Molecular Replacement

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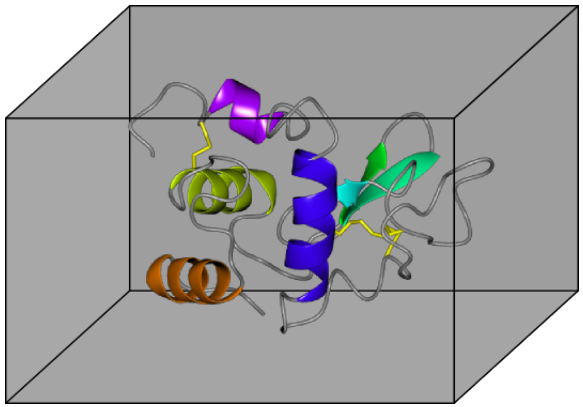
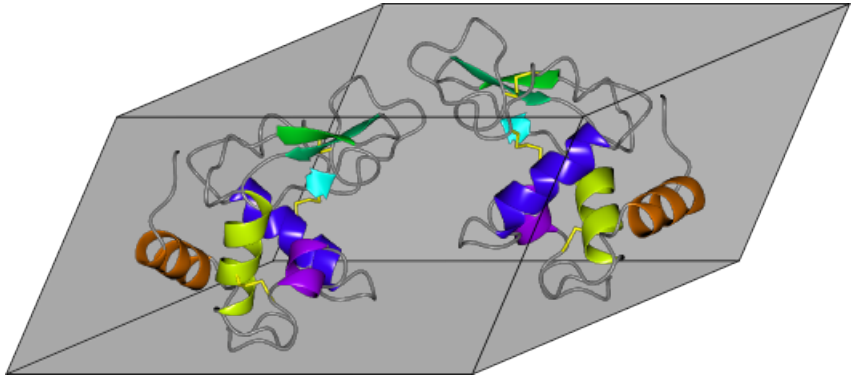
CCP4

MR Problem

Known crystal structure



New crystal structure

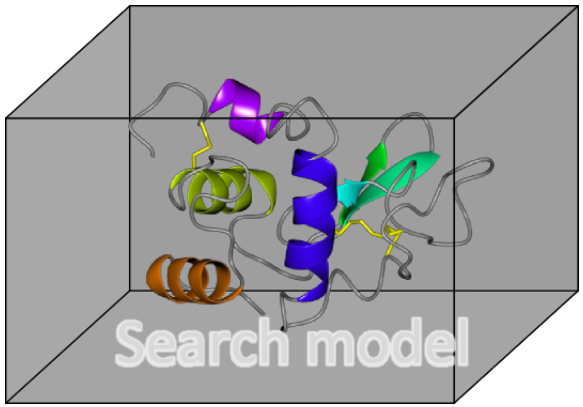


Given:
- Crystal structure of a homologue
- New X-ray data

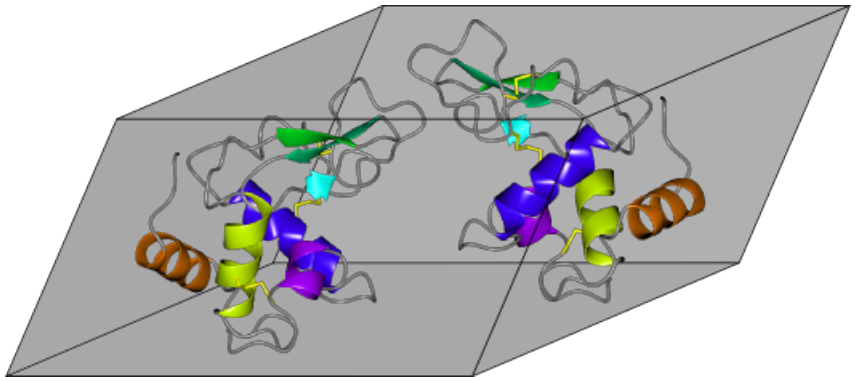
Find:
- The new crystal structure

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:

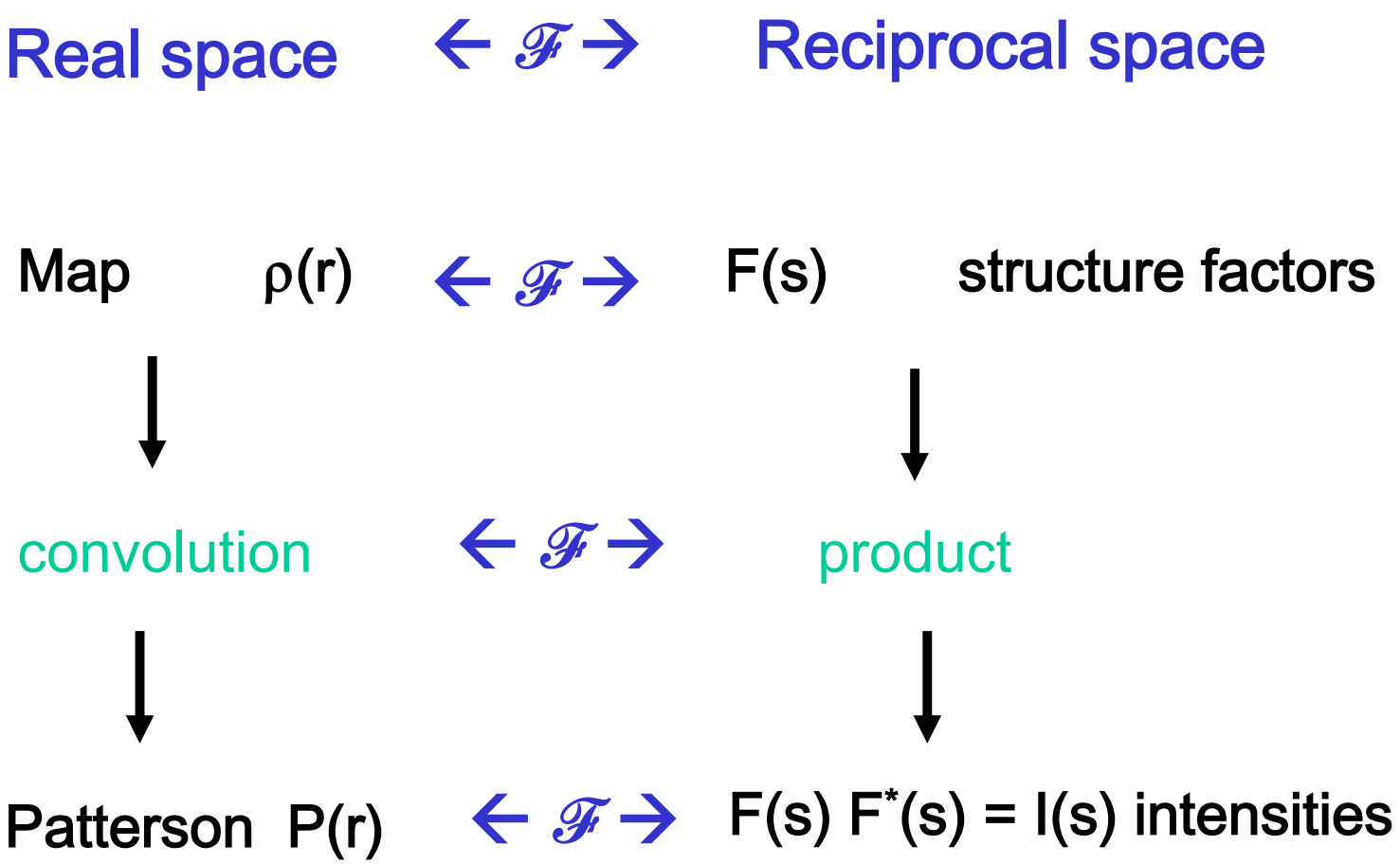
- Scoring
  - the match between the data and an (incomplete) crystal model
  - ideally: the highest score = correct solution

Real and Reciprocal spaces

- Terms may refer real space but actual calculations may be performed in the reciprocal space:
  - "Search in the electron density"
  - "Patterson search"

- The concepts formulated in real space are more intuitive

Functions in Real and Reciprocal spaces



Structure factors and Electron density map

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

– A discrete complex function in the reciprocal space

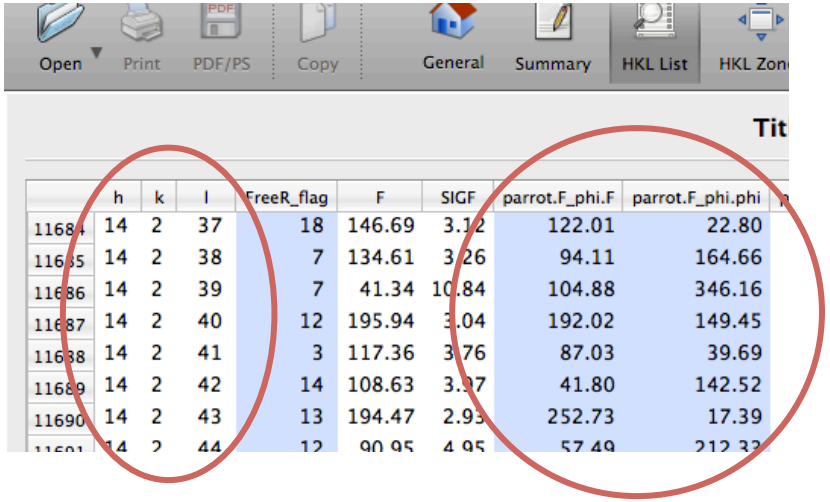
At given *h, k, l*

– Complex number:

$$F = A + iB$$

– Can be expressed via structure amplitude and phase

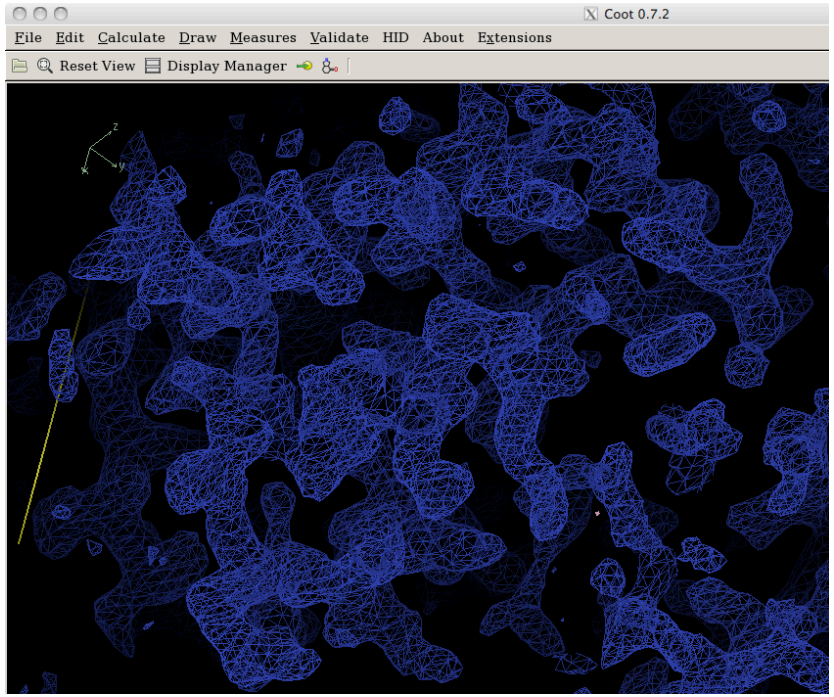
$$F = |F| \exp(i\phi)$$



	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	3.97	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	212.33

Electron density map

– periodic 3-d function in real space



is directly interpretable

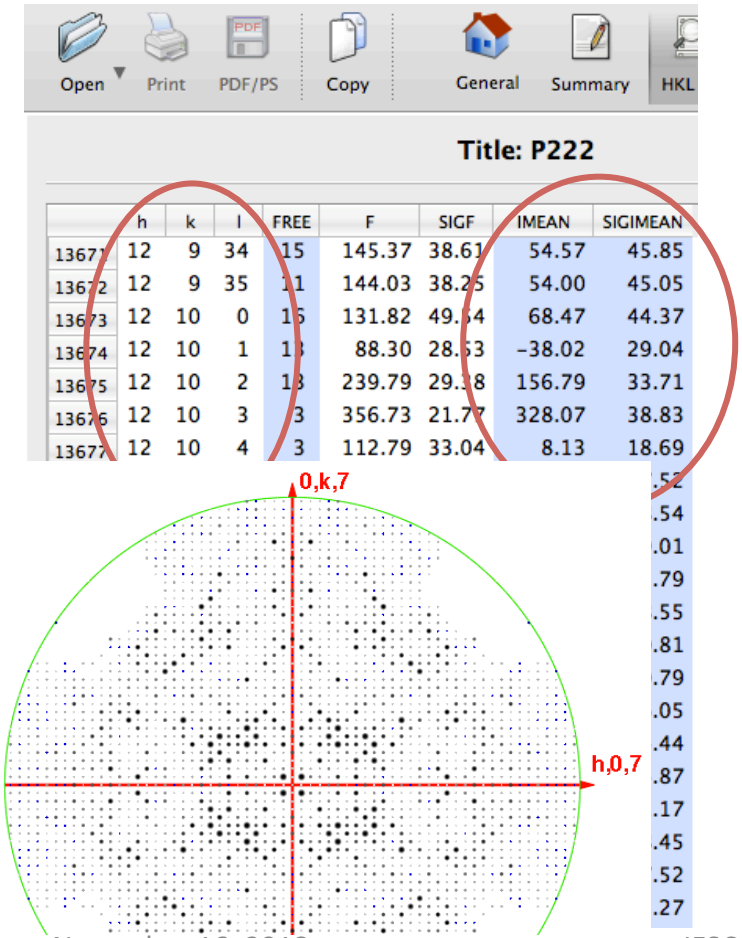
– model building

– real-space fitting of fragments

Intensities and Patterson map

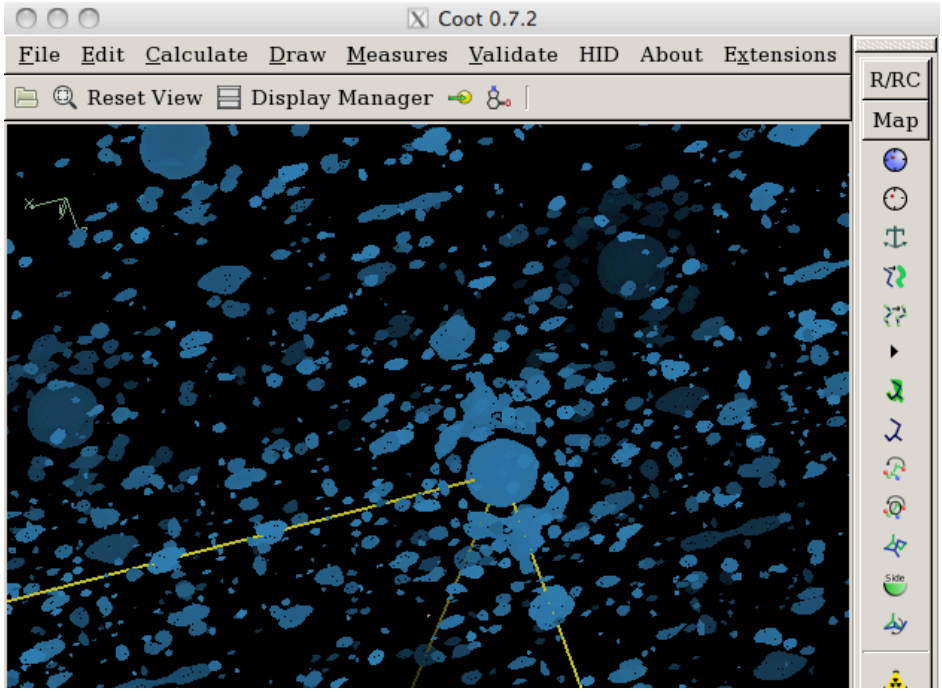
Intensities $I(h,k,l)$

– 3-d discrete real function in the reciprocal space



Patterson map:

– 3-d function in real(*) space



– No features resembling a protein molecule

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

Data and MR

MR: Two distinct cases dependent on availability of phases

- Data = structure factors (include phases)
  - "Search in the electron density"
    - Electron density maps are compared: calculated *vs*. observed
    - Model building is a more straightforward approach

  » Useful in special cases

- Data = observed intensities (no phases)
  - "Patterson search"
    - Patterson maps are compared: calculated *vs*. observed
    - Direct model building is impossible in the absence of phases

  » The most common case of MR

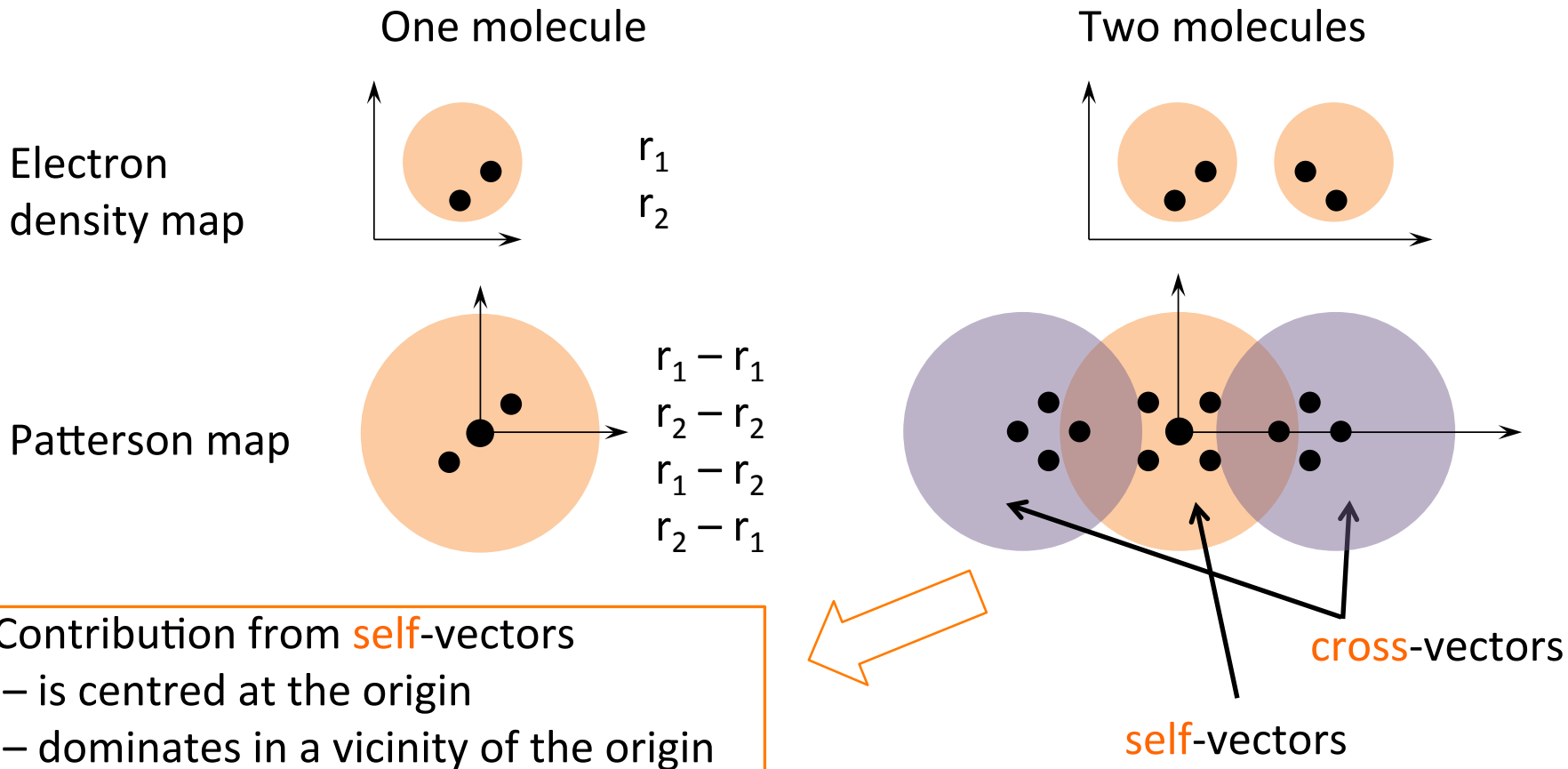
As a rule, all computations are in the reciprocal space

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

Patterson search

Patterson map:

- Contribution from self-vectors is centred at the origin

- Self-vectors are, in average, shorter than cross-vectors
  - Peaks from self-vectors dominates in a vicinity of the origin
  - Peaks from cross-vector dominates away from the origin

- One 6-dimensional search splits into
  - Rotation Function: 3-dimensional search (using self-vectors)
  - Translation Function: 3-dimensional search (using cross-vectors)

Rotation Function

$$RF(\alpha,\beta,\gamma) = \iiint P^{\text{obs}}(\mathbf{r}) \times P^{\text{calc}}_{\text{self}}(\alpha,\beta,\gamma,\mathbf{r})\,\mathrm{d}\mathbf{r}^{3}$$

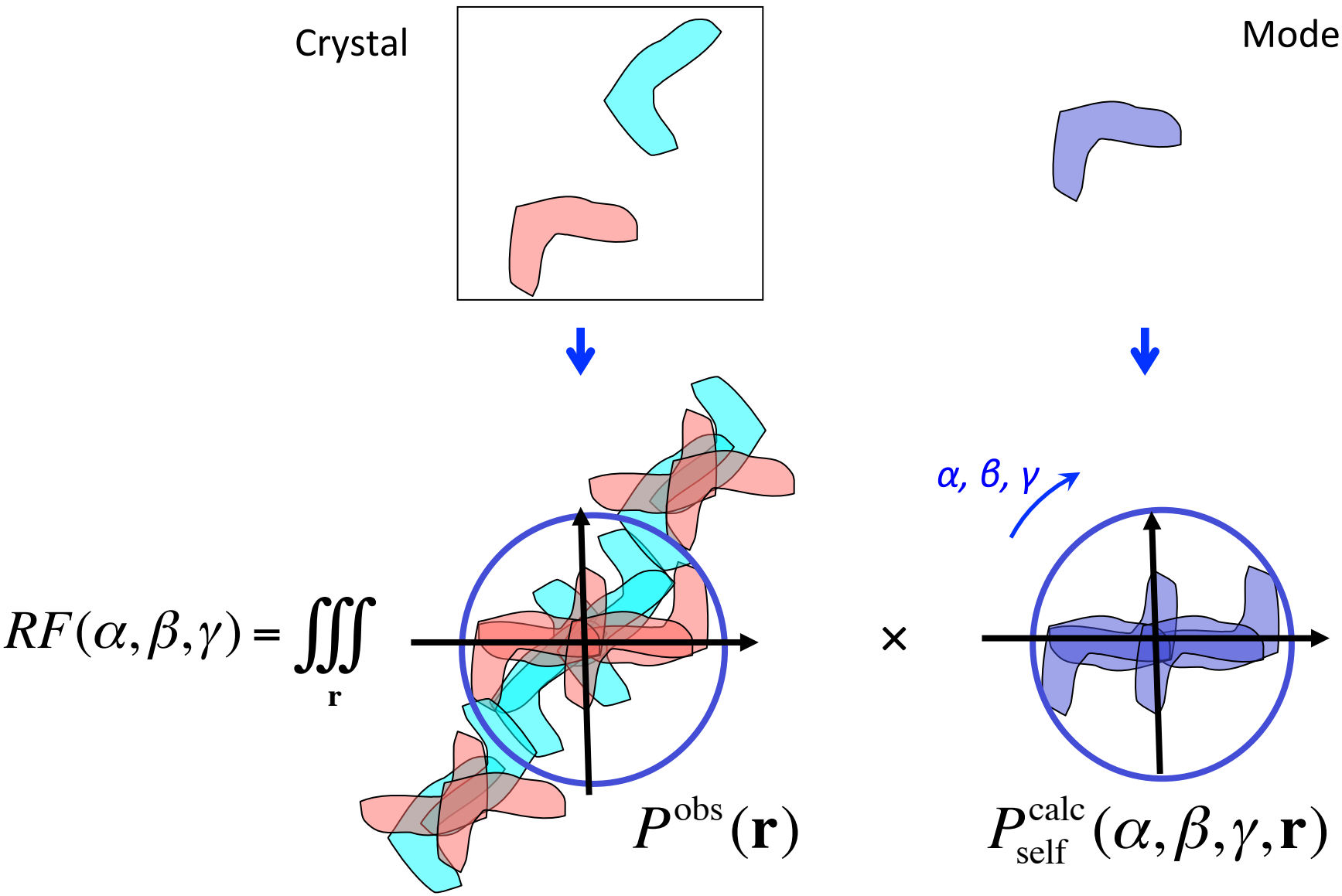
$P^{\text{calc}}_{\text{self}}(\alpha,\beta,\gamma,\mathbf{r})$ contains only

- self-vectors

$P^{\text{obs}}(\mathbf{r})$ contains

- self-vectors (signal from one of the orientations in the crystal)
- cross-vectors (noise)

Rotation Function



Translation Function

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

$P^{\text{calc}}_{\text{cross}}(\mathbf{t},\mathbf{r})$ contains

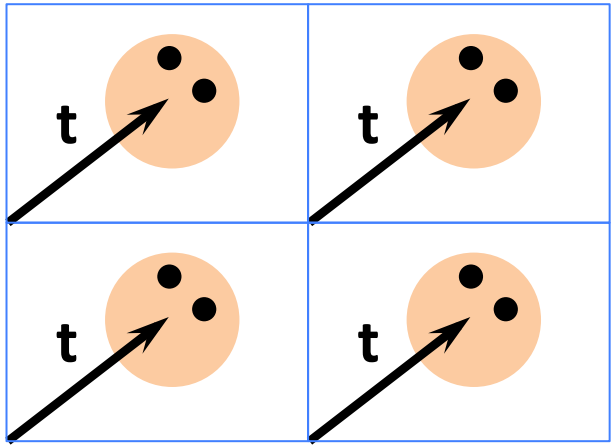
- self-vectors (background)
- cross-vectors

$P^{\text{obs}}(\mathbf{r})$ contains

- self-vectors (background or noise)
- cross-vectors (relevant vectors: signal, others: noise)

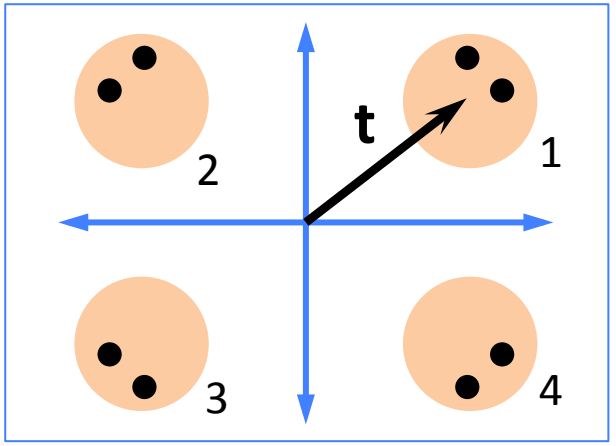
Translation Function

P 1



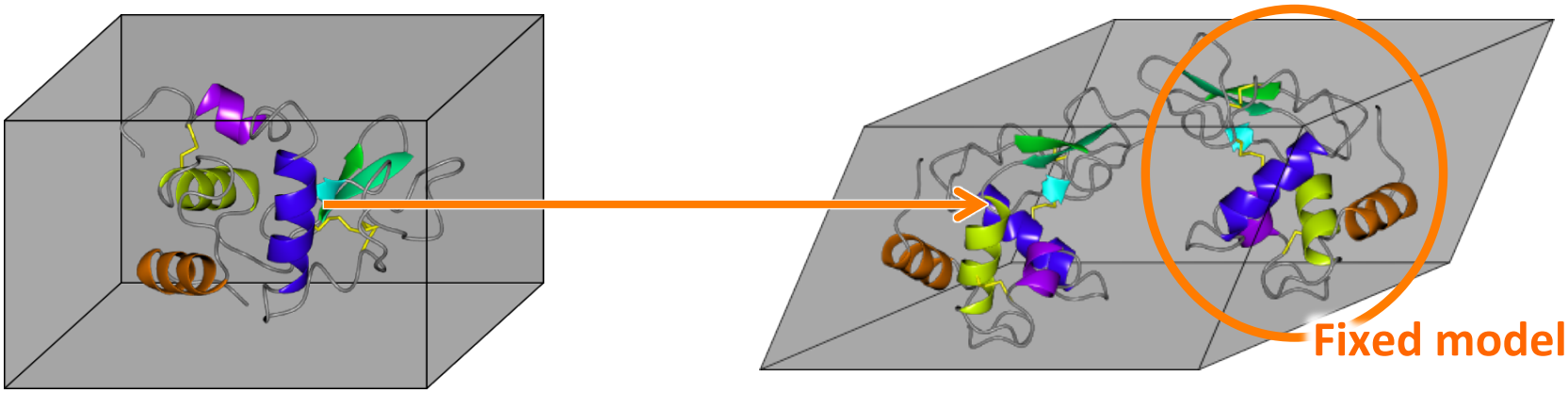
- Translation **t** does not change the structure
  - can be compensated with shift of crystallographic origin
- TF step is not needed

*P*222



- The centre of molecule 1:
  - parameter **t**
- Centres of molecules 2, 3 and 4
  - from symmetry operation
- MR program matches $P_{calc}(t)$ to P_{obs} to find the best matching t

Fixed partial model



Almost the same equation as for a single molecule search,

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

- Again FFT technique can be used

- No exception for the space group *P*1 anymore!
  - translation of the second copy relative to the first one cannot be compensated by a shift of crystallographic origin

Packing considerations

Molecules in the crystal do not overlap

How can we use this information?

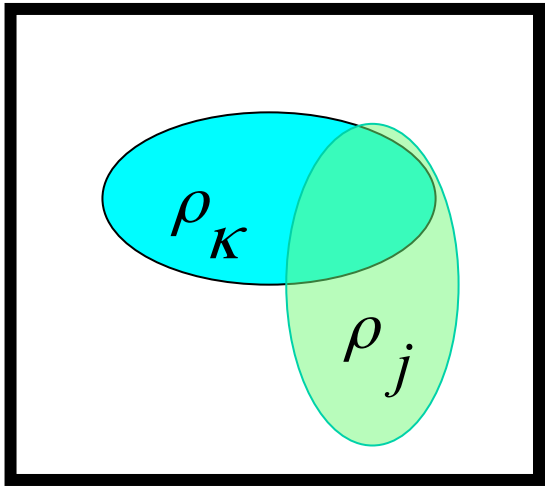
» Patterson map does not explicitly reveal molecular packing

Reject MR solutions

- Restrict distance between centres of molecules
- Count close interatomic contacts

Modify TF

- Divide TF by Overlap Function
- Multiply TF by Packing function



Specialised MR techniques

- Search in the density (phased MR)
- Handling Translational Non-Crystallographic symmetry
  - Non-origin peaks in the Patterson map indicate the presence of TNCS
  - Requires special handling of model errors (Phaser)
  - Molecules related by TNCS can be found in one go as they have nearly the same orientation
- Self Rotation Function
- Locked RF and TF
  - Using point symmetry of oligomers
- Exhaustive searches
- Stochastic searches

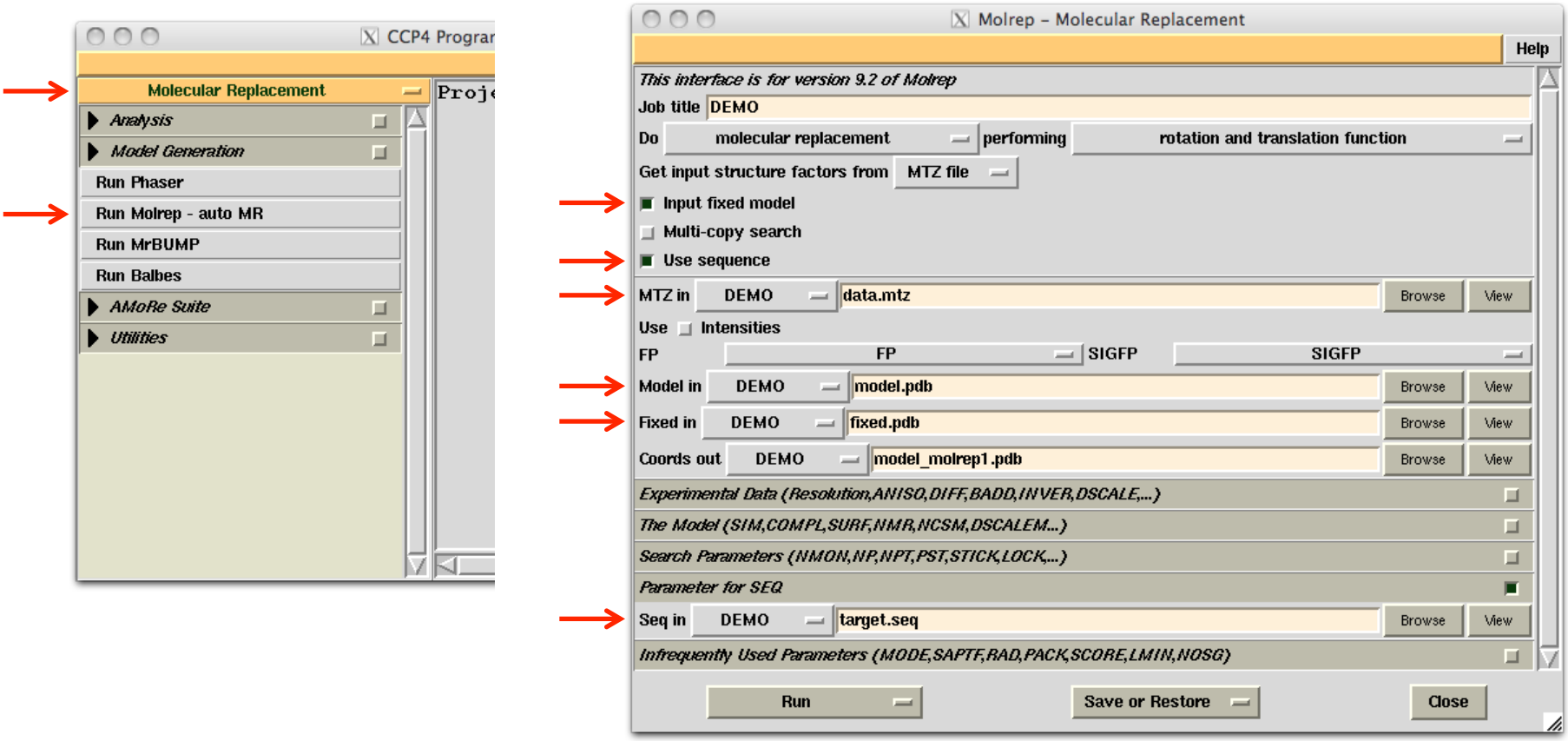
Molrep

Alexey Vagin

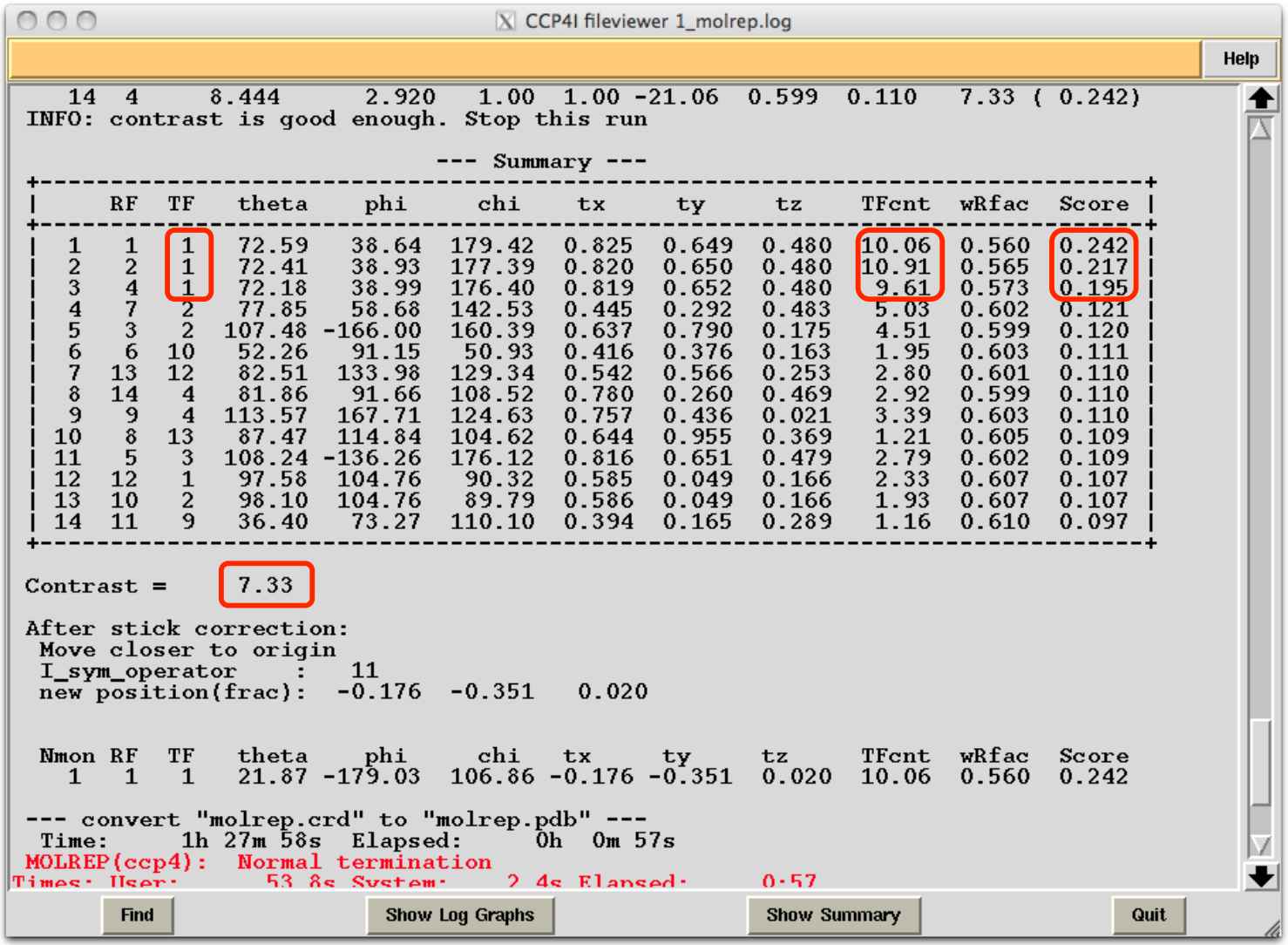
YSBL University of York

Molrep

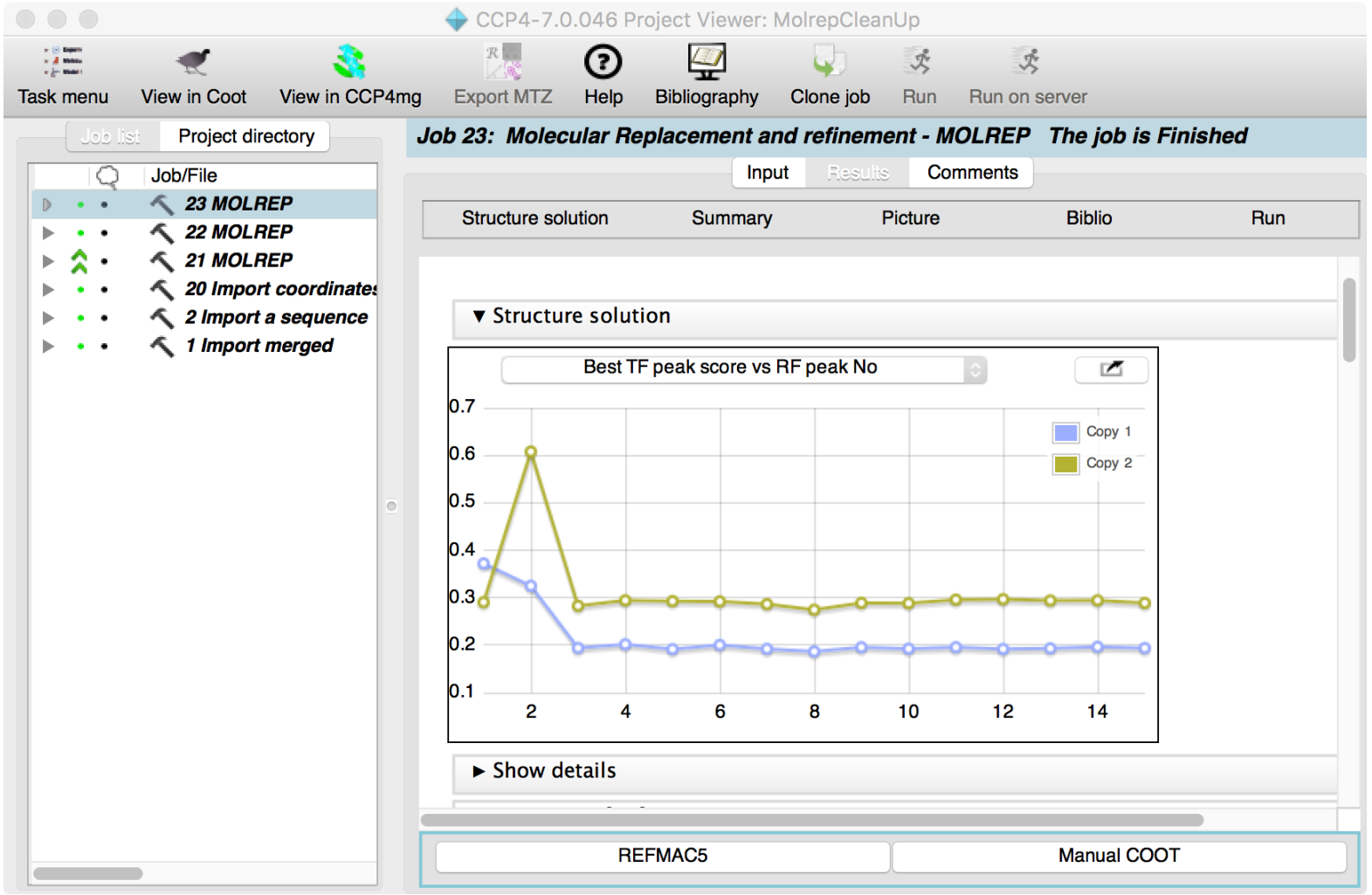
```
molrep -f data.mtz -m model.pdb -mx fixed.pdb -s target.seq
```



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
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
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▶ Show details
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Manual COOT

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)

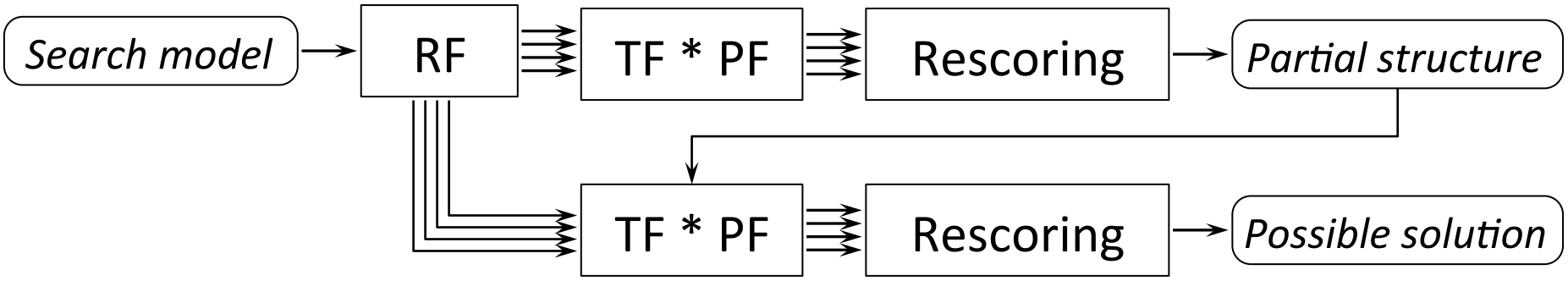
Model modification in MOLREP

```
molrep -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

Molrep protocol for two copies of a model

X-ray data ⟶ all steps



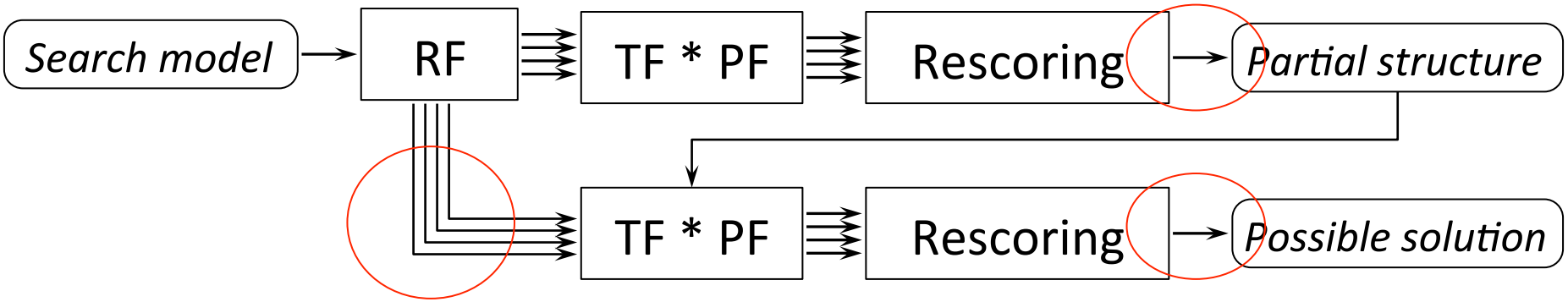
$RF = \sum_{hkl} w * I_O * I_C(\alpha\beta\gamma)$

$TF = \sum_{hkl} w * I_O * I_C(xyz)$

Rescoring: Correlation Coefficient* PF

Molrep protocol for two copies of a model

X-ray data → all steps



$RF = \sum_{hkl} w * I_O * I_C(\alpha\beta\gamma)$

$TF = \sum_{hkl} w * I_O * I_C(xyz)$

Rescoring: Correlation Coefficient* PF

Molrep vs Phaser

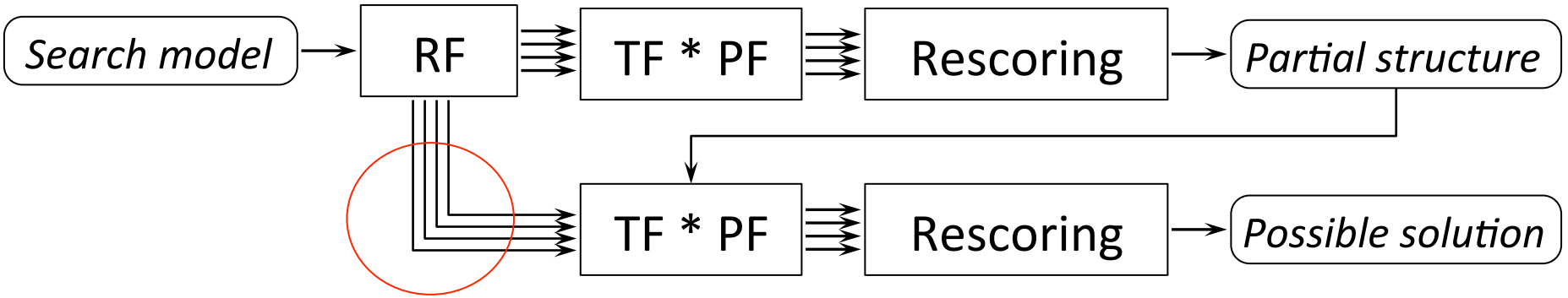
Single solution is taken forward	>>	More sophisticated search strategy
TF/ sig(TF)	=	TFZ
CC	>>	Log-likelihood gain (LLG)
LS rigid body refinement	>>	LL-based rigid body refinement
and more		

Improved scoring

- is crucial for using distant homologues successfully in MR method
- allows correct placement of small fragment models (even single atom)

Molrep protocol for two copies of a model

X-ray data → all steps

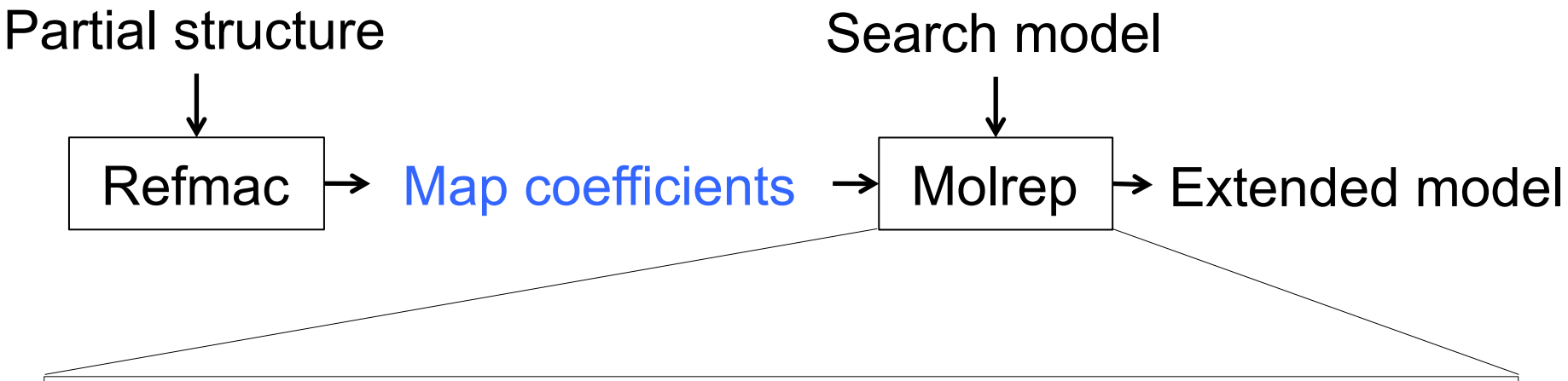


$RF = \sum_{hkl} w * I_O * I_C(\alpha\beta\gamma)$

$TF = \sum_{hkl} w * I_O * I_C(xyz)$

Rescoring: Correlation Coefficient* PF

Search in the electron density map



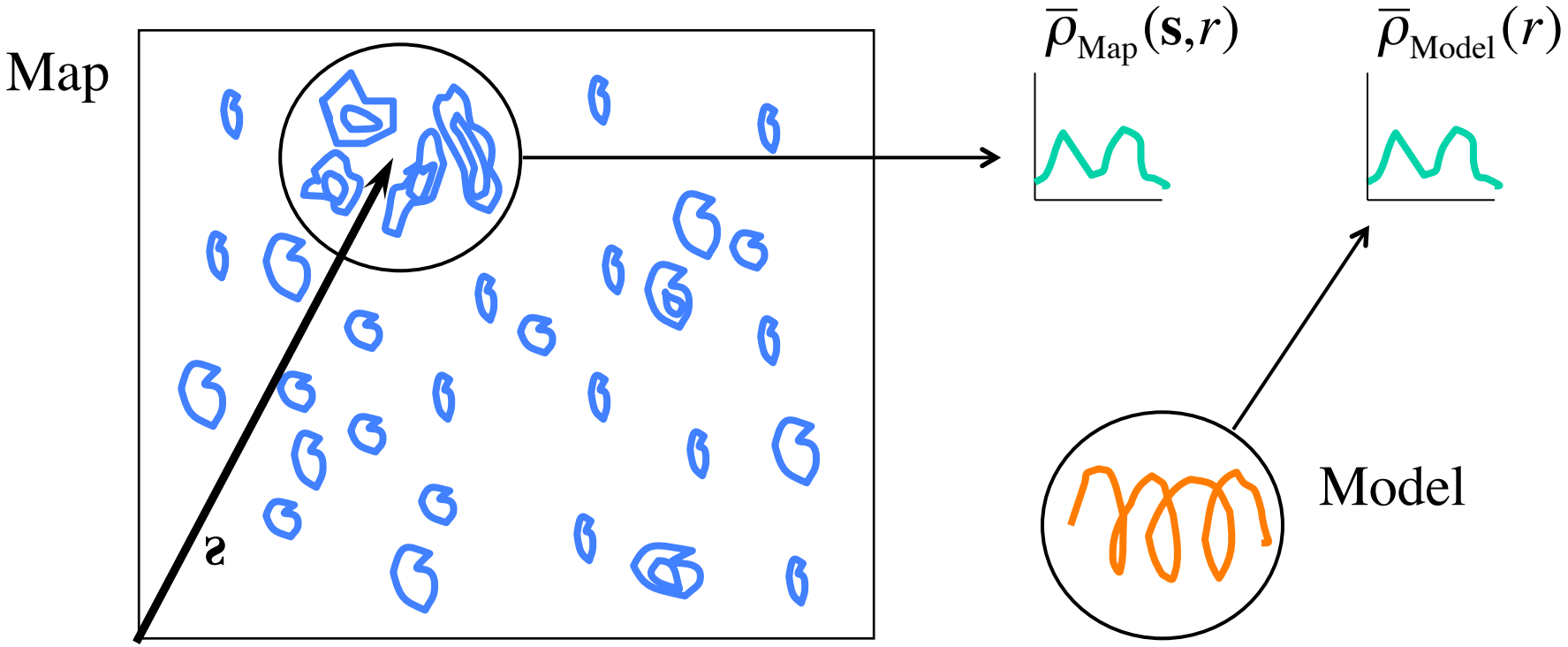
Search in the map

- Calculate 2-1 or 1-1 maps after restrained refinement of partial structure
- Flatten the map corresponding to the known substructure
- Calculate structure amplitudes from the modified map
- Use these modified amplitudes in Rotation Function
- And finally – Phased TF

Molrep: SAPTF

Spherically Averaged Phased Translation Function
(FFT based algorithm)

$$\mathrm{SAPTF}(\mathbf{s}) = \int \overline{\rho}_{\mathrm{Map}}(\mathbf{s},r)\ \overline{\rho}_{\mathrm{Model}}(r)\ r^2\,\mathrm{d}r$$



Molrep: Search in the map with SAPTF

1. Find approximate position:
   Spherically Averaged Phased Translation Function

2. Find orientation:
   Local Phased Rotation Function
   - Local search of the orientation in the density

3. Verify and adjust position:
   Phased Translation Function

Molrep: Search in the map with SAPTF

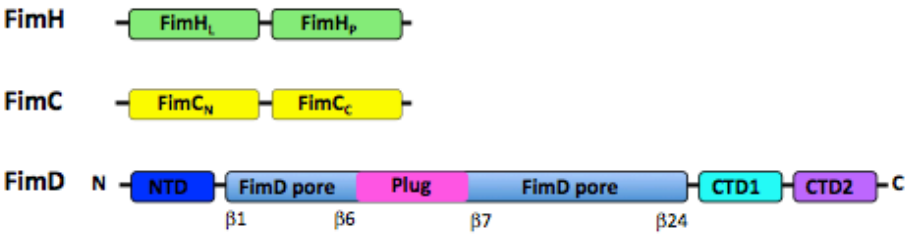
1. Find approximate position:
   Spherically Averaged Phased Translation Function

2. Find orientation:
   Local Rotation Function
   - Structure amplitudes from the density within the SAPTF sphere

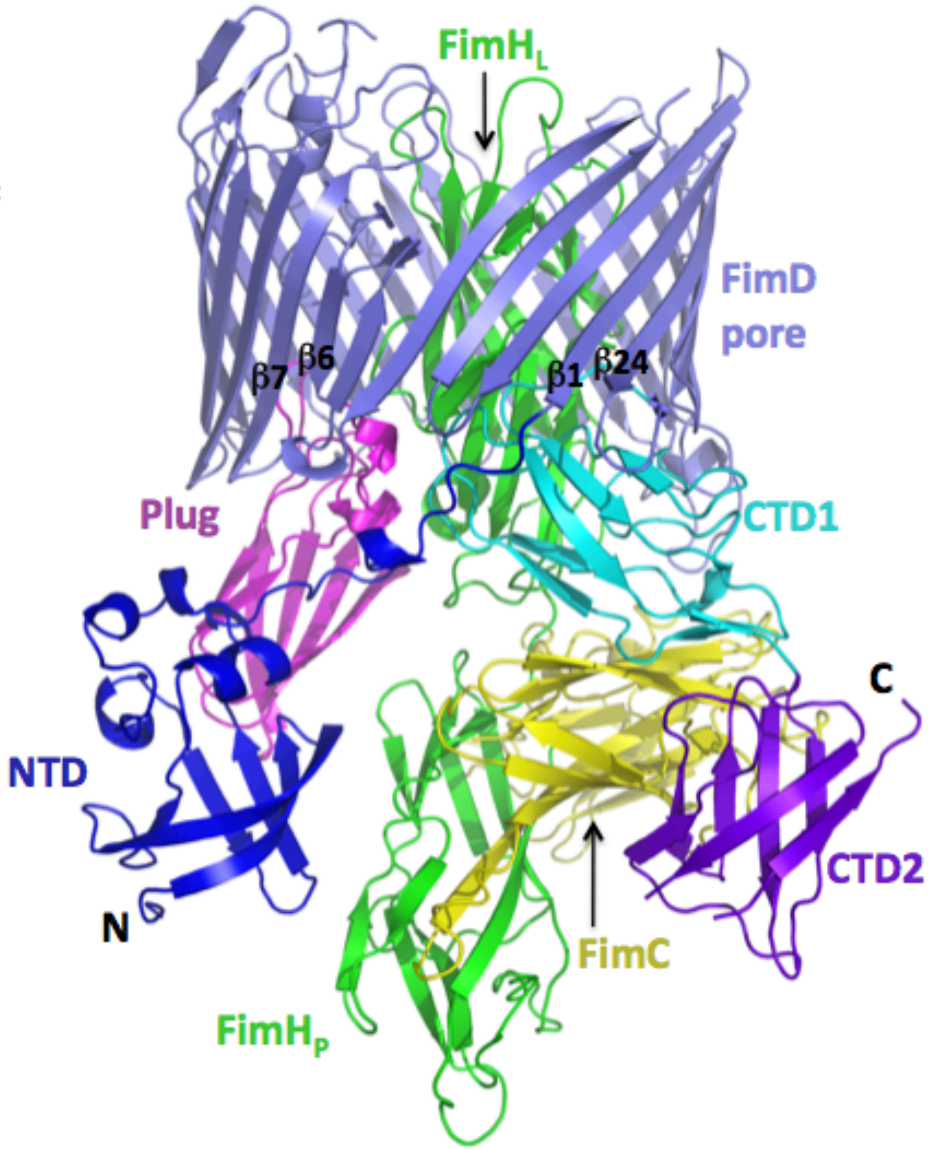
3. Verify and adjust position:
   Phased Translation Function

- Local RF is less sensitive than Phased RF to inaccuracy of the model position

Example



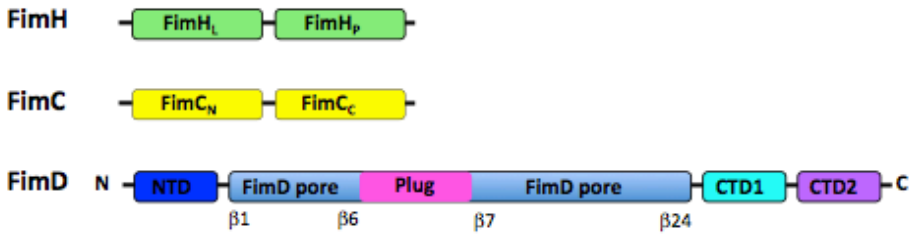
- Asymmetric unit two copies
- Resolution 2.8 Å



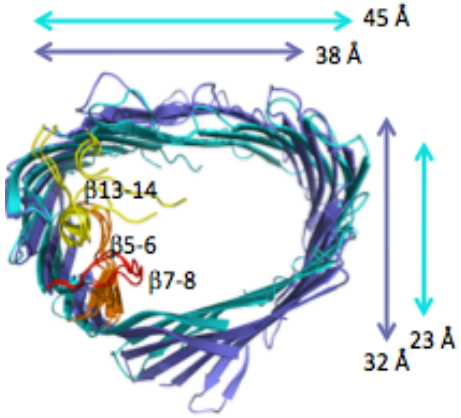
Phane et. al (2011) Nature, 474, 50-53

Usher complex structure solution

1. Conventional MR
   - FimC-N + FimC-C
   - FimH-L + FimH-P
   - FimD-Pore



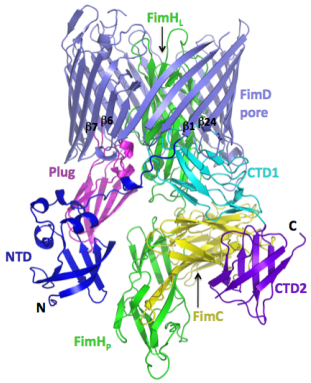
2. Jelly body refinement (Refmac)
   - FimD-Pore

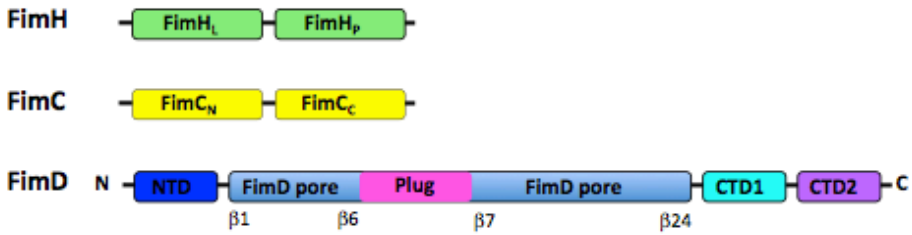


3. Fitting into the electron density
   - FimD-Plug
   - FimD-NTD
   - FimD-CTD-2

4. Manual building
   - FimD-CTD-1

Performance of fitting methods

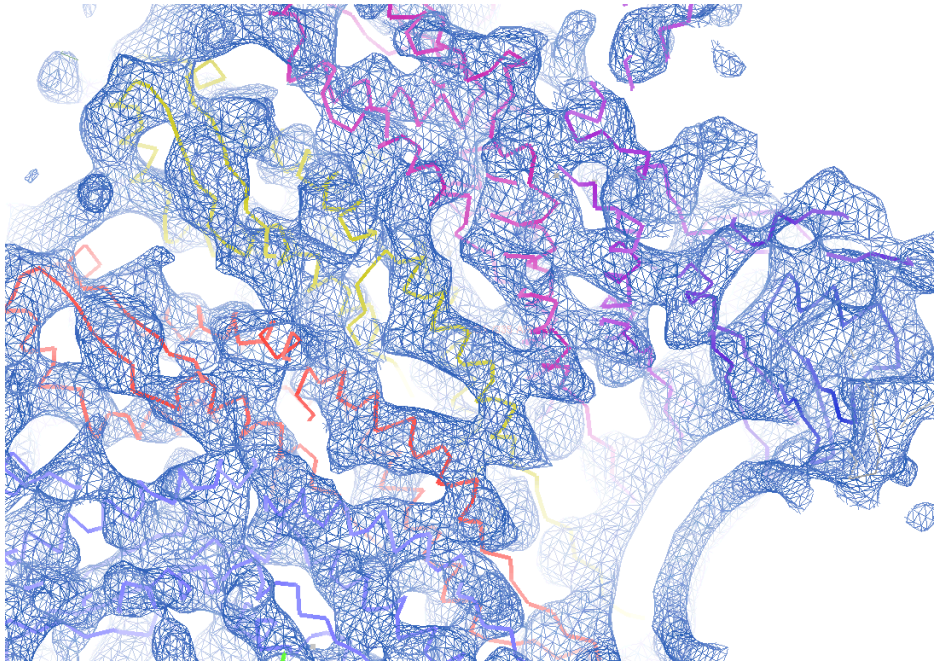


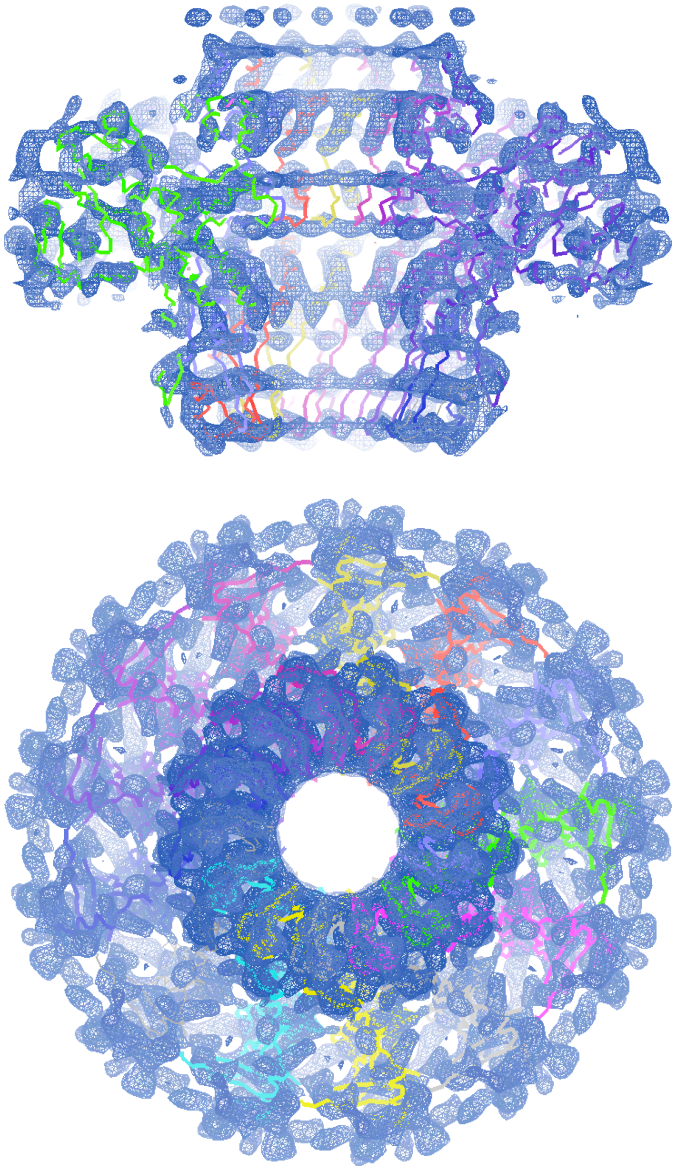


	search model	sequence identity	"Masked" RF PTF *prf n*	SAPTF PRF PTF *prf y*	SAPTF Local RF PTF *prf s*
FimD-Plug	3fip_A	38.5%	2 (2)	– (–)	1 (2)
FimD-NTD	1ze3_D	100%	2 (2)	1 (2)	2 (2)
FimD-CTD-2	3l48_A	33.3%	– (–)	2 (2)	– (–)

Trying several methods is a good practice (also because of cross-validation)

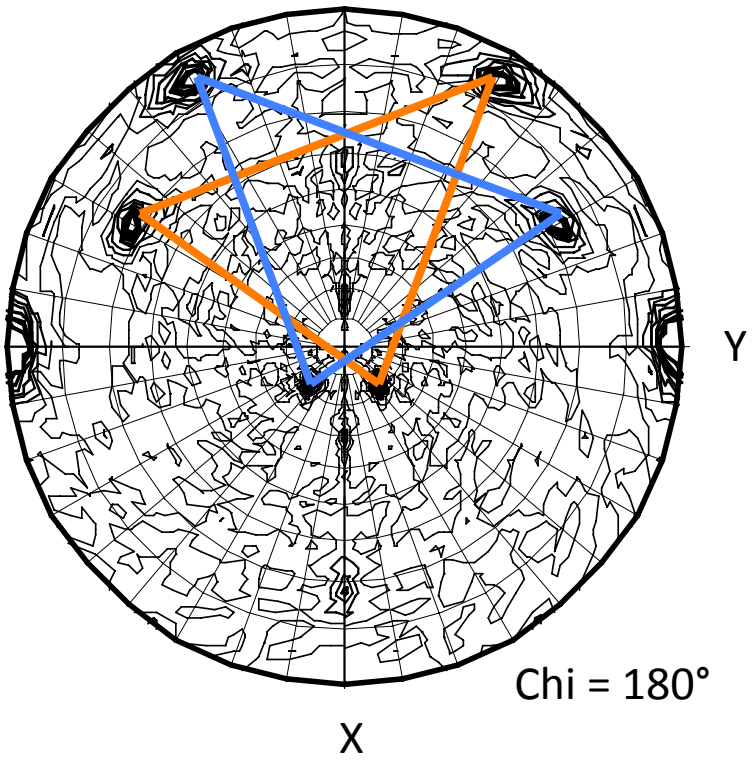
Fitting into EM maps





SPP1 portal protein

Self Rotation Function (SRF)



Example of SRF

- Space group P21
- One 222-tetramer in the AU

Preliminary analysis of X-ray data

- Oligomeric state of the protein in crystal
- Selection of oligomeric search model

Limited use

- No clear interpretation or even artifact peaks in high symmetry point groups (e.g. 622)
- different oligomers with the same symmetry

Locked Rotation Function

- Uses SRF to derive NCS operations
- Averages RF over NCS operations
- In favorable cases Improves signal to noise ratio in RF

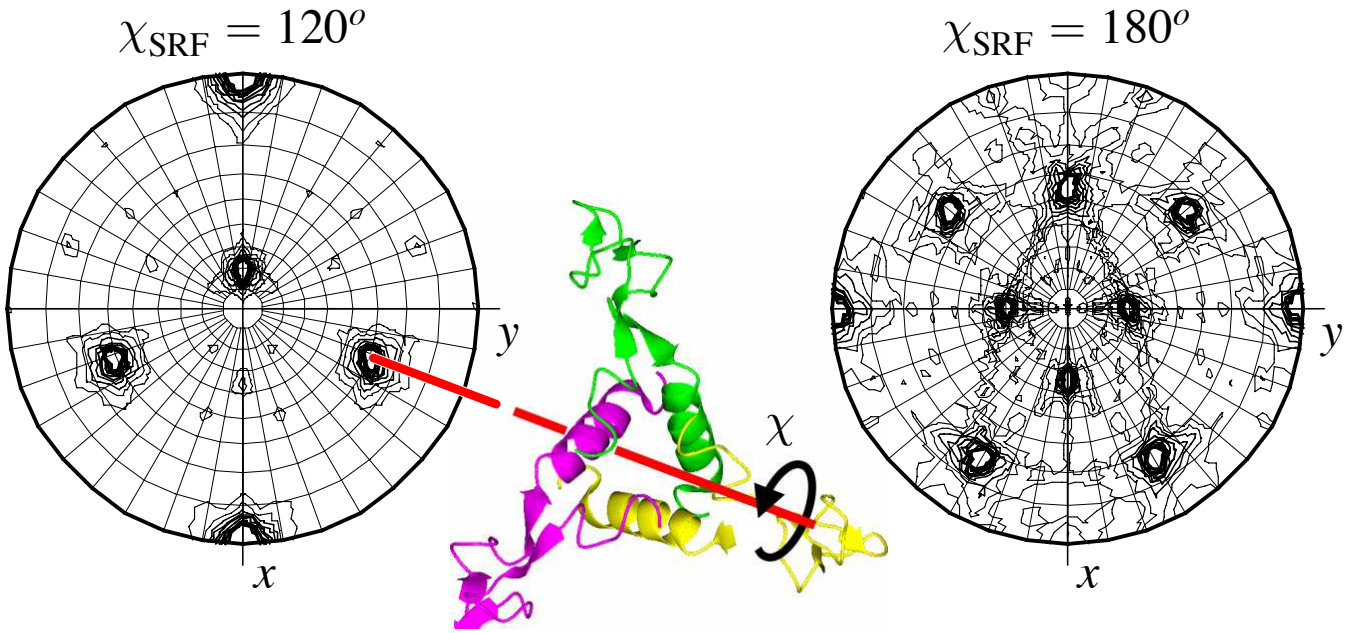
Automatic mode:

```
molrep -f s100.mtz -m monomer.pdb -s s100.seq –i <<+
lock y
+
```

There is an option of selecting specific SRF peaks

Available from CCP4I

One-dimensional exhaustive search (exotic case)

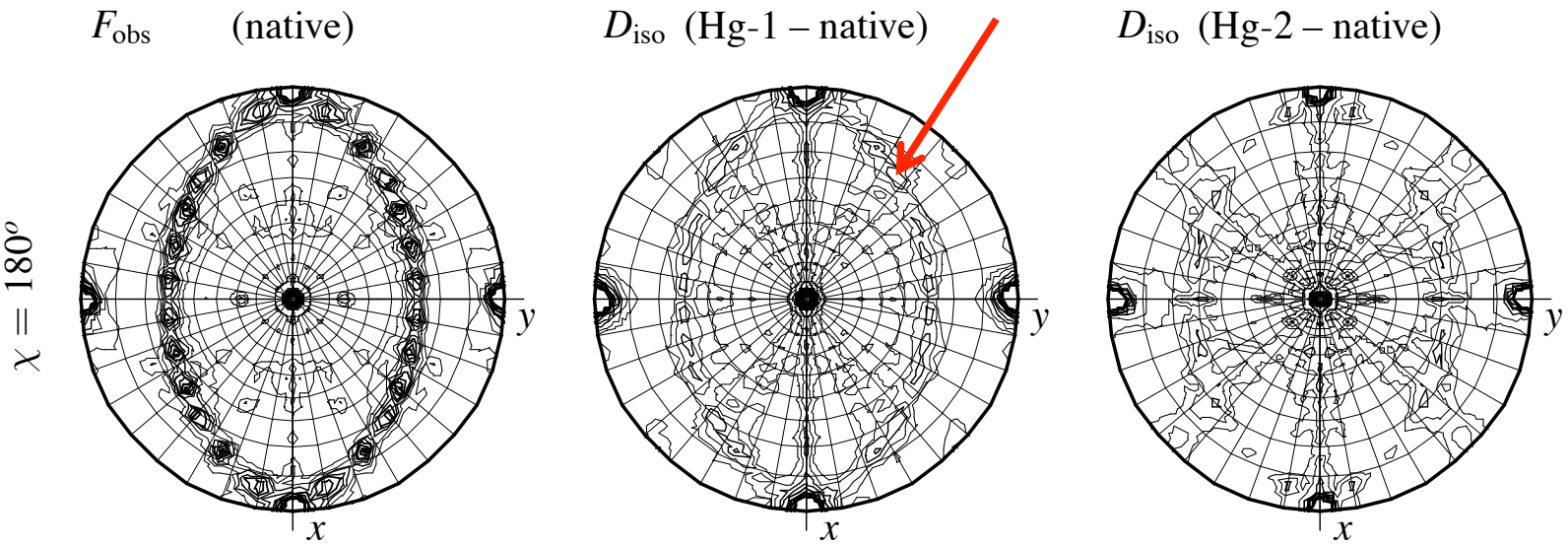


SRF helps restrict dimensionality in an exhaustive search

- Orientation of the trimer is known from the analysis of SRF
- Unknown parameter: rotation about 3-fold axis
- One-parametric exhaustive search using TF as score function

MR substructure solution (exotic case)

- Select isomorphous derivative
  - by comparing native SRF and SRF from D-iso



- Hg-substructure is a 13-atom ring (from native SRF analysis)
  - Orientation of the ring is known from the analysis of SRF
  - Unknown parameters: radius of the ring, rotation about 13-fold axis
- Two-parametric exhaustive search

Which direction does MR go?

Automation:

✖ Collection of tricks

✔ Improvement of "standard" methods
✔ Better scoring system

✔✔ Models