MR introduction

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Outline of this talk

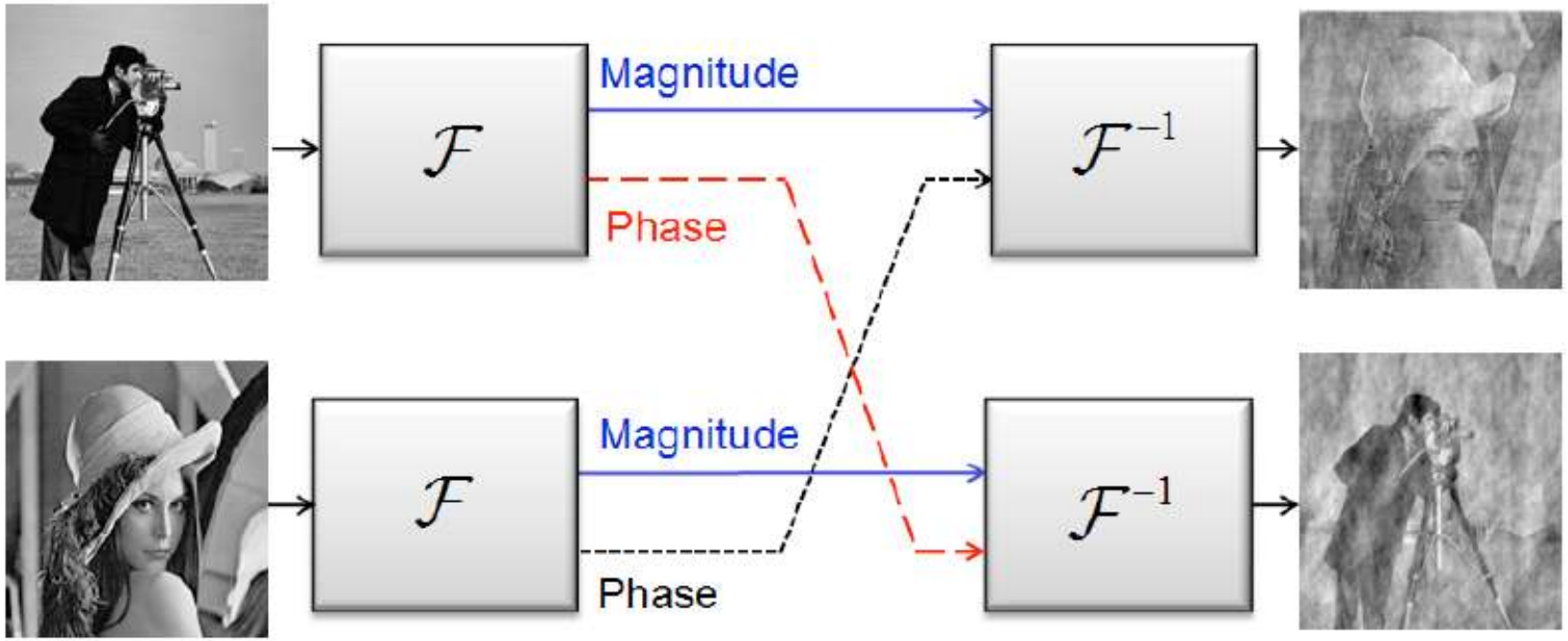
- **What is Molecular replacement**
- **Functions for Molecular Replacement in Molrep**
- **Solution indicators and signs**
- **MR pipelines in CCP4: examples**

Importance of phases

It is well known that phases are very important.

This is an example from image processing demonstrating it

Real examples given later



MOLECULAR REPLACEMENT

If a structure is known of a homologue molecule (for proteins > 30 % sequence identity) or predicted model (RosettaTTAfold. Alphafold 2) molecular replacement (MR) is the method of choice. MR positions the known structure into unknown crystal cell and this gives a set of initial phases which can be improved by structure refinement and density modification.

Some structures are solved with sequence identity as low as 14-20 %. Proportion of structures solved by MR grows with an increase in number of PDB entries and development of MR software.

Overall results reported in PDB (2006)

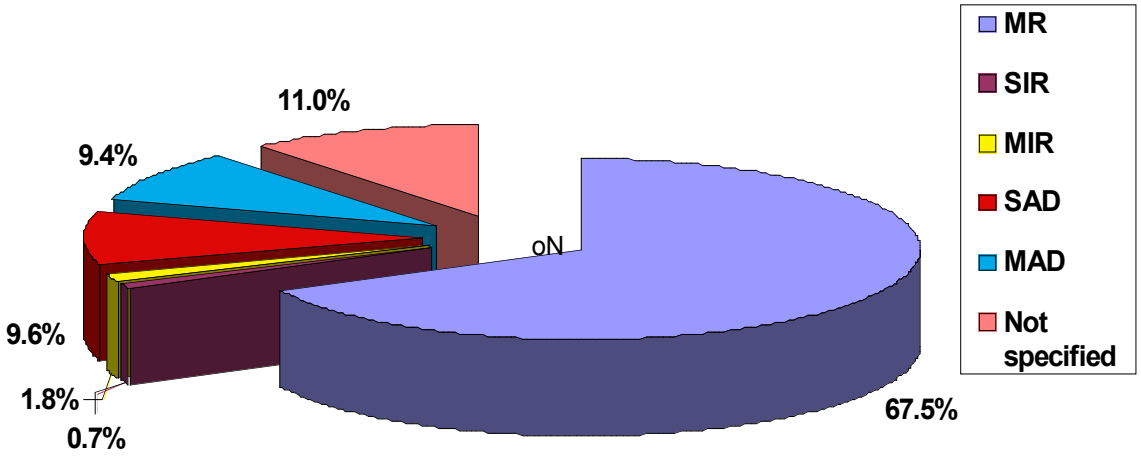


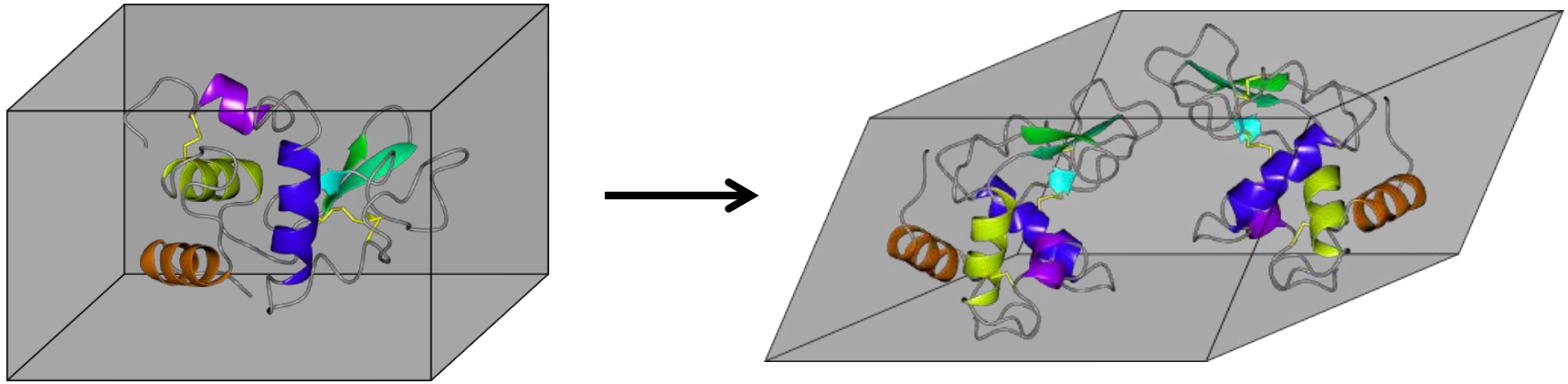
Diagram showing the percentage of structures in the PDB solved by different techniques

At least 80% of structures are solved by Molecular Replacement (MR) (2016)
Around 10% of structures are solved by experimental phasing
AlphaFold 2 prediction is likely to increase number of MR solved entries

MR Problem

Known crystal or predicted structure

New crystal structure



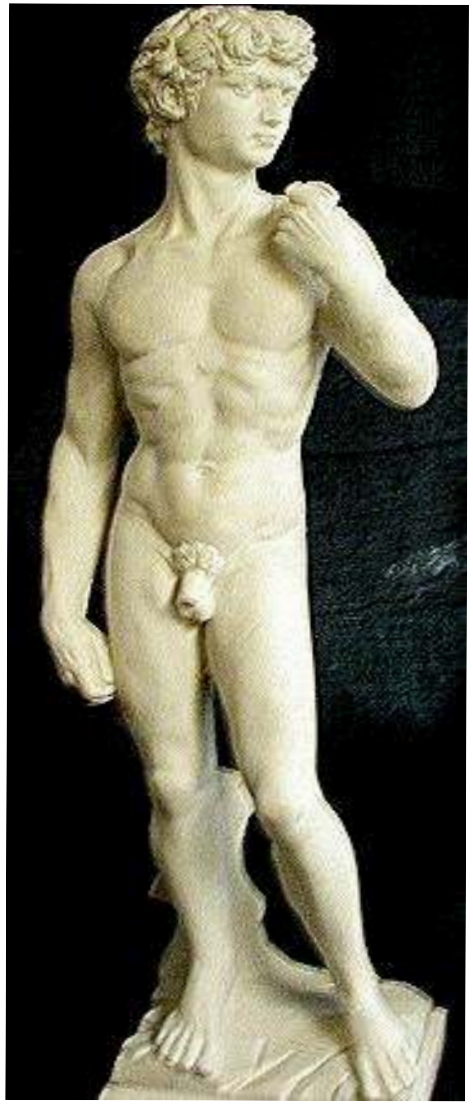
Given:
- Crystal (predicted) structure of a homologue
- New X-ray data

Find:
- The new crystal structure

Model

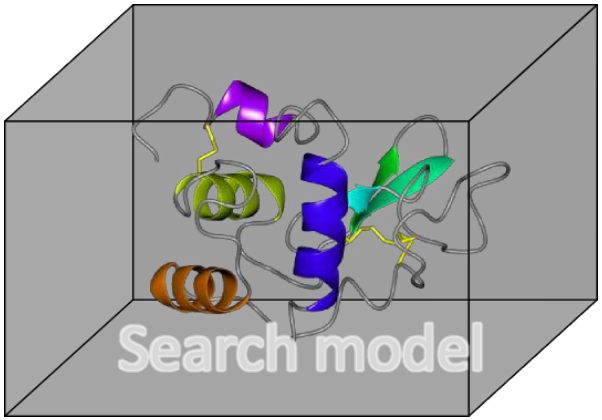
Corrected model

MR solution after refinement

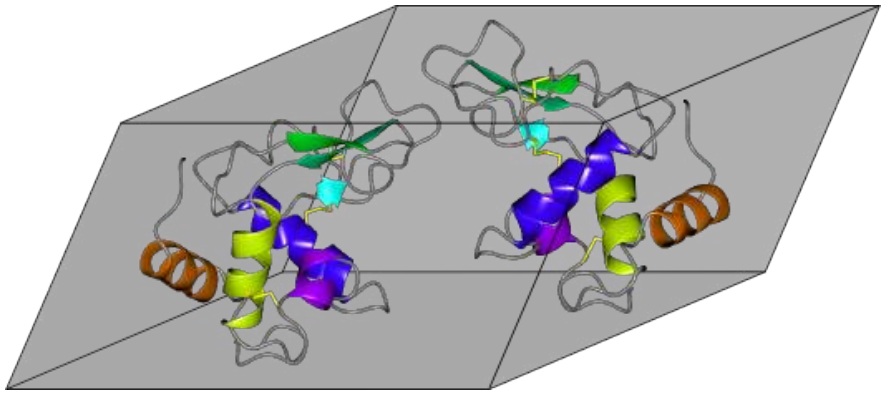


MR Technique

Known crystal structure



New crystal structure



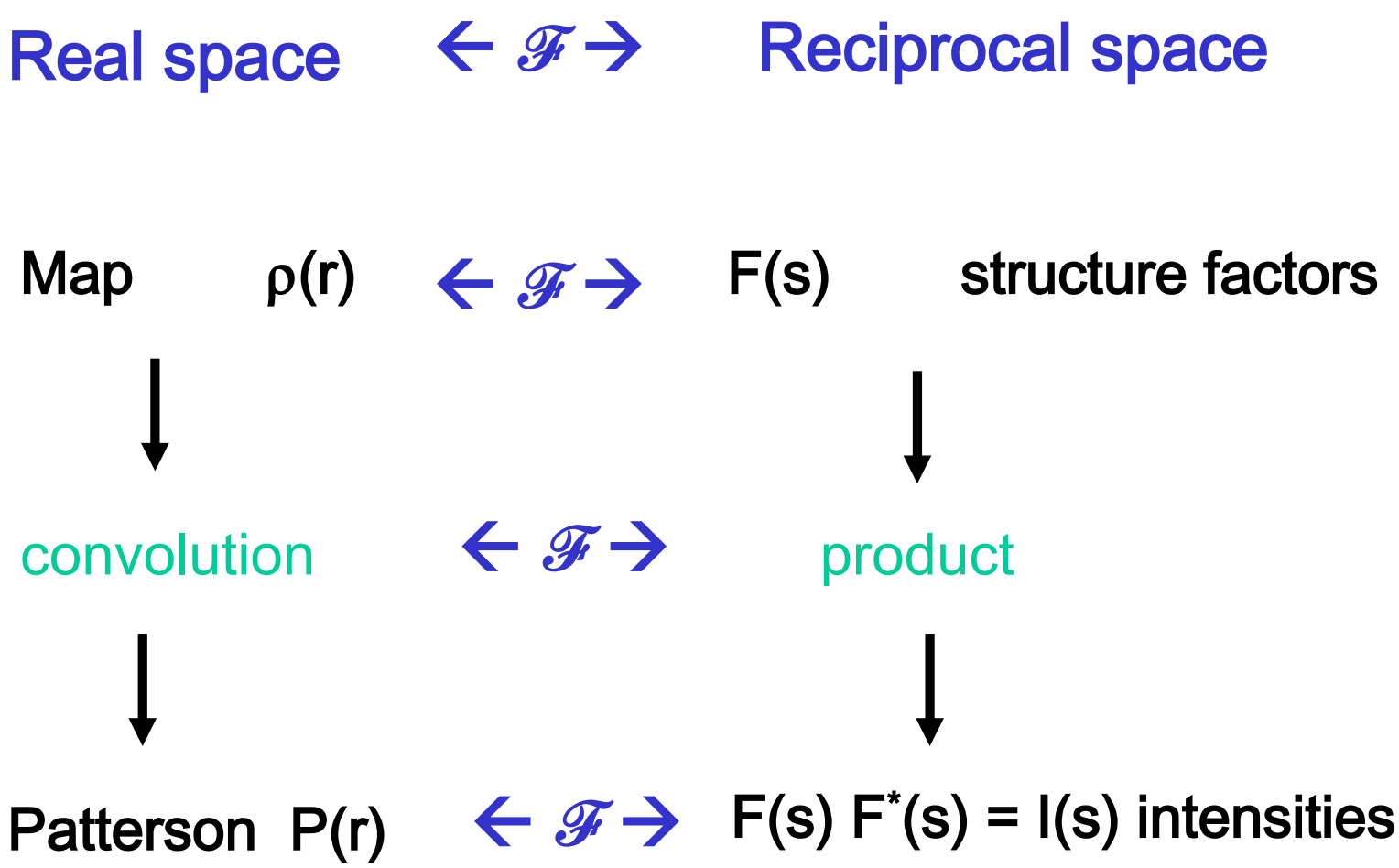
Method:

- 6 × N - dimensional global optimisation
  - one 6-d search for each molecule in the AU
    - >> split further to orientation + translation searches = 3 + 3
    - >> fast search step using FFT

Required:

- Scoring
  - the match between the data and an (incomplete) crystal model
  - ideally: the highest score = correct solution

Functions in Real and Reciprocal spaces



Structure factors and Electron density map

Structure factors $F(h,k,l)$

– A discrete complex value function in the reciprocal space

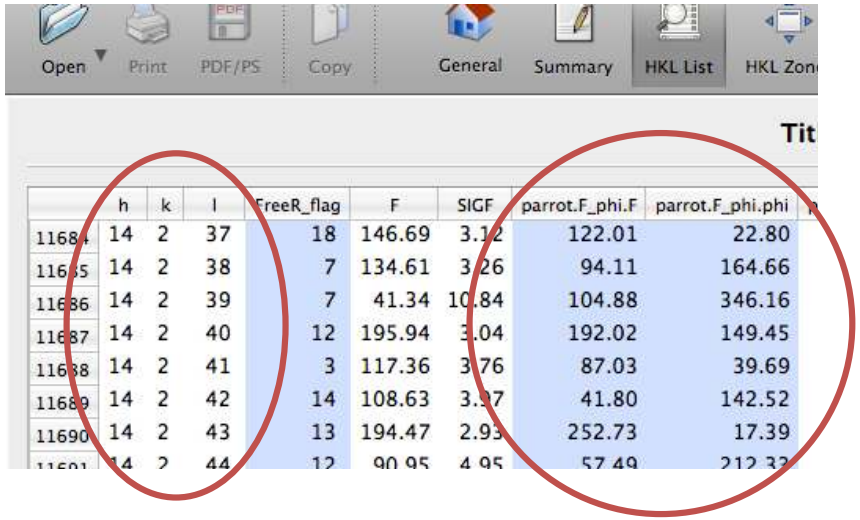
At given *h, k, l*

– Complex number:

$$F = A + iB$$

– Can be expressed via structure amplitude and phase

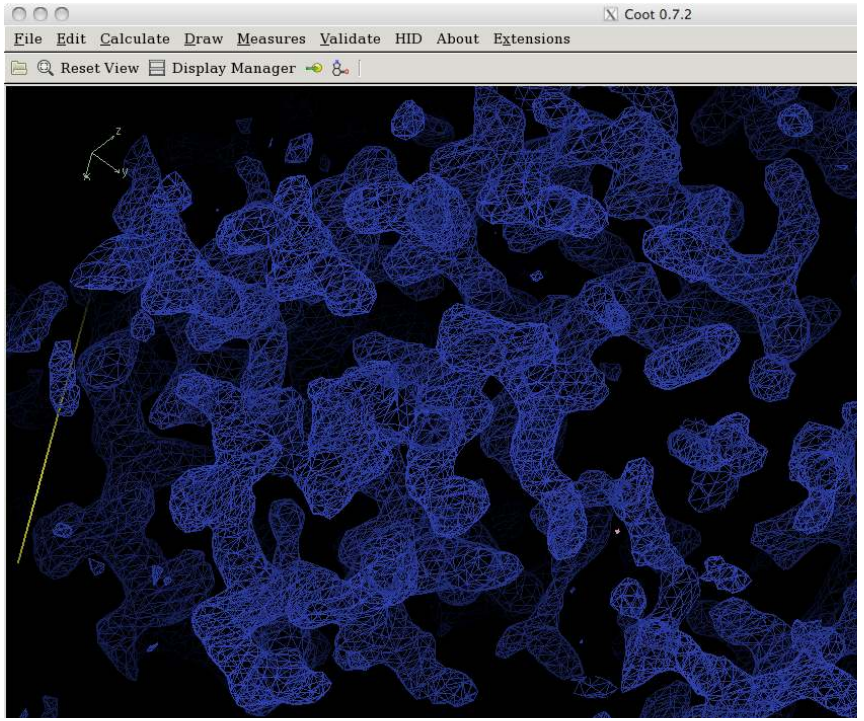
$$F = |F| \exp(i\phi)$$



	h	k	l	FreeR_flag	F	SIGF	parrot.F_phi.F	parrot.F_phi.phi
11684	14	2	37	18	146.69	3.12	122.01	22.80
11685	14	2	38	7	134.61	3.26	94.11	164.66
11686	14	2	39	7	41.34	10.84	104.88	346.16
11687	14	2	40	12	195.94	3.04	192.02	149.45
11688	14	2	41	3	117.36	3.76	87.03	39.69
11689	14	2	42	14	108.63	3.57	41.80	142.52
11690	14	2	43	13	194.47	2.93	252.73	17.39
11691	14	2	44	12	90.95	4.95	57.49	212.32

Electron density map

– periodic 3-d function in real space



is directly interpretable

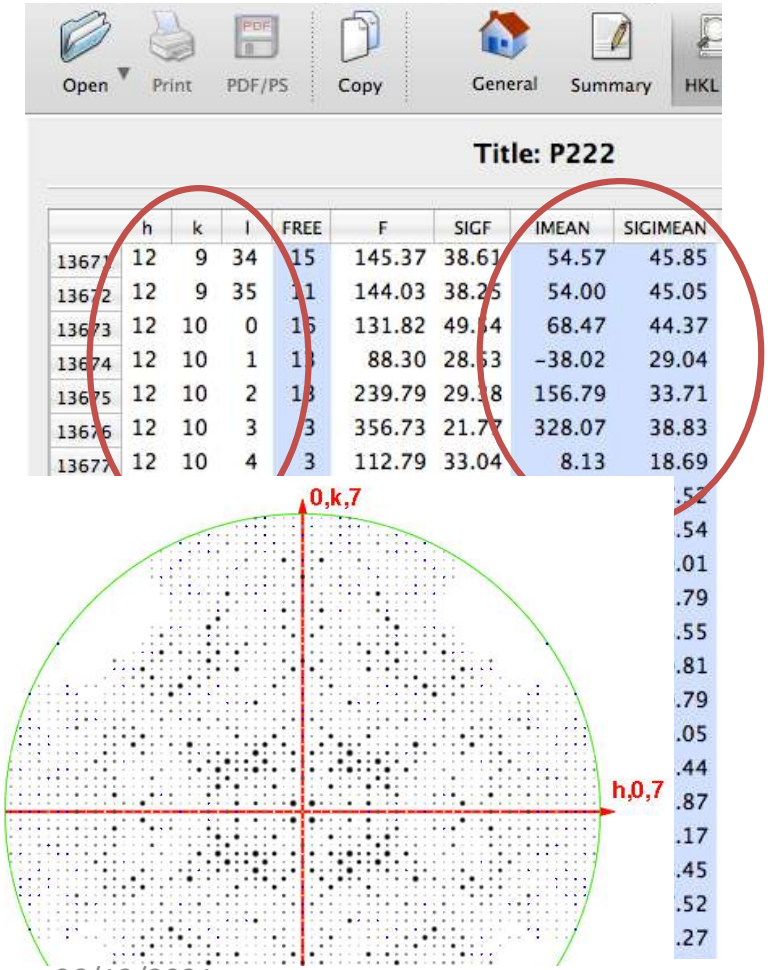
– model building

– real-space fitting of fragments

Intensities and Patterson map

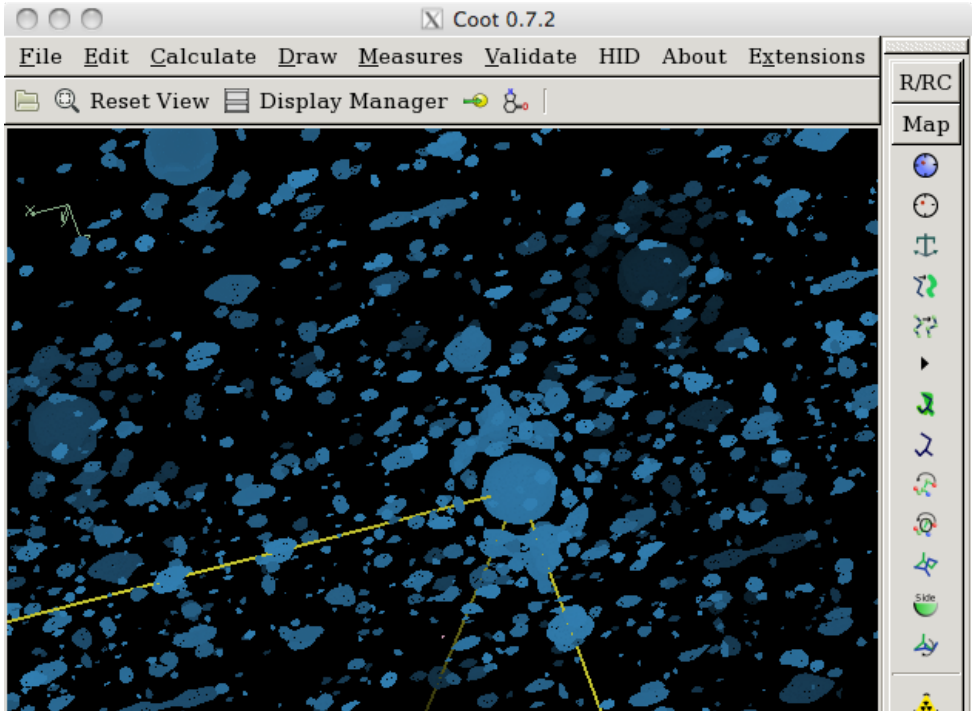
Intensities $I(h,k,l)$

– 3-d discrete real function in the reciprocal space



Patterson map:

–periodic 3-d function in real(*) space



– Nothing reminds of a protein molecule

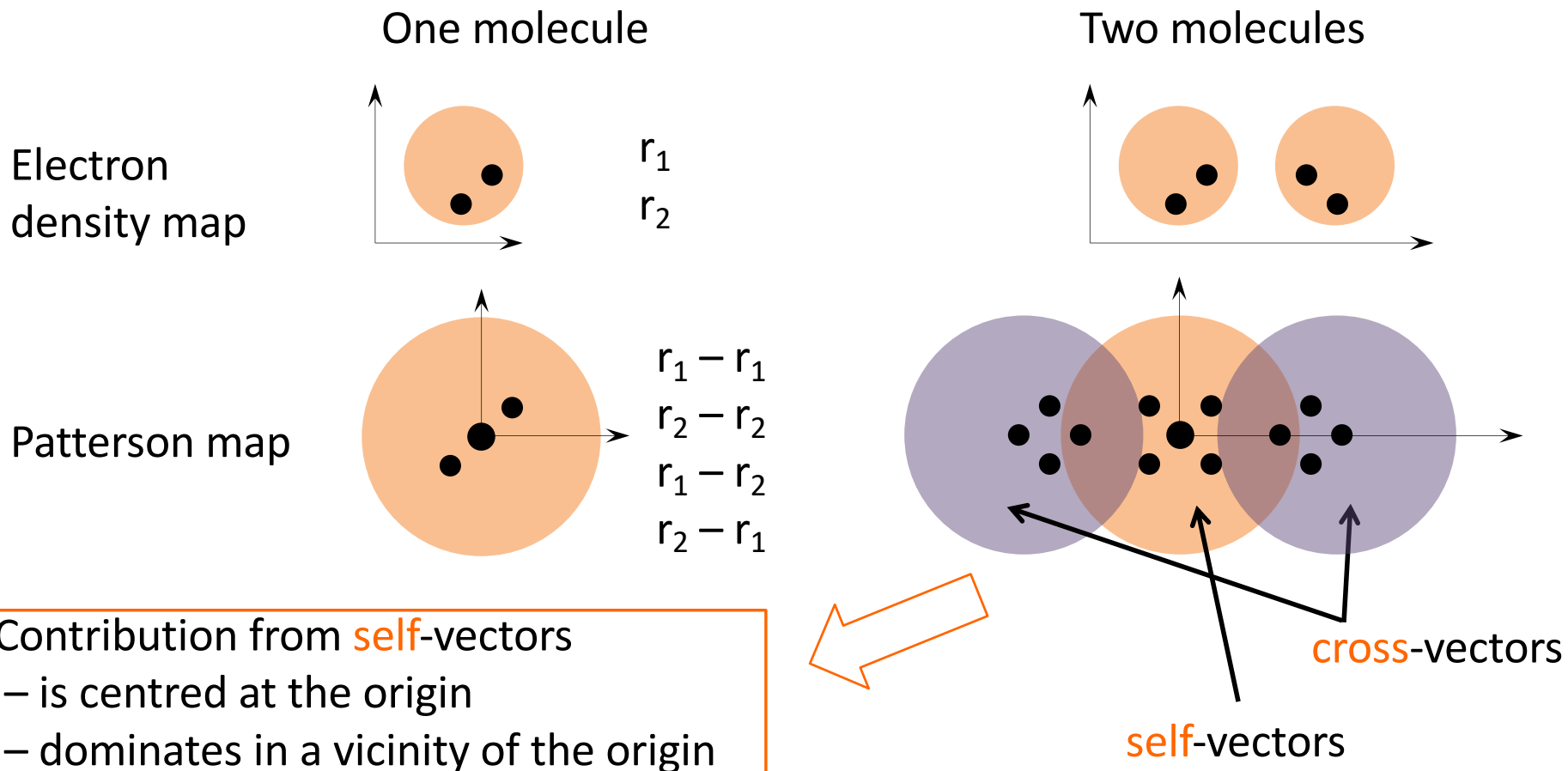
– **Model building**, residue by residue, is **impossible**

Self and cross vectors

Electron density map = peaks from all atoms

Patterson map = peaks from all interatomic vectors

- self-vectors: vectors between atoms belonging to the same molecule
- cross-vectors: vectors between atoms belonging to different molecules



Contribution from self-vectors
– is centred at the origin
– dominates in a vicinity of the origin

Molecular Replacement Programs in CCP4

- AMoRe
- Molrep
- Phaser

CCP4 programs for model preparation

Single model correction:

- Chainsaw
- Molrep
- Sculptor

Preparation of ensemble models – fitting models:

- Lsqkab
- ProSMART
- SSM (also in Coot)
- Gesamt

Automated preparation of ensemble models:

- Ensembler
- MrBUMP-CCP4MG

Molrep

Alexey Vagin

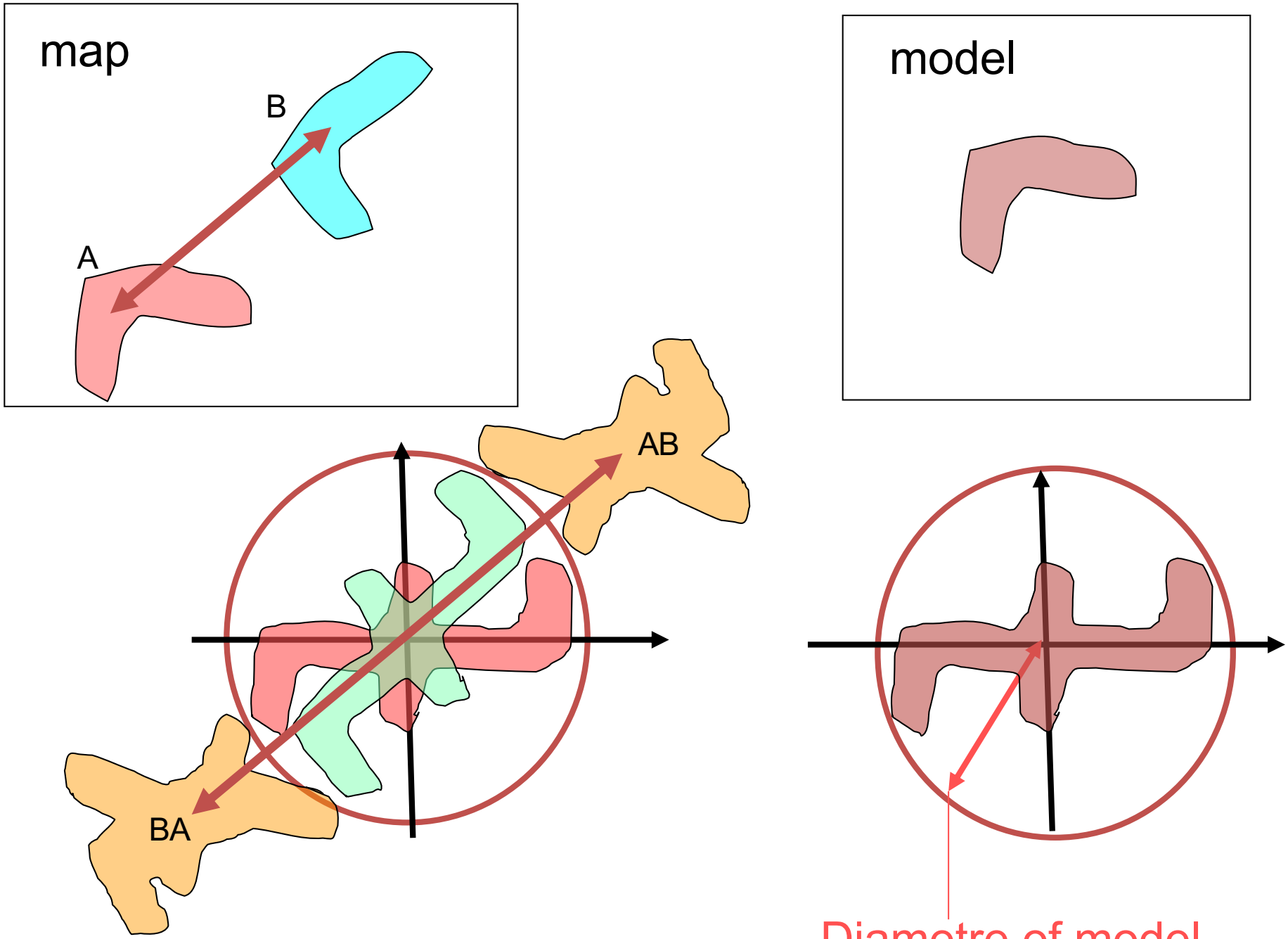
YSBL University of York

Default protocol

```
molrep -f data.mtz -m model.pdb -mx fixed.pdb -s target.seq
```

- model correction based on sequence and structure information
- defines the number of molecules per AU
- anisotropic correction of the data
- weighting the data according to model completeness and similarity
- check for pseudotranslation and use it if present
- 30+ peaks in Cross RF for use in TF (accounts for close peaks)
- applied packing function
- make use of partial structure (fixed model)

Rotation function



Translation function

$$TF(\mathbf{t}) = \iiint P^{\text{obs}}(\mathbf{r}) \times P^{\text{calc}}_{\text{cross}}(\mathbf{t},\mathbf{r})\,\mathrm{d}\mathbf{r}^3$$

- Conventional (Patterson based) TF:
  - absence of any phases

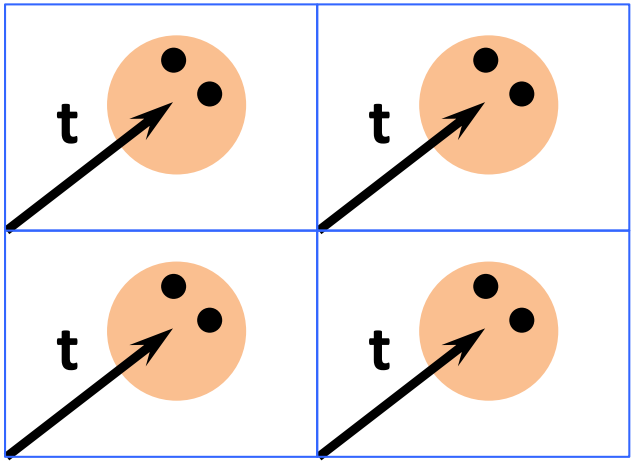
$$TF(\mathbf{t}) = \iiint \rho^{\text{obs}}(\mathbf{r}) \times \rho^{\text{calc}}(\mathbf{t},\mathbf{r})\,\mathrm{d}\mathbf{r}^3$$

- Phased TF:
  - (poor) experimental phases
  - phases from partial model
  - Fitting into EM reconstruction

t - vector of translation

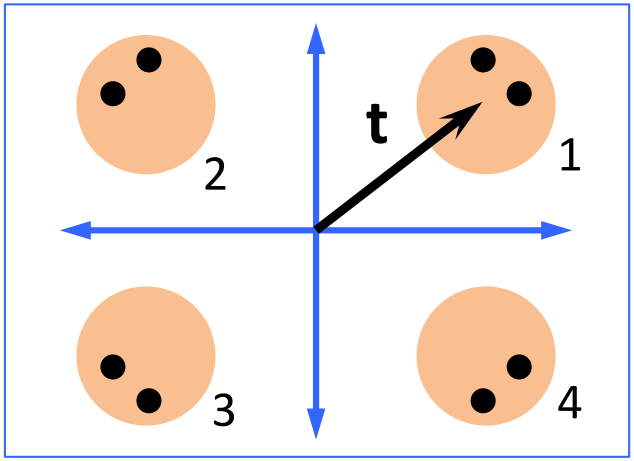
Translation Function

P 1



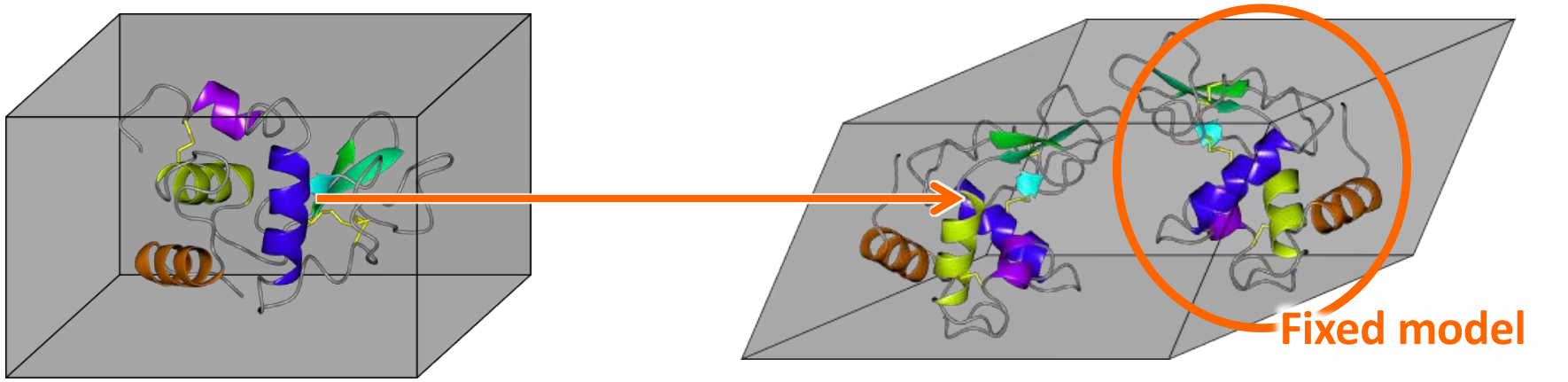
- Translation **t** does not change the structure!
  - can be compensated with shift of crystallographic origin
- TF step is not needed

Other space groups *e.g. P*222



- The centre of molecule 1:
  - parameter **t**
- Centres of molecules 2, 3 and 4
  - from symmetry operation
- $TF(\mathbf{t})$: Fourier coefficients, FFT
- Best **t**: peak search in $TF(\mathbf{t})$

Fixed partial model in Patterson search



Almost the same equation as for a single molecule search,

$$TF(\mathbf{t}) = \sum_{\mathbf{h}} I_{\mathbf{h}}^{\text{obs}} \times \left| F_{\mathbf{h}}^{\text{fixed}} + F_{\mathbf{h}}^{\text{calc}}(\mathbf{t}) \right|^2$$

Translation function

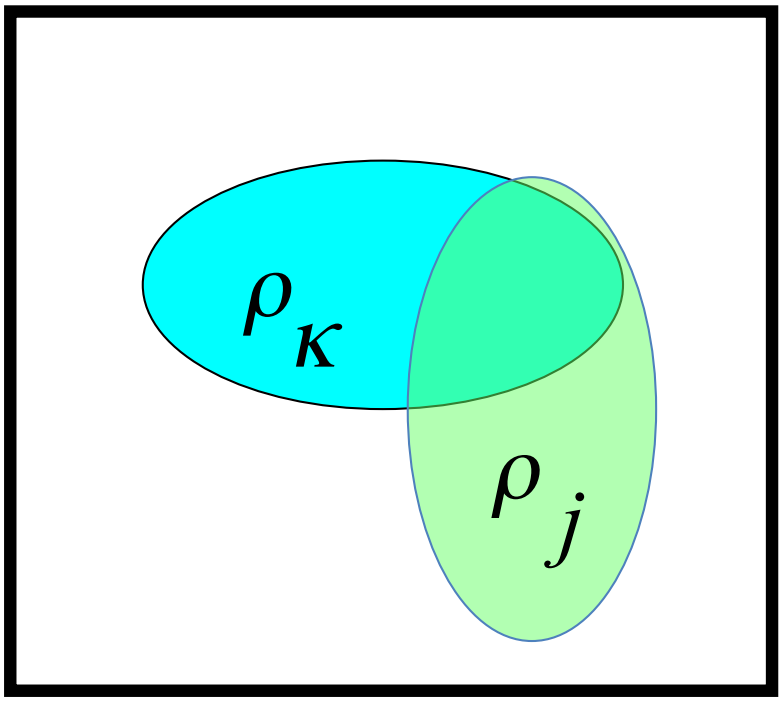
Patterson based TF

- Space group P1
  - » First copy: no translation function needed
  - » All subsequent copies: 3-d translation function
- Polar space groups (P2, $P2_1$, C2, P3, $P3_1$, $P3_2$, …, $P6_5$)
  - » First copy: translation function is 2-dimensional
  - » All subsequent copies: 3-d translation function
- Other space groups
  - » All copies: 3-d translation function

Phased TF

- » All copies: 3-d translation function

Fast Packing Function



Estimation of overlap:

$$\int \rho_k(\mathbf{r},\mathbf{s})\,\rho_j(\mathbf{r},\mathbf{s})\,d\mathbf{r}$$

Packing function:

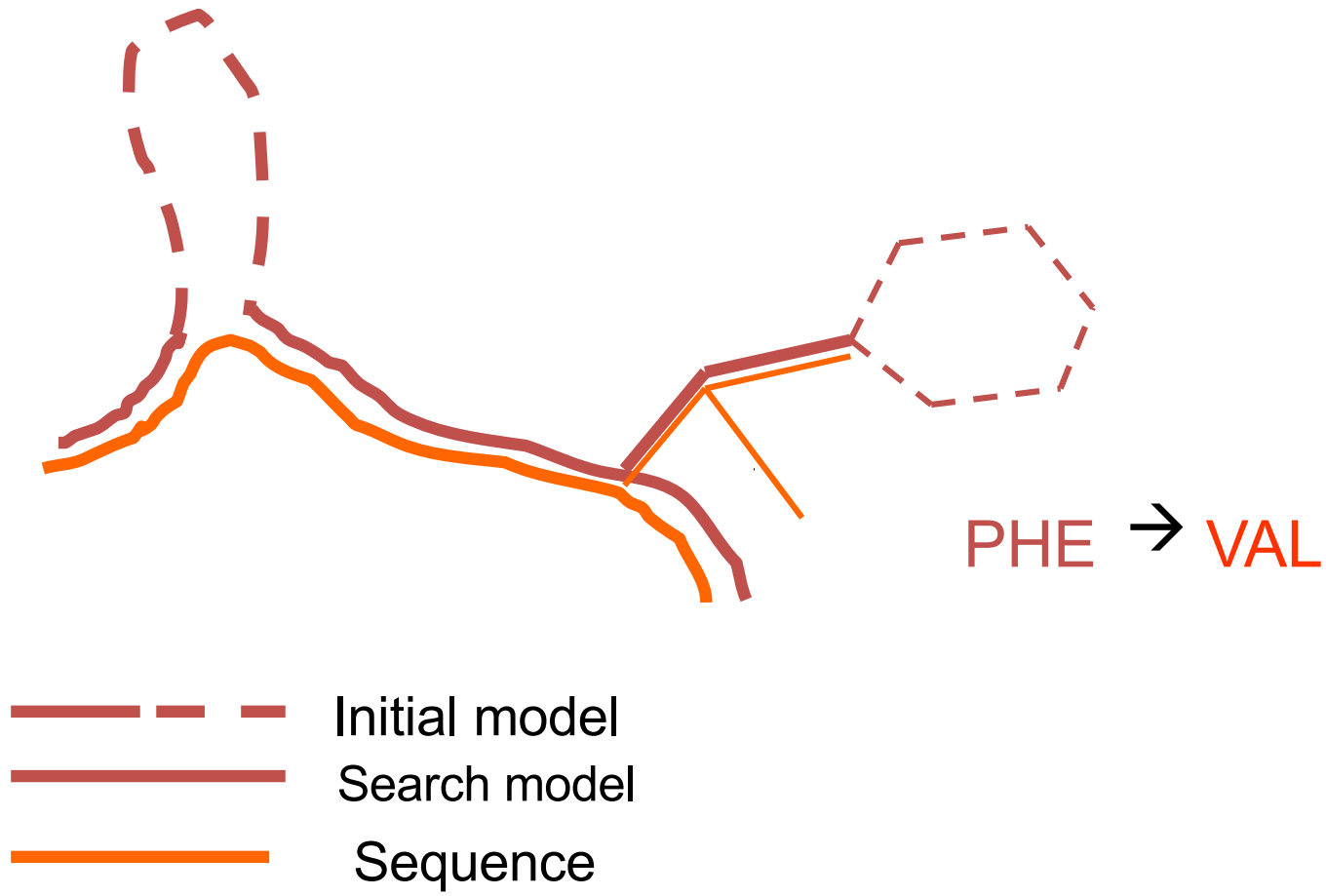
$$P(\mathbf{s}) = \mathbf{1} - \sum_k \sum_{j \neq k} \int \rho_k(\mathbf{r},\mathbf{s})\,\rho_j(\mathbf{r},\mathbf{s})\,d\mathbf{r}$$

Model modification in MOLREP

```
molrep -f data.mtz -m model.pdb -s target.seq
```

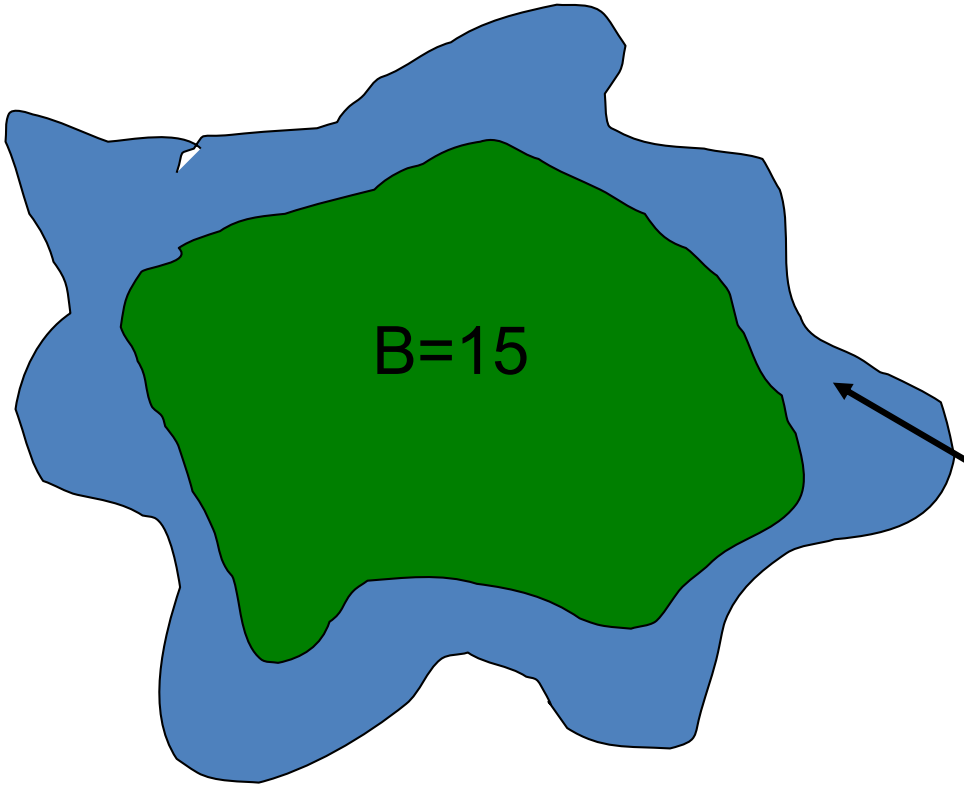
- Performs model correction:
  - Identifies secondary structure in the model
  - Aligns target and model sequences
    - » no deletions or insertions in α-helixes or β-strands
  - Retains aligned residues
  - Retains "aligned" atoms in aligned residues
- Adds B-factor to residues exposed to solvent
- Uses sequence identity to down-weight high resolution data

Automatic correction of the model using sequence alignment



Model improvement

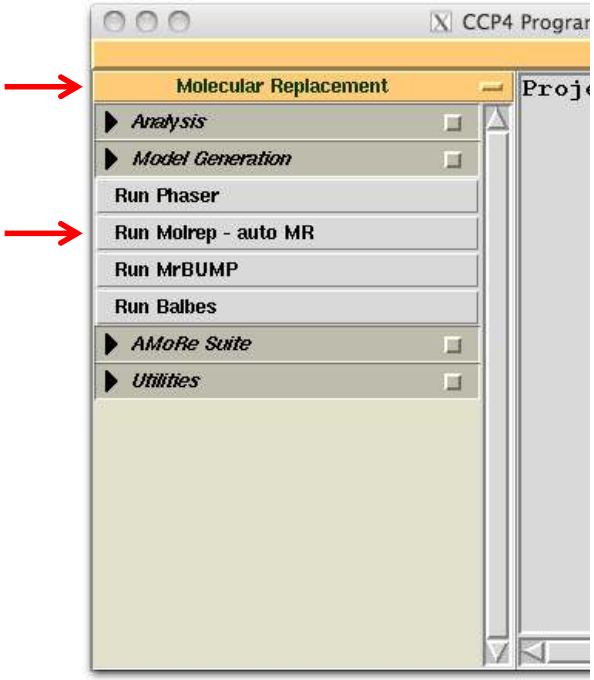
Set atomic B values according to accessible surface area

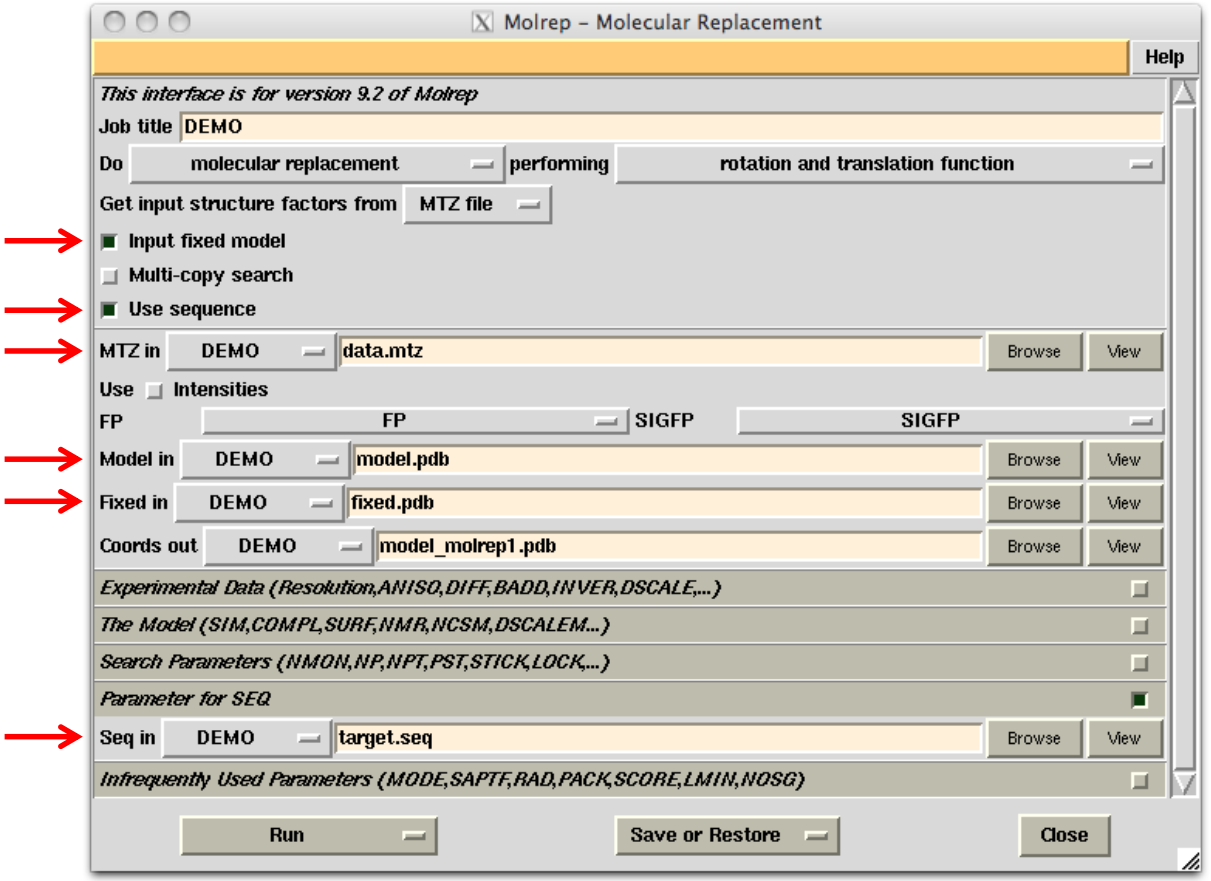


$B\ (A^2) = 15\ (A^2) + 10\ SA\ (A^2)$

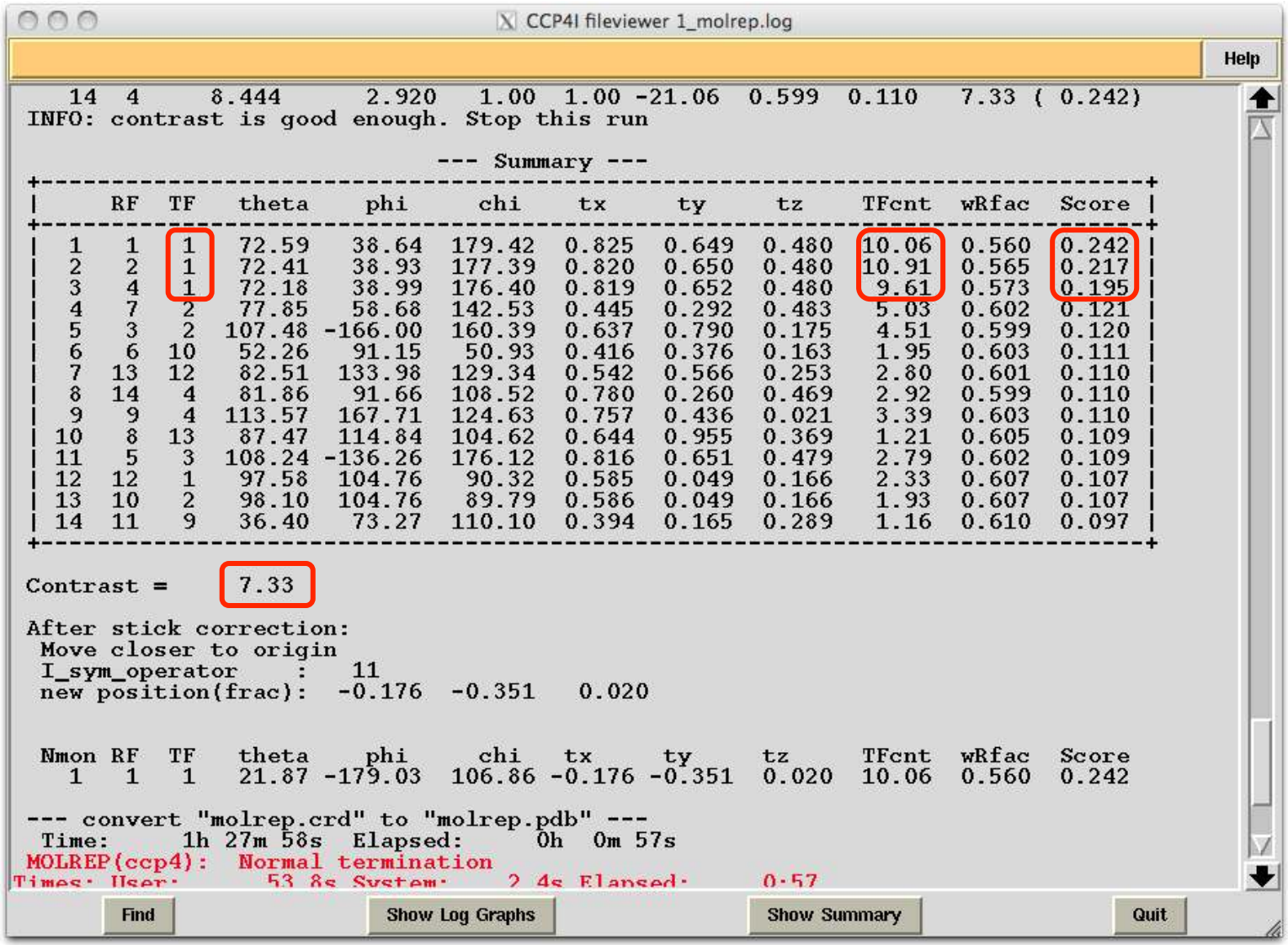
SA – surface area

Molrep in CCP4I

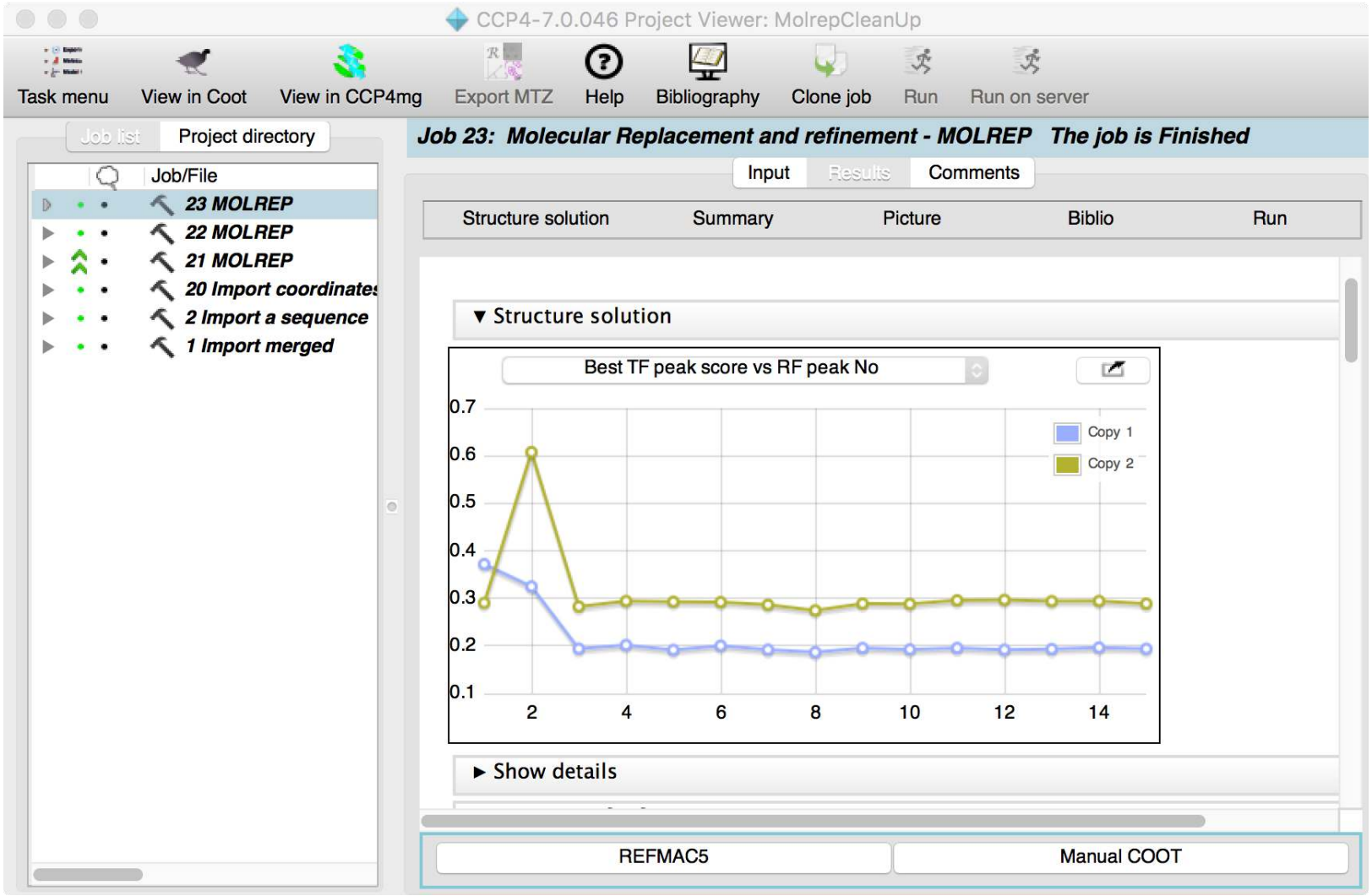




Log-file


CCP4I fileviewer 1_molrep.log
Help
14 4 8.444 2.920 1.00 1.00 -21.06 0.599 0.110 7.33 (0.242)
INFO: contrast is good enough. Stop this run
--- Summary ---
| RF TF theta phi chi tx ty tz TFcnt wRfac Score |
| 1 1 1 72.59 38.64 179.42 0.825 0.649 0.480 10.06 0.560 0.242 |
| 2 2 1 72.41 38.93 177.39 0.820 0.650 0.480 10.91 0.565 0.217 |
| 3 4 1 72.18 38.99 176.40 0.819 0.652 0.480 9.61 0.573 0.195 |
| 4 7 2 77.85 58.68 142.53 0.445 0.292 0.483 5.03 0.602 0.121 |
| 5 3 2 107.48 -166.00 160.39 0.637 0.790 0.175 4.51 0.599 0.120 |
| 6 6 10 52.26 91.15 50.93 0.416 0.376 0.163 1.95 0.603 0.111 |
| 7 13 12 82.51 133.98 129.34 0.542 0.566 0.253 2.80 0.601 0.110 |
| 8 14 4 81.86 91.66 108.52 0.780 0.260 0.469 2.92 0.599 0.110 |
| 9 9 4 113.57 167.71 124.63 0.757 0.436 0.021 3.39 0.603 0.110 |
| 10 8 13 87.47 114.84 104.62 0.644 0.955 0.369 1.21 0.605 0.109 |
| 11 5 3 108.24 -136.26 176.12 0.816 0.651 0.479 2.79 0.602 0.109 |
| 12 12 1 97.58 104.76 90.32 0.585 0.049 0.166 2.33 0.607 0.107 |
| 13 10 2 98.10 104.76 89.79 0.586 0.049 0.166 1.93 0.607 0.107 |
| 14 11 9 36.40 73.27 110.10 0.394 0.165 0.289 1.16 0.610 0.097 |
Contrast = 7.33
After stick correction:
Move closer to origin
I_sym_operator : 11
new position(frac): -0.176 -0.351 0.020
Nmon RF TF theta phi chi tx ty tz TFcnt wRfac Score
1 1 1 21.87 -179.03 106.86 -0.176 -0.351 0.020 10.06 0.560 0.242
--- convert "molrep.crd" to "molrep.pdb" ---
Time: 1h 27m 58s Elapsed: 0h 0m 57s
MOLREP(ccp4): Normal termination
Times: User: 53.8s System: 2.4s Elapsed: 0:57
Find
Show Log Graphs
Show Summary
Quit

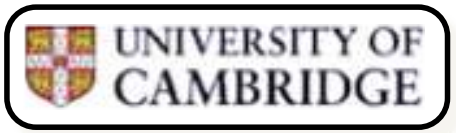
CCP4I2


CCP4-7.0.046 Project Viewer: MolrepCleanUp
Task menu
View in Coot
View in CCP4mg
Export MTZ
Help
Bibliography
Clone job
Run
Run on server
Job list
Project directory
Job/File
23 MOLREP
22 MOLREP
21 MOLREP
20 Import coordinates
2 Import a sequence
1 Import merged
Job 23: Molecular Replacement and refinement - MOLREP The job is Finished
Input
Results
Comments
Structure solution
Summary
Picture
Biblio
Run
▼ Structure solution
Best TF peak score vs RF peak No
Copy 1
Copy 2
0.7
0.6
0.5
0.4
0.3
0.2
0.1
2
4
6
8
10
12
14
► Show details
REFMAC5
Manual COOT

Modern MR: Phaser

Phaser implements all the above,
but revised in proper statistical terms
and with advanced search strategies based on these stats.

Randy Read, Airlie McCoy,
Gabor Bunkoczi, Rob Oeffner



Correct MR solution

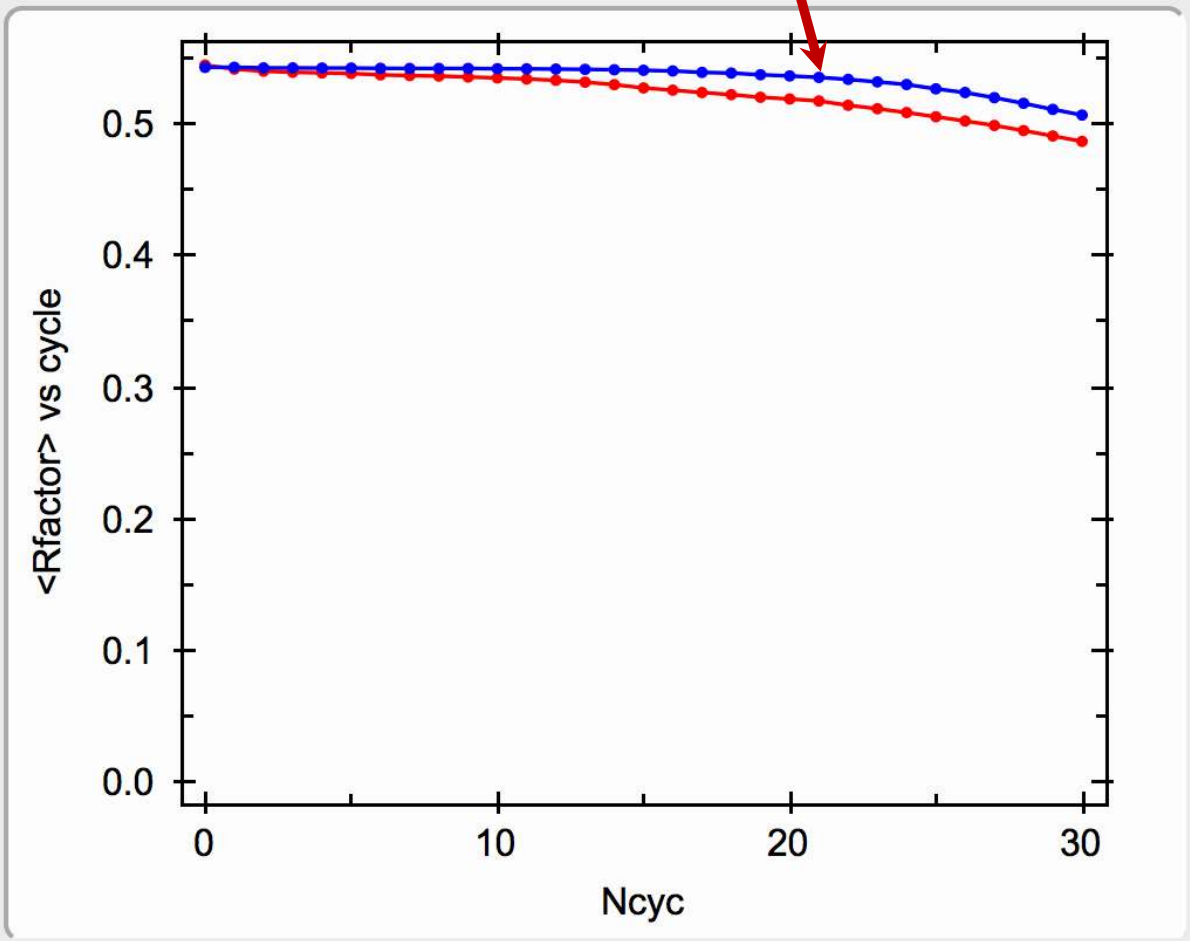
	Initial	Final
R factor	0.5443	0.4863
R free	0.5425	0.5063
Rms BondLength	0.0454	0.0110
Rms BondAngle	2.9234	1.9581
Rms ChirVolume	0.8050	0.6442

R-free > 0.5 but going down

Graph Data

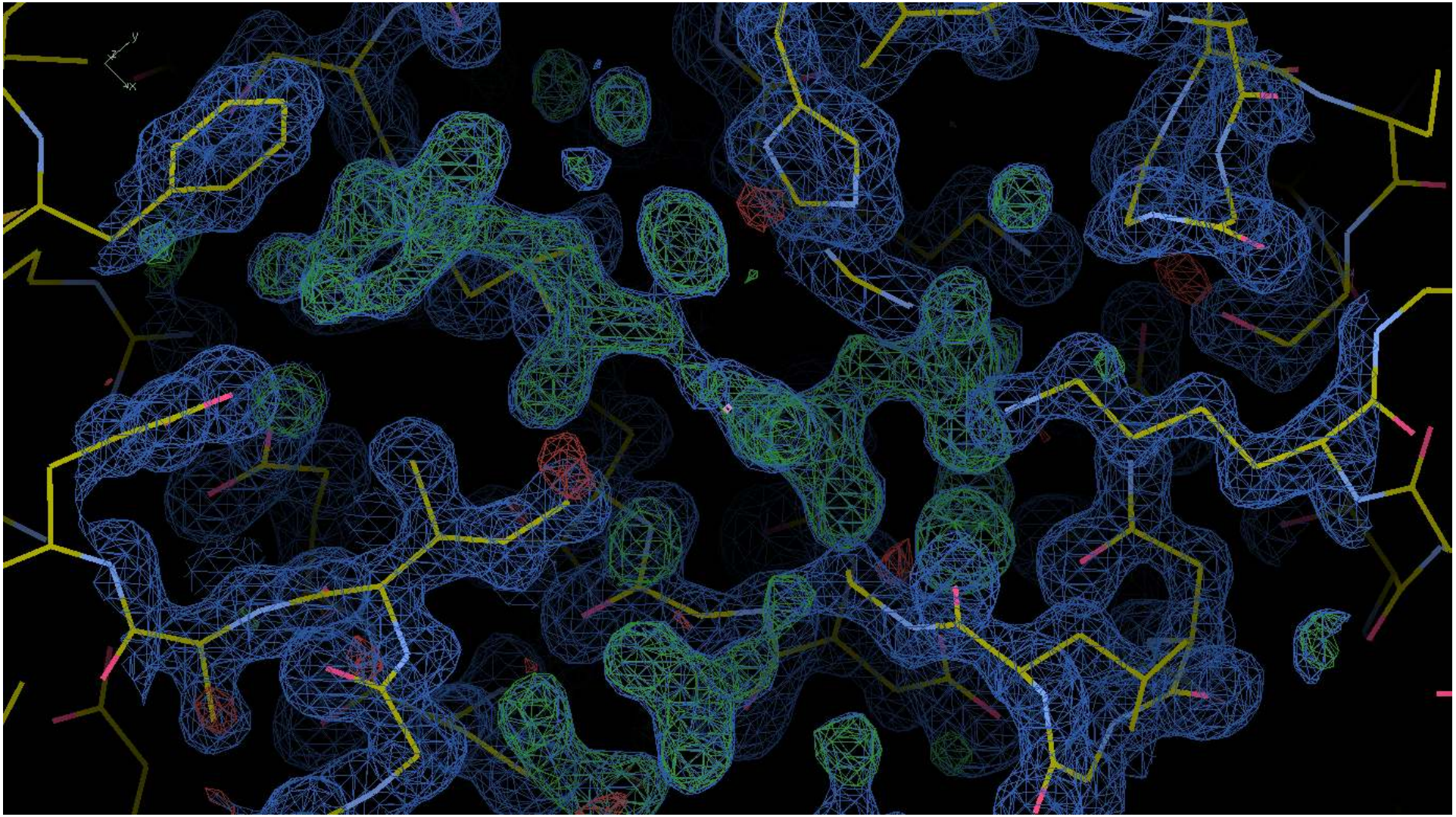
- Cycle 1. Rfactor analysis, F distribution v resln
- Cycle 1. FSC and Fom(<cos(DelPhi)>-acentric, …
- Cycle 30. Rfactor analysis, F distribution v resln
- Cycle 30. FSC and Fom(<cos(DelPhi)>-acentric…
- Cycle 31. Rfactor analysis, F distribution v resln
- Cycle 31. FSC and Fom(<cos(DelPhi)>-acentric…
- Rfactor analysis, stats vs cycle
  - <Rfactor> vs cycle
    - Rfact
    - Rfree
  - FOM vs cycle
  - -LL vs cycle
  - -LLfree vs cycle
  - Geometry vs cycle

raw data | Print | Export | Copy



Correct MR solution map (1.4 Å; 100 %)

Missing side chains are visible
New features (ligands and solvent molecules).



Correct solution at 1.6Å, very remote model.

Result

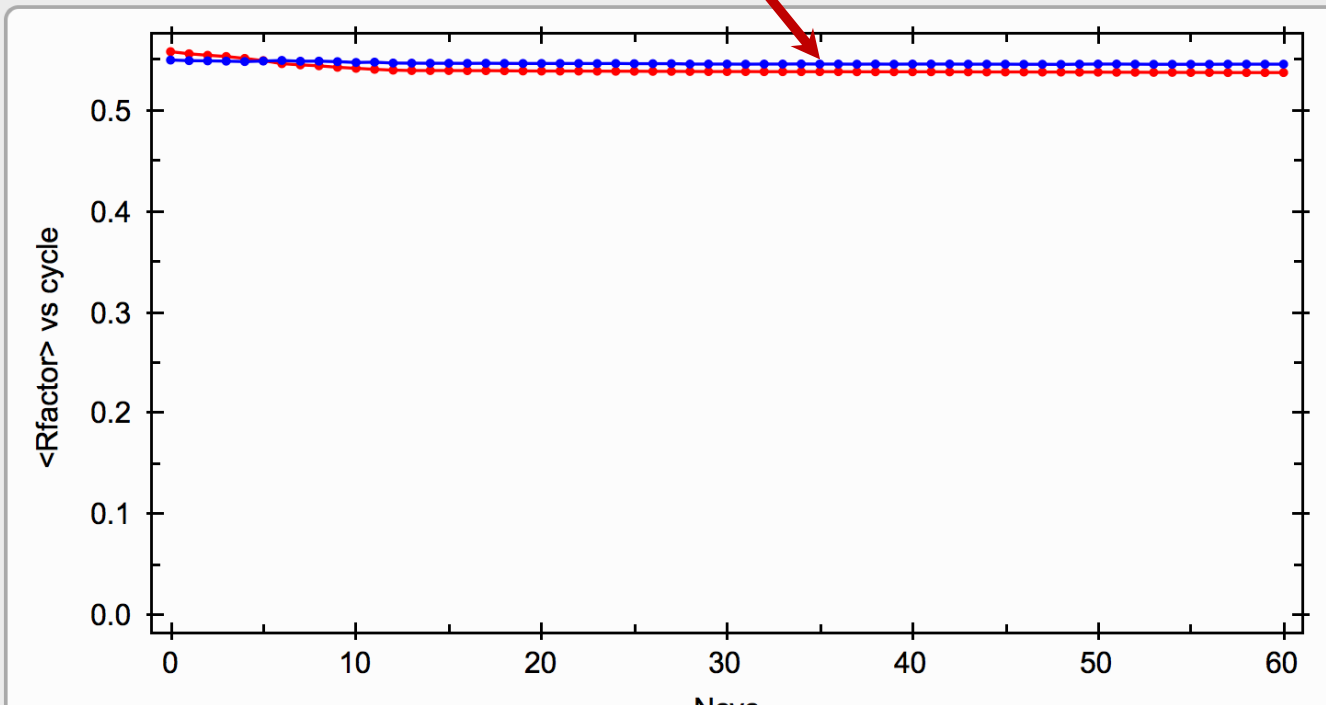
	Initial	Final
R factor	0.5575	0.5368
R free	0.5492	0.5451
Rms BondLength	0.0148	0.0081
Rms BondAngle	1.7169	1.8411
Rms ChirVolume	0.1337	0.0684

R-free > 0.5 and going down very slowly

Graph Data

- Cycle 1. Rfactor analysis, F distribution v resln
- Cycle 1. FSC and Fom(<cos(DelPhi)>-acentric, ...
- Cycle 60. Rfactor analysis, F distribution v resln
- Cycle 60. FSC and Fom(<cos(DelPhi)>-acentric...
- Cycle 61. Rfactor analysis, F distribution v resln
- Cycle 61. FSC and Fom(<cos(DelPhi)>-acentric...
- Rfactor analysis, stats vs cycle
  - <Rfactor> vs cycle
    - Rfact
    - Rfree
  - FOM vs cycle
  - -LL vs cycle
  - -LLfree vs cycle
  - Geometry vs cycle

raw data | Print | Export | Copy



Very remote model initial map

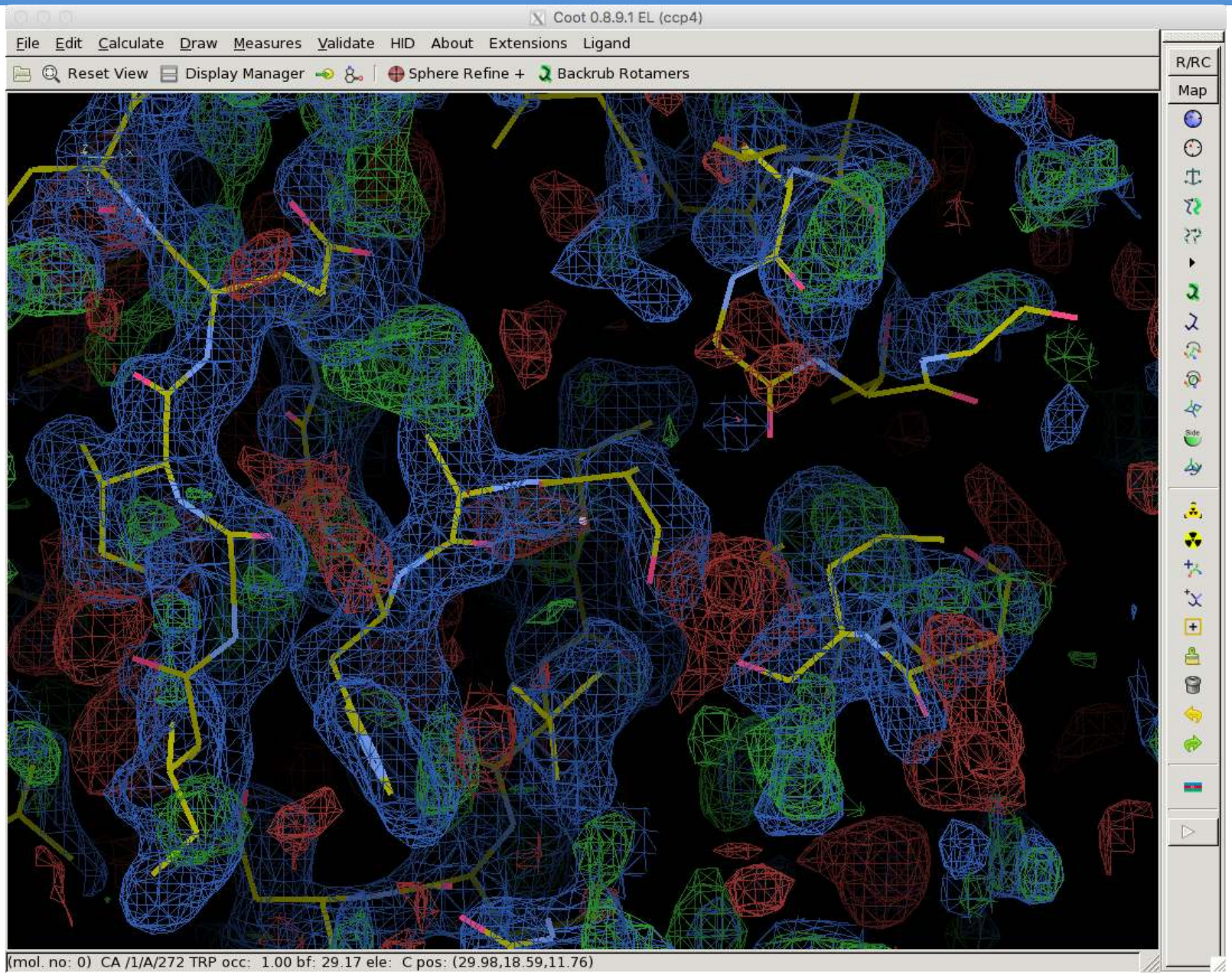
ED maps:

- Good long fragments with smoothly shaped density
- Clearly bad fragments with model in red density
- Clear green density for extension of main chain and building side chains

Refinement:

- R-free does not grow (or drops and slowly grows not higher than initial value)

Very remote model initial map



Wrong MR solution at 1.6 Å resolution.

Result

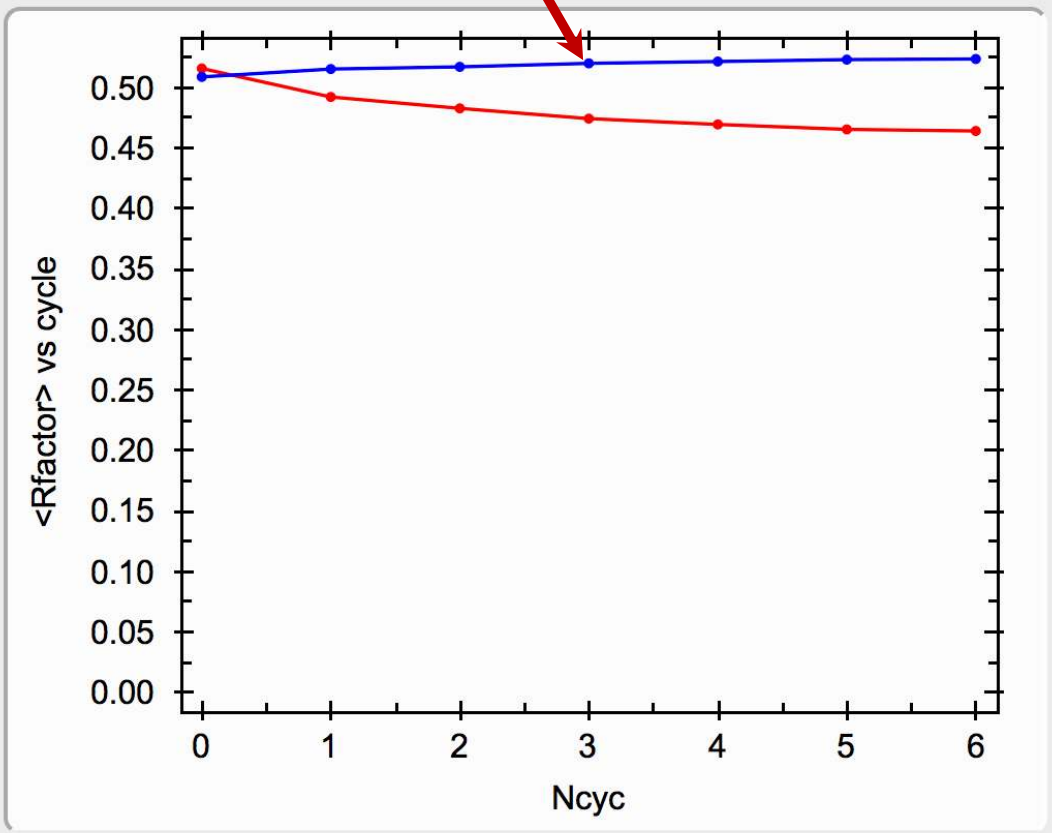
	Initial	Final
R factor	0.5154	0.4637
R free	0.5084	0.5232
Rms BondLength	0.0089	0.0510
Rms BondAngle	1.3596	7.1730
Rms ChirVolume	0.0929	0.6472

R-free > 0.5 and growing

Graph Data

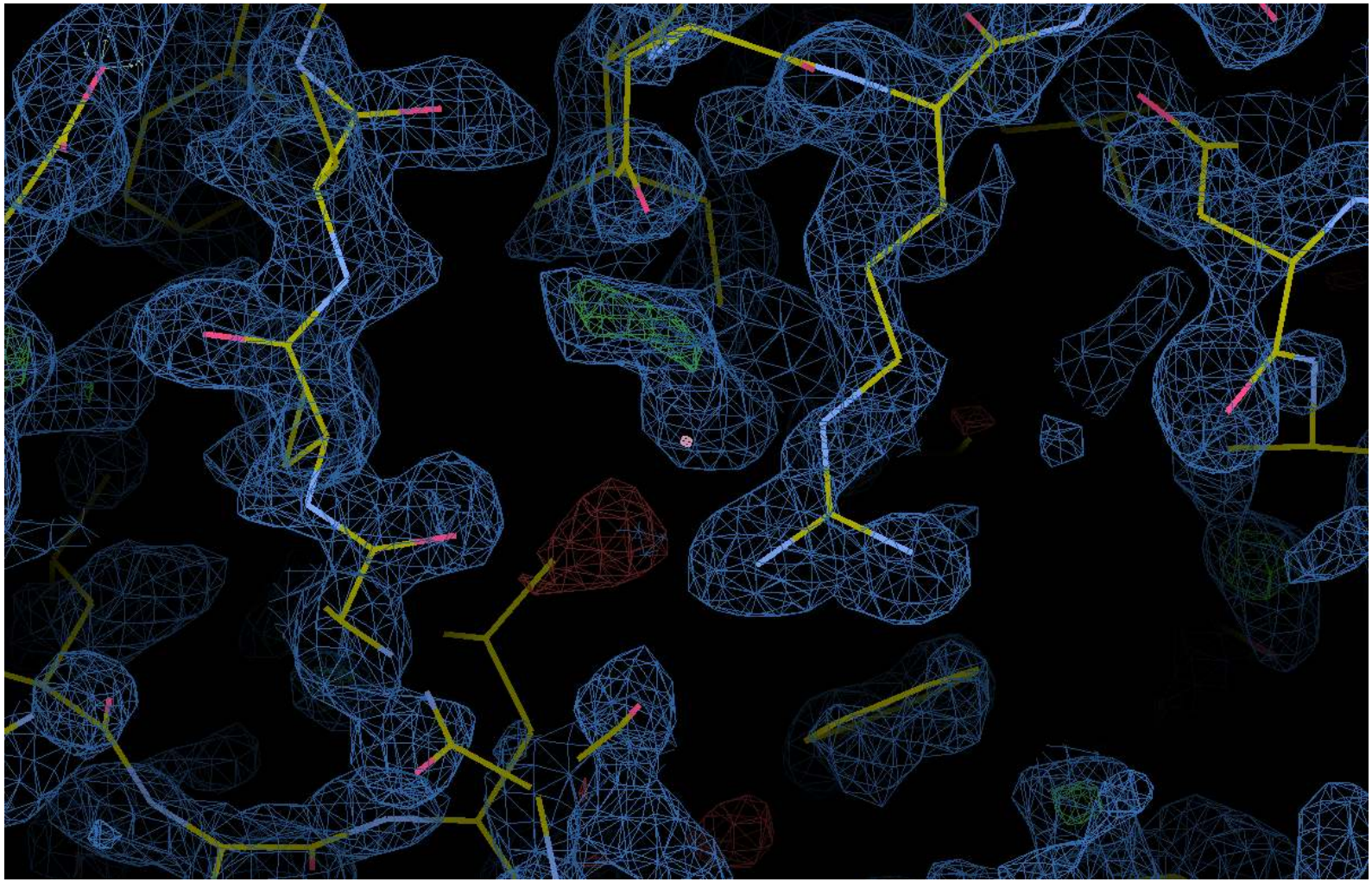
- Rfactor analysis, stats vs cycle
  - <Rfactor> vs cycle
    - Rfact
    - Rfree
  - FOM vs cycle
  - -LL vs cycle
  - -LLfree vs cycle
  - Geometry vs cycle

raw data | Print | Export | Copy



How does wrong MR solution map looks like at 1.6Å?

The maps look more similar to the model the lower the resolution ‘Model bias’
Main chain breaks
No useful features for rebuilding



35 % complete model at 2.5 Å resolution.

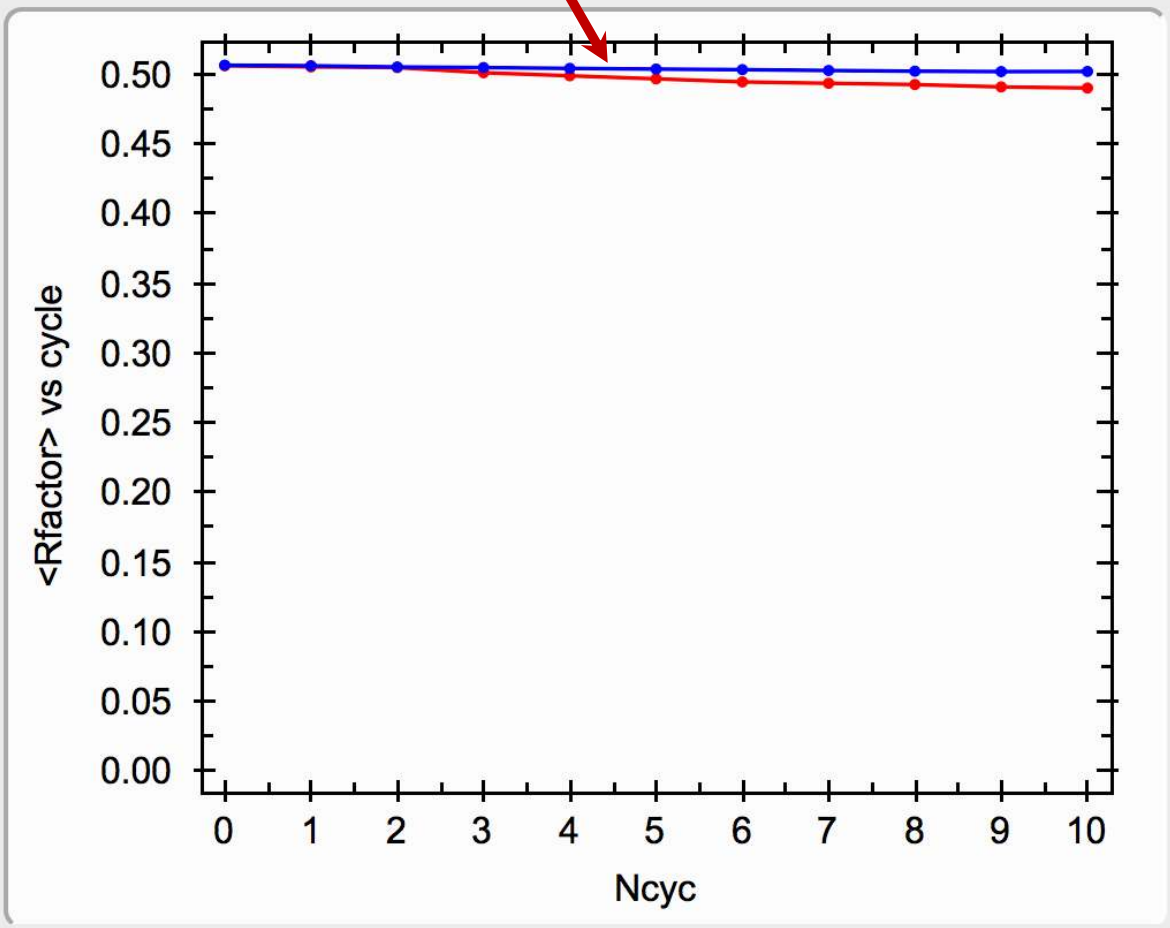
Initial	Final		
	R factor	0.5059	0.4899
	R free	0.5063	0.5018
Rms	BondLength	0.0134	0.0041
Rms	BondAngle	1.5788	1.0578
Rms	ChirVolume	0.1075	0.0943

R-free > 0.5 and slowly going down

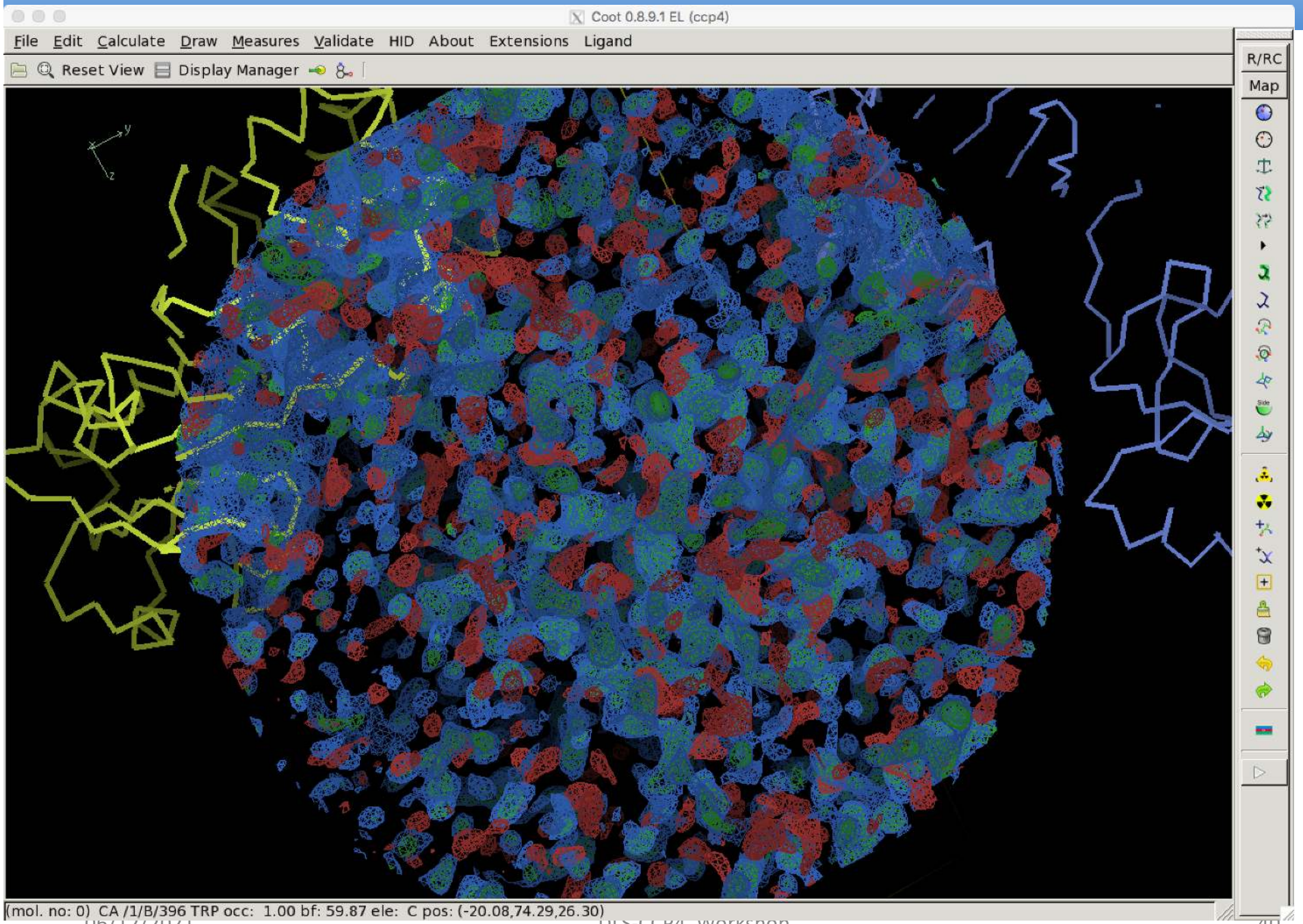
Graph Data

- Cycle 1. Rfactor analysis, F distribution v resln
- Cycle 1. FSC and Fom(<cos(DelPhi)>-acentric, …
- Cycle 10. Rfactor analysis, F distribution v resln
- Cycle 10. FSC and Fom(<cos(DelPhi)>-acentric…
- Cycle 11. Rfactor analysis, F distribution v resln
- Cycle 11. FSC and Fom(<cos(DelPhi)>-acentric…
- Rfactor analysis, stats vs cycle
  - <Rfactor> vs cycle
    - Rfact
    - Rfree
  - FOM vs cycle
  - -LL vs cycle
  - -LLfree vs cycle
  - Geometry vs cycle

raw data | Print | Export | Copy



35 % complete model at 2.5 Å resolution



Structure solution: User's viewpoint

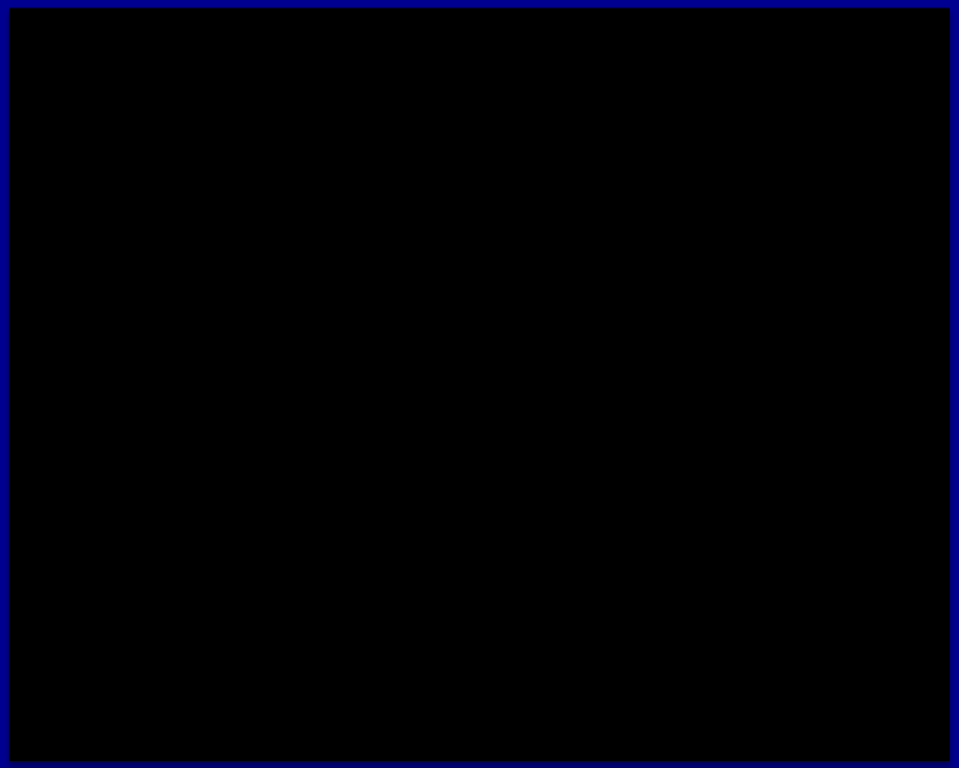
Structural biologists often present difficult cases solved by a combination of techniques and unusual approaches to combat problems and pathologies such as twinning, wrong SG/origin assignment, OD-structure, disorder of large parts of the structure etc.

These examples are highly educational. But…

The fact that well over half of the structures can be (are being) solved by 'a black box' MR or EP pipelines is often underreported.

A Black Box crystallography pipeline

Data, sequence



Refined Model

User does not have to know what is inside

Practical approach to phasing : always start with pipelines.

CCP4 suite contains a number of pipelines both for MR and EP phasing. These are available from CCP4 online, CCP4 cloud and two interfaces – ccp4i and ccp4i2.

Pipelines should be tried first for phasing of new structures.

For learning purposes it may be more useful to analyze pipeline result (post-mortem) than waste time trying to unsuccessfully solve a challenging case.

I have tried a number of phasing pipelines in all CCP4 interfaces.

CCP4 MR Phasing pipelines.

MR Pipelines

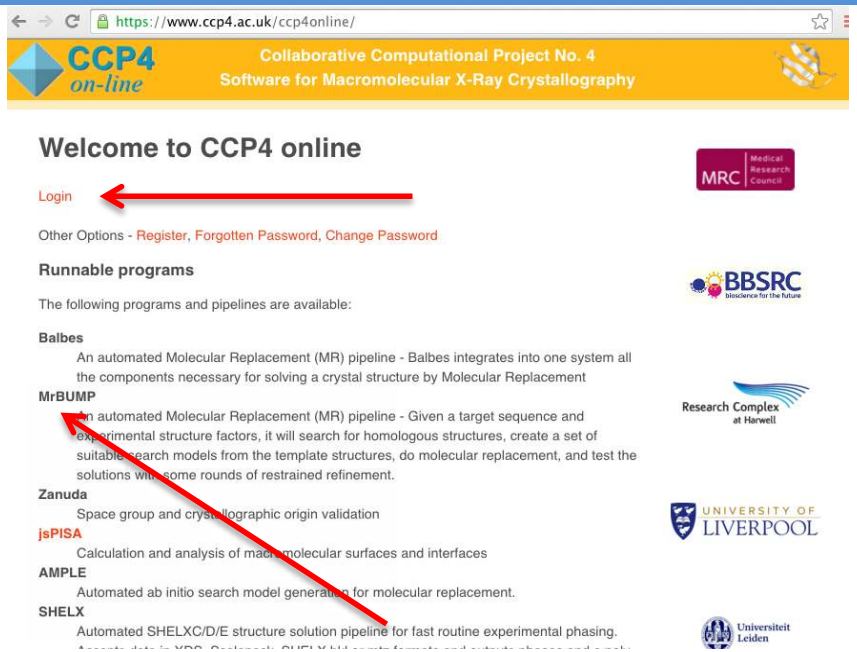
MRBump	CCP4online, CCP4i, CCP4i2, CCP4cloud
	(ca 230 publications in WoS)
BALBES	CCP4online, CCP4i
	(550 publications)
MORDA	CCP4online, CCP4i, CCP4i2, CCP4cloud
	(57 publications)

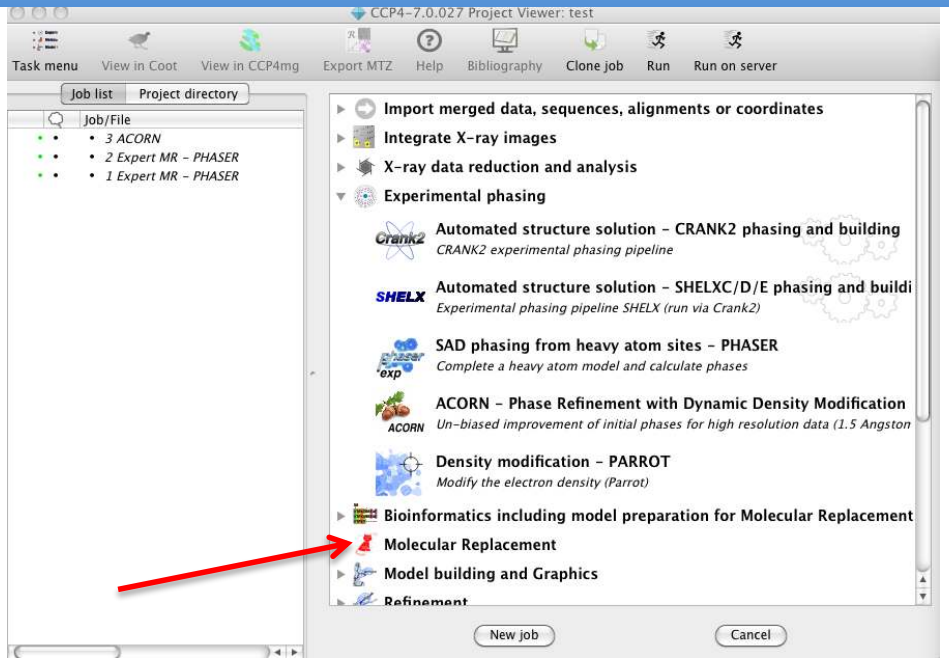
Ab-initio/exhaustive phasing pipelines for difficult cases

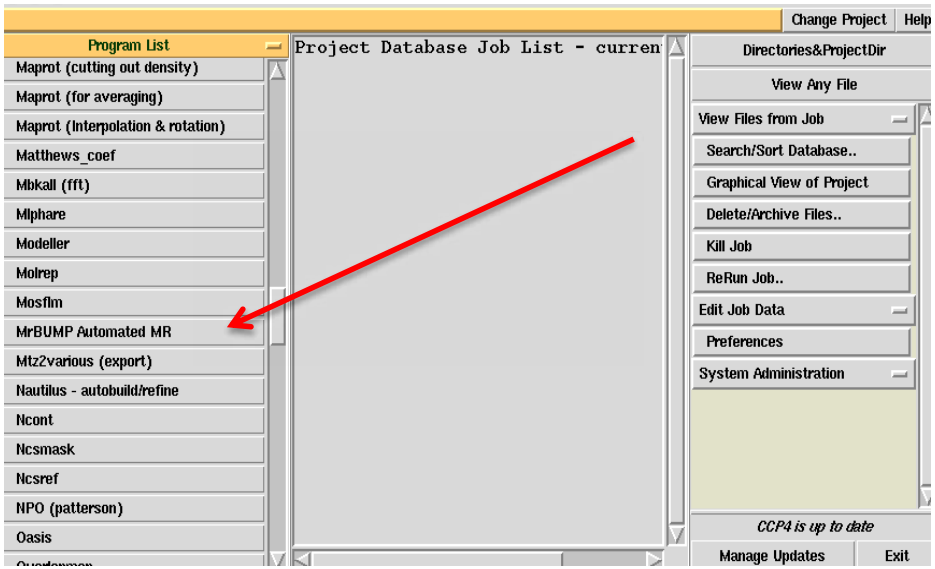
(not discussed in this talk)

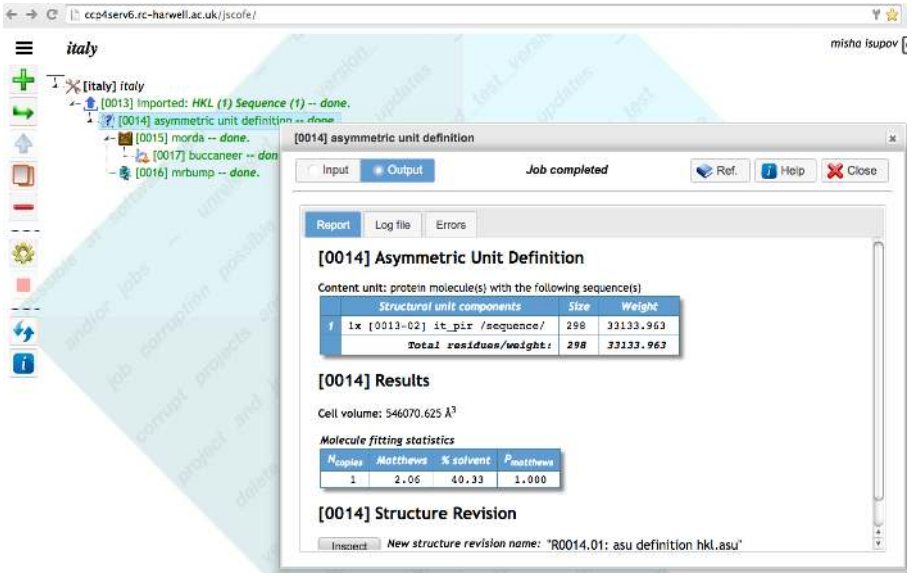
AMPLE	CCP4online, CCP4i, CCP4i2
ARCHIMBOLDO	CCP4i, CCP4i2
SIMBAD	CCP4online, CCP4i, CCP4cloud
FRAGON	CCP4i2

CCP4 interfaces









MR pipelines in CCP4.

- There is a very good chance that a new protein structure can be solved by straight MR.
- A putative MR solution needs to be validated by the downstream refinement, this may require data or model manipulations which may be not trivial for a beginner structural biologist.
- MR phasing pipelines refine putative solutions for several models in all candidate space groups and earmark the best of them .

MR pipelines.

- MR Phasing pipelines approach structure solution by using several models (including oligomers and separate domains) and model ensembles.

- The search parameters are optimized on many cases, therefore these pipelines are very efficient and sometimes it is challenging even to reproduce the pipeline results by running stand-alone MR programs.

- According to a Morda author Alexei Vagin up to 95% of deposited X-ray structures in PDB this year can be solved by an MR pipeline.

MR pipeline test.

- To test existing MR pipelines in CCP4, data and sequences of 5 unpublished (not present in PDB) medium–sized structures with better than 2 Å resolution were submitted to each of CCP4 MR pipelines (MrBump, BALBES, MORDA) on CCP4 online server. Three of these cases have medium difficulty and two are easy.

- All three pipelines have found the solution and built the correct model for each project, as can be verified by final R-factors of the partially refined models.

- Presented are important points of these structure determinations.

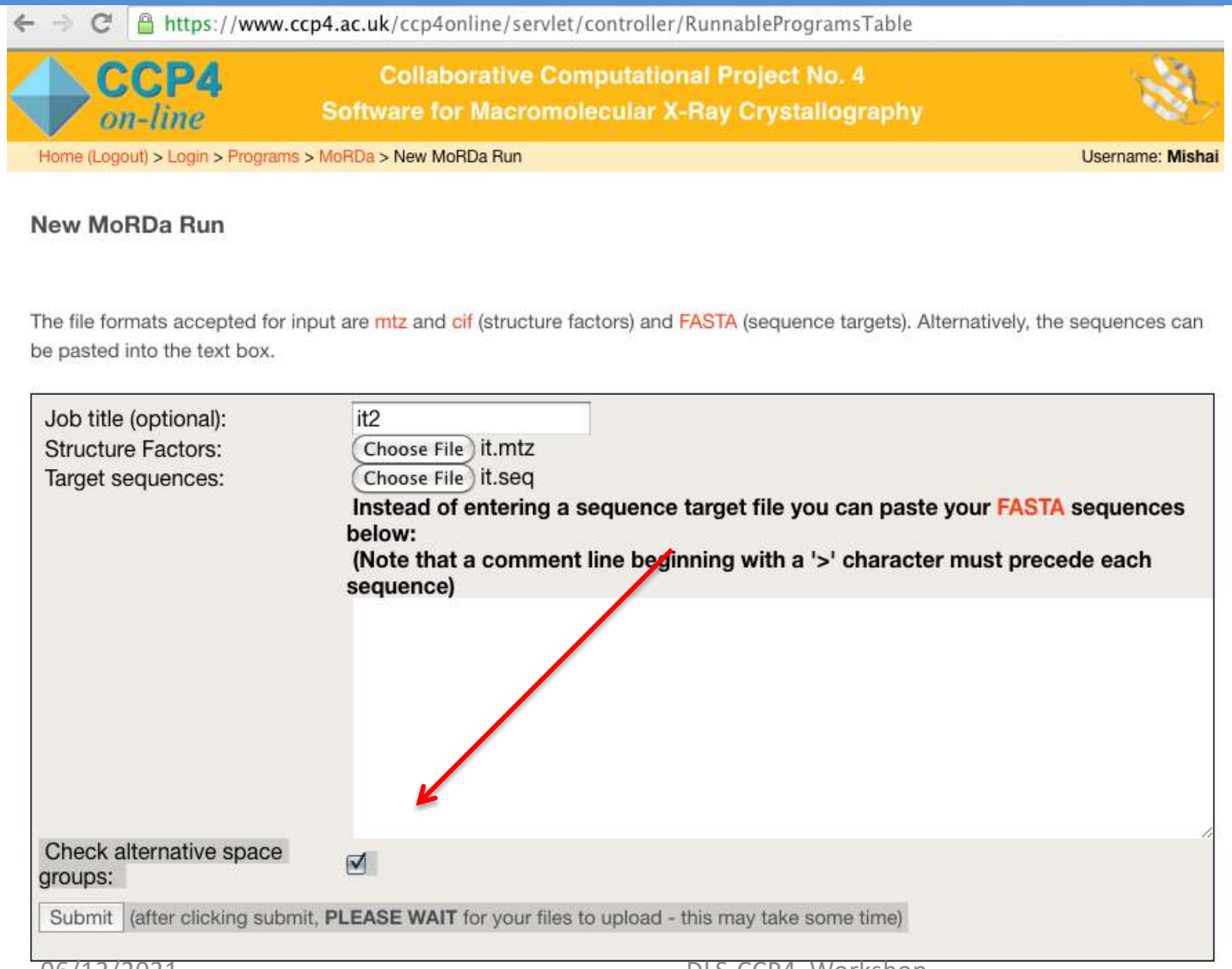
MR pipeline trial proteins.

Project	Resolution Å	Highest identity model %	Space group	Unit cell a,b,c Å, α, β, γ °	Monomers in a.u.
Serine hydroxy-methyl transferase *Thermoanaerobacter*	1.6	70	$P2_1$	49.7, 108.2, 73.5 90, 90.3, 90	2 x 413 aa
Metagenomic epoxide hydrolase Sample Tomsk55	1.6	31	$C222_1$	41.2, 84.2, 157.5, 90, 90, 90	1 x 297 aa
Metagenomic epoxide hydrolase Sample China65	1.4	34	C2	163.9, 46.2, 73.9, 90, 106.9, 90	2 x 293 aa
Sugar transaminase *Archaeoglobus*	1.3	53	$P2_12_12_1$	70.2, 105.9, 111.2 90, 90, 90	2 x 371 aa
Sugar transamina-se*Thermoanaerobacter*	1.5	39	$P3_221$	76.4, 76.4, 134.4 90, 90, 120	1 x 375 aa

Treatment of space group ambiguity in CCP4 phasing pipelines.

- Space group assignment is done at the data integration/scaling stage (POINTLESS).
- Enantiomorph ambiguity arises when systematic absences used for the space group assignment, are the same for two distinct space groups, e.g for both $P4_3$ and $P4_1$ general condition for reflection of type 00*l* is *l=4n, i.e.* both space groups are possible and can only be resolved by phasing.
- Unfavourable orientation of the crystal used for data collection may result in the intensities not being measured along crystallographic axis, e.g. if the reflections 0*k*0 in an orthorhombic space group are not measured, the axis y can be either screw or rotational.
- Pseudo-symmetry may result in pseudo-absences in the diffraction pattern, affecting assignment of a correct space group.
- Straightforward solution is to try phasing in all possible space groups.

Do not forget to tick the box if space group ambiguity is expected. Not present in MrBump online – run jobs for all candidate space groups.



Enantiomorph choice (sugar transaminase)

MoRDa

Please cite the following paper, if you used a solution from MoRDa:
A. Vagin, A. Lebedev, *Acta Cryst.* (2015). A71, s19

MoRDa homepage contains more details and instructions for local installation.

PROCESS **8644678102** HAS ENDED

Check alternative space groups: ☐

Report | Prep | Details

- Job Details
- Input Data
- Best Solution

No	DB code	ens	ident	Nres	Nexp	Nfound	Q	correct	space group
1	3dr4A	+	0.386	363	1	1	0.480		P 31 2 1

PROCESS **7506573332** HAS ENDED

Check alternative space groups: ☑

Report | Prep | Details

- Job Details
- Input Data
- Best Solution

No	DB code	ens	ident	Nres	Nexp	Nfound	Q	correct	space group
1	3dr4A	+	0.386	363	1	1	0.830	***	P 32 2 1

The best solution for the project epoxide hydrolase Tomsk55 by MORDA is based on the PDB entry which is not present in not-renewed BALBES database.

```
#-------------------------------------------------------------------------------#
#                          A structure is suggested by BALBES                   #
#                      Its probability to be a solution is 99.0%                #
#-------------------------------------------------------------------------------#
The solution model index is sq1st3m1
|-------------------------------------------------------------------------------|
| ITS PDB FILE       |              results/refmac_final_result.pdb             |
|-------------------------------------------------------------------------------|
| ITS MTZ FILE       |              results/refmac_final_result.mtz             |
|-------------------------------------------------------------------------------|
| R_ini/R_fin        |  0.5460/0.4300  |  Rfree_ini/Rfree_fin  |  0.5450/0.4570 |
|-------------------------------------------------------------------------------|
| ITS Q FACTOR       |                          0.630                          |
|-------------------------------------------------------------------------------|
```

MoRDa homepage contains more details and instructions for local installation.

PROCESS **1265910401** HAS ENDED

Report | Prep | Details

Searches for sequence 1

No	DB code	ens	ident	Nres	Nexp	Nfound	Z score	initial R-Rfree	final R-Rfree	Q	correct
1	4inzA	+	0.311	286	1	1	11.687	0.562-0.568	0.369-0.402	0.736	***
2	4inzA_1	+	0.379	195	1	1	11.485	0.562-0.568	0.407-0.443	0.667	***
3	2e3jA	+	0.280	293	1	1	7.740	0.562-0.576	0.448-0.470	0.611	**
4	2e3jA_1	+	0.333	204	1	1	8.262	0.560-0.565	0.450-0.476	0.597	**

Comparison of MR pipelines in CCP4

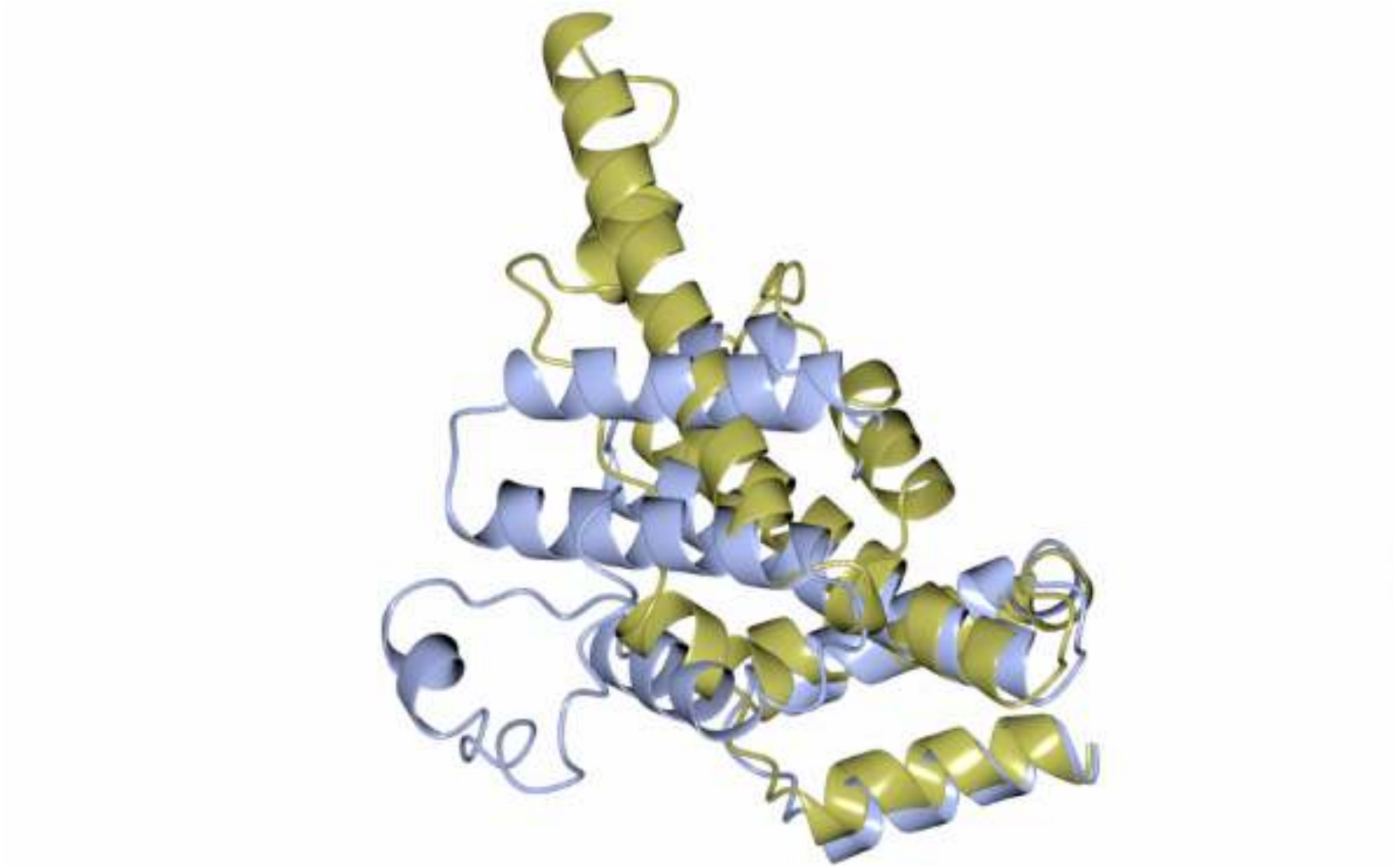
- The three CCP4 MR pipelines are consistently solving medium difficulty cases and are likely to be mutually complementary in more difficult projects.
- Balbes and Morda have their own internal databases, this speeds up calculations, MrBump relies on external resources.
- Own database may be a weakness, since regular update is required. Balbes database is few years behind. Morda database is updated monthly. Databases used by MrBump are renewed externally except for the sequence database.
- MrBump uses both PHASER and MOLREP increasing solution chances, but takes longer to run.
- Analyse the output of pipelines. If no solution is found your project presents a challenging case.

How to approach a new project.

1. Submit data to all CCP4 MR phasing pipelines. Add Alphafold 2/RosettaTTAfold models. If a solution is found, proceed to finalize refinement/validation.
2. Not conclusive - try refinement/ density modification/model rebuilding of putative solutions (Shelxe, Buccaneer, Arp/Warp), especially if FreeR is below 45-48%.
3. Try to address space group/origin mis-assignment (Zanuda).

4. If Q-factors of putative MR solutions are low and FreeR values are well over 50% it is worth trying MR pipelines using data collected on similar crystals which may happen to be better (more ordered, not-twinned, have less radiation damage etc).
5. Consider experimental phasing, crystallisation of separate domains, try stand-alone MR programs (Phaser, Molrep, Amore) with manually edited search models, ab-initio phasing pipelines, seek help of expert crystallographers or outsource.

A pinch of salt: not all Alpahfold 2 structures will solve your phase problem.



Acknowledgements

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