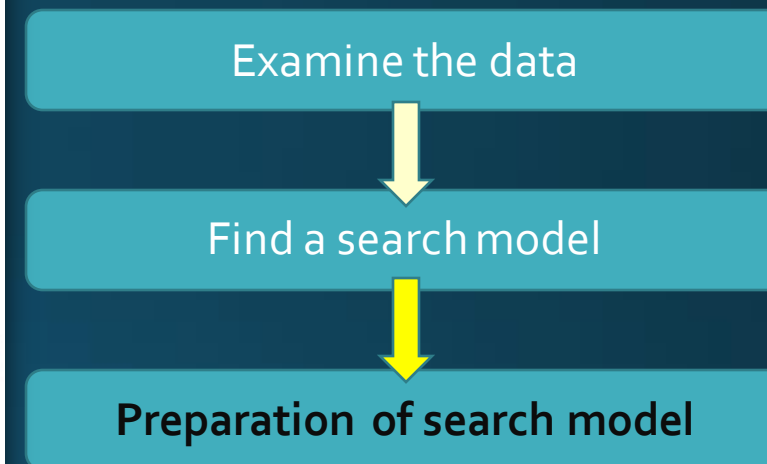
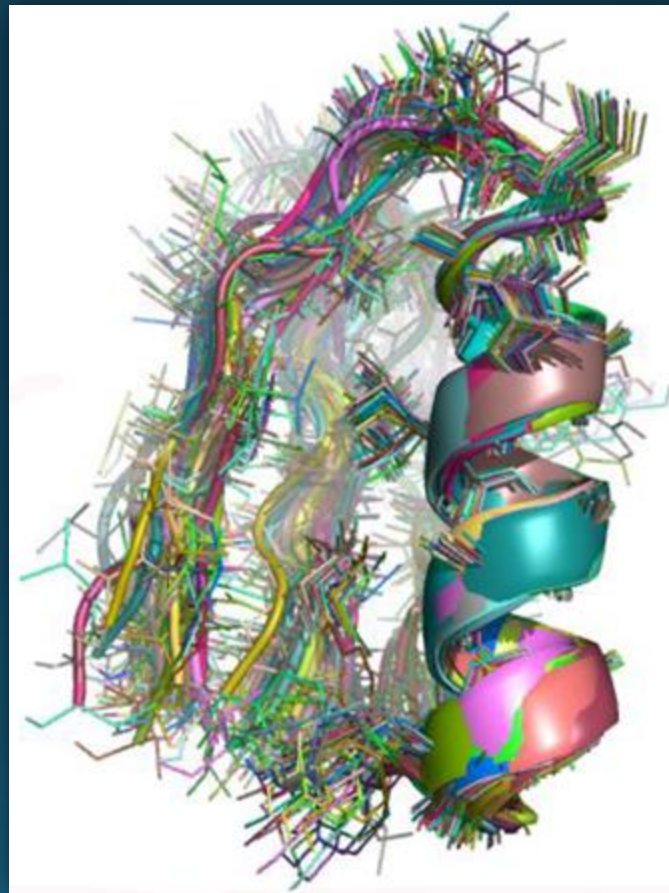
Examine the data

Find a search model

Preparation of search model

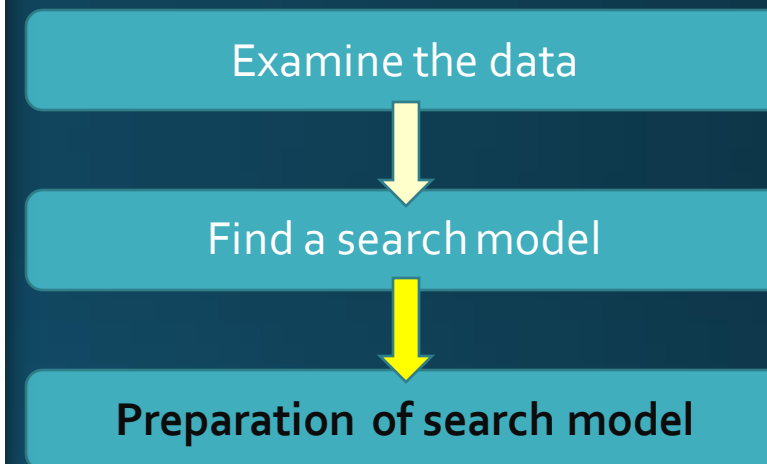
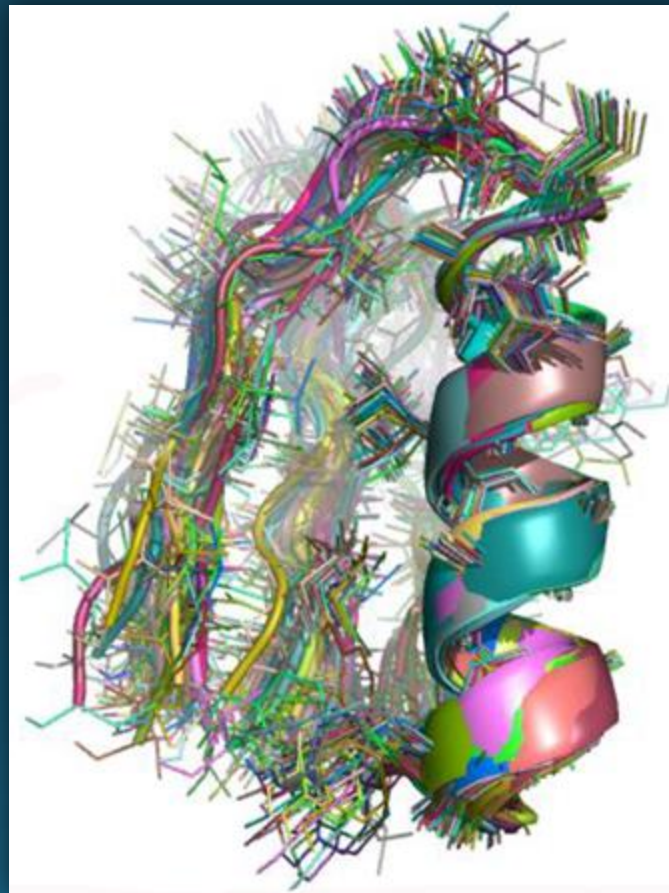
Step-by-Step MR: *Preparation of search model*

- Ensembles: alignment of search models
 1. Variance across members can guide experimental data weighting in Phaser



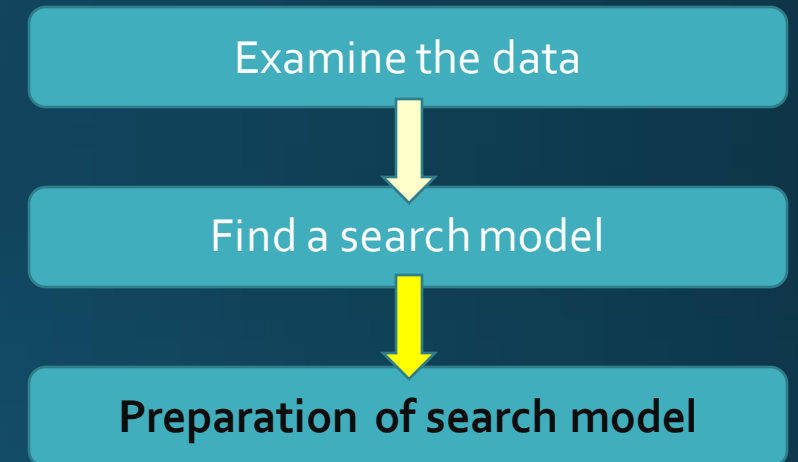
Step-by-Step MR: *Preparation of search model*

- Ensembles: alignment of search models
 1. Variance across members can guide experimental data weighting in Phaser
 2. Truncation based on alignment variance can identify conserved regions or core



Step-by-Step MR: *Preparation of search model*

- Ensembles: Generating ensembles in CCP4
 - Ensembler:
 - Align models and truncate based on a variance threshold
 - General alignment tools:
 - Gesamt
 - Superpose
 - Can also be run through CCP4mg and Coot graphical interfaces



Step-by-Step MR: *Preparation of search model*

The screenshot displays the CCP4mg-MrBUMP software interface. The main window shows a 3D ribbon diagram of a protein structure, labeled "A/35(ASP)/CA". A blue arrow points to a "slider" for ensemble truncation, with a scale bar indicating 15.3 Å. The "Display Table" on the right lists objects like "sculpt_1gxt_A1" and "sculpt_1gxu_A1". A "Finished MrBUMP search" dialog box is open, showing a list of domains (1gxt_A, 1gxu_A, 4g9i_G, 4g9i_B, 4g9i_E, 4g9i_F, 4g9i_D, 4g9i_A, 3vth_A, 3vti_A) and a "Display models for" section. A red dashed box highlights the "Display models for" section, and a blue arrow points to it. The "Sequence Viewer" at the bottom shows the protein sequence in PIR format.

Ensemble generation with CCP4mg-MrBUMP

Ensemble truncation "slider"

15.3 Å

Finished MrBUMP search

Display models for

Show residues with gesamt variance

Show MrBUMP models

Show unsculpted PDB models

Close MrBUMP models

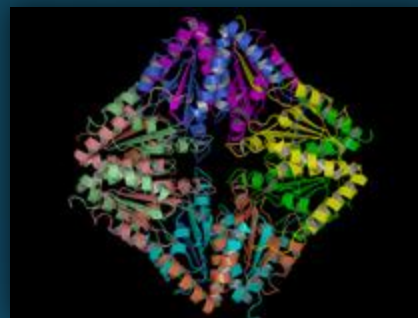
Close

Cancel

mp_1\logs\phmmmerALN.log from 3 mrbump_basic

Step-by-Step MR: *Preparation of search model*

- Multimers as search models
 - A single chain search model can be too small if the target has crystallised in multimeric form
 - The signal for the correct position is too weak against the background noise of incorrect positions
 - Particularly a problem at lower resolutions and crystals with high symmetry



Examine the data



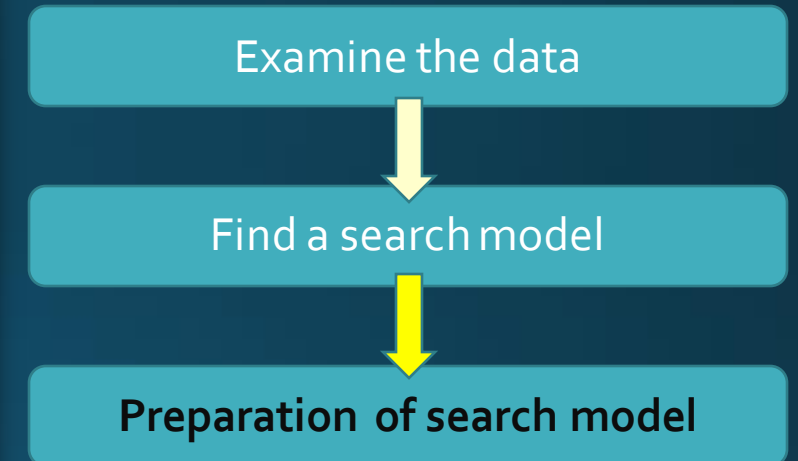
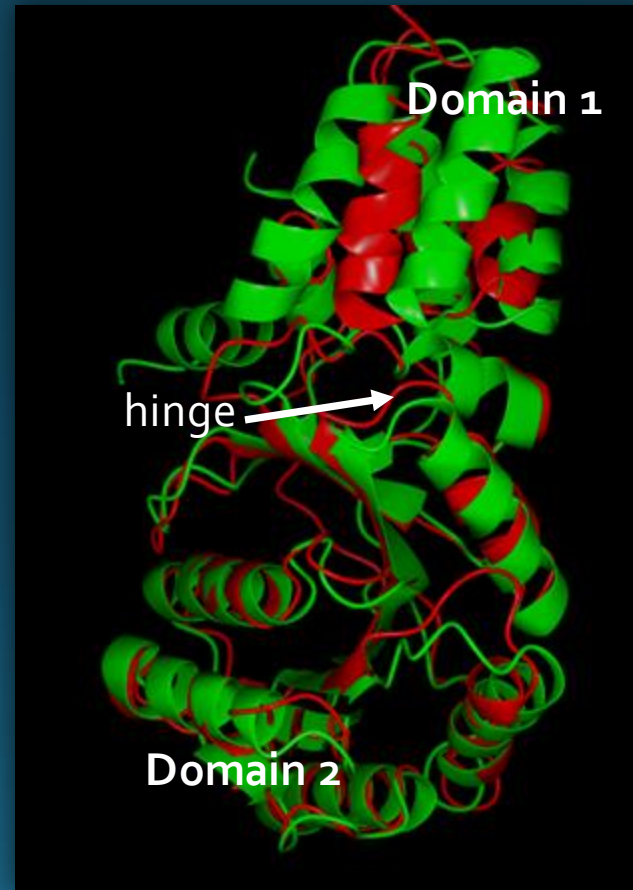
Find a search model



Preparation of search model

Step-by-Step MR: *Preparation of search model*

- Domains as search models
 - A hinge motion may alter the relative orientation of domains within a chain
 - Domain models should be isolated from the parent search model and used separately in MR



Step-by-Step MR: *Preparation of search model*

- Domains as search models

Evolutionary Classification of Protein Domains

[Browse](#) ECOD classification as tree or [download](#) distributable files. The classification currently includes only proteins with experimentally determined spatial structures.

[Search by keyword](#)
[Search by PDB ID](#)
[Search by sequence](#)
[Search by structure](#)
[Search by COMPADRE](#)

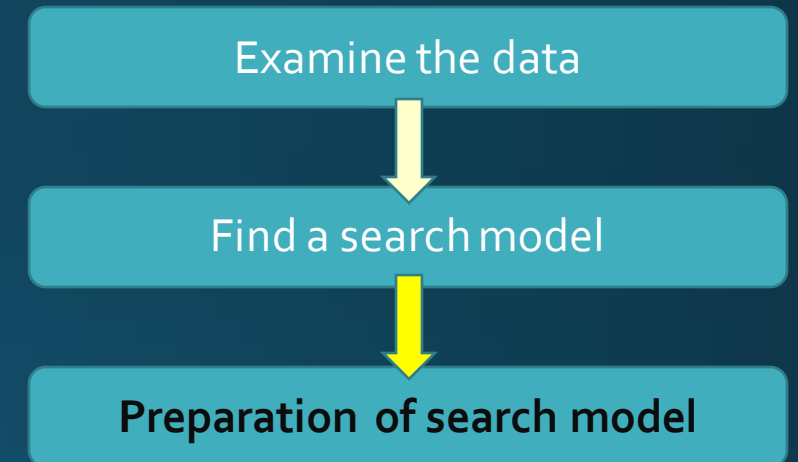
Search by keyword

Submit

ECOD was updated on **Fri Sep 4 11:55:06 CDT 2020** and contains **166,956** PDB structures released before **08/14/2020**.

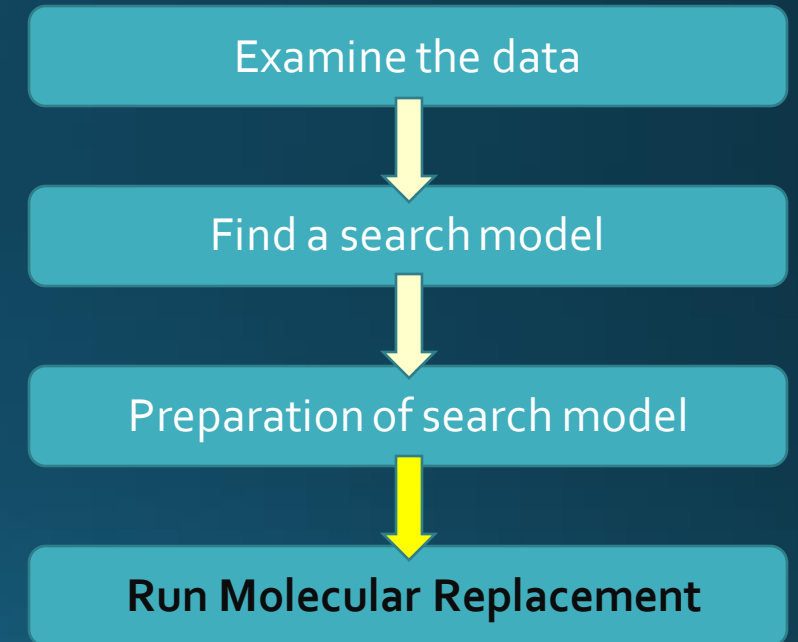
ECOD is a hierarchical classification of protein domains according to their evolutionary relationships. Only proteins with experimentally determined spatial structures from the [PDB](#) database are currently classified in ECOD. Compared with other classification databases, such as [SCOP](#) and [CATH](#), ECOD emphasizes on distant evolutionary relationships and updates every week to include new structures released in PDB. Due to the delay of PDB FTP release and time needed to classify new domains, ECOD usually falls two to three weeks behind the PDB. Next update is expected on or before 09/10/2020.

ECOD statistics (history)	
166,956	PDBs
551,431	PDB chains
773,538	Domains
2,460	X-groups
3,716	H-groups
15,218	F-groups



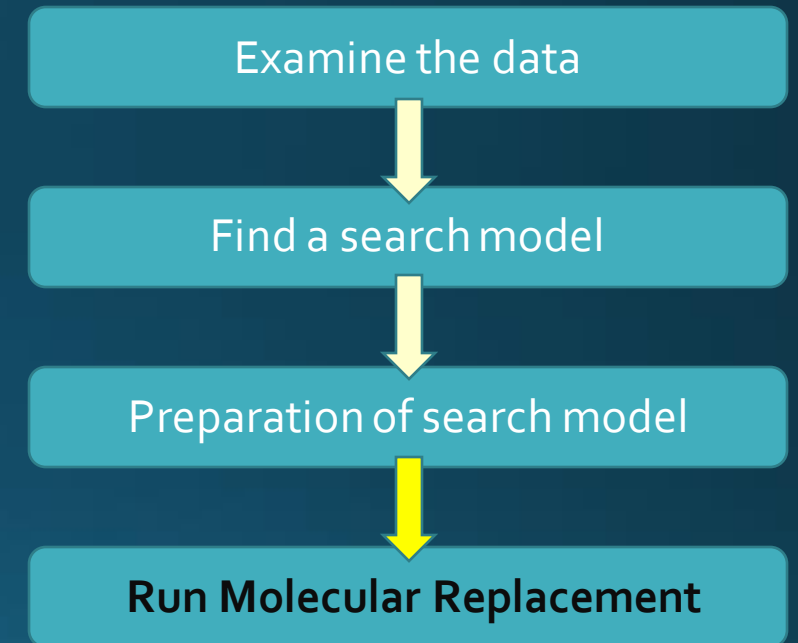
Step-by-Step MR: *Run Molecular Replacement*

- CCP4 has several programs for doing Molecular Replacement
 - Amore
 - Manual steps but very fast
 - Molrep
 - Automated MR
 - Several useful features e.g. searching a map
 - Phaser
 - Maximum likelihood approach
 - Accounts for potential model errors
 - Best for difficult cases and for correctly positioning fragment search models



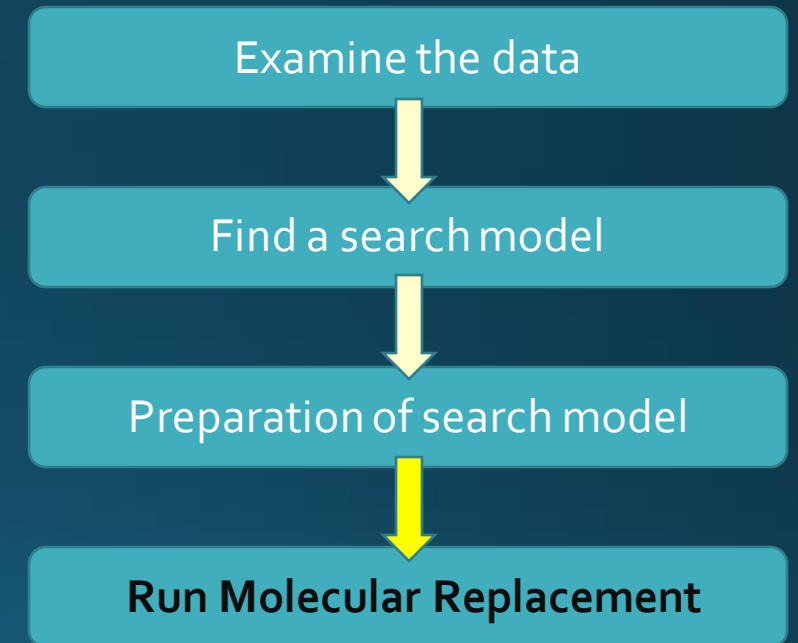
Step-by-Step MR: *Run Molecular Replacement*

- Important points on using Phaser



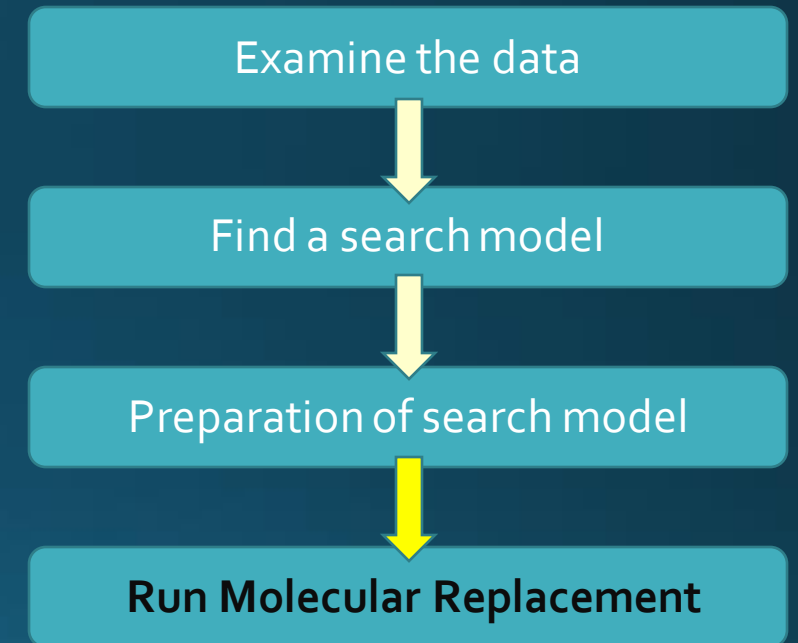
Step-by-Step MR: *Run Molecular Replacement*

- Important points on using Phaser
 - Phaser accounts for errors in:



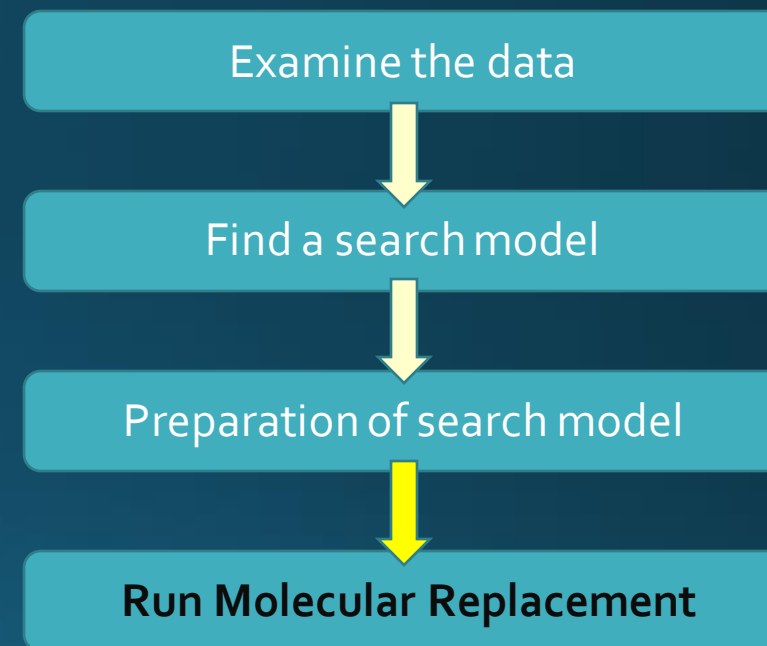
Step-by-Step MR: *Run Molecular Replacement*

- Important points on using Phaser
 - Phaser accounts for errors in:
 1. Model
 - Provide accurate details of AU composition
 - Use RMS value rather than sequence identity and try different values if first attempt doesn't work



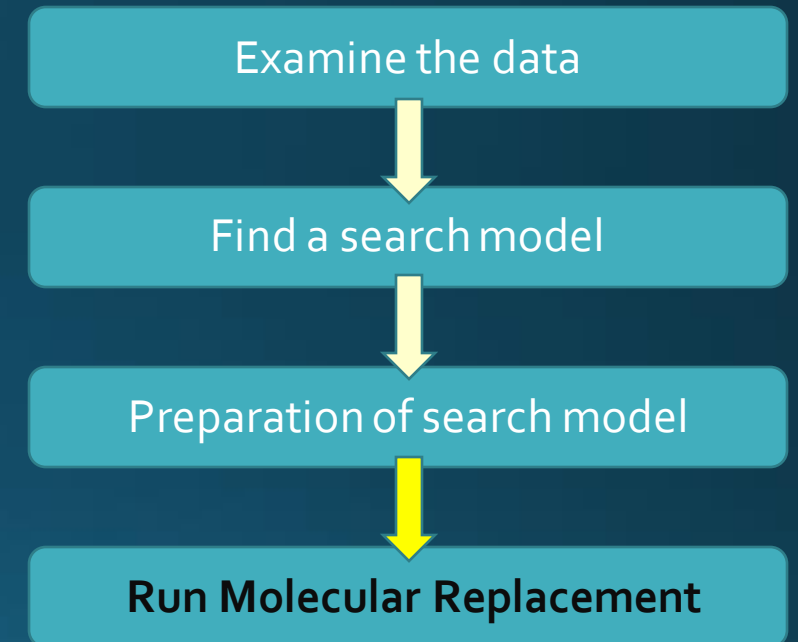
Step-by-Step MR: *Run Molecular Replacement*

- Important points on using Phaser
 - Phaser accounts for errors in:
 1. Model
 - Provide accurate details of AU composition
 - Use RMS value rather than sequence identity and try different values if first attempt doesn't work
 2. Data
 - Provide intensities – internally works out amplitudes accounting for experimental errors

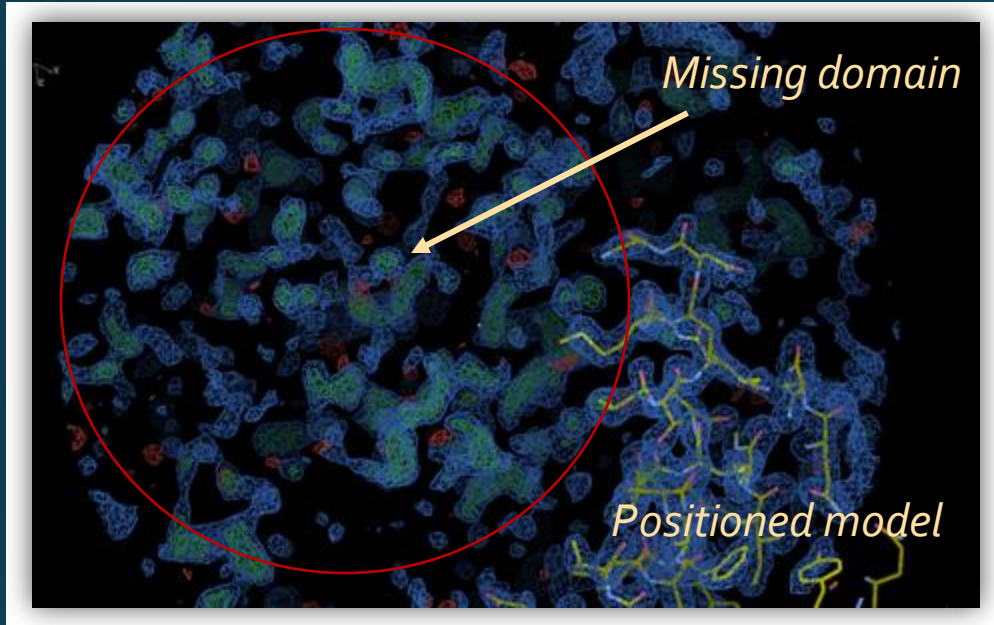


Step-by-Step MR: *Run Molecular Replacement*

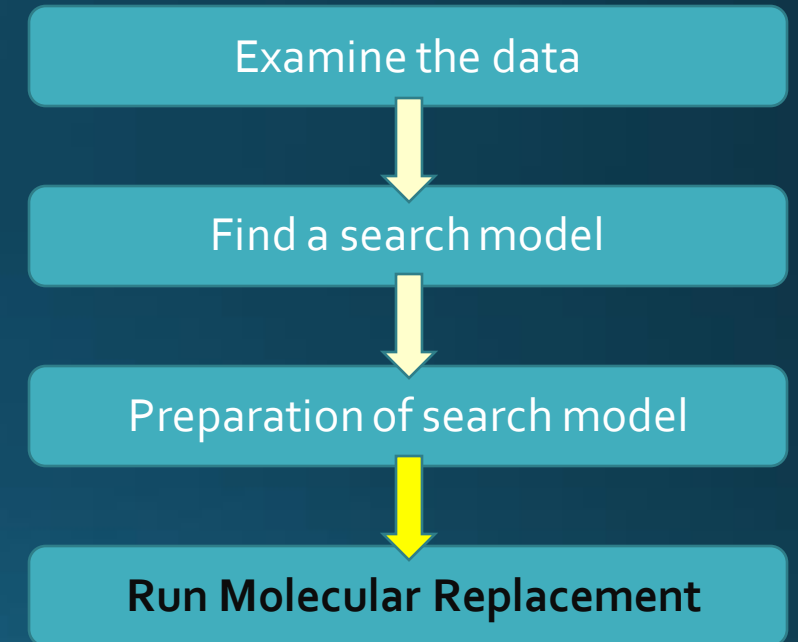
- Important points on using Phaser
 - Phaser accounts for errors in:
 1. Model
 - Provide accurate details of AU composition
 - Use RMS value rather than sequence identity and try different values if first attempt doesn't work
 2. Data
 - Provide intensities – internally works out amplitudes accounting for experimental errors
 - Phaser performs clever decision making for automation
 - Provide minimal details and let Phaser make it's own decisions e.g. search order, search all possible space groups
 - If it doesn't work take step-by-step approach – 1 copy at a time



Step-by-Step MR: *Run Molecular Replacement*



- Phased translation search
 - Used when searching for several copies or dealing with a complex
 - Available through MOLREP (3 protocols) and Phaser
 - Often more successful than standard MR search approach particularly when looking for small domains

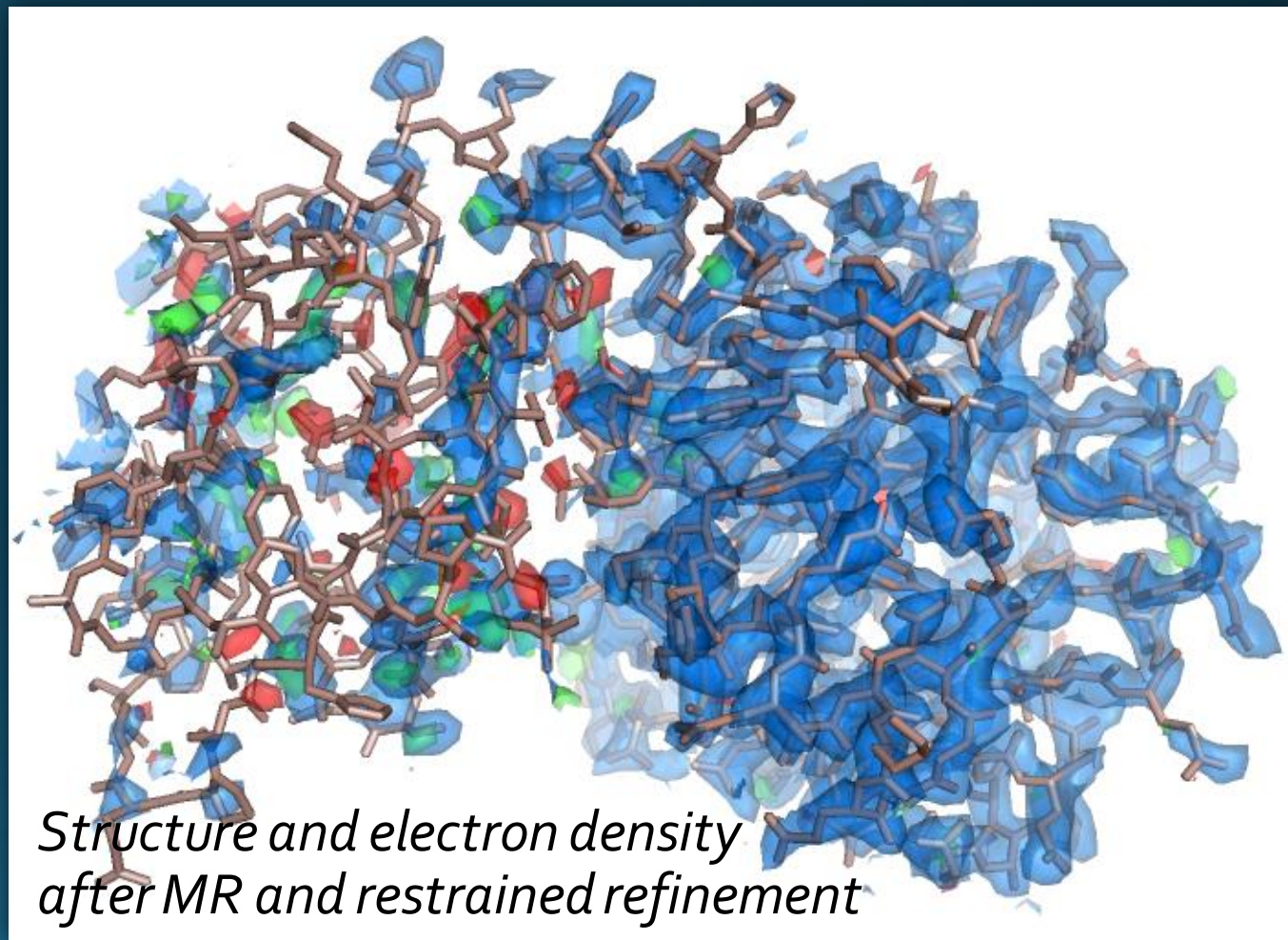


Step-by-Step MR: *Run Molecular Replacement*

Phased translation search:

Example: 1tj3

Search model: 1s20, chain A



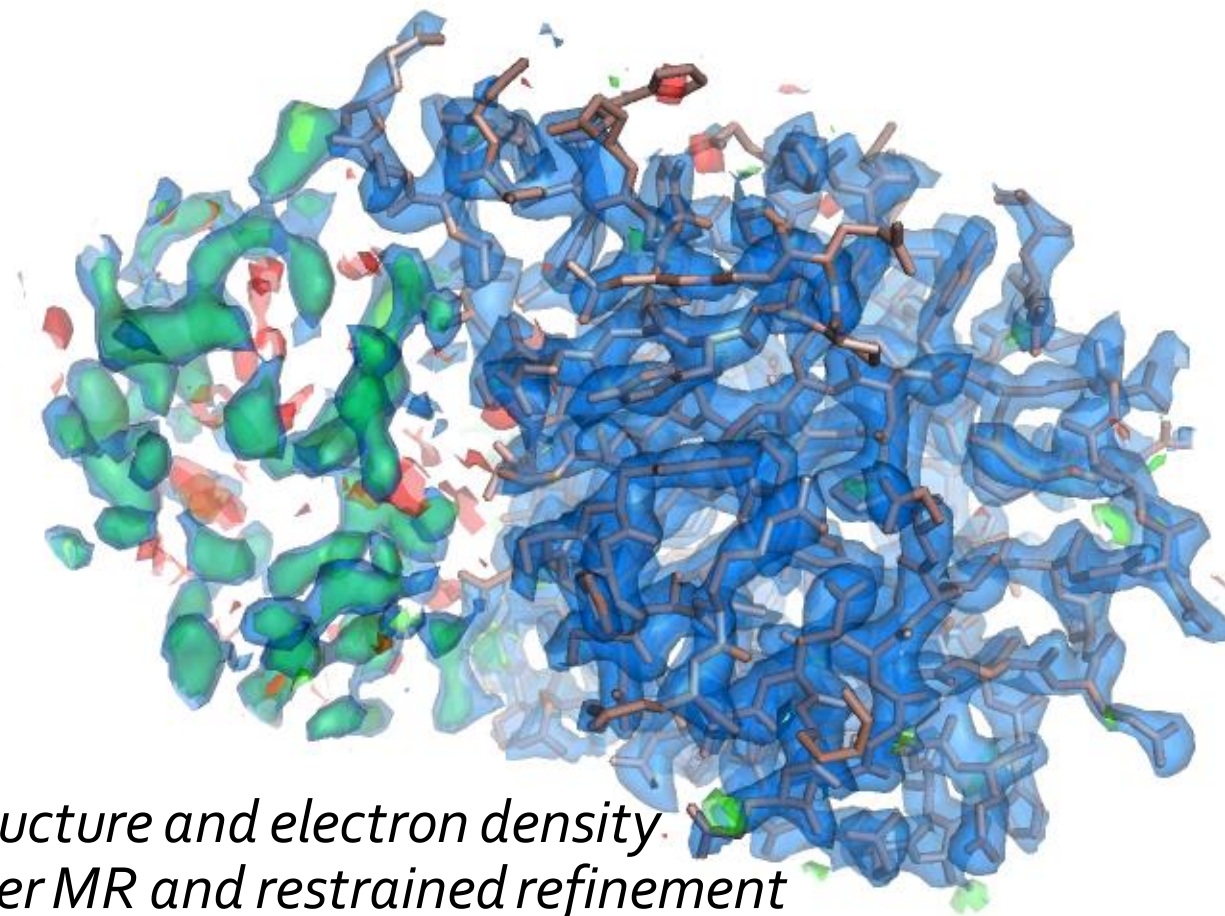
Step-by-Step MR: *Run Molecular Replacement*

Phased translation search:

Example: 1tj3

Search model: 1s20, chain A

Large Domain only



*Structure and electron density
after MR and restrained refinement*

Step-by-Step MR: *Run Molecular Replacement*

Phased translation search:

Example: 1tj3

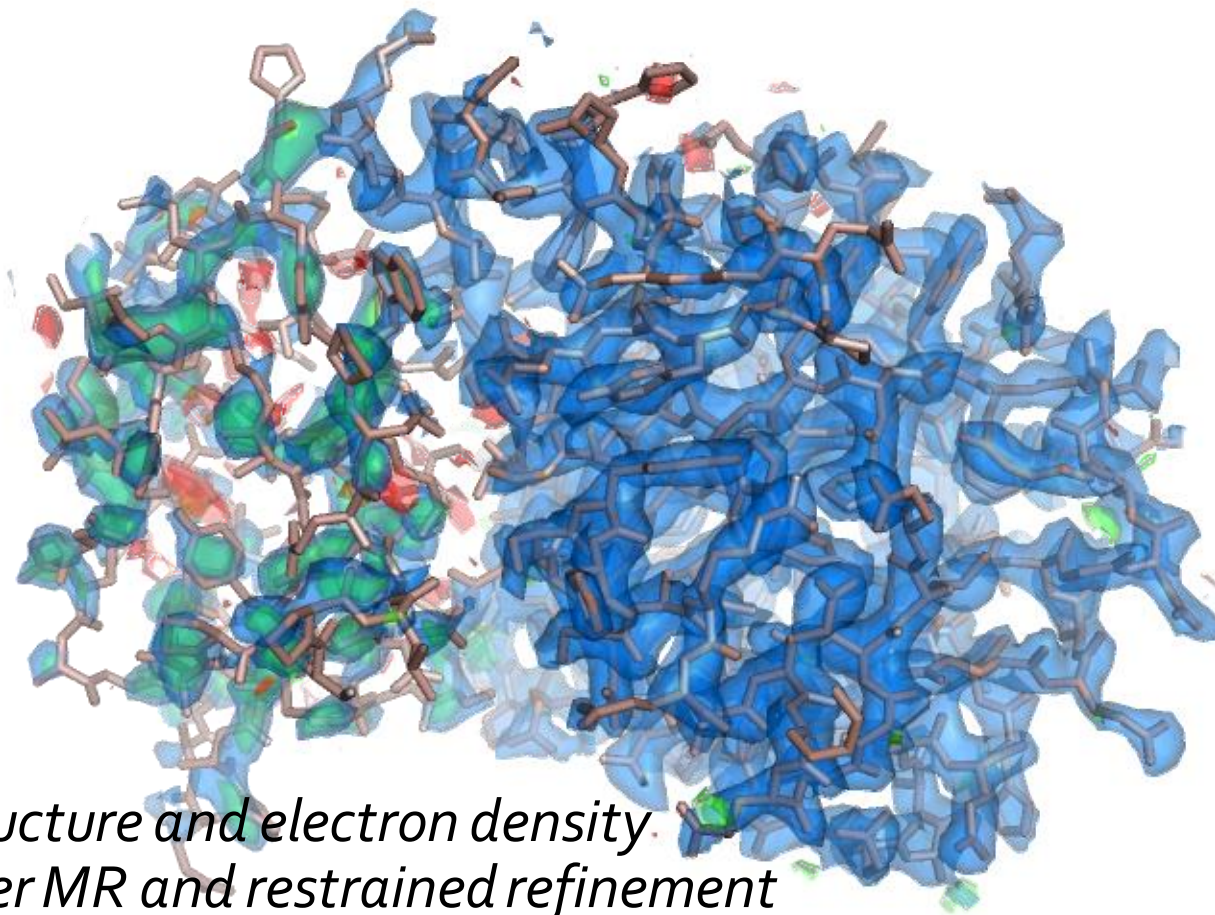
Search model: 1s20, chain A

Large Domain fixed

Small Domain search

Fit into density

(Molrep & Phaser)



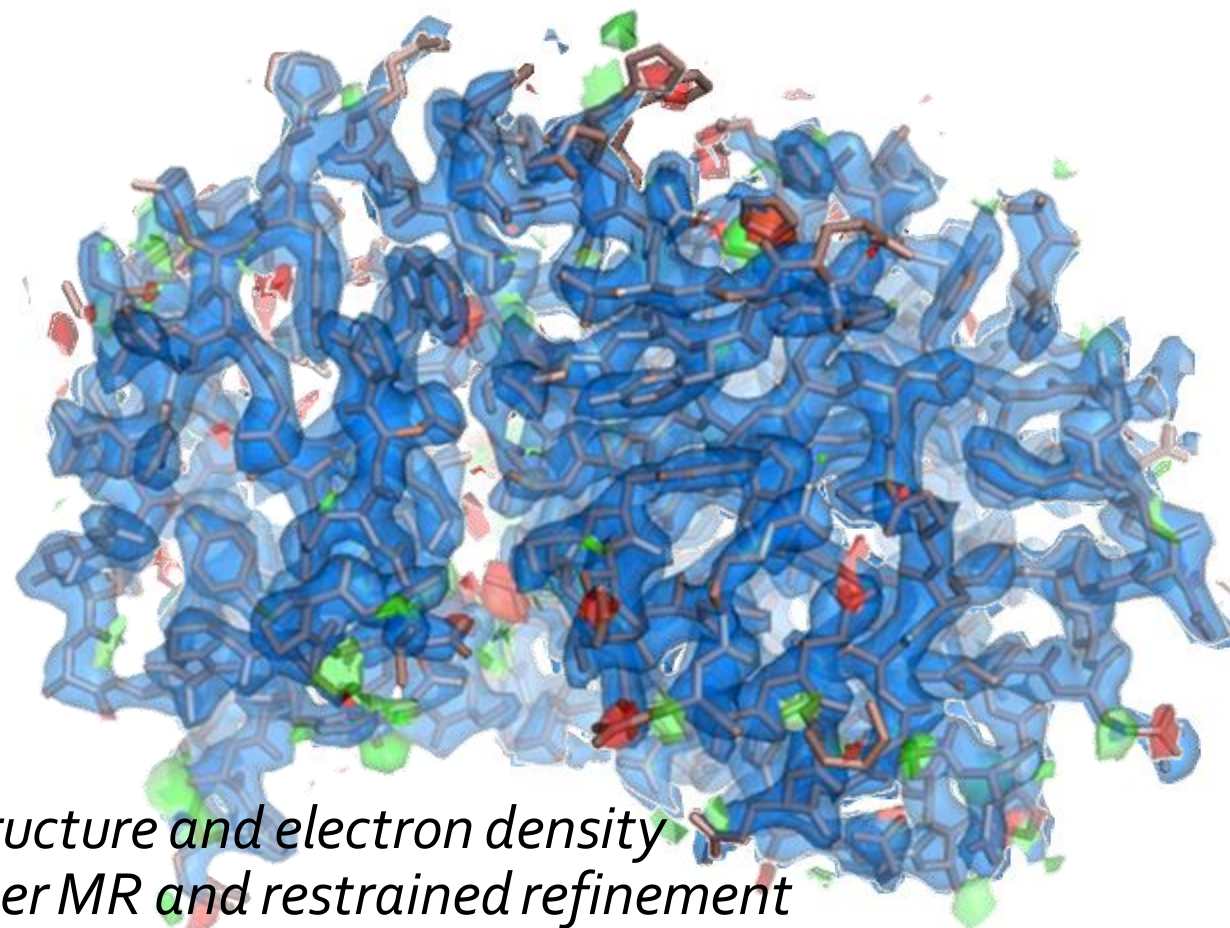
*Structure and electron density
after MR and restrained refinement*

Step-by-Step MR: *Run Molecular Replacement*

Phased translation search:

Example: 1tj3

Complete structure and
electron density after
restrained refinement

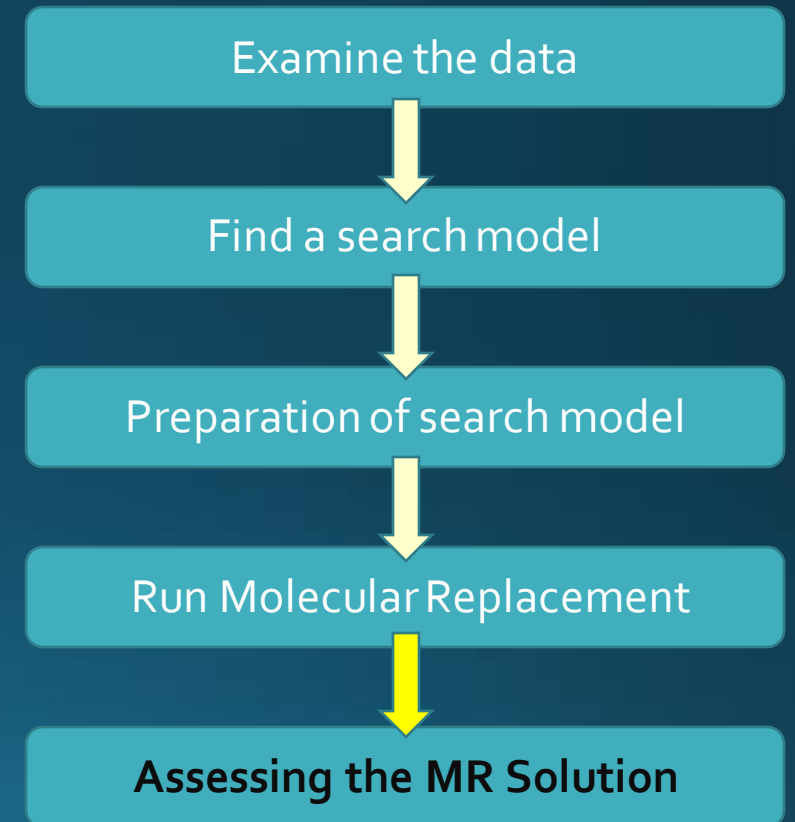


*Structure and electron density
after MR and restrained refinement*

How do I know my MR solution is successful?

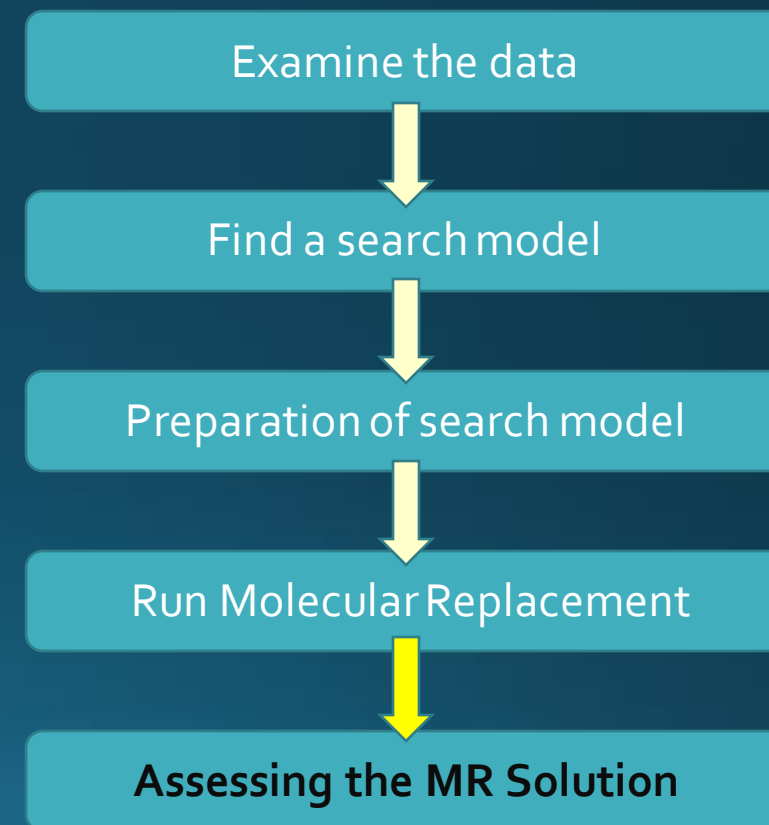
Step-by-Step MR: *Assessing the MR solution*

- Terminology
 - MR solution
 - Successful MR solution
- What is a successful MR solution?
 - A search model placed in the target unit cell such that it will provide us with sufficiently accurate phase estimates for the target
 - Enables us to complete the model through model building and refinement

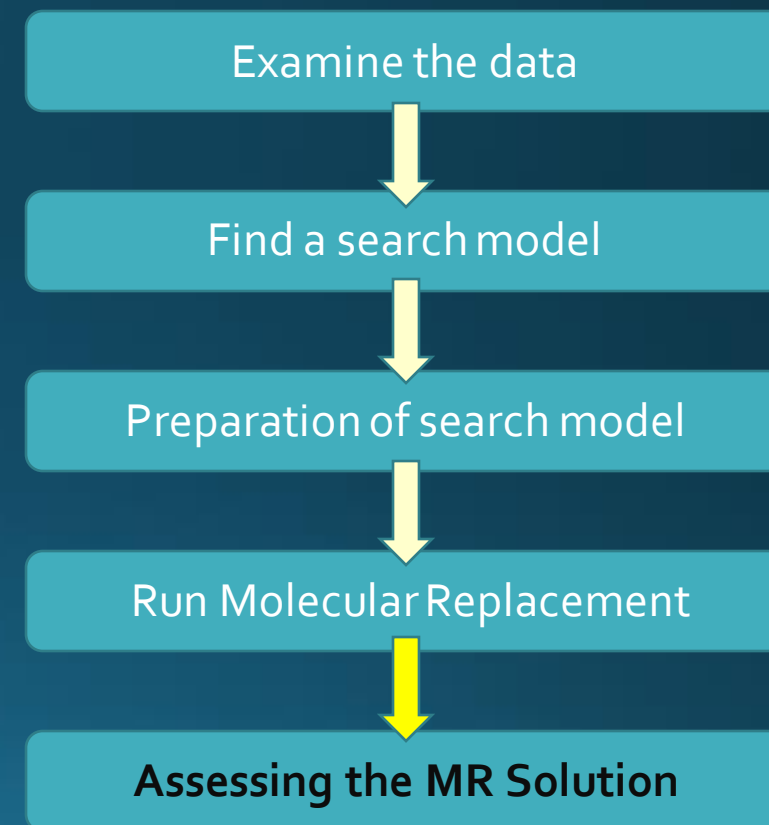
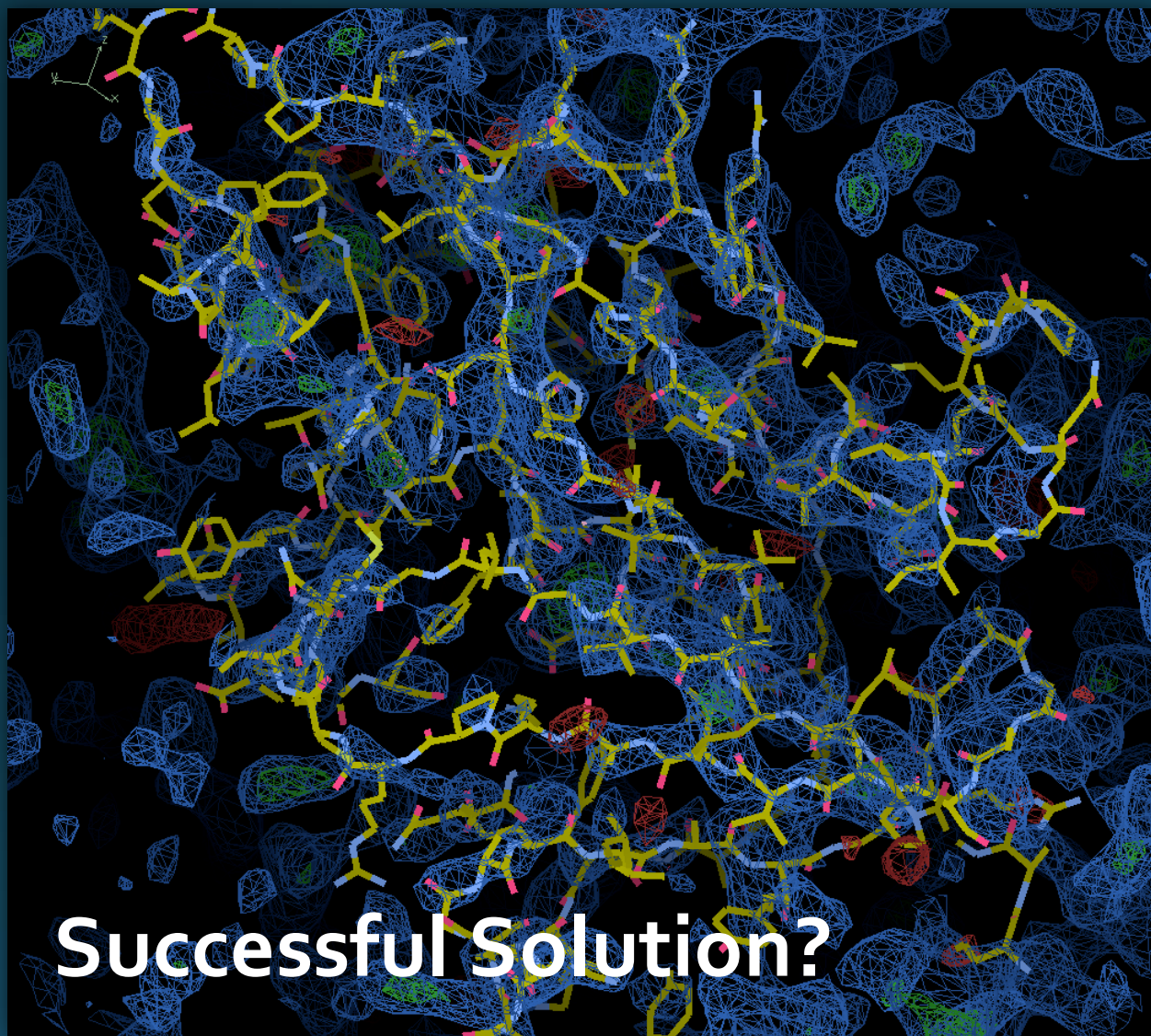


Step-by-Step MR: *Assessing the MR solution*

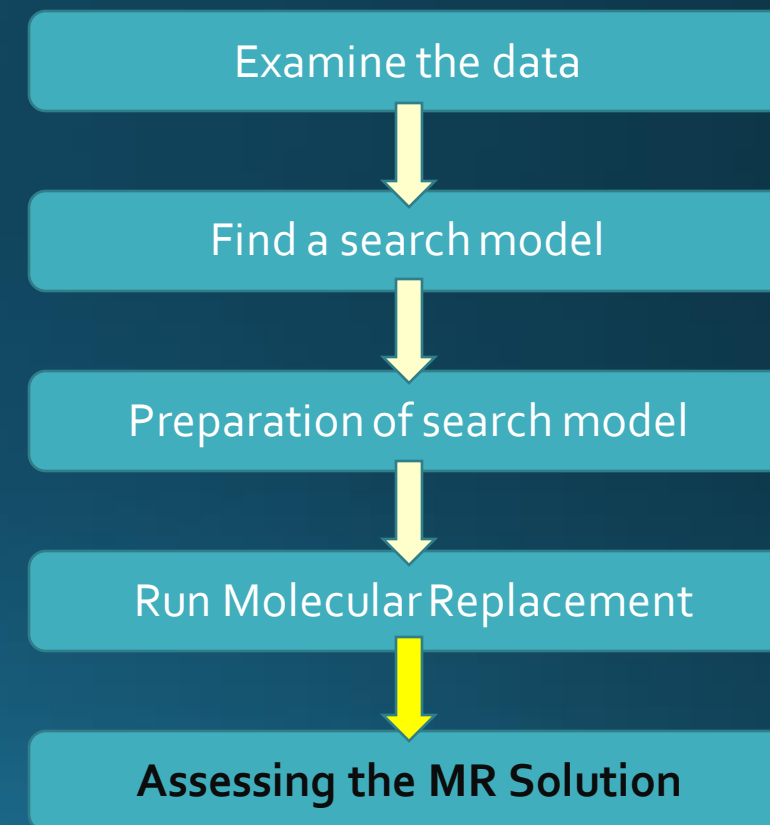
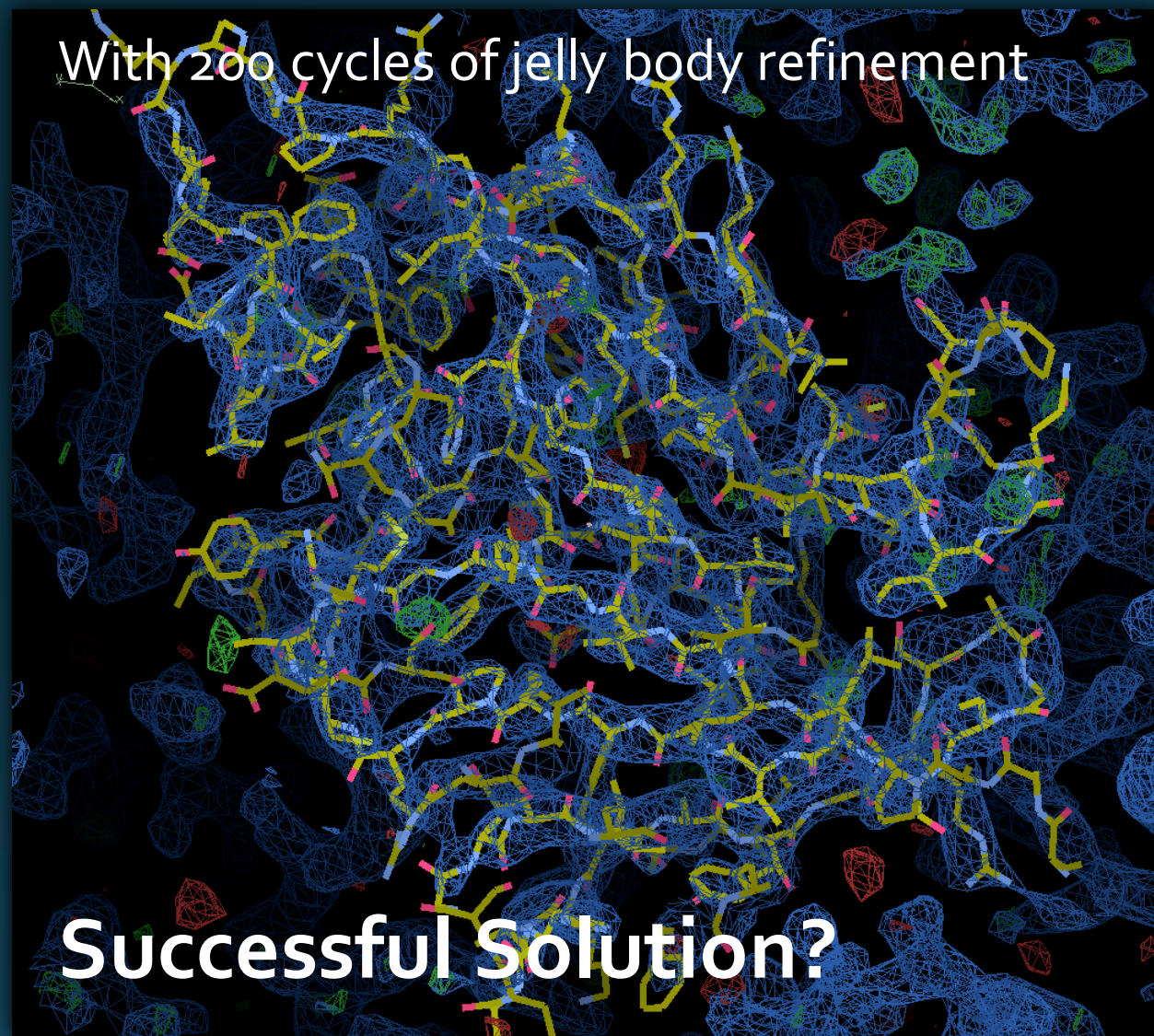
- In difficult cases the position may be correct but getting from the MR solution to a complete model may not be straight forward
- Assessment often involves performing additional structure solutions steps such as refinement and density modification



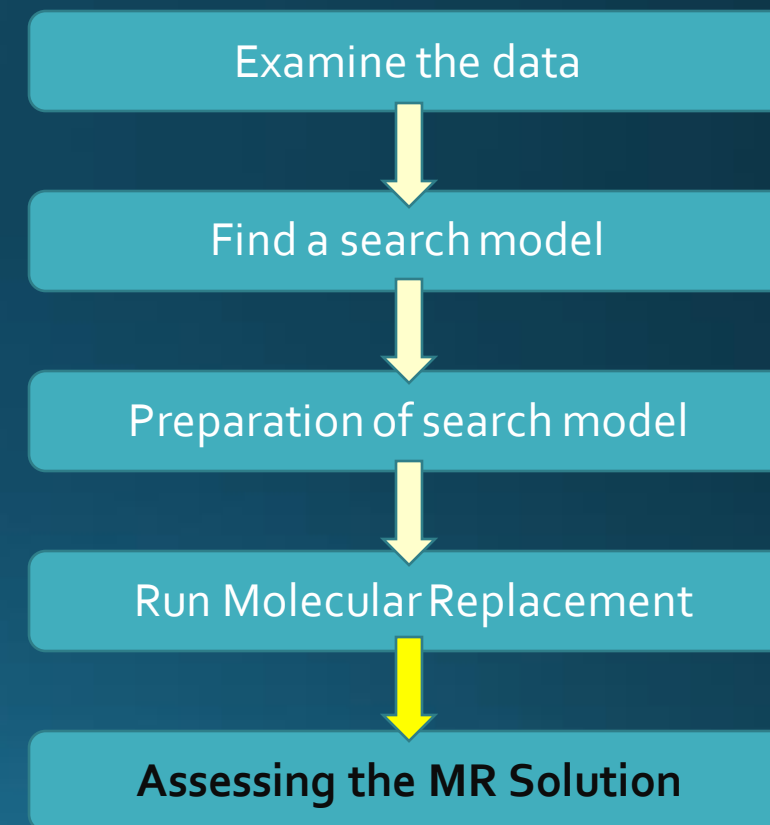
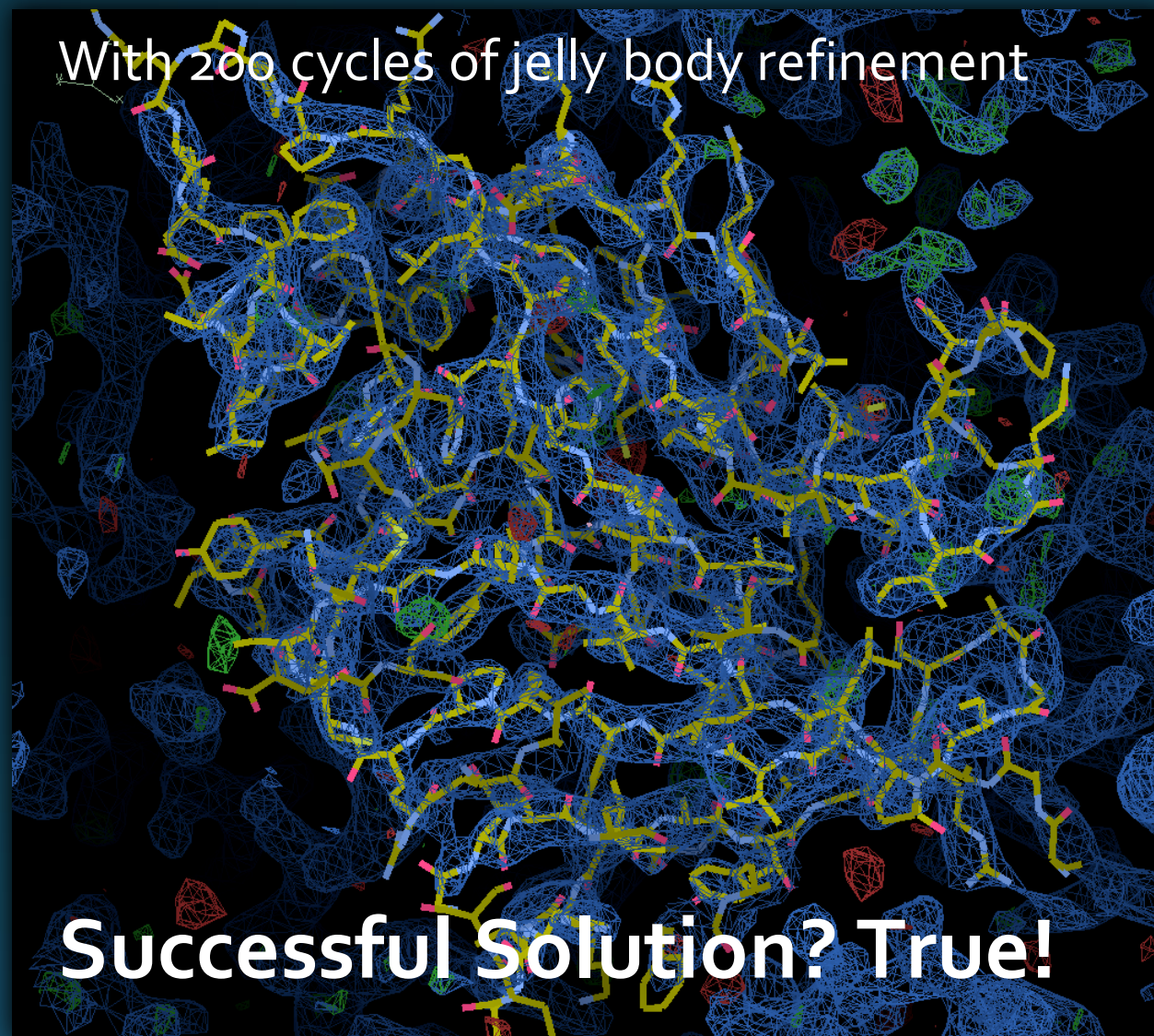
Step-by-Step MR: *Assessing the MR solution*



Step-by-Step MR: *Assessing the MR solution*

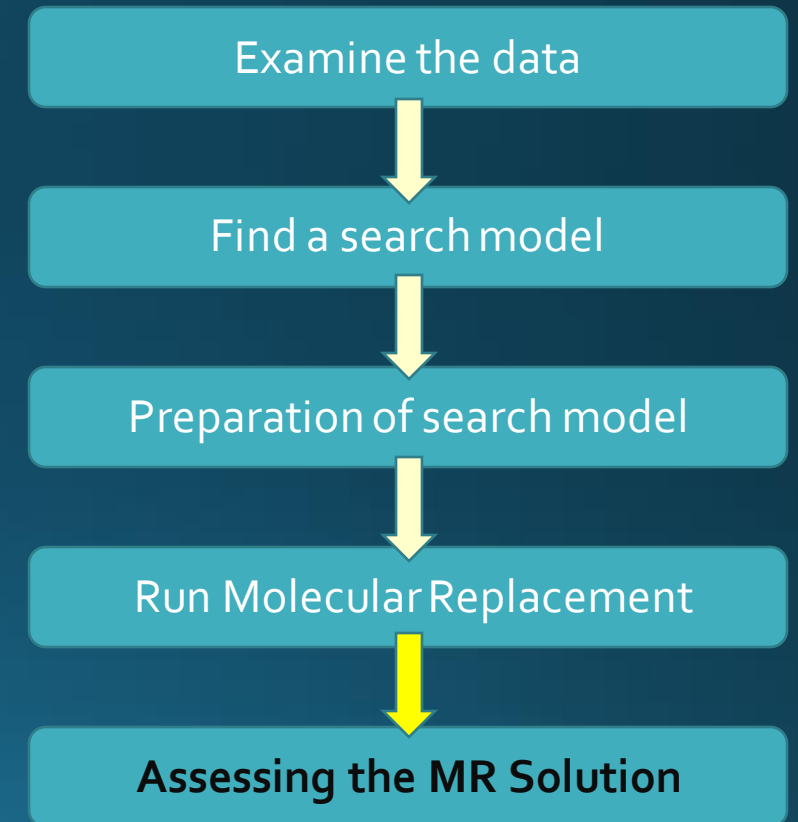


Step-by-Step MR: *Assessing the MR solution*



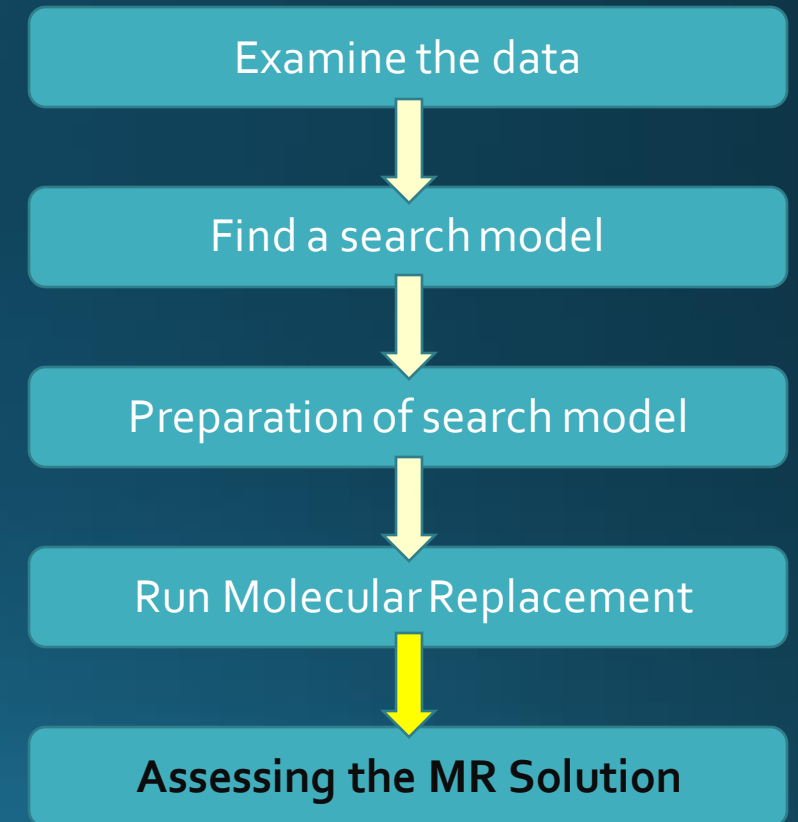
Step-by-Step MR: *Assessing the MR solution*

- Rough guide to MR program scoring



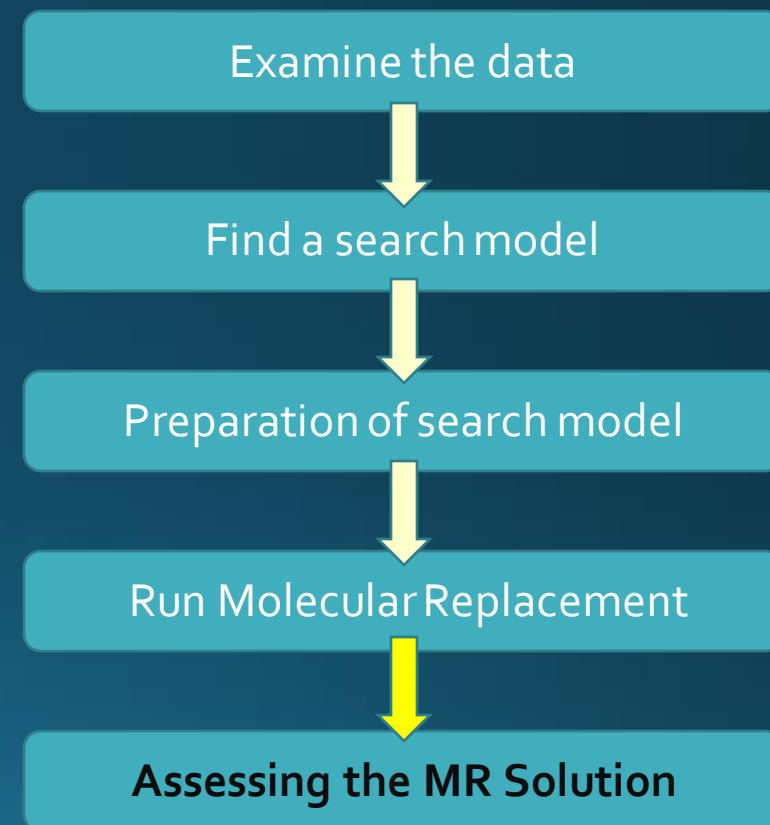
Step-by-Step MR: *Assessing the MR solution*

- Rough guide to MR program scoring
 - Phaser scores
 - LLG scores – has it increased by 60 or more after the placement of a new molecule?
(resolution and space group dependent)
 - TFZ – greater than 8?
 - Few or single solution almost always indicative of success



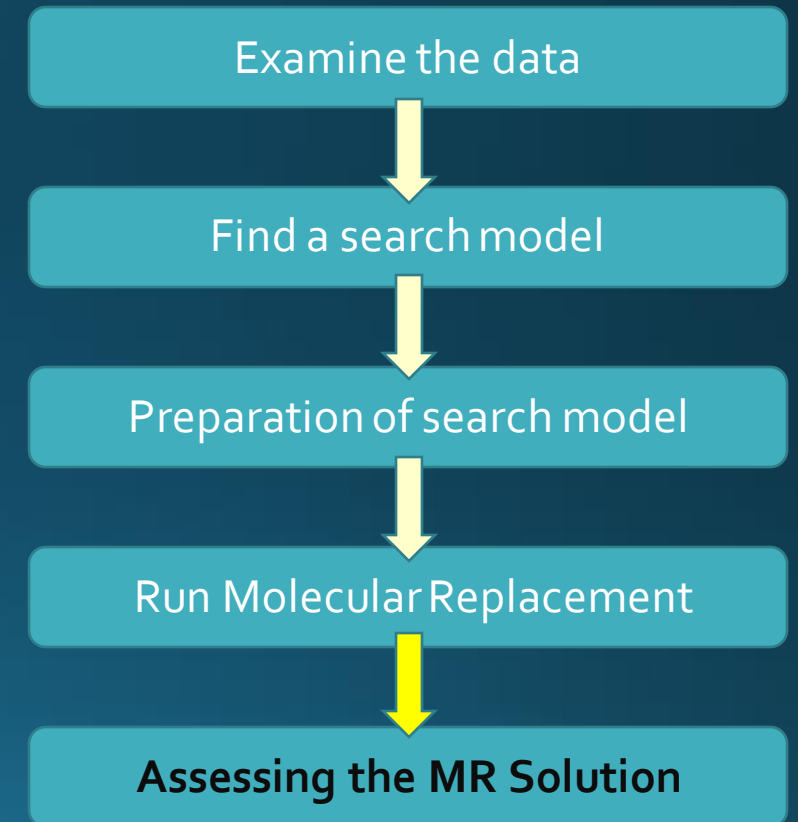
Step-by-Step MR: *Assessing the MR solution*

- Rough guide to MR program scoring
 - Phaser scores
 - LLG scores – has it increased by 60 or more after the placement of a new molecule?
(resolution and space group dependent)
 - TFZ – greater than 8?
 - Few or single solution almost always indicative of success
 - Molrep scores
 - RFZ – rotation search score greater than 5 – is there a clear peak?
 - TFZ – translation search score – is there a clear peak?



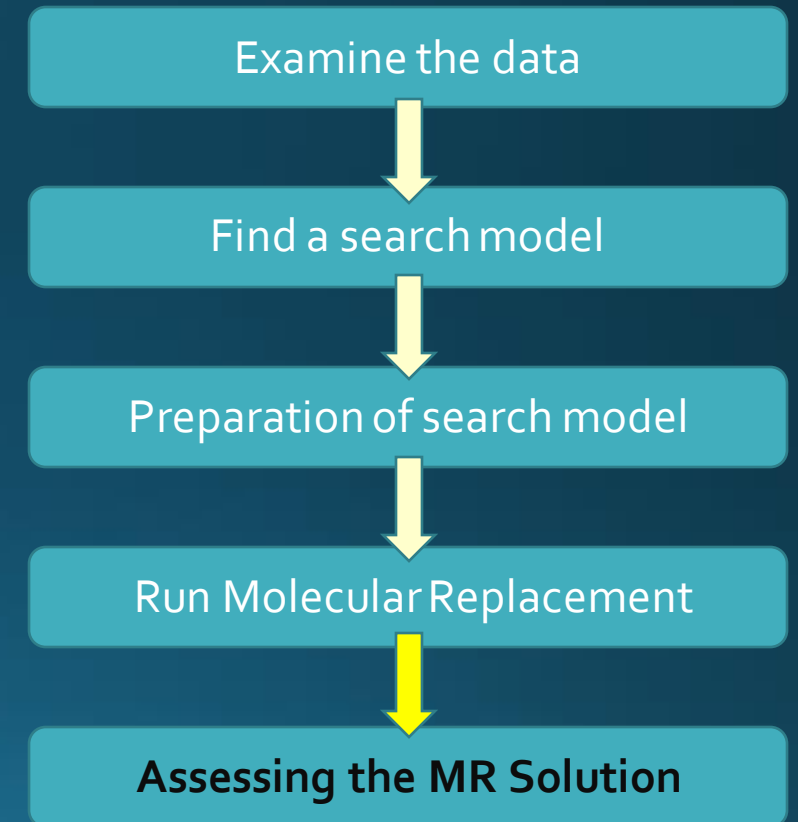
Step-by-Step MR: *Assessing the MR solution*

- Refinement



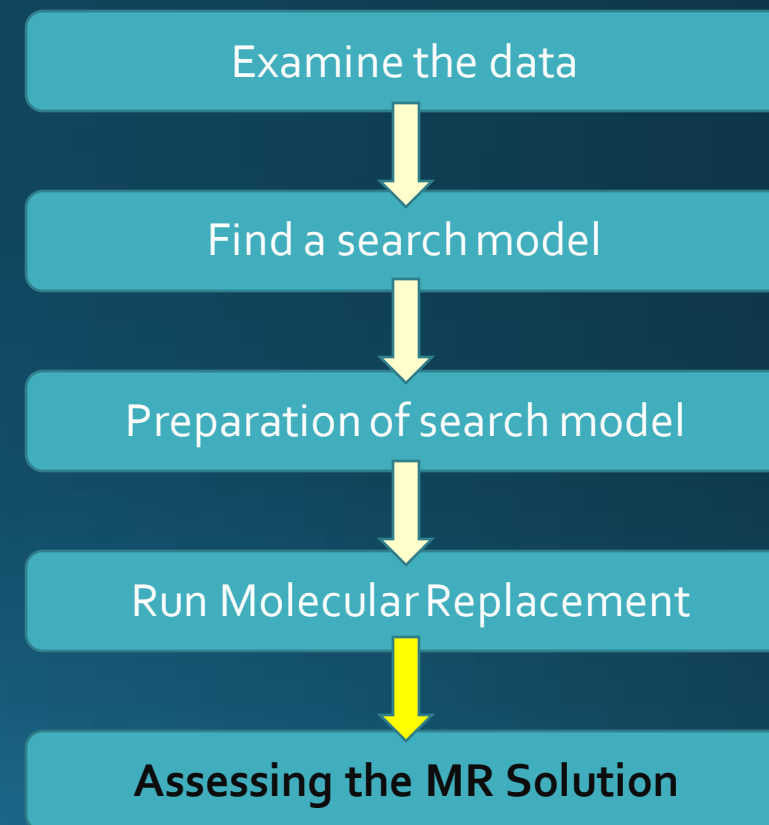
Step-by-Step MR: *Assessing the MR solution*

- Refinement
 - Look at Rfactor/Rfree
 - are they falling? Is Rfree below 0.5?



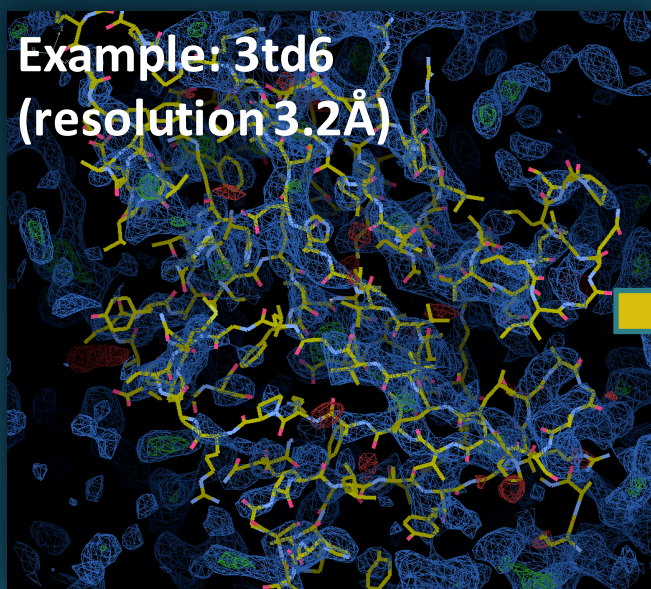
Step-by-Step MR: *Assessing the MR solution*

- Refinement
 - Look at Rfactor/Rfree
 - are they falling? Is Rfree below 0.5?
 - Use 200 cycles of jelly body refinement option in Refmac post MR

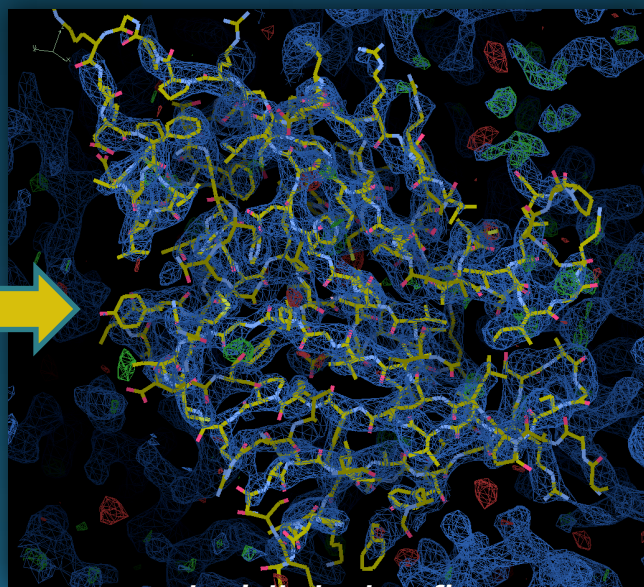


Step-by-Step MR: *Assessing the MR solution*

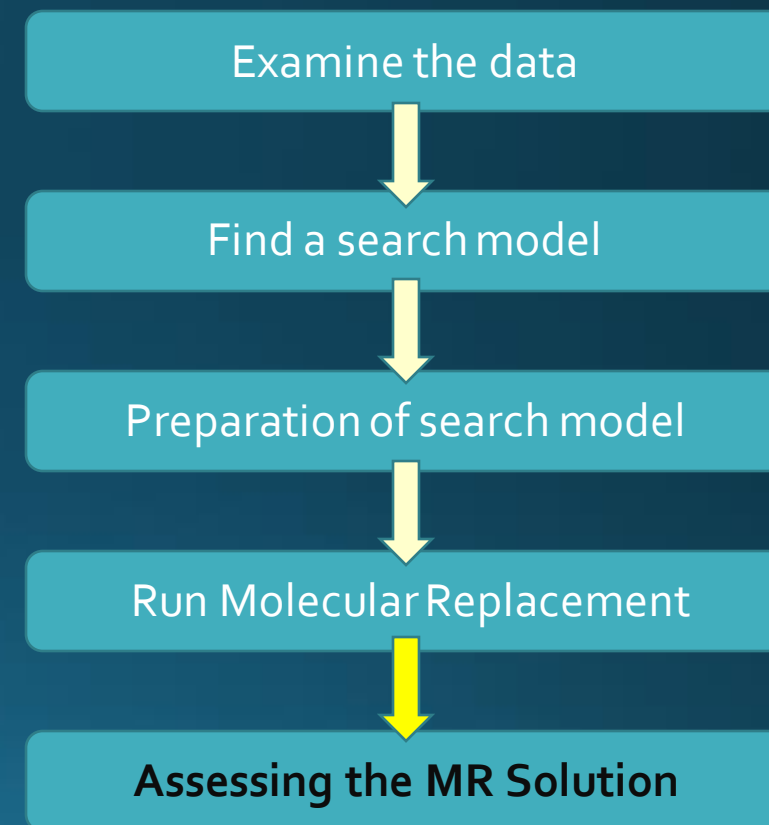
- Refinement
 - Look at Rfactor/Rfree
 - are they falling? Is Rfree below 0.5?
 - Use 200 cycles of jelly body refinement option in Refmac post MR



Phaser positioned model
R/Rfree=0.52/0.56
(model RMSD to target of 1.5Å)

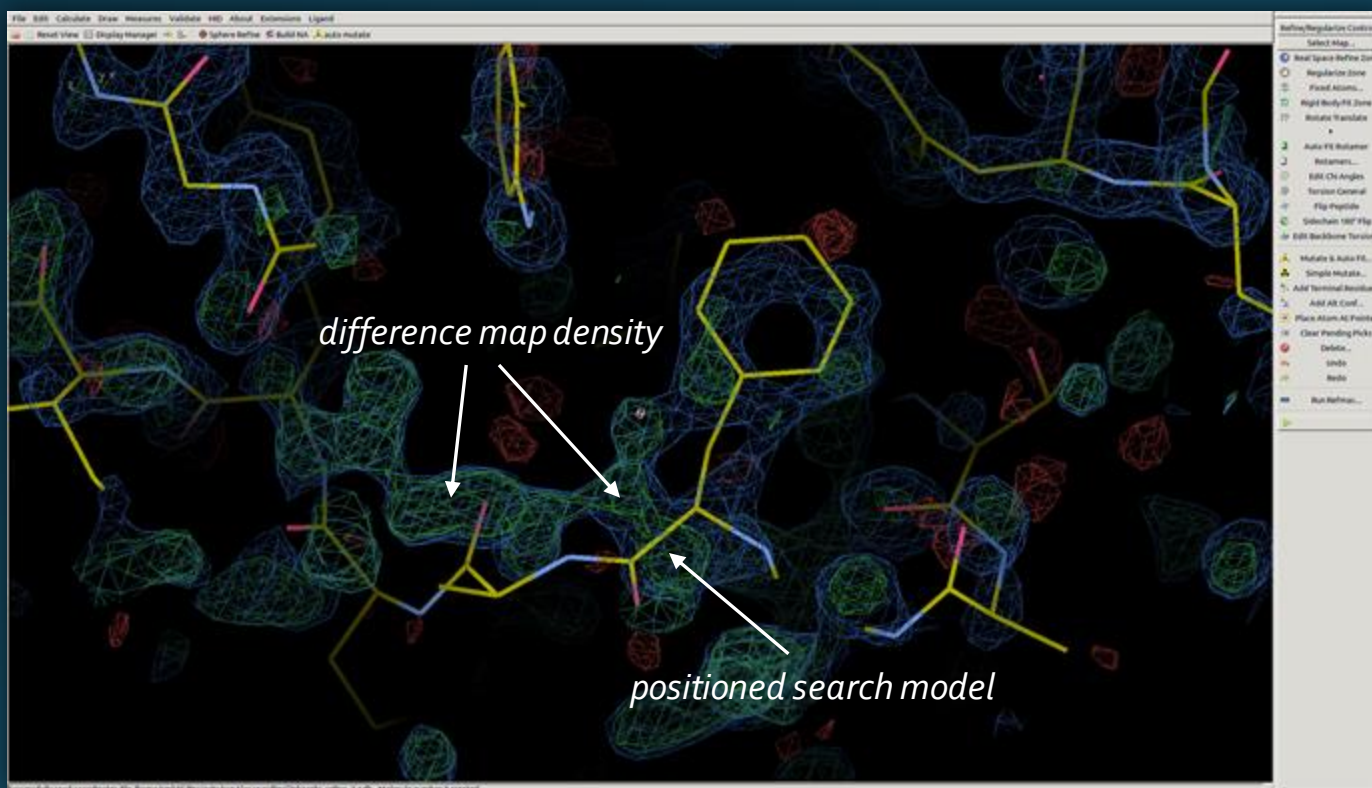


200 cycles jelly body refinement
R/Rfree=0.44/0.48
(model RMSD to target of 0.9Å)



Step-by-Step MR: *Assessing the MR solution*

- Examine solution by eye
 - Use Coot to examine positioned models & maps



Examine the data

Find a search model

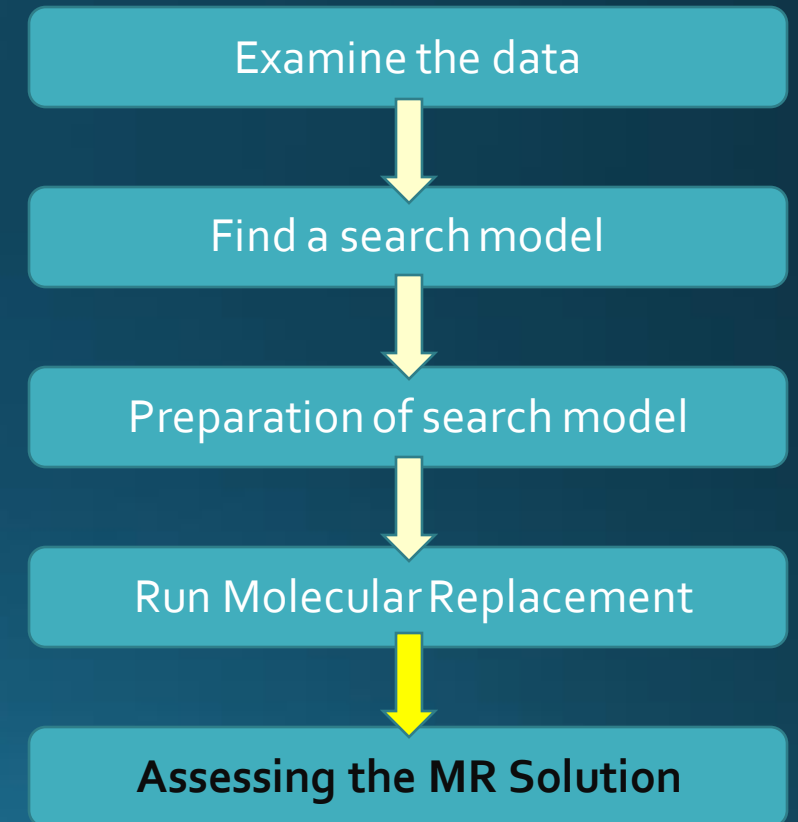
Preparation of search model

Run Molecular Replacement

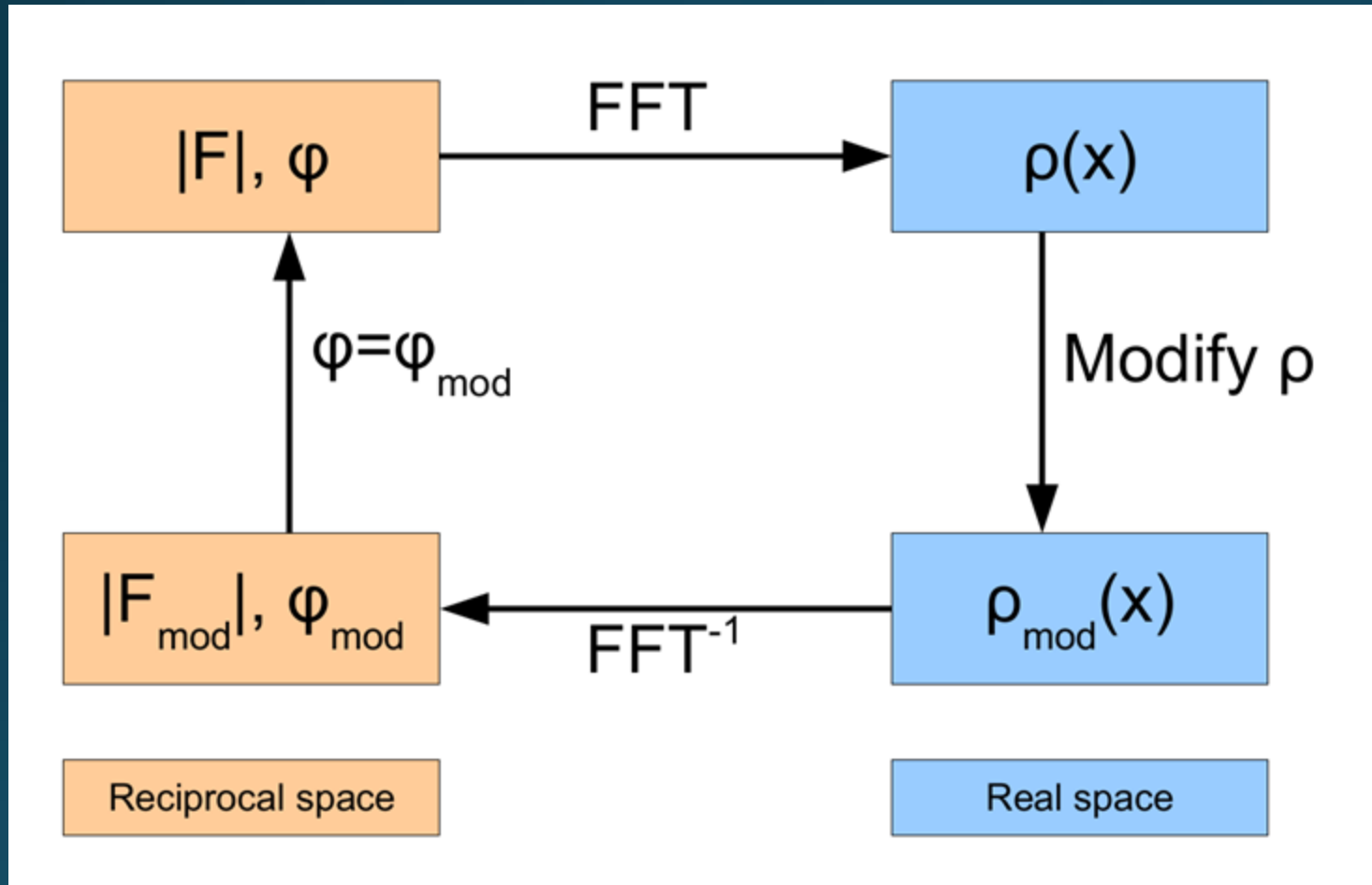
Assessing the MR Solution

Step-by-Step MR: *Assessing the MR solution*

- Density Modification & C-alpha tracing



Density Modification

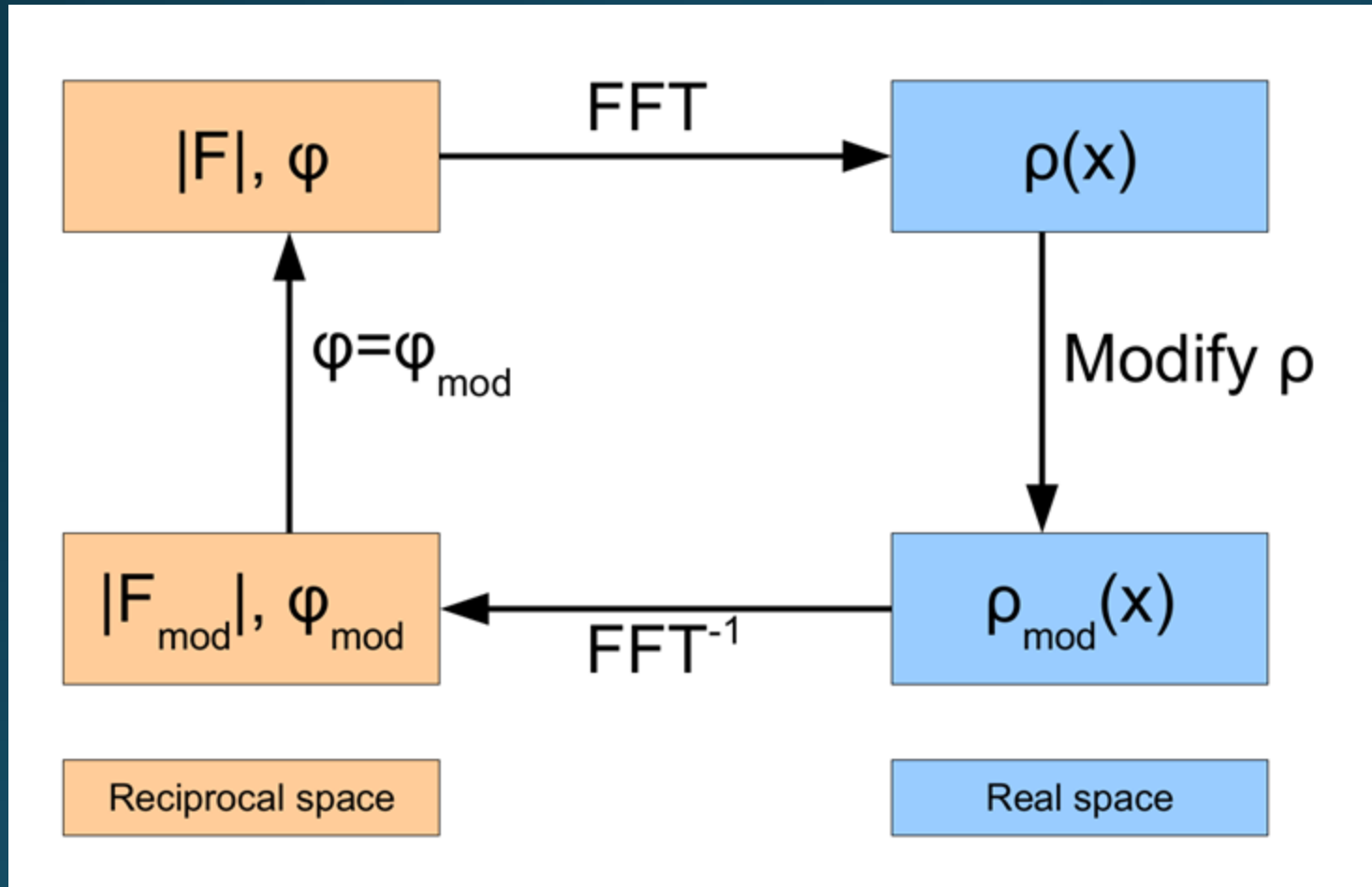


Main techniques:

1. Solvent flattening
2. Histogram matching
3. NCS averaging
4. C-alpha tracing

(slide from Kevin Cowtan)

Density Modification



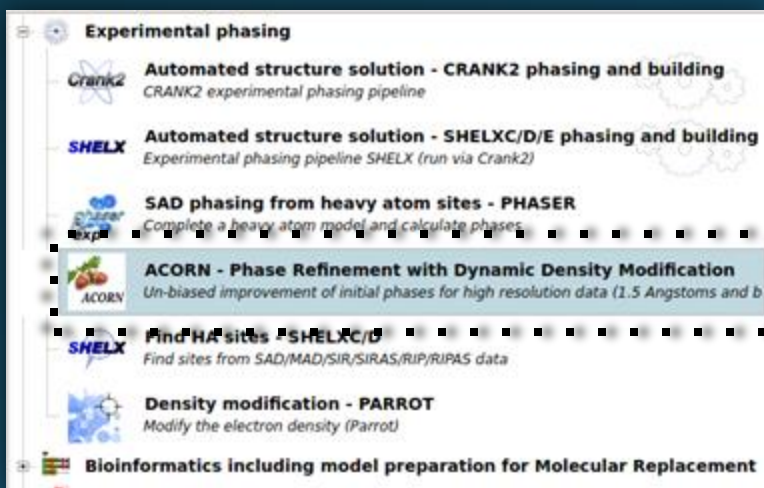
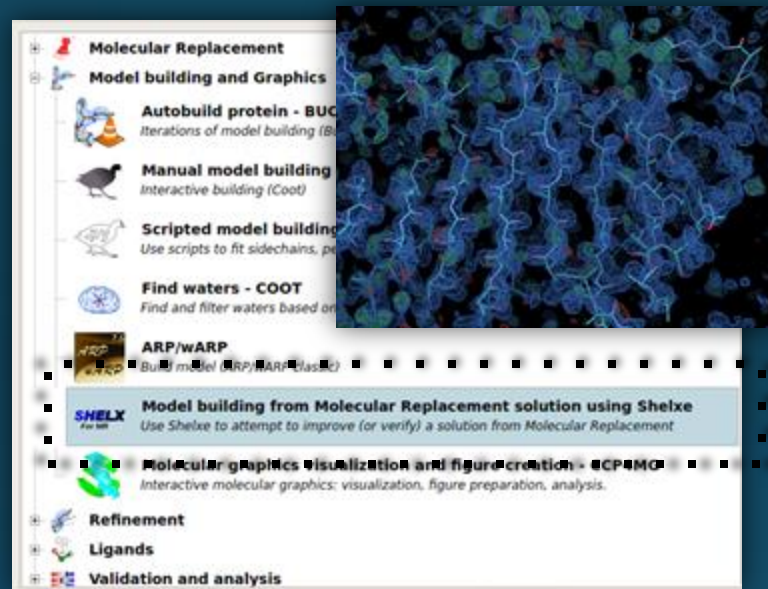
CCP4 Applications:

- Parrot
- SHELXE
- ACORN
- Pirate
- Solomon
- DM

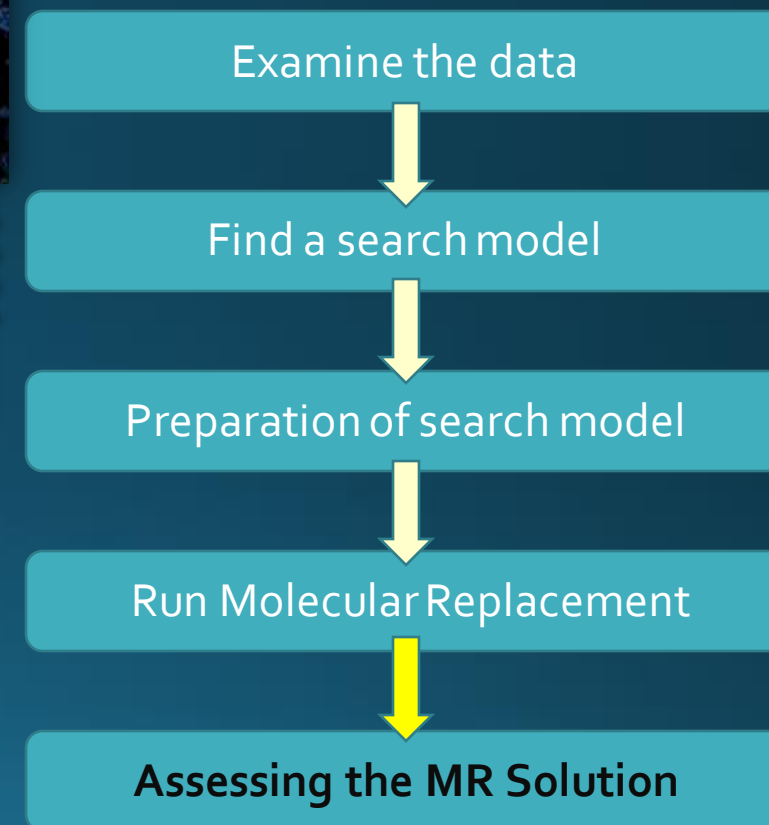
(slide from Kevin Cowtan)

Step-by-Step MR: *Assessing the MR solution*

- Density Modification & C-alpha tracing
 - SHELXE for MR (*resolution better than 2.5Å*)
 - CC better than 25%

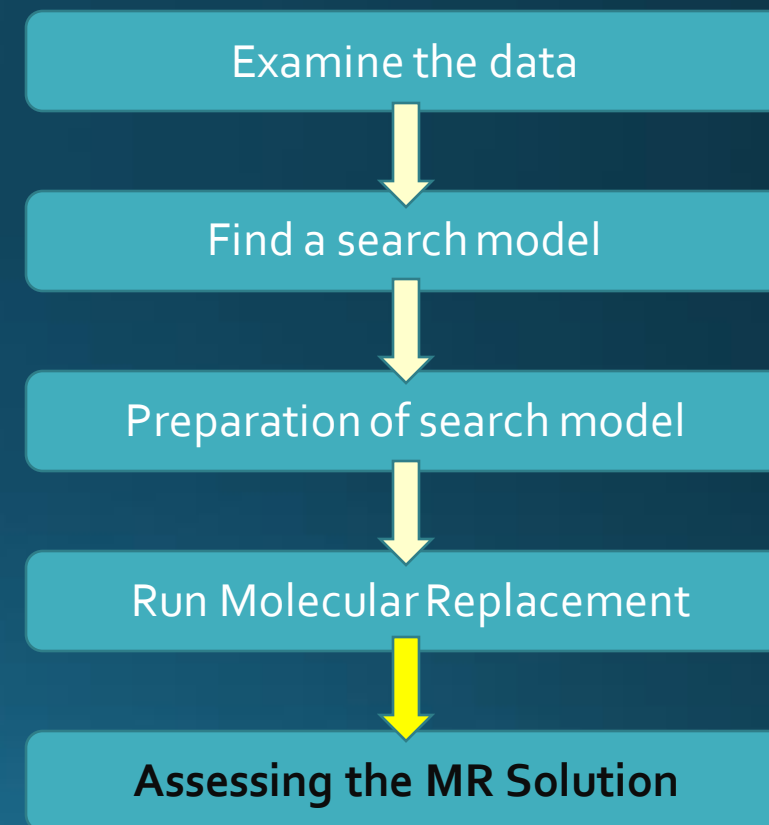


- ACORN (*resolution better than 1.7Å*)



Step-by-Step MR: *Assessing the MR solution*

- Automatic model building: Buccaneer & ARP/wARP
 - Can be used post-MR for generation of better model and phases for the target
- Rebuilding parts that may not be present in search model
- Useful for assessing whether or not your positioned MR model is true – eliminates bias

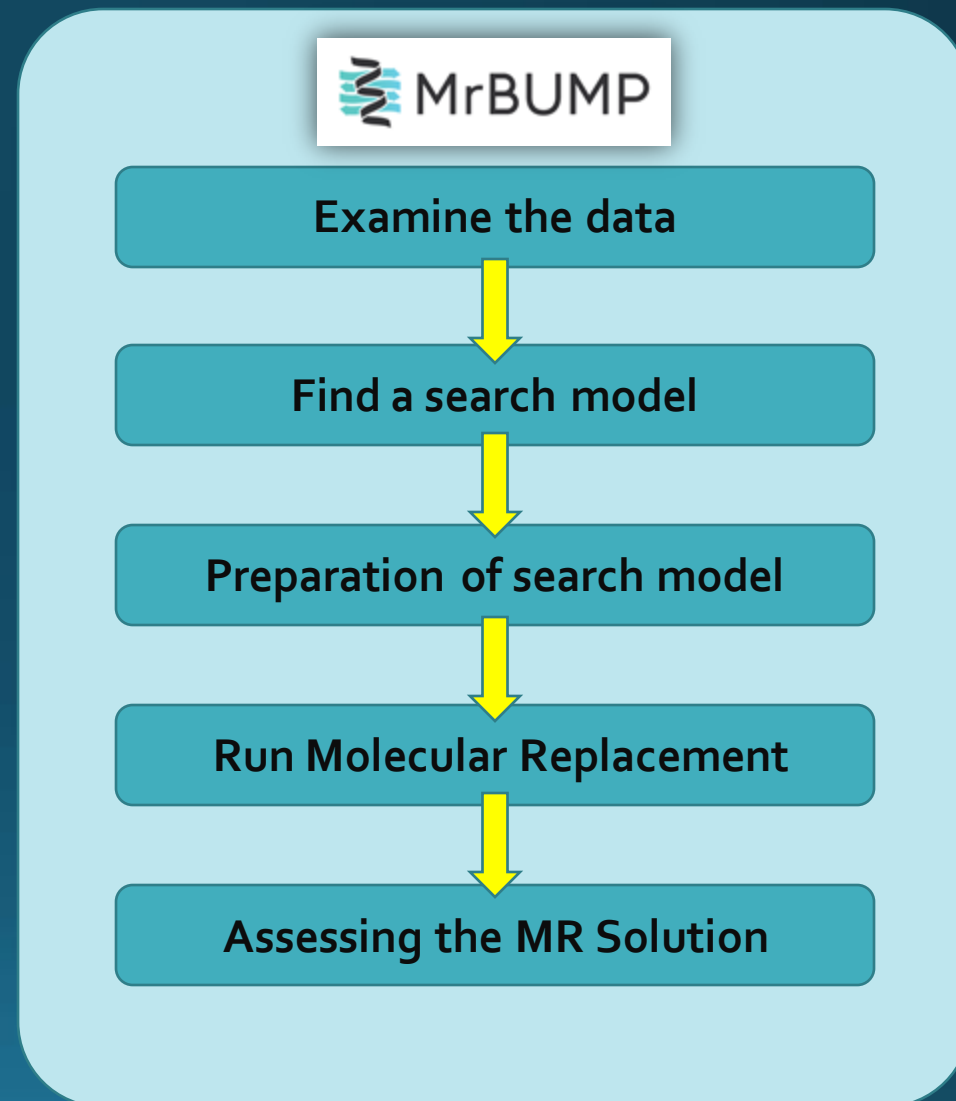


Automated Molecular Replacement in CCP4

- Several automation pipelines for MR in CCP4:
 - *MrBUMP* – model search, preparation, MR and refinement
 - *BALBES* – model search in custom version of PDB database
 - *MoRDa* – similar to BALBES

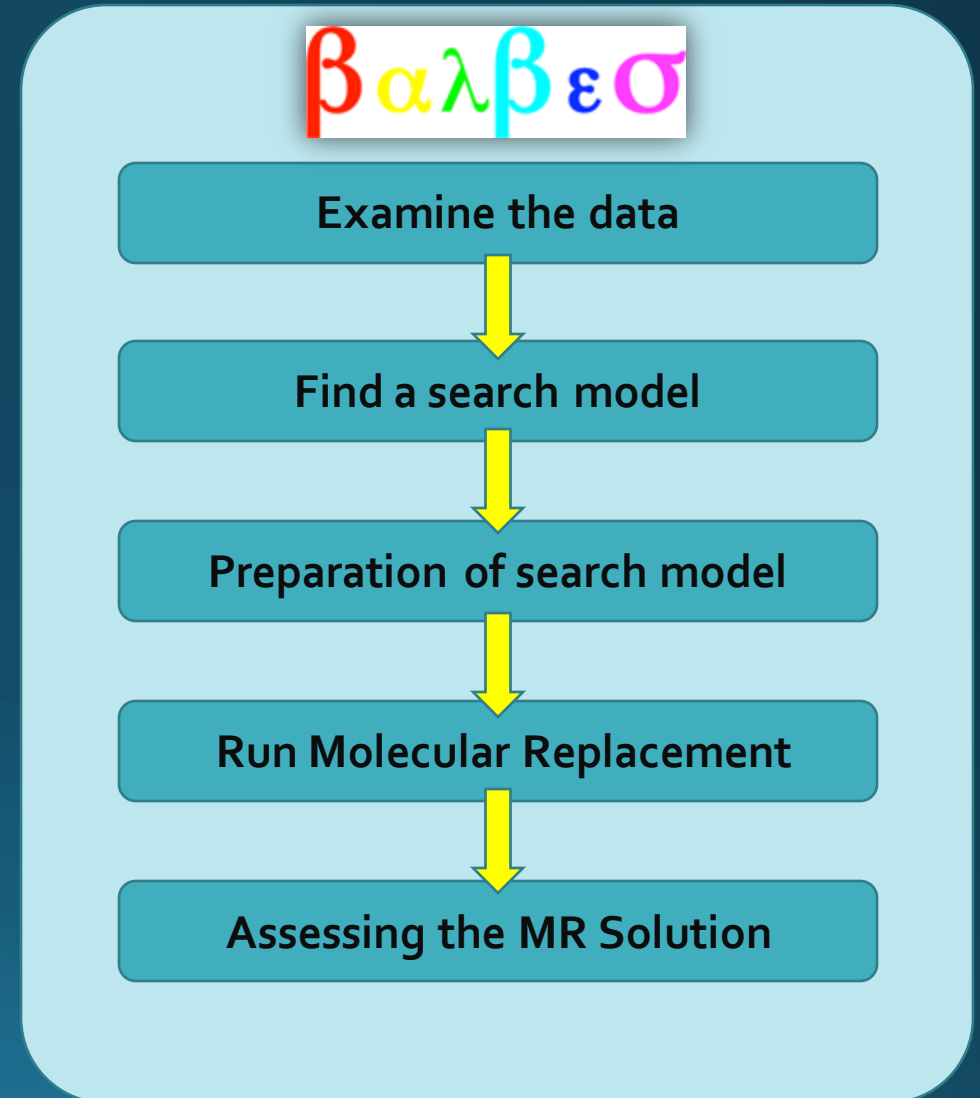
MrBUMP

- Covers all stages from data examination through to initial model building
- Two modes:
 1. Model generation only
 2. Model generation and Molecular Replacement through to model building
- Can be run from:
 - CCP4i2, CCP4 Cloud & CCP4 Online
 - CCP4mg – model generation step with graphical view



BALBES/MoRDa

- Covers all stages from data examination through to refinement
- Custom database:
 1. Non-redundant version of PDB
 2. Basic entry is a domain with definitions for constructing full chains, multimers and ensembles
- Other features:
 - Search all spacegroups
 - Uses Molrep for MR and Refmac for refinement
 - Available in CCP4i2, CCP4 Cloud & on CCP4 Online



CCP4 Online: *MrBUMP*, *BALBES*, *MoRDa* & more

CCP4 on-line Collaborative Computational Project No. 4 Software for Macromolecular X-Ray Crystallography

Home (Logout) > Login > Programs > MrBUMP > New MrBUMP Run Username: testUser

New MrBUMP Run

The file formats accepted for input are **mtz** and **cif** (structure factors) and **FASTA** (sequence target).

Structure Factors: No file chosen

Sequence Target: No file chosen

Instead of entering a Sequence Target file you can paste your **FASTA** sequence below:
(Note that a comment line beginning with a ';' character must precede each sequence)

I have the SHELX licence (optional): ☐ (You can get the licence from the [SHELX website](#).)

(after clicking submit, PLEASE WAIT for your files to upload - this may take some time)

Monomer 1		Monomer 2	
F	E	F	E
Protein	Protein		
X, Y, Z	X, Y, Z		
1_555	1_555		
232	7.3%	221	6.9%
1925	60.5%	1921	60.4%
3184	100.0%	3183	100.0%
66	16.1%	65	15.9%
380	92.9%	385	94.4%
409	100.0%	408	100.0%
2146.6	9.6%	2345.5	10.6%
2253.4	100.0%	22072.6	100.0%
-331.9		-334.3	
-7.5		-5.0	

Interface parameters

Parameter	Value
IA : Interface area, Å²	2246
DG : Solvation Energy, kcal/mol	-12.46
BE : Total Binding Energy, kcal/mol	-26.65
PV : Hydrophobic P-value	0.3542
HB : Number of Hydrogen Bonds	11
SB : Number of Salt Bridges	25
DS : Number of Disulphide Bonds	0

CCP4 STFC Rutherford Appleton Laboratory, Harwell Campus
Didcot Oxon OX11 0FA
Tel: (+44) 1235 567726 | Fax: +44 (+44) 1235 567720 | Email: ccp4@stfc.ac.uk

[About](#) | [Legal Statements](#)

<http://www.ccp4.ac.uk/ccp4online>

MR on the CCP4 Cloud

- Excellent interface for tackling difficult MR problems
- Explore broad range of search models, preparation protocols and refinements
- Exploit vast computational resources

The screenshot displays the CCP4 Cloud web interface. The main panel shows a project named 'venom2019' with a list of processing steps, including importing sequences, ensemble preparation, phasing, and refinement. A sidebar on the left contains icons for various functions. A pop-up window titled '[0005] phaser MR' shows 'Job completed' and options to export or view logs. Another window titled '0005-02_phaser-mr Structure and electron density' displays a 3D molecular model with a blue mesh representing the electron density map. The bottom of the interface includes logos for CCP4 on-line, Science & Technology Facilities Council, and DBSRC, along with the version number 'CCP4 Cloud v.1.4.001 [07.10.2019]'.

<https://cloud.ccp4.ac.uk>

What to do if Molecular Replacement doesn't work

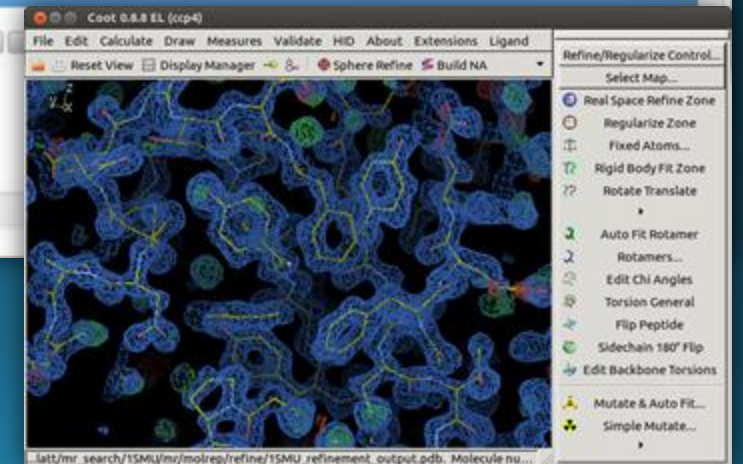
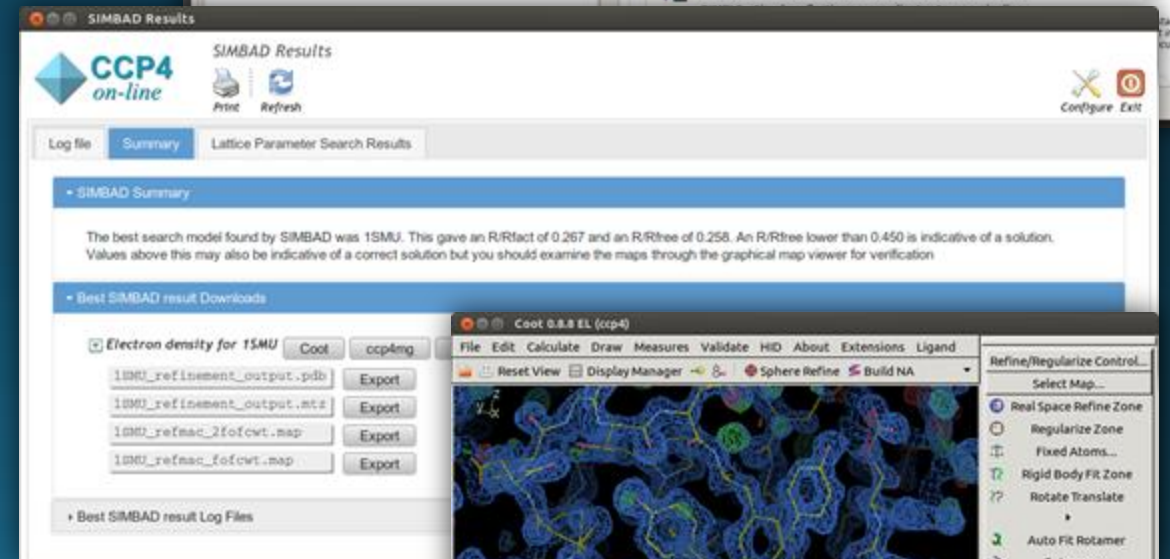
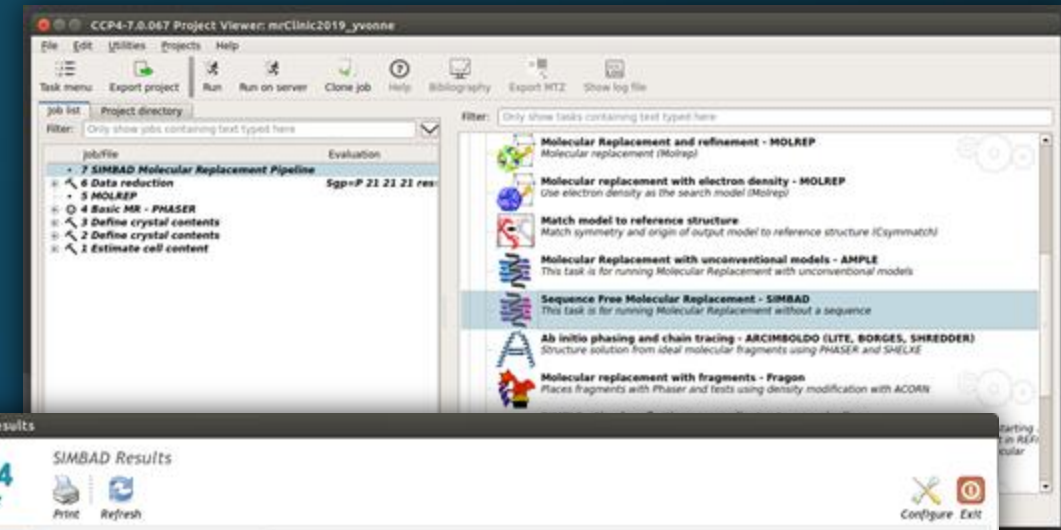
What to do if Molecular Replacement doesn't work

- **SIMBAD: Sequence-less MR**

1. nearest cell (lattice)
2. contaminants
3. entire non-redundant domain database (90000 models)

- Accessible from *Molecular Replacement* menu

- CCP4 Online
- CCP4i2

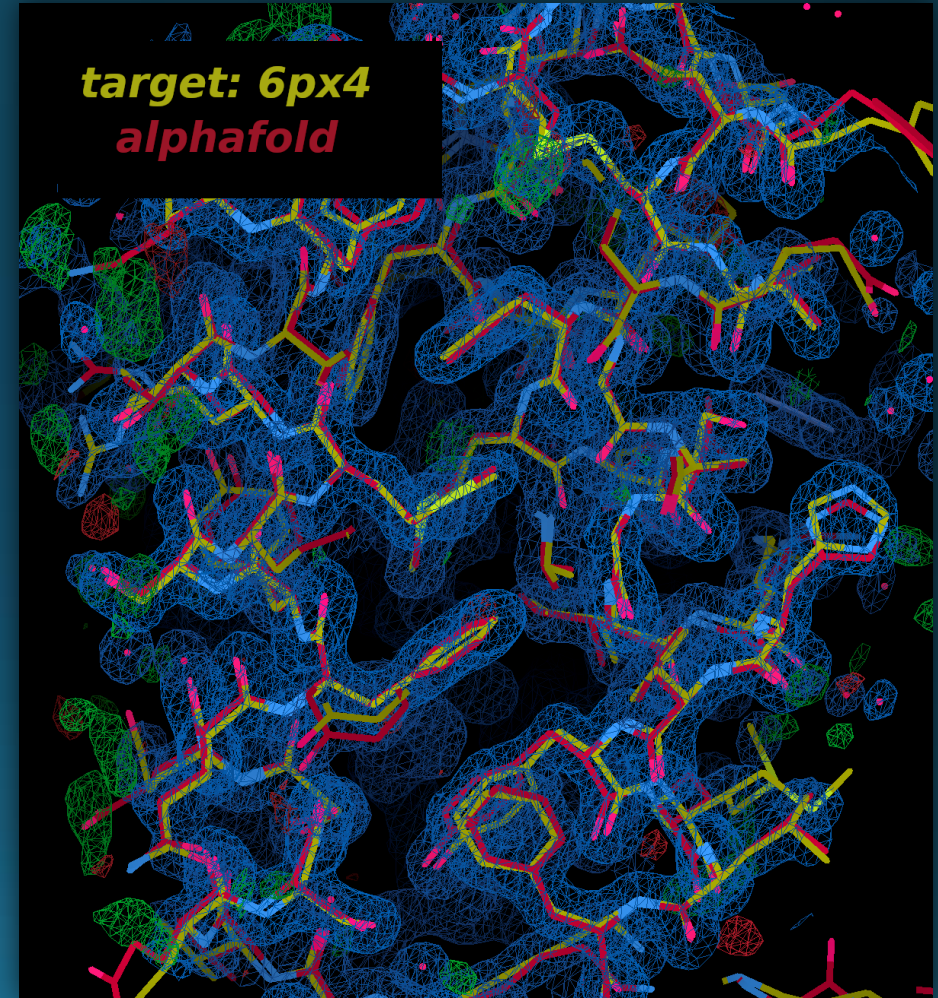
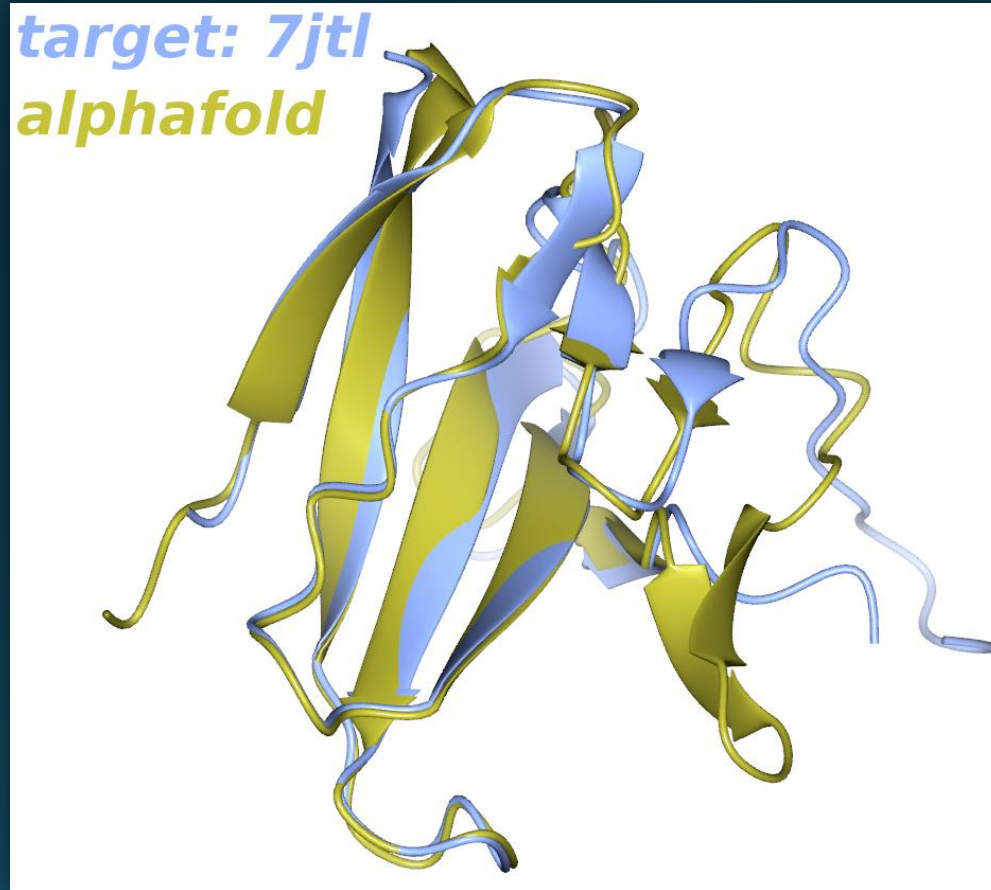


What to do if Molecular Replacement doesn't work

- *ARCIMBOLDO*
- Lite
 - Makes use of simple secondary structure elements such as helices in MR
 - Attempts to position fragments and build up the rest of the c-alpha backbone using SHELXE
- Borges
 - Similar to Lite but draws on a library of common motifs from the PDB as search models e.g. Zinc fingers or common beta sheet fragments
- Shredder
 - Cuts distant homologues into fragments and will use them as search models in ARCIMBOLDO
- <http://chango.ibmb.csic.es/ARCIMBOLDO/>

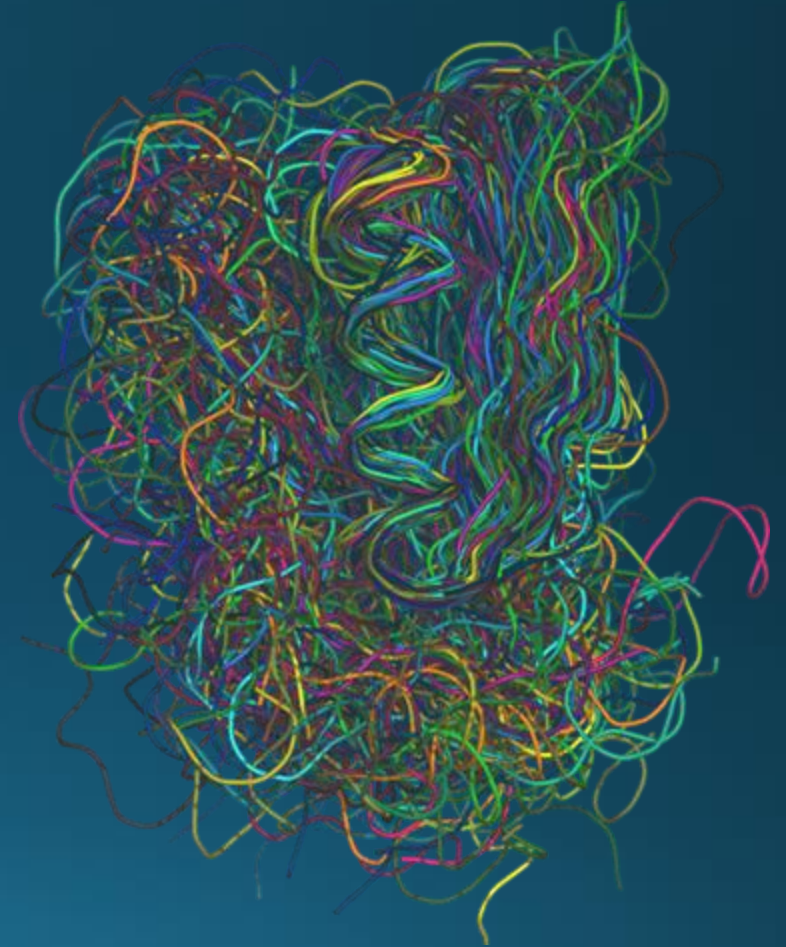
What to do if Molecular Replacement doesn't work

- *Ab initio* model prediction from sequence



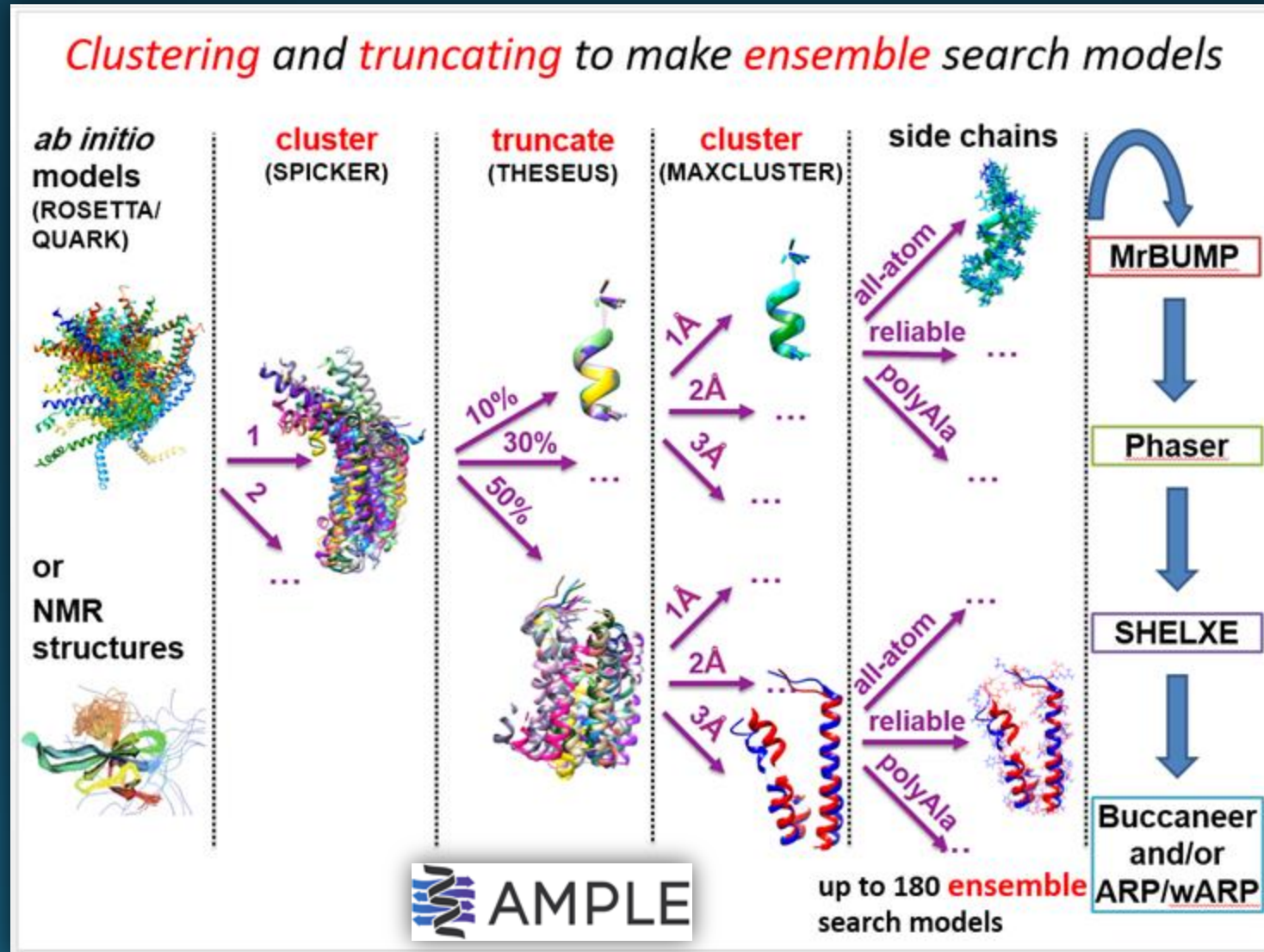
What to do if Molecular Replacement doesn't work

- **AMPLE**: *Ab initio* modelling to generate models for molecular replacement
- Uses programs like *Rosetta* and *Quark* to generate search models from the target sequence
- Can exploit sequence alignment derived residue contact predictions



What to do if Molecular Replacement doesn't work

AMPLE Pipeline



(image from
Jens
Thomas)

Acknowledgements

- Alexey Vagin & Andrey Lebedev – ***MoRDa and Molrep***
- Daniel Rigden, Filo Sanchez Rodriguez & Adam Simpkin - ***University of Liverpool***
- Stuart McNicholas, Keith Wilson & Christian Roth - ***University of York***
- Randy Read, Airlie McCoy & Gabor Bunkozci - ***Phaser***
- Martyn Winn, Charles Ballard, Eugene Krissinel, Ville Uski & Kyle Stevenson - ***CCP4***
- Marcin Wojdyr - ***Global Phasing***
- Isabel Uson, Andrea Thorn, Tim Gruene & George Sheldrick – ***SHELXE & ARCIMBOLDO***
- Garib Murshudov, Rob Nicholls & Fei Long – ***Refmac and BALBES***
- Kevin Cowtan & Paul Bond – ***Buccaneer & DM***
- Victor Lamzin & Grzegorz Chojnowski - ***ARP/wARP***
- Thanks to authors of all other underlying programs