Collaborative Computational Project Number 4
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MrBUMP – Automated Molecular Replacement

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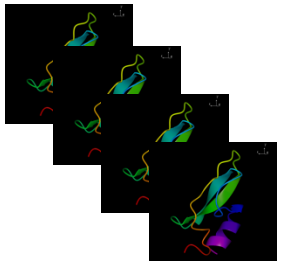
BBSRC bioscience for the future

Science & Technology Facilities Council

The aim of MrBUMP



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

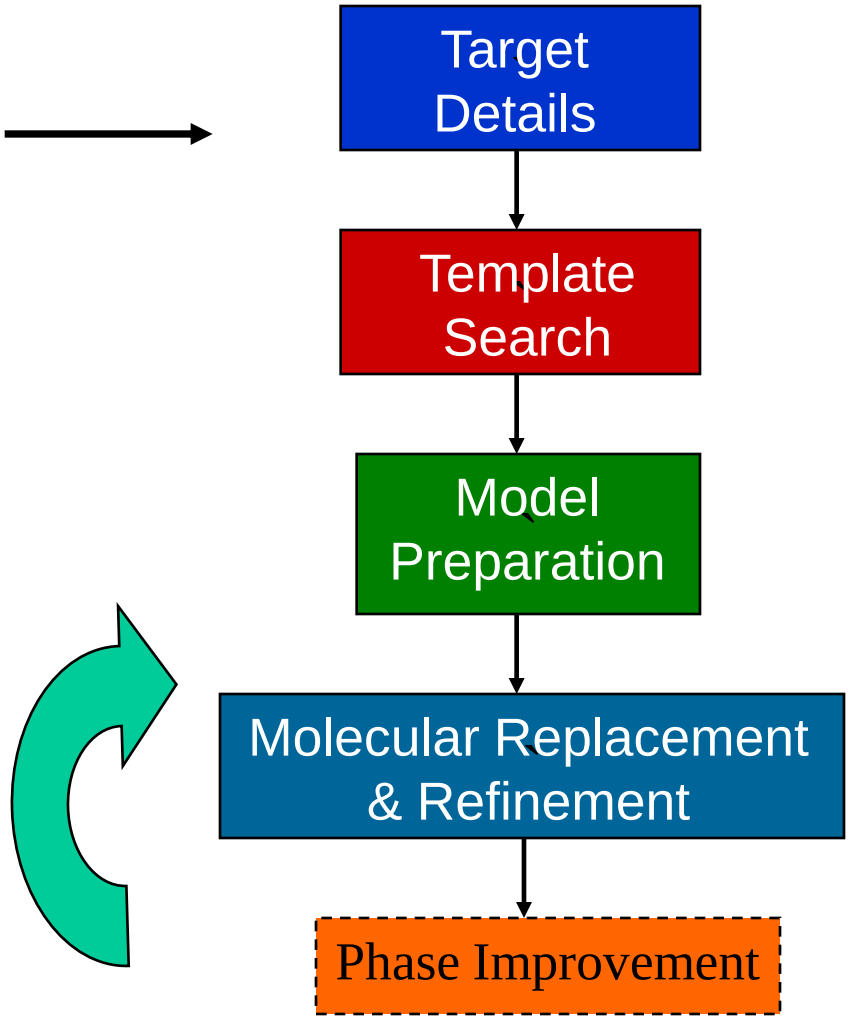
Wraps **Phaser** and/or **Molrep**.

- 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



Check scores and exit or select the next model

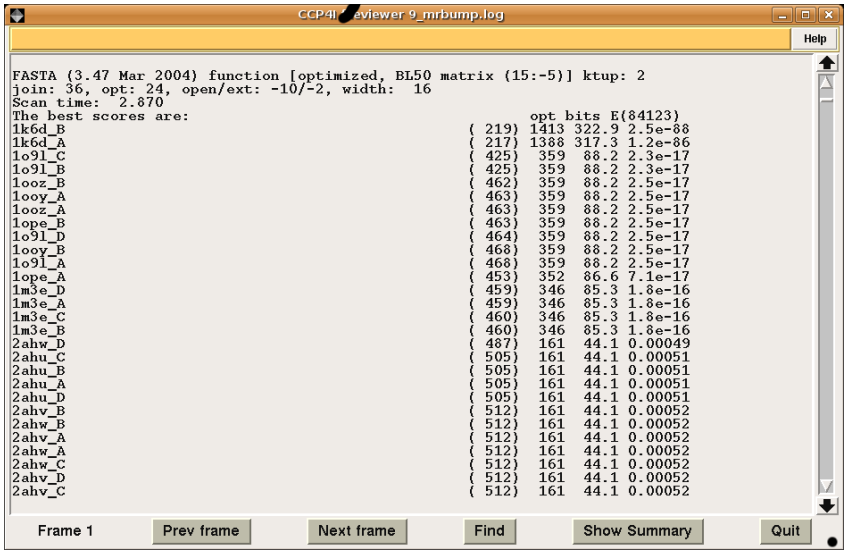
Search for model templates

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

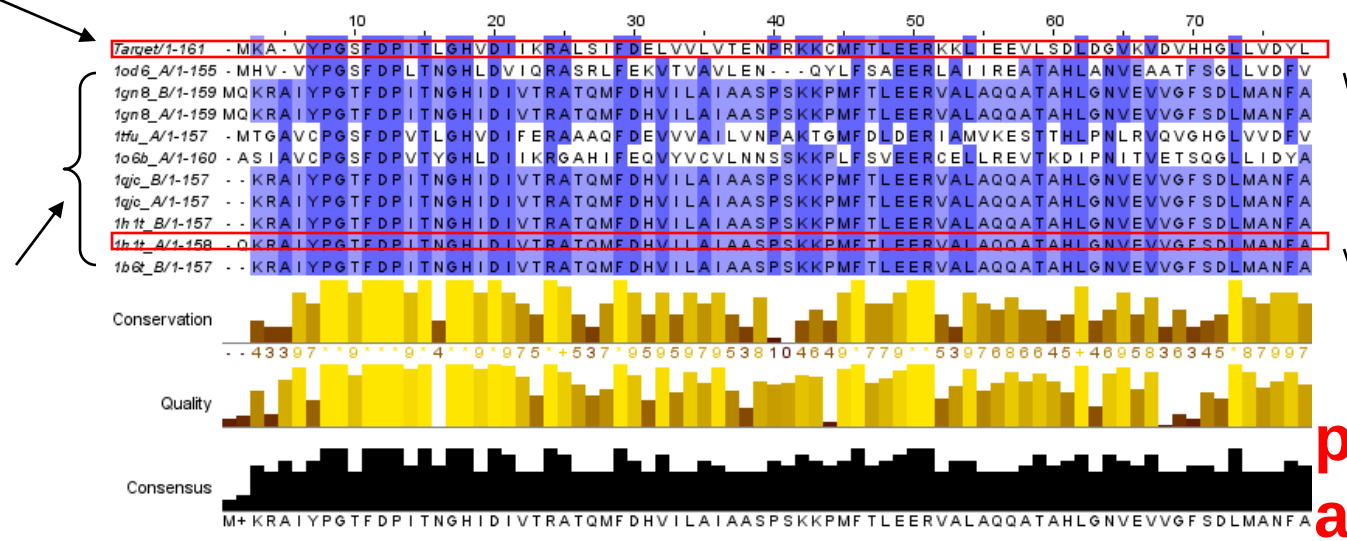


Other templates from:

- 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

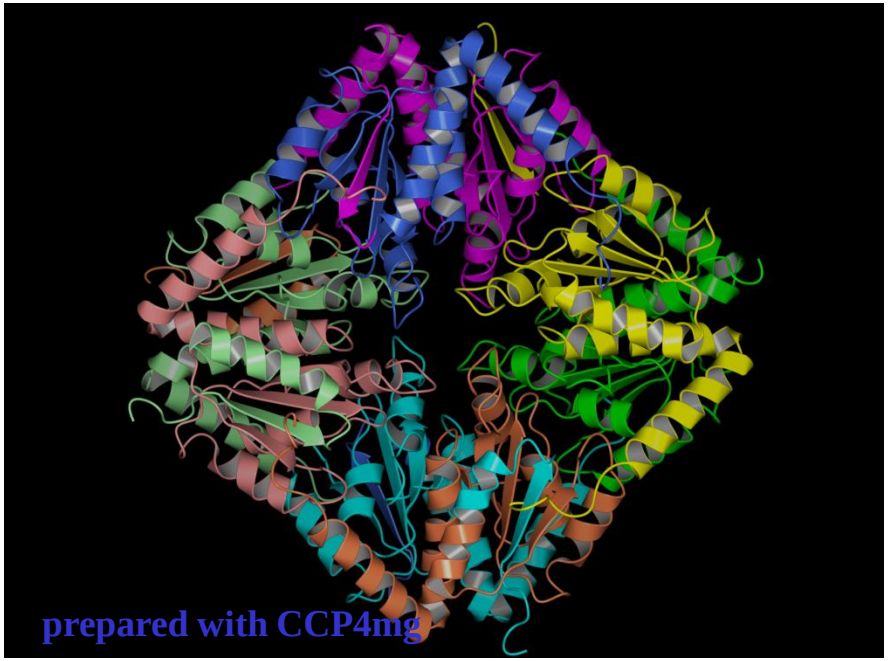
Model template scoring: score = sequence identity X alignment quality

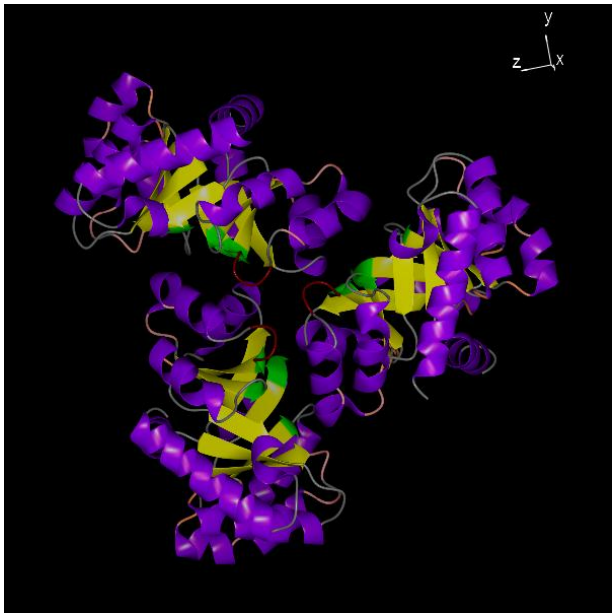
Domains

- 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



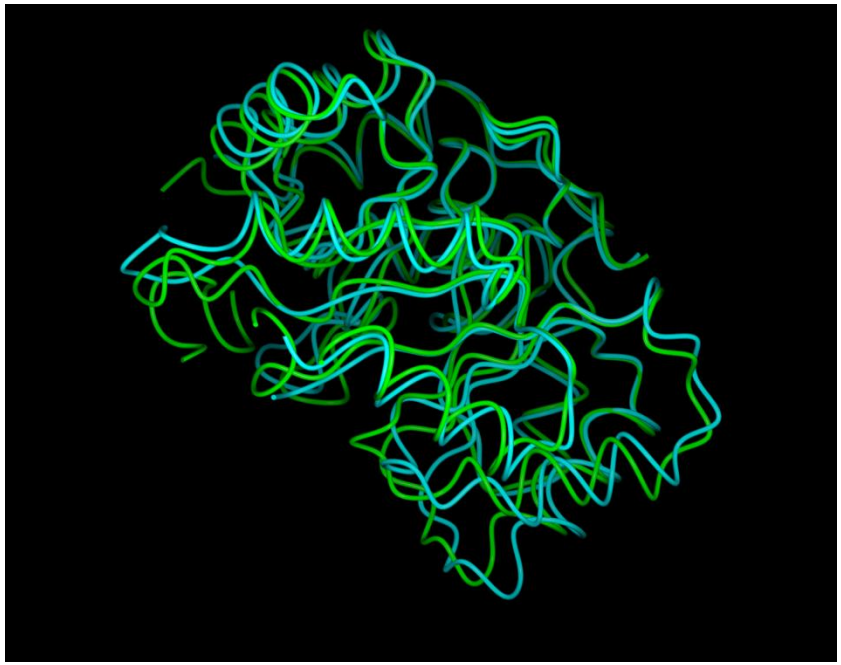


Ensemble model

- Create ensemble 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:

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

more side chain truncation

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:

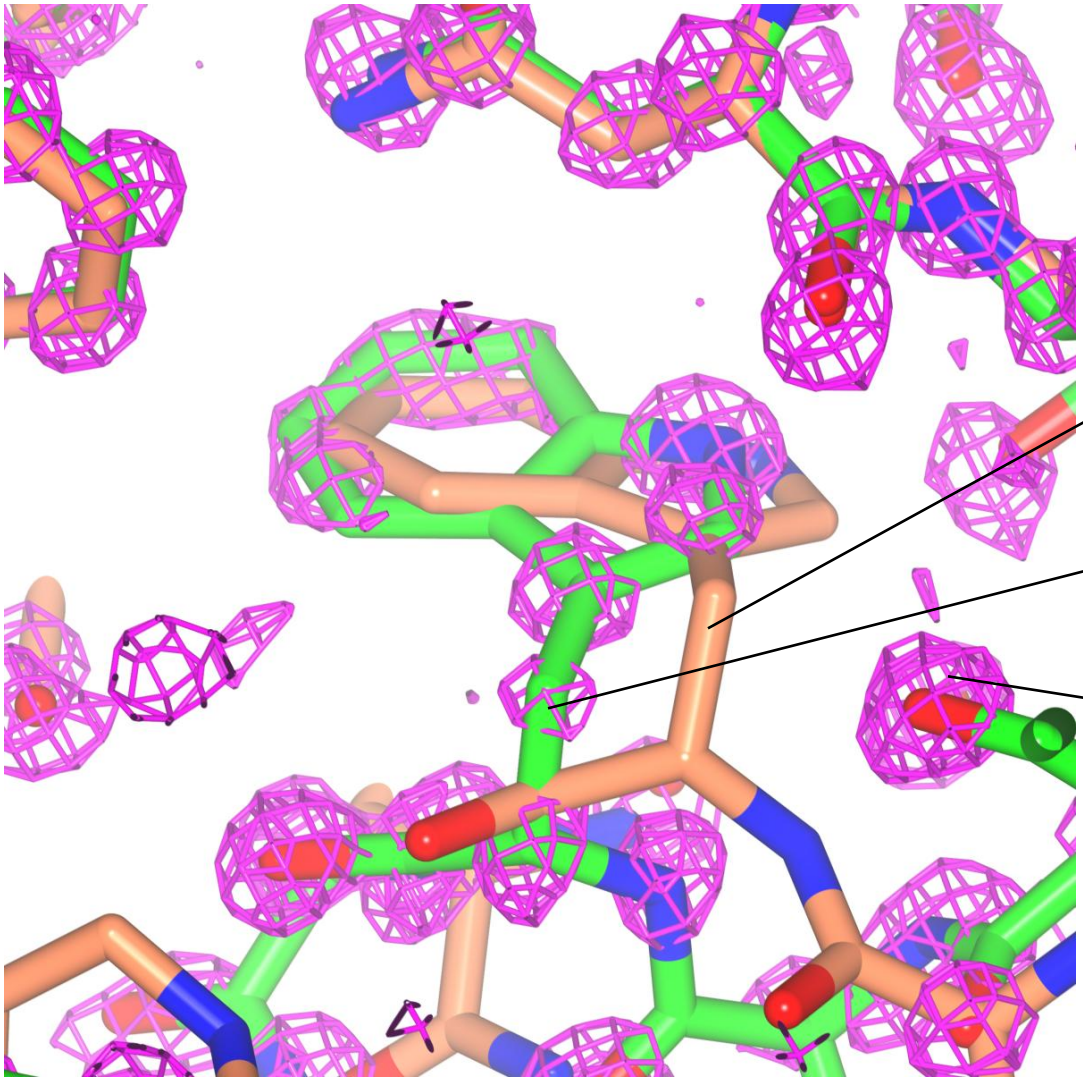
initial phase set from refined MR solution
artificial phase extension to 1.0Å
dynamic density modification

Result:

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

At lower resolutions:

Use parrot (todo!)



ccp4mg

dUTPase from C.jejuni
data to 1.65Å

positioned/refined
search model

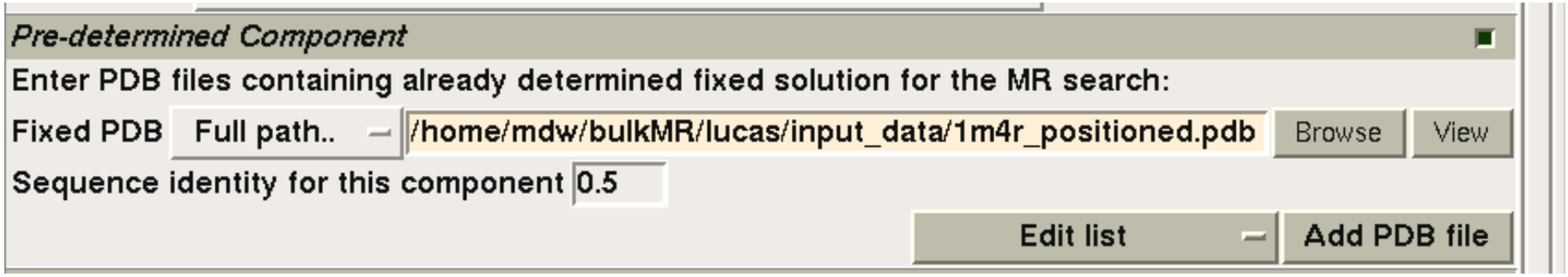
final model (1w2y)

Acorn map (as
generated by
MrBUMP)

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

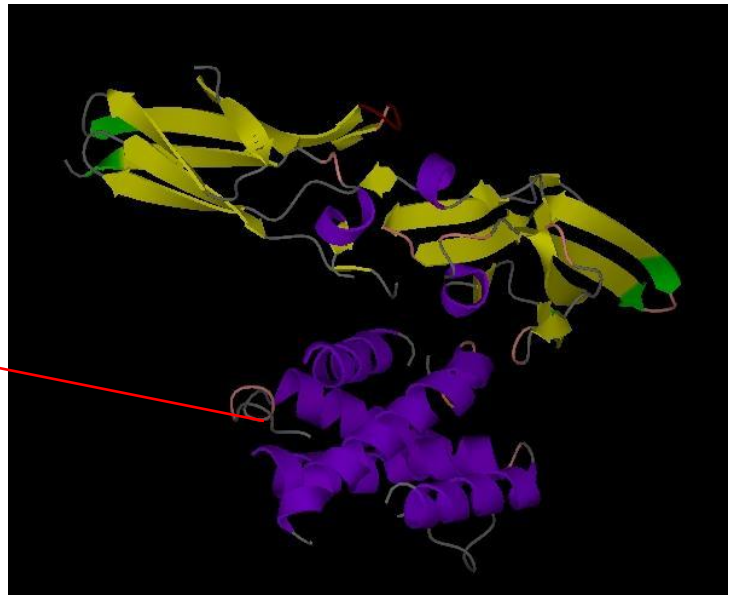
Inclusion of fixed models

- 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

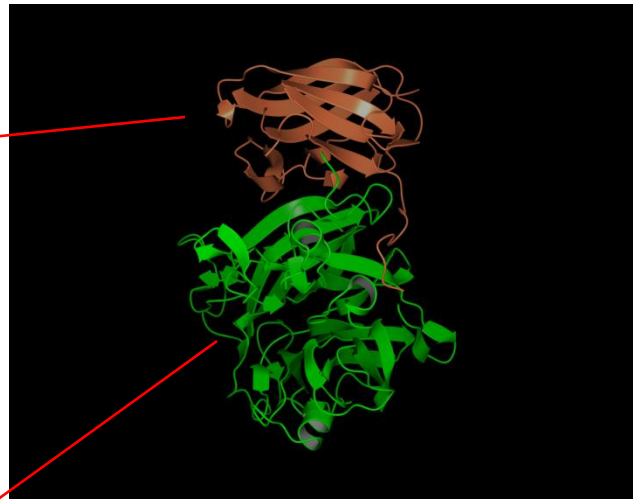
Small protein (151 res) already solved, easy to locate in complex.

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

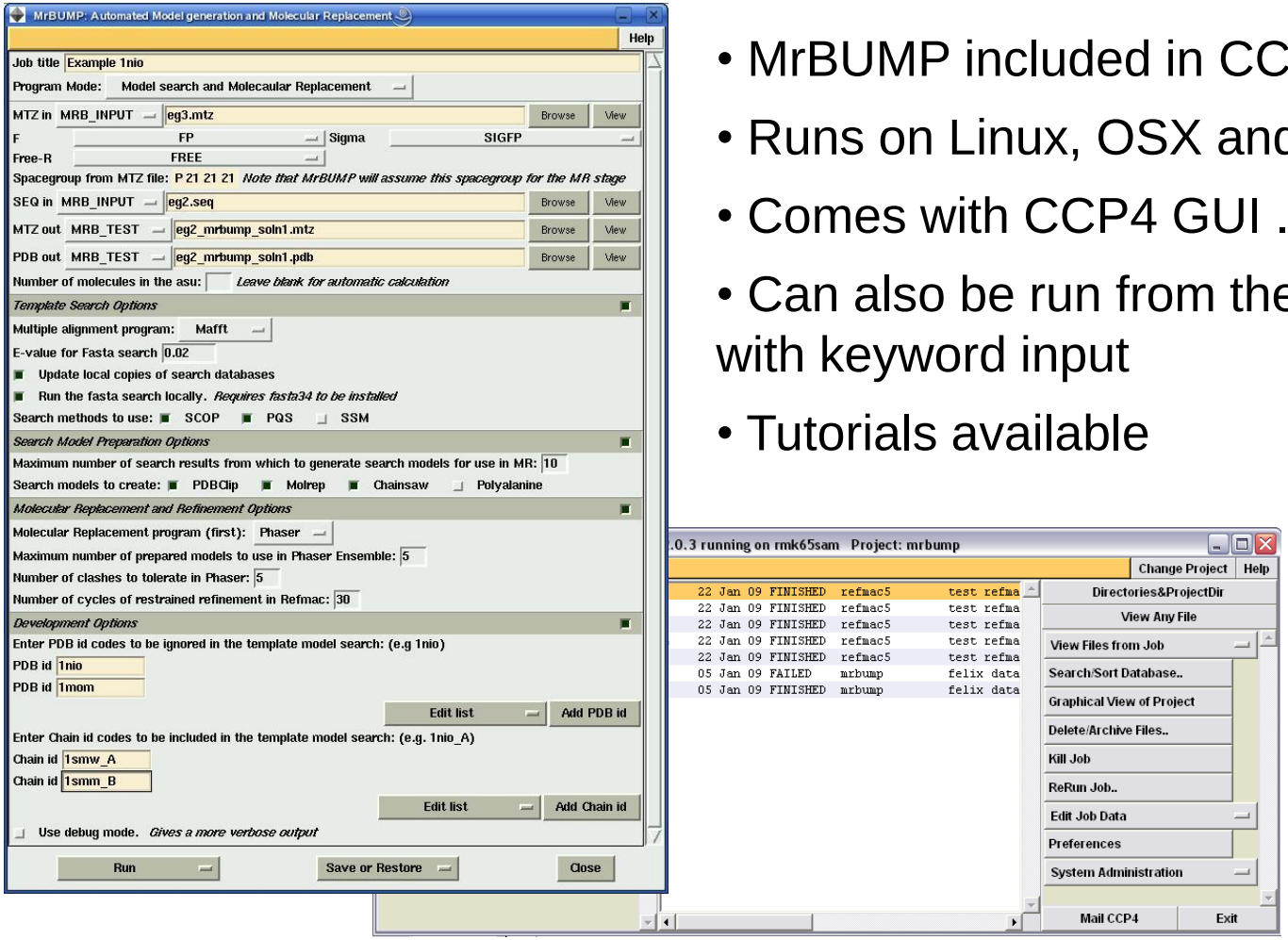


Small C domain (144 res) solved with 34% seq ident model
(1w9t_B_MOLREP best out of 4 solutions)

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

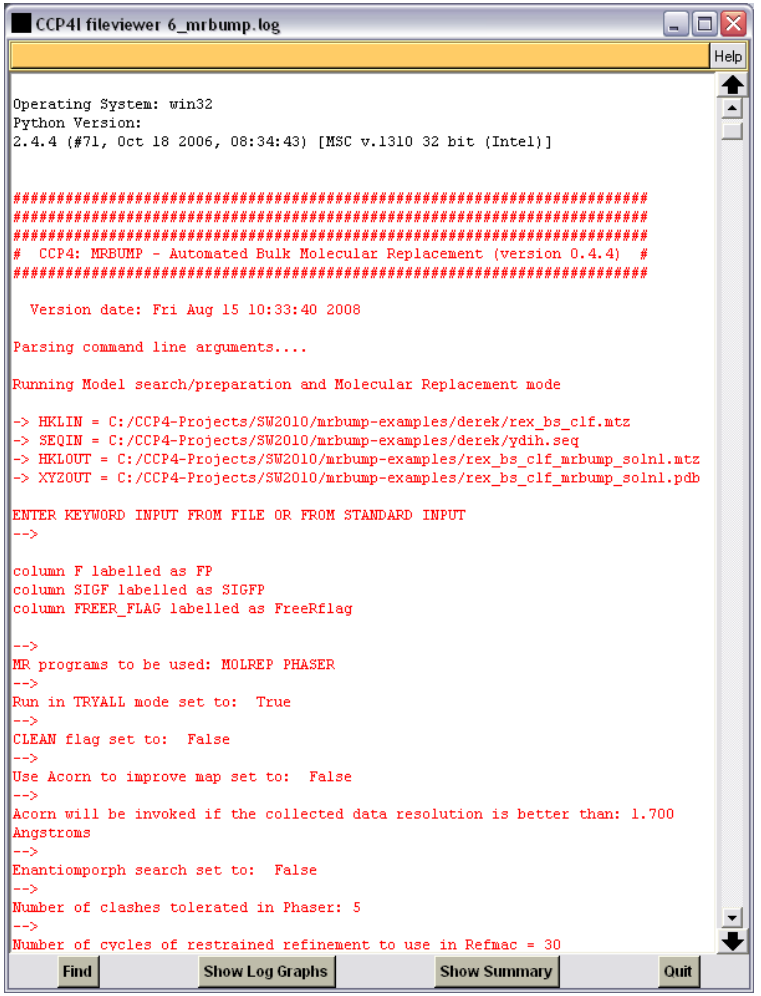
MrBUMP in CCP4i



- 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>**
- 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***".

Acknowledgements

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  (see references in MrBUMP log file)
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