MrBUMP – Automated Molecular Replacement

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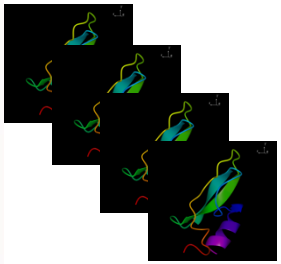
BBSRC bioscience for the future

Science & Technology Facilities Council

CCP4

The aim of MrBUMP



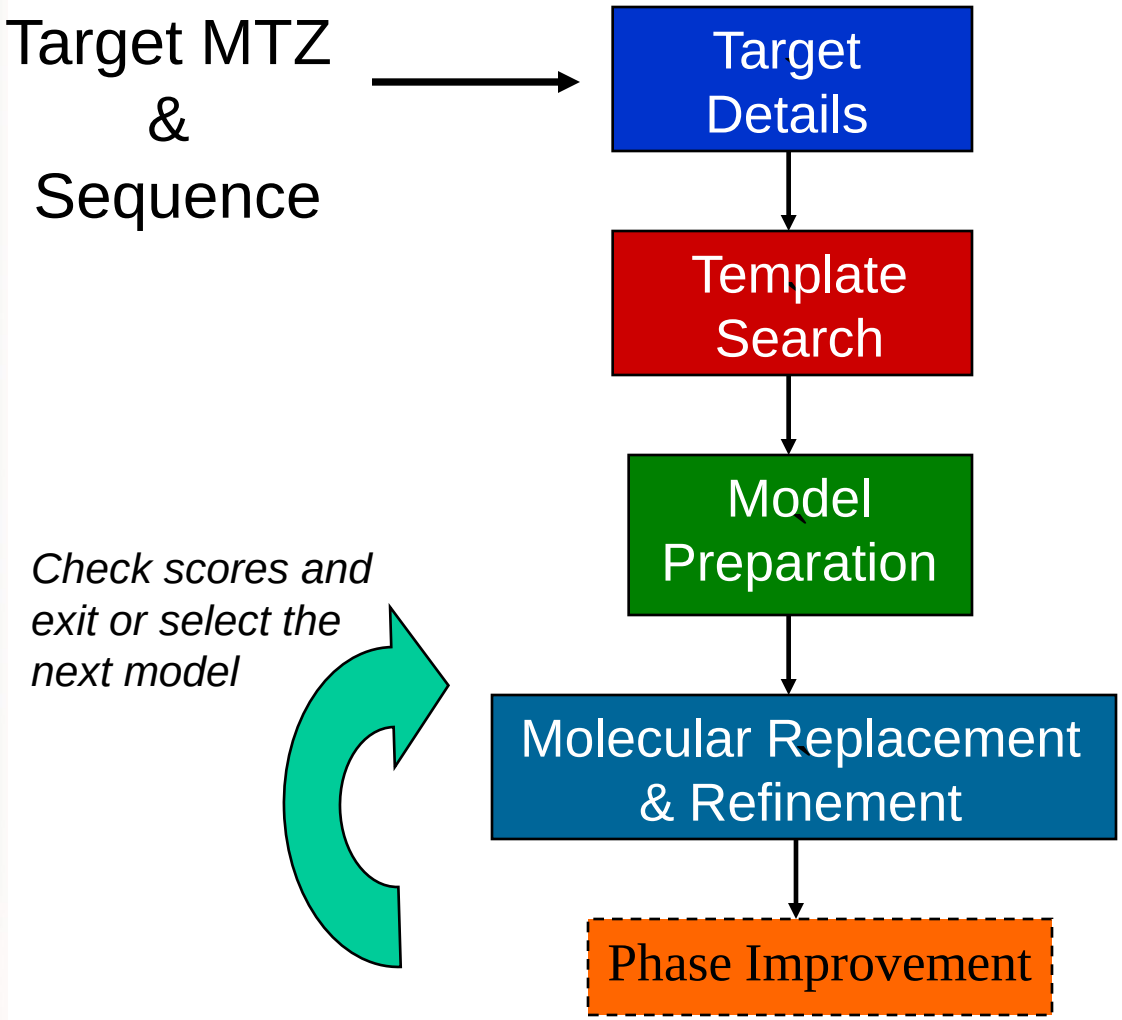
- An automation framework for Molecular Replacement.
- Particular emphasis on generating a variety of search models.

Wraps **Phaser** and/or **Molrep**.

- Also uses a variety of helper applications (e.g. Chainsaw) and bioinformatics tools (e.g. Fasta, Mafft)
- Uses on-line databases (e.g. PDB, Scop)

- In favourable cases, gives “one-button” solution
- In Complicated Cases, will suggest likely search models for manual investigation (lead generation)

Pipeline

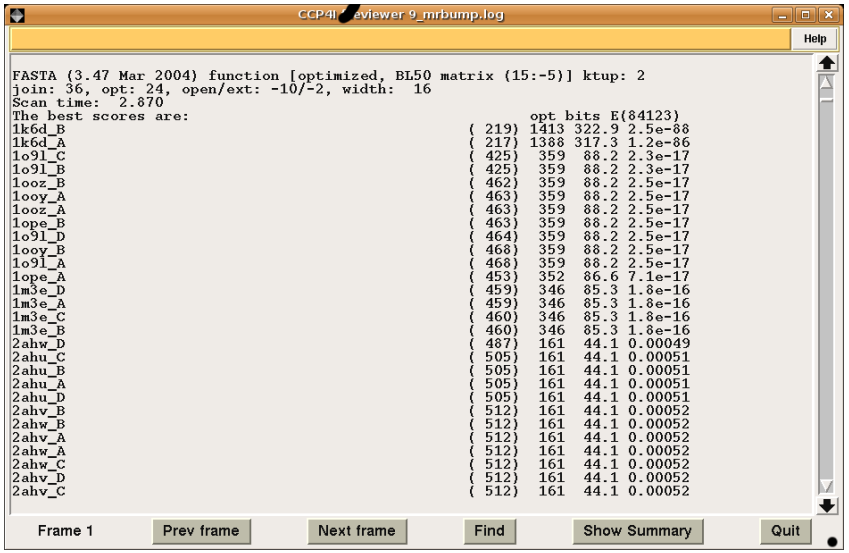

Target MTZ
&
Sequence
Target Details
Template Search
Model Preparation
Molecular Replacement & Refinement
Phase Improvement
Check scores and exit or select the next model

Search for model templates

FASTA search of PDB

– Sequence based search using sequence of target structure

All of the resulting PDB id codes are added to a list



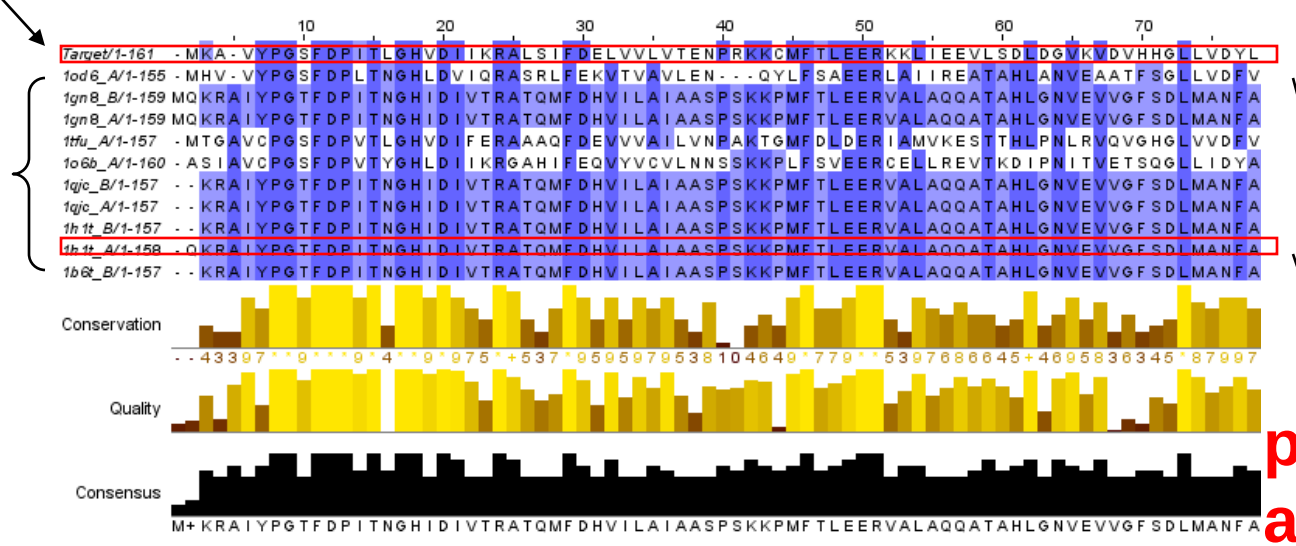
These structures are called model templates

Other templates from:

- SSM search using top hit from the FASTA search
- Can add additional PDB id codes to the list, e.g. from FFAS or psiBLAST searches
- Can add local PDB files

Multiple Alignment step

target



Jalview 2.08.1 Barton group, Dundee

pairwise alignment

(used in Chainsaw)

currently support ClustalW, MAFFT, probcons or T-coffee for multiple alignment

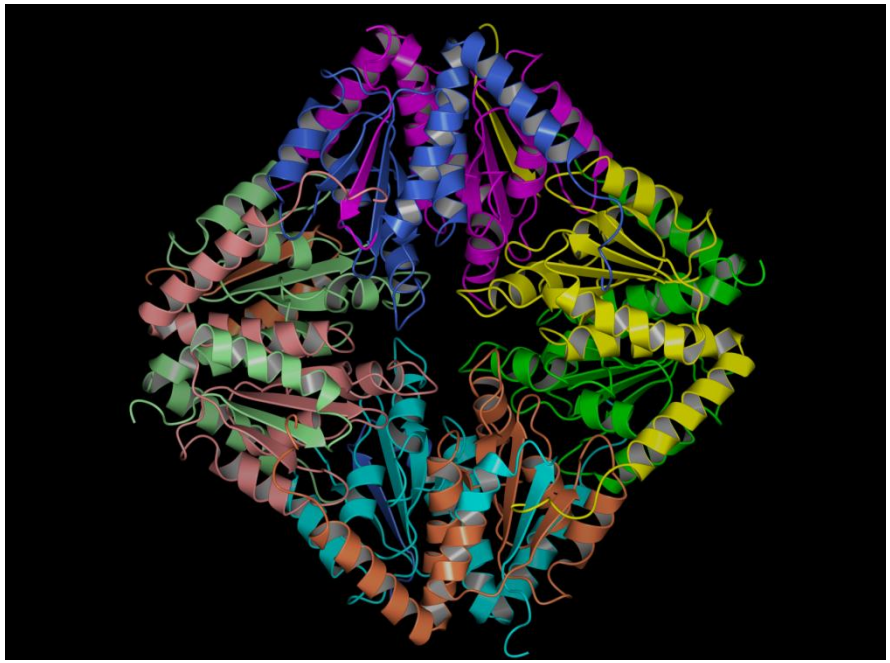
Model template scoring: score = sequence identity X alignment quality

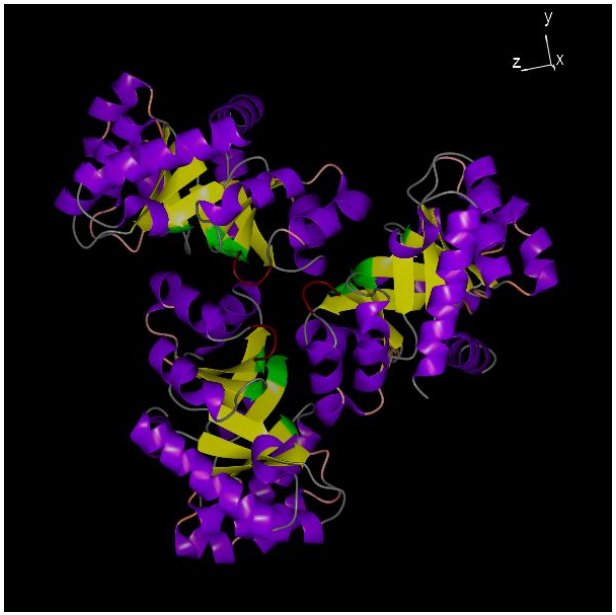
Domains

- Suitable templates for target domains may exist in isolation in PDB, or in combination with dissimilar domains
- In case of relative domain motion, may want to solve domains separately
- SCOP database is scanned to see if domains exist for each of the PDBs in the list of templates
- Domains are then extracted from the parent PDB structure file and added to the list of template models as additional search models for MR.

Multimers

- Use template multimer as model for target multimer (currently uses PQS, will use PISA)
- Better signal-to-noise ratio than monomer, ***if*** assembly is correct for the target.
- Biologically relevant multimers more likely transferable





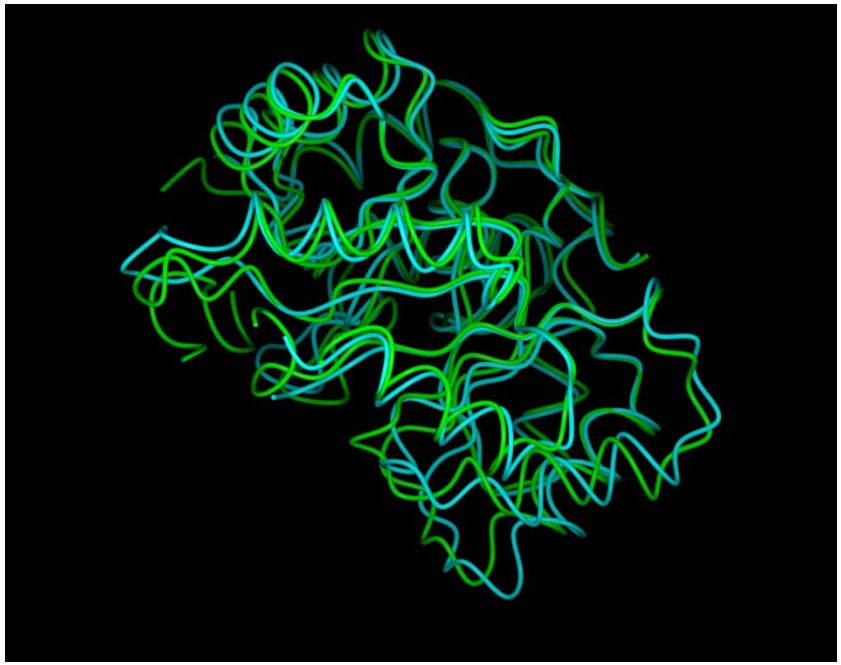
prepared with CCP4mg

Ensemble model

- Create ensembles of top search models, for use in additional run of Phaser.

- Models must be sufficiently similar (MW and rmsd)

- Molrep can also use ensembles (not yet implemented)



Search Model Preparation

Search models prepared in four ways:

more side chain truncation

PDBclip
- original PDB with waters removed, most probable conformations selected and format tidied (e.g. chain ID added)

Molrep
- Molrep contains a model preparation function which will align the template sequence with the target sequence and prune the non-conserved side chains accordingly.

Chainsaw
- Can be given any alignment between the target and template sequences. Non-conserved residues are pruned back to the gamma atom.

Polyalanine
- Created by excluding all of the side chain atoms beyond the CB atom using the Pdbset program

deal with deletions

Also create an ensemble model based on top 5 models

Molecular Replacement Step

Running MR

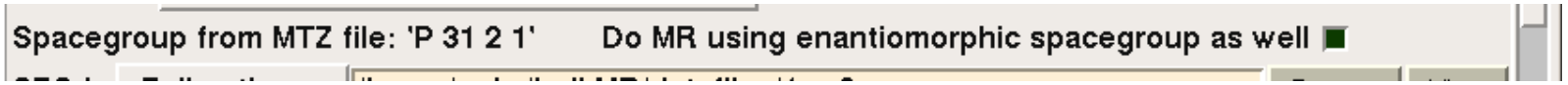
- For each search model, MR done with **Molrep** or **Phaser** or both.
- MR programs run mostly with defaults
- MrBUMP provides LABIN columns, MW of target, sequence identity of search model, number of copies to search for, number of clashes tolerated
- Allow Molrep / Phaser to set resolution limits and weights

MR output

- MR scores and un-refined models available for later inspection
  ⇒ assess quality of solution, extent of model bias
- MrBUMP doesn't use MR scores, but checks for output file with positioned model, and passes to Refmac

Testing enantiomorphic spacegroups

- 11 pairs of enantiomorphic spacegroups containing screw axes of opposite handedness, e.g. $P4_1$ and $P4_3$)
- usually both need to be tested in MR
- correct spacegroup indicated by TF and packing



- MrBUMP can test both in Molrep and/or Phaser.
- For each search model, best MR results used to fix spacegroup for subsequent steps.
- Discrimination good for good search model + correct MR solution

Restrained Refinement Step

- The resulting models from molecular replacement are passed to **Refmac** for restrained refinement.
- The change in the Rfree value during refinement is used as rough estimate of how good the resulting model is.

final Rfree < 0.35 or
final Rfree < 0.5 and dropped by 20% ⇒ "good"

final Rfree < 0.48 or
final Rfree < 0.52 and dropped by 5% ⇒ "marginal"

otherwise ⇒ "poor"

conservative .....

Phase improvement

If resolution better than 1.7Å use Acorn procedure:

initial phase set from refined MR solution
artificial phase extension to 1.0Å
dynamic density modification

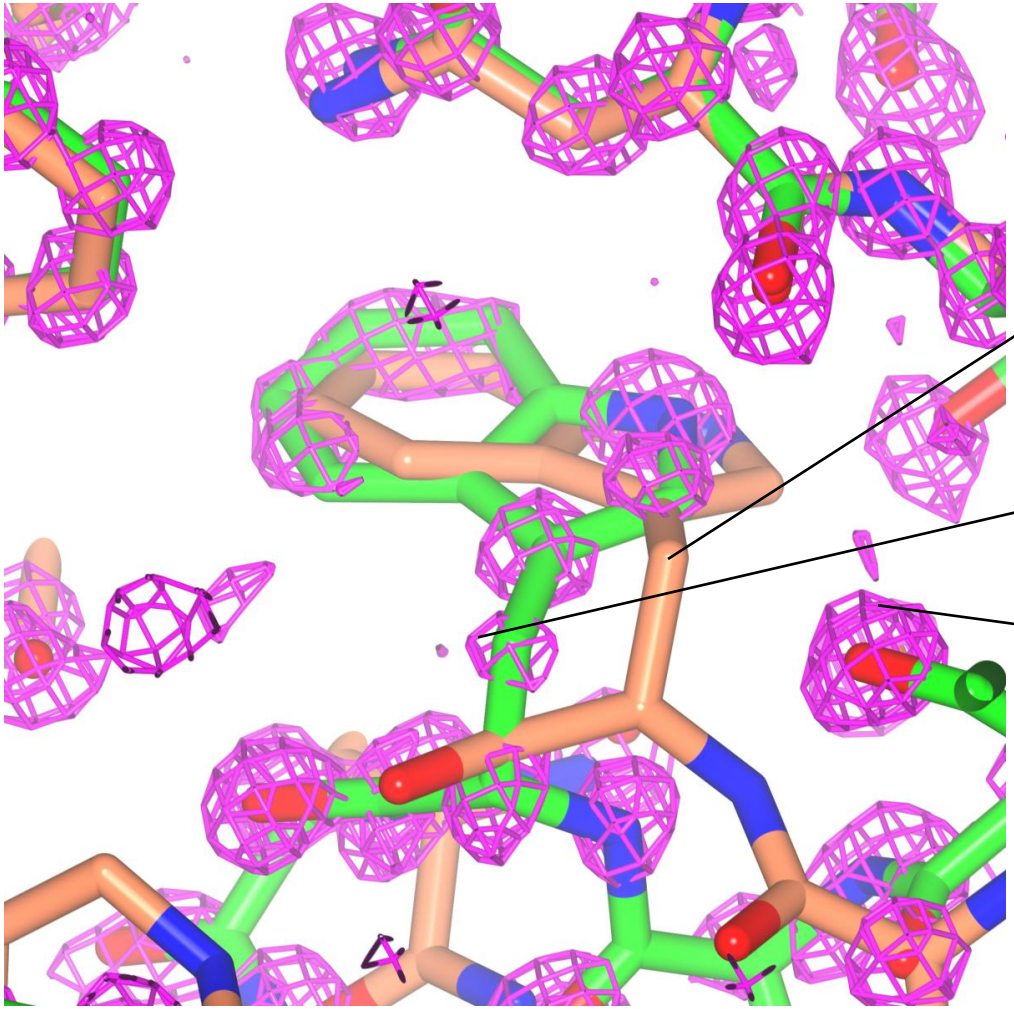
Result:

CC for medium Es good indicator of solution
Use E-maps for re-building

At lower resolutions:

Use parrot (todo!)

dUTPase from C.jejuni
data to 1.65Å



positioned/refined search model

final model (1w2y)

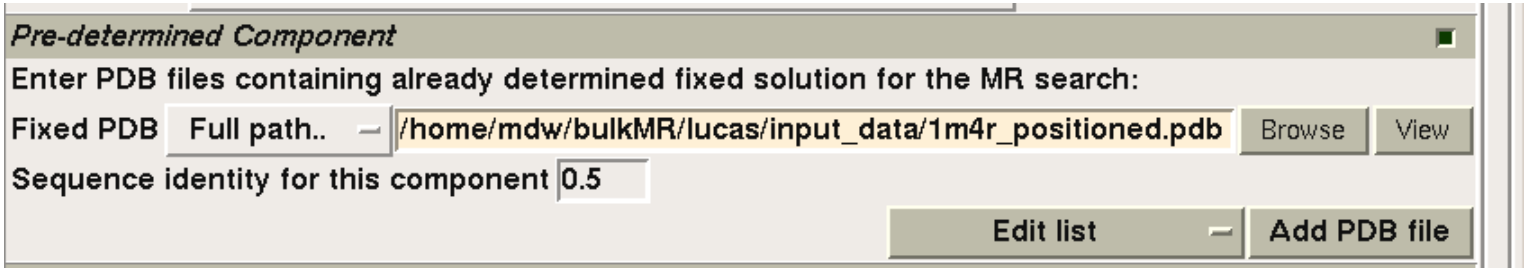
Acorn map (as generated by MrBUMP)

CC: 0.078 → 0.156
ARP/wARP re-builds into Acorn map

ccp4mg

Inclusion of fixed models

- MrBUMP will now accept one or more positioned models.
- These are included as fixed models in all MR jobs.



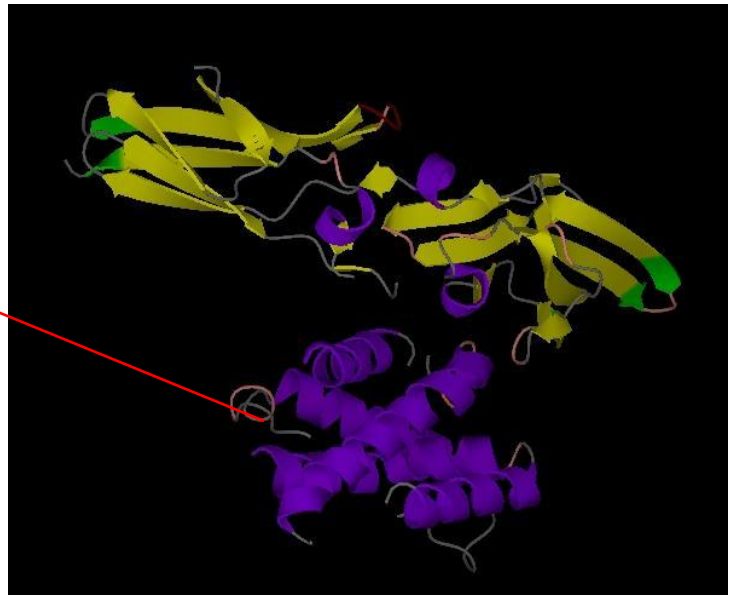
- Thus, solve complexes through consecutive runs of MrBUMP.
- Automation of this in progress ....

Example (with thanks to Lucas Bleicher)

1:1 complex (1 copy in a.s.u.)
data to 1.9Å in C2

Small protein (151 res) already solved, easy to locate in complex.

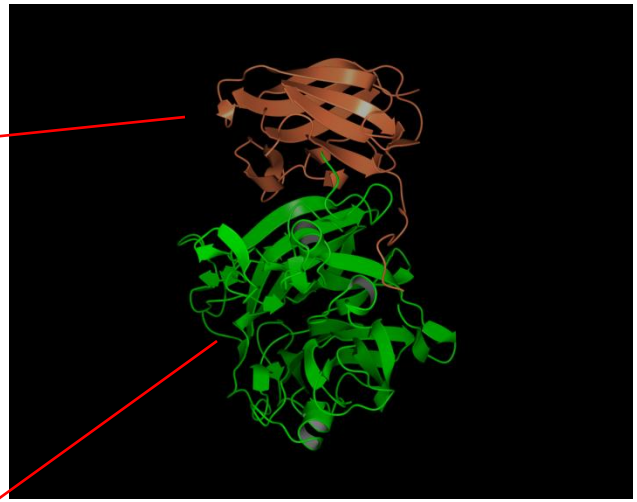
Larger protein (217 res), 2 domains, nearest homologs around 26%



Run MrBUMP with small protein fixed.

Example (thanks to Elien Vandermarliere)

Target is an arabinofuranosidase
Data to 1.55Å in P212121

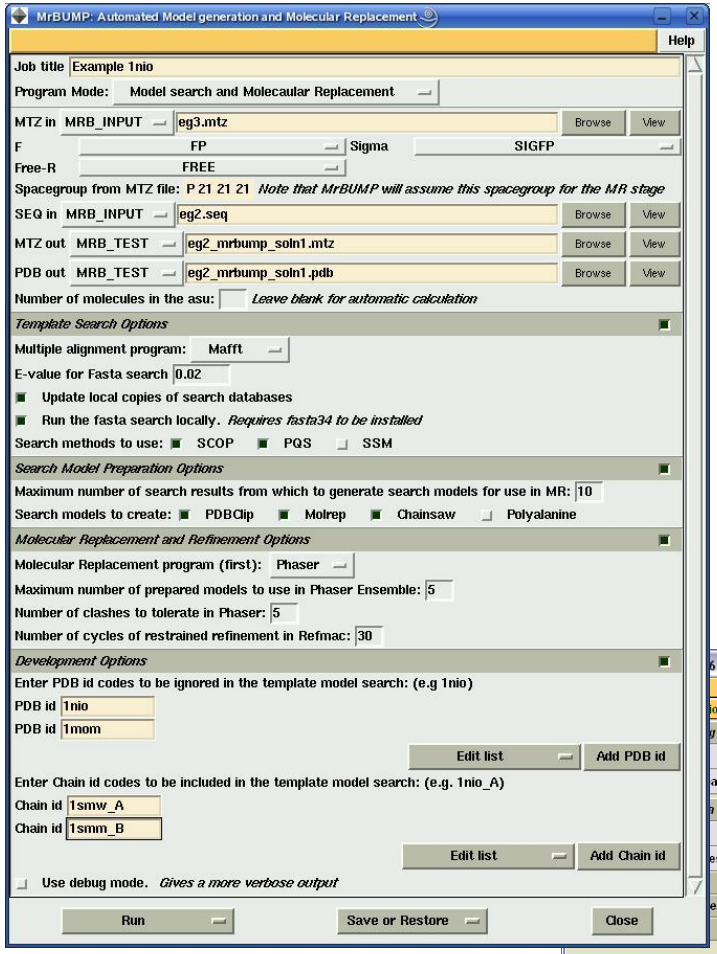


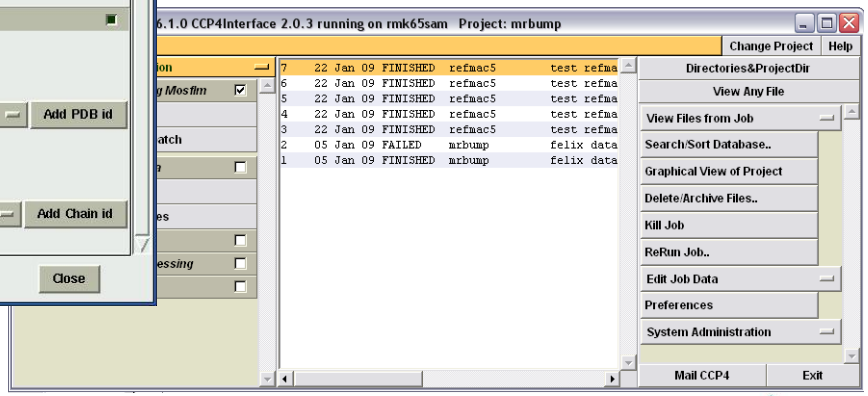
Small C domain (144 res) solved with 34% seq ident model
(1w9t_B_MOLREP best out of 4 solutions)

With C domain solution fixed, large N domain (345 res) solved with 28% seq ident model
(1gyh_C_CHNSAW best out of 7 solutions)

Acorn: CC increases from 0.04 to 0.18
ARP/wARP then builds 457/493 residues to R/Rfree 0.185/0.225

MrBUMP in CCP4i

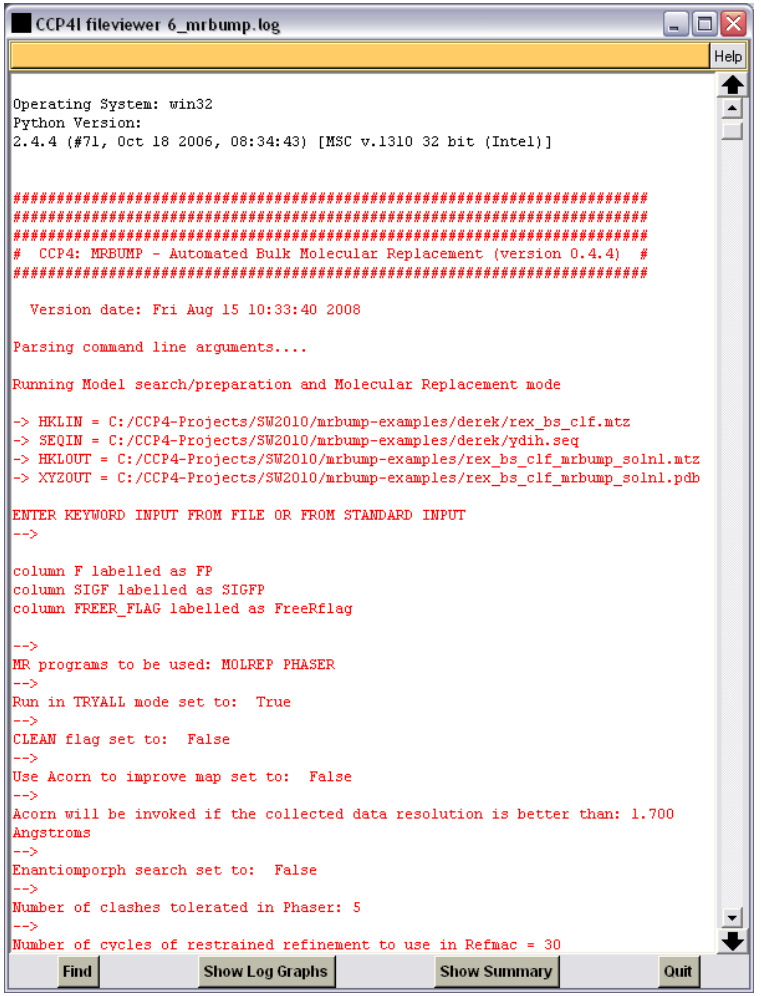




- MrBUMP included in CCP4 6.1 series
- Runs on Linux, OSX and Windows.
- Comes with CCP4 GUI .
- Can also be run from the command line with keyword input
- Tutorials available

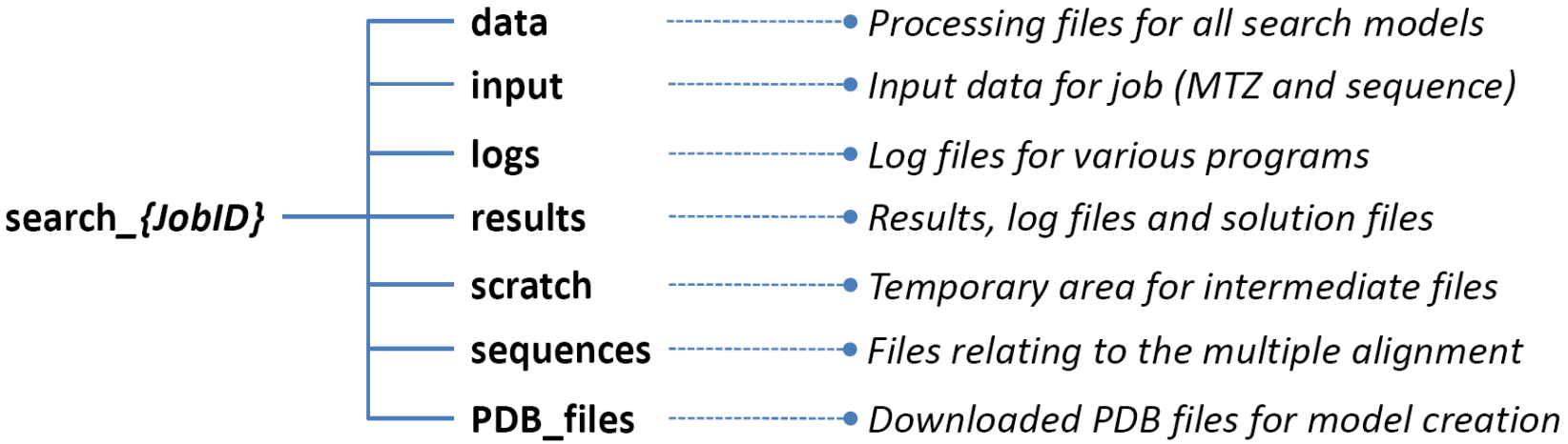
MrBUMP Output

- Log file gives summary of models tried and results of MR
- May get several putative solutions
- Ease of subsequent model re-building, model completion may depend on choice of solution
- Worth checking “failed” solutions
- Top solution available from ccp4i



Output files

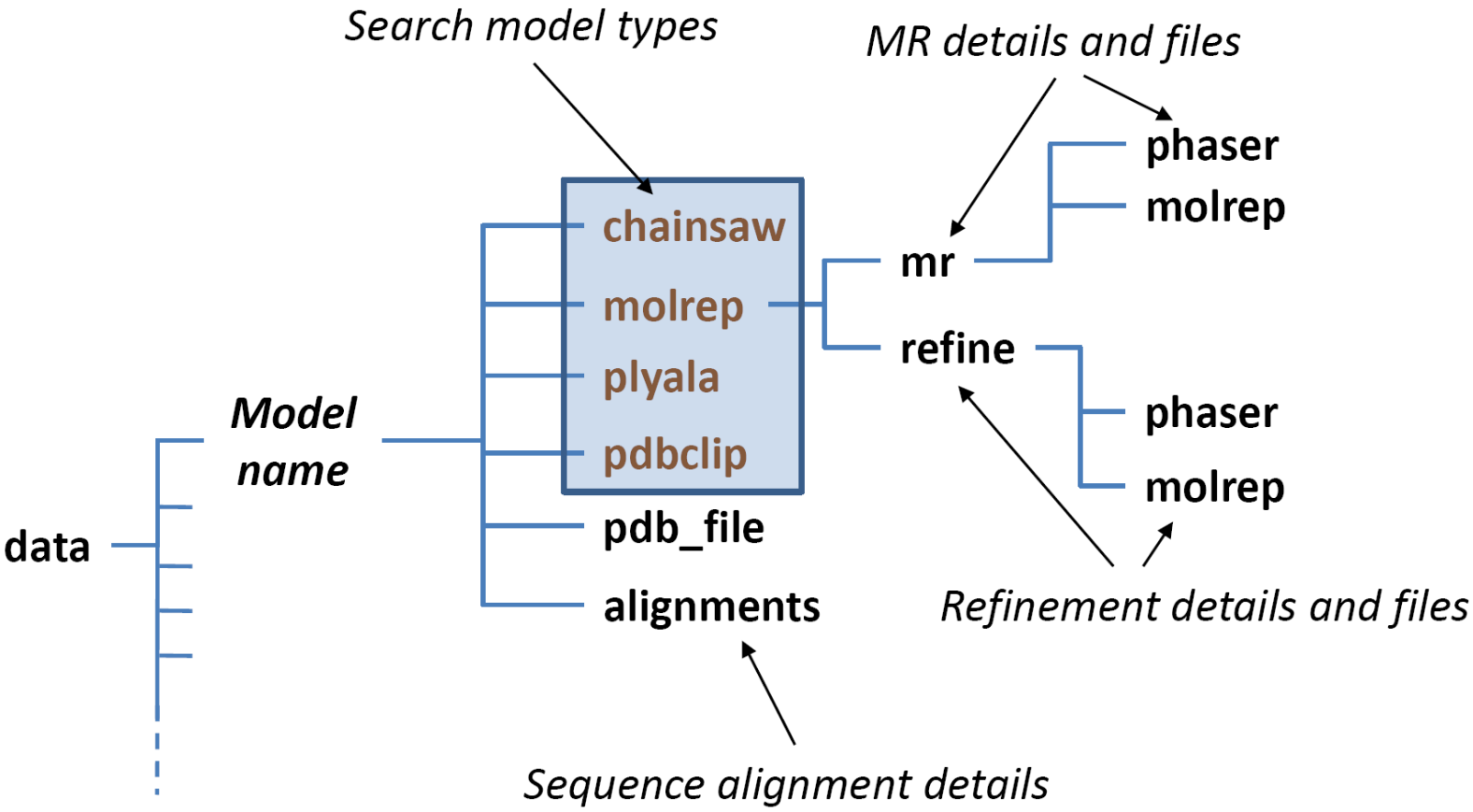
- Detailed results located in:
  - <ccp4i project directory>/***search*_<job number>**



Output files

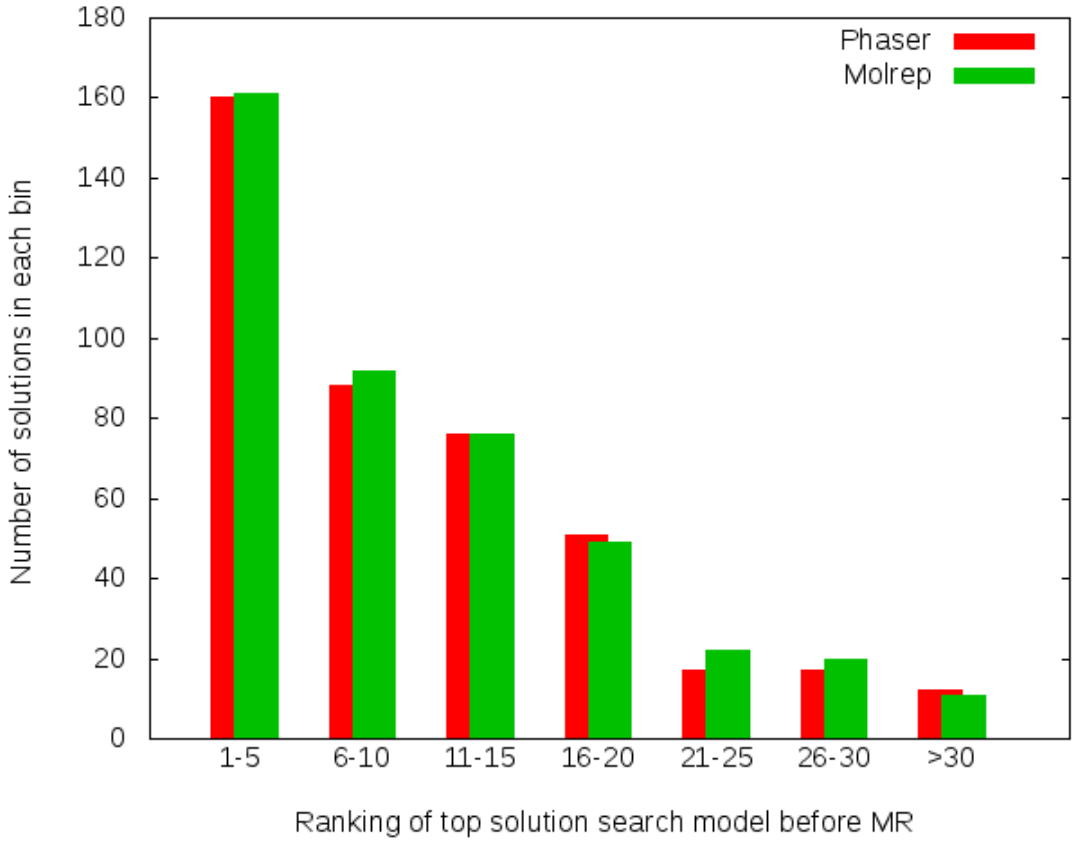
- In this directory, there are a number of subdirectories, including:
  - ***data***
    - Contains the data files and log files from all jobs run. The directory hierarchy is of the form <template>/<search model>/<pipeline step>
    - e.g:

      <ccp4i projectdirectory>/***search_55/data/loc0_A/chainsaw/mr***
  - ***results***
    - Results from the successful search model are placed into subdirectory "***solution***".
    - Other results are placed into subdirectory "***marginal_solns***".


Search model types
MR details and files
phaser
molrep
chainsaw
molrep
plyala
pdbclip
mr
refine
phaser
molrep
Model name
data
pdb_file
alignments
Refinement details and files
Sequence alignment details

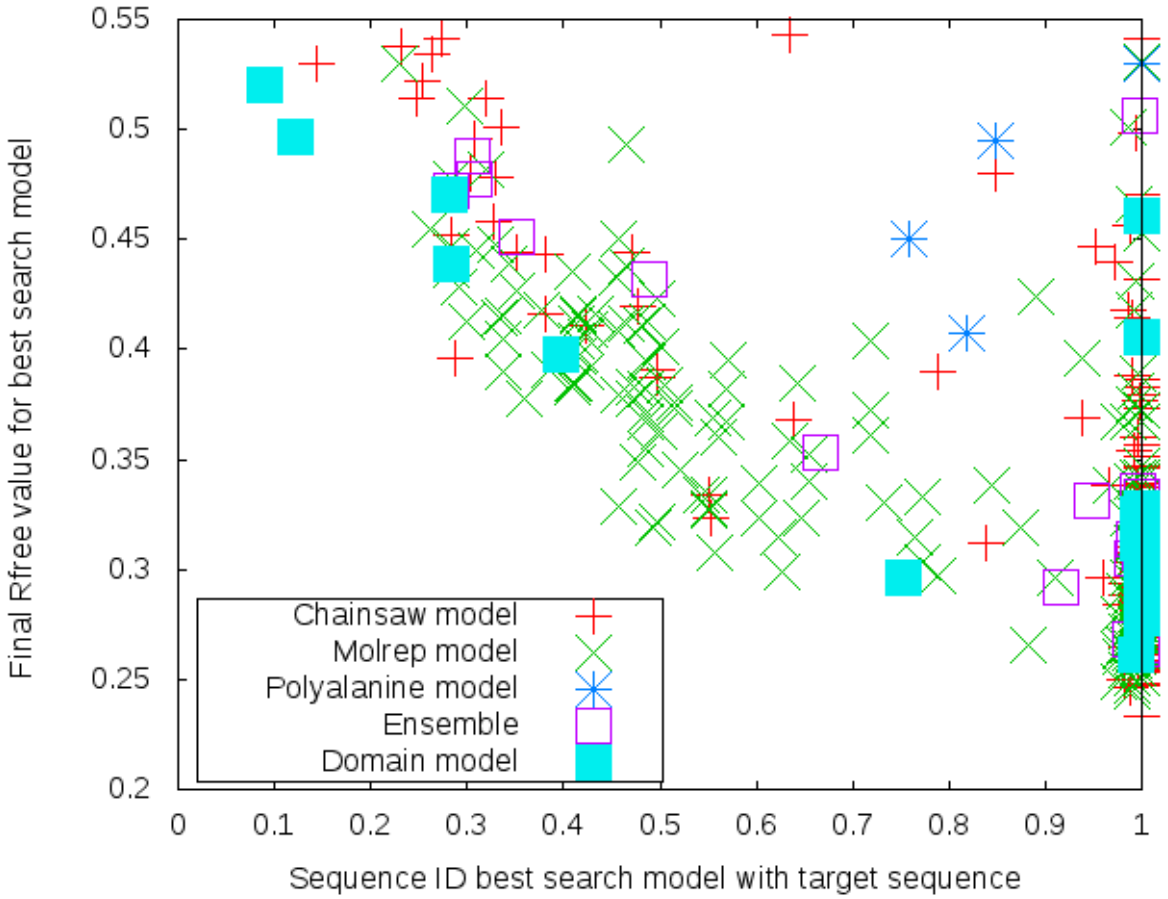
MrBUMP Testing Results

- Sample set from 2009 PDB depositions processed
- Best MR solutions based against initial scoring of the search model used



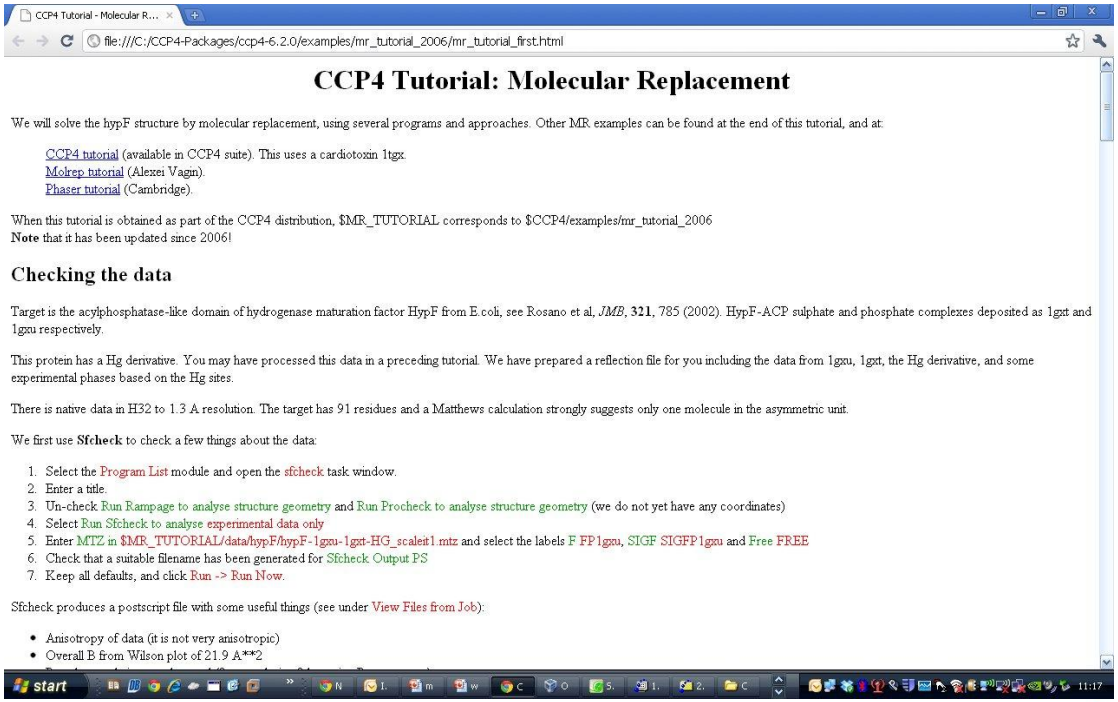
MrBUMP Testing Results

- Model type for best solution



MR/MrBUMP Tutorial

- Handout in folder *or*
- In the CCP4 installation under:
  - *$CCP4/examples/mr_tutorial_2006*
  - 2 HTML pages - beginners and advanced



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- Thanks to authors of all underlying programs and services
  (see references in MrBUMP log file)
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  - Vincent Fazio, CSIRO, Australia
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  - Geoff Barton, Charlie Bond, University of Dundee
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http://www.ccp4.ac.uk/MrBUMP

e-Science Resource for Protein Crystallography

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