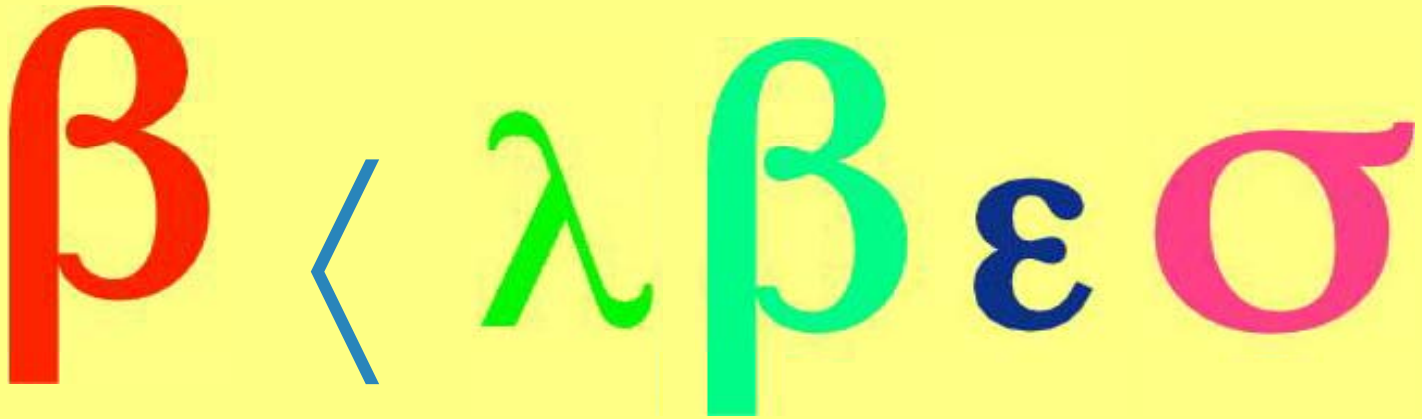


# A Molecular Replacement Pipeline

Garib Murshudov

Chemistry Department, University of York

# Introduction

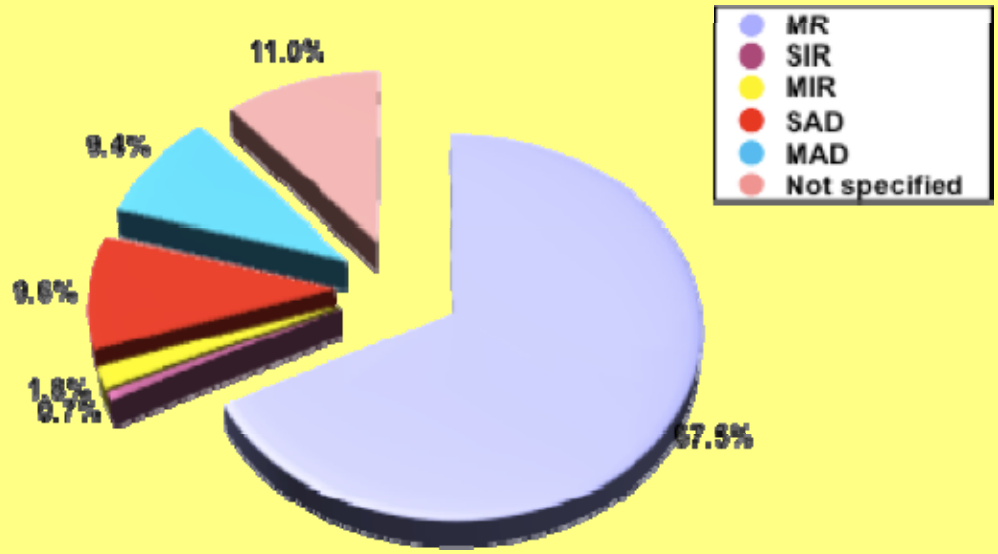


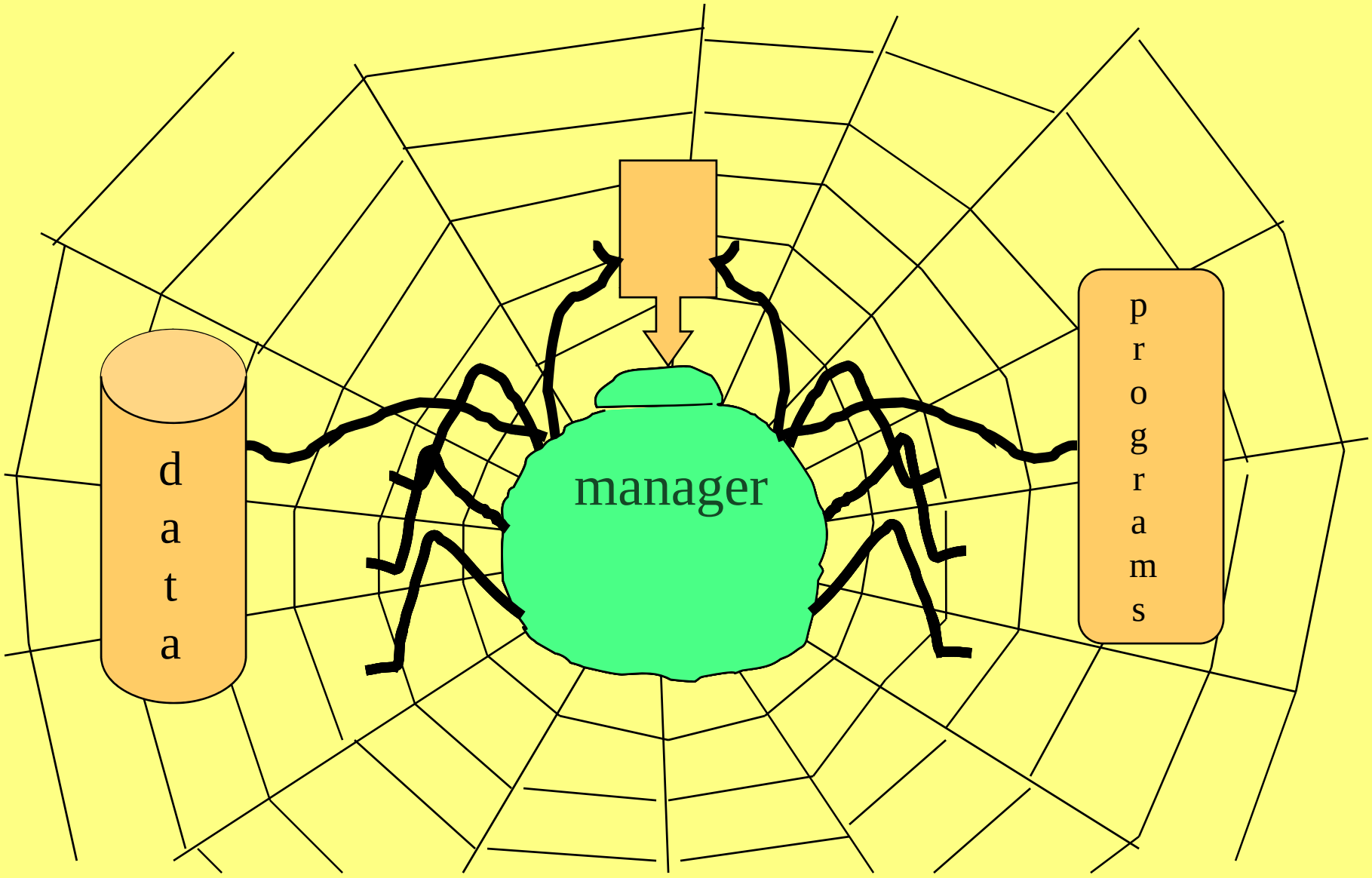

Diagram showing the percentage of structures in the PDB solved by different techniques

67.5% of structures are solved by Molecular Replacement (MR)

21% of structures are solved by experimental phasing

# Organisation of BALBES

## BALBES consists of three essential components



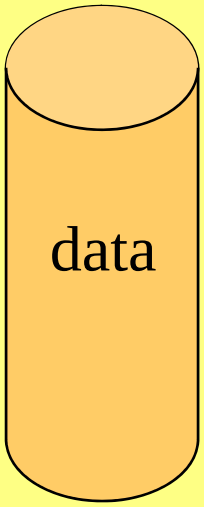
# Manager

It is written using PYTHON and relies on files of XML format for information exchange:

1. Data
   - Resolution for molecular replacement
   - Data completeness and other properties
   - Twinning
   - Pseudo translation
2. Sequence
   - Finds template structures with their domain and multimer organisations
   - Estimates number of molecules in the asymmetric unit
   - “Corrects” template molecules using sequence alignment
3. Protocols
   - Runs various protocols with molecular replacement and refinement and makes decisions accordingly

# Manager

makes decisions on different protocols according to search models available in the internal database.




# Database

Chains . The internal database has around 20000 unique entries selected from more than 45,000 present in the PDB. All entries in the PDB are analysed according to their identity. Only non-redundant sets of structures are stored.

Domains. About 8000 of them have two or more domains. The DB contains 30000 domain definitions Loops and other flexible parts are removed from the domain definitions.

Multimers of structures (using PISA)

Hierarchy is organized according to sequence identity and 3D similarity (rmsd over Ca atoms).

programs

# Programs

MOLREP - molecular replacement

Simple molecular replacement, phased rotation function (PRF), phased translation function (PTF), spherically averaged phased translation function (SAPTF), multi-copy search, search with fixed partial model

REFMAC

Maximum likelihood refinement, phased refinement, twin refinement, rigid body refinement, handling ligand dictionary, map coefficients

SFCHECK

Optical resolution, optimal resolution for molecular replacement, analysis of coordinates against electron density, twinning tests, pseudo translation

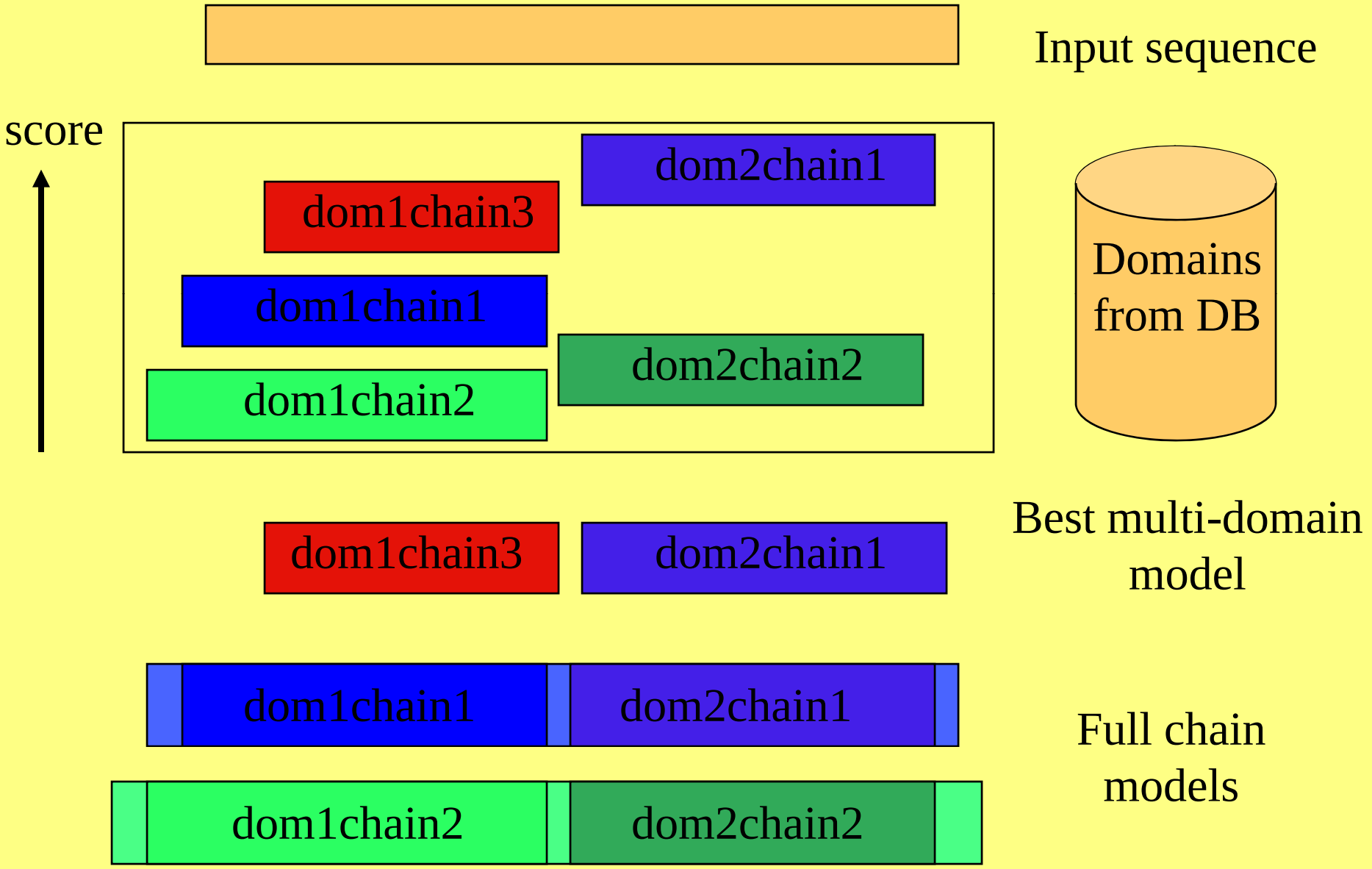
Other programs:

Alignment, search in DB, analysis of sequence and data to suggest number of expected monomers, semiautomatic domain definition

# Molrep

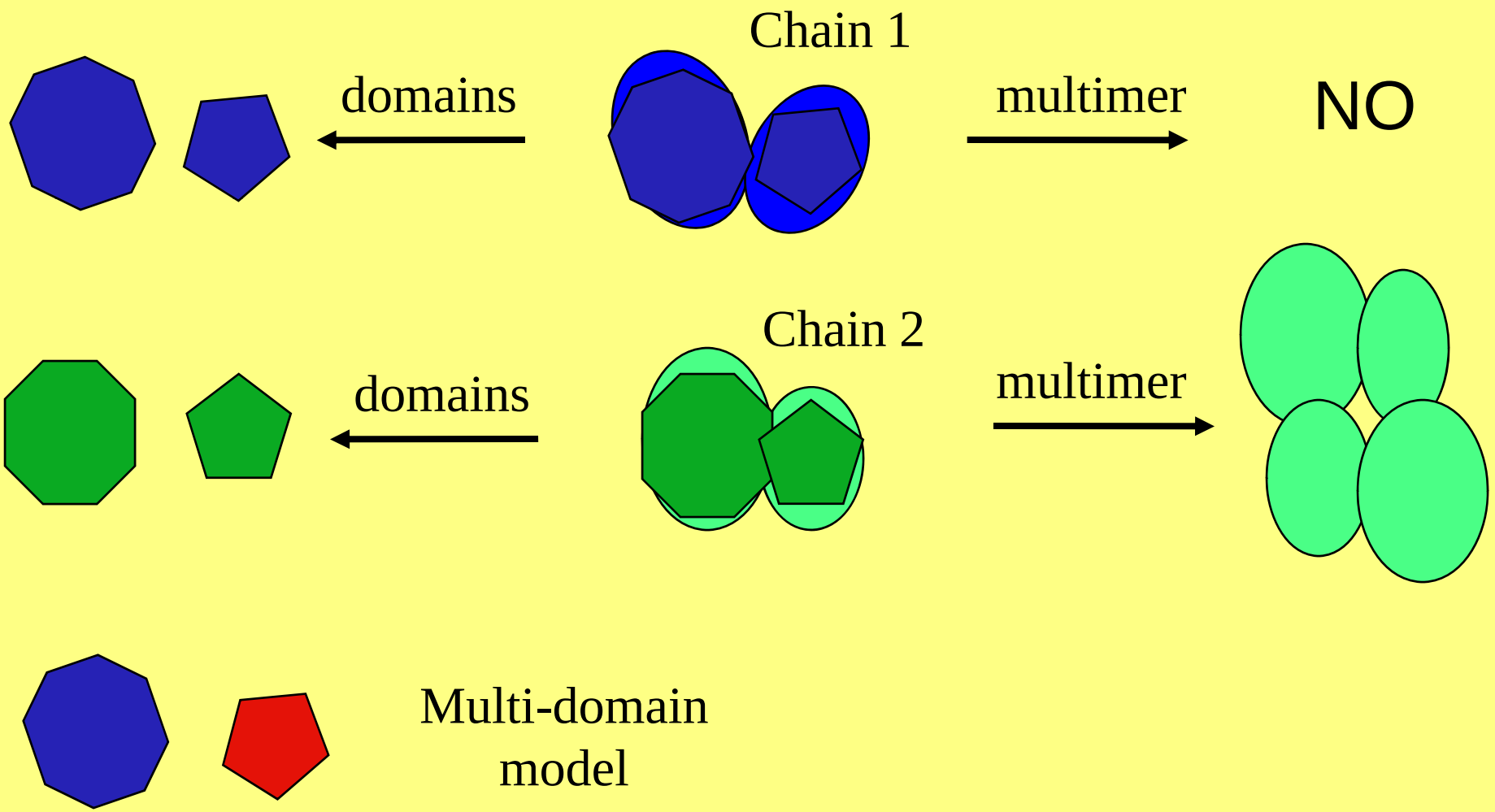
- Molrep is highly automated molecular replacement program. With given data, pdb and sequence (if available) it tries to solve molecular replacement problems.
- It automatically decides amount of molecule you expect in the asymmetric unit
- It uses packing function to remove false solutions
- It does anisotropic correction when it is necessary before starting molecular rotation and translation search
- It can handle pseudo-translations
- It can search small fragment in the electron density
- It can do multi-copy search for cases when there are several domains or several subunits.
- It can do fit of Xray model to EM and EM to Xray using full point group constraints

# Search models



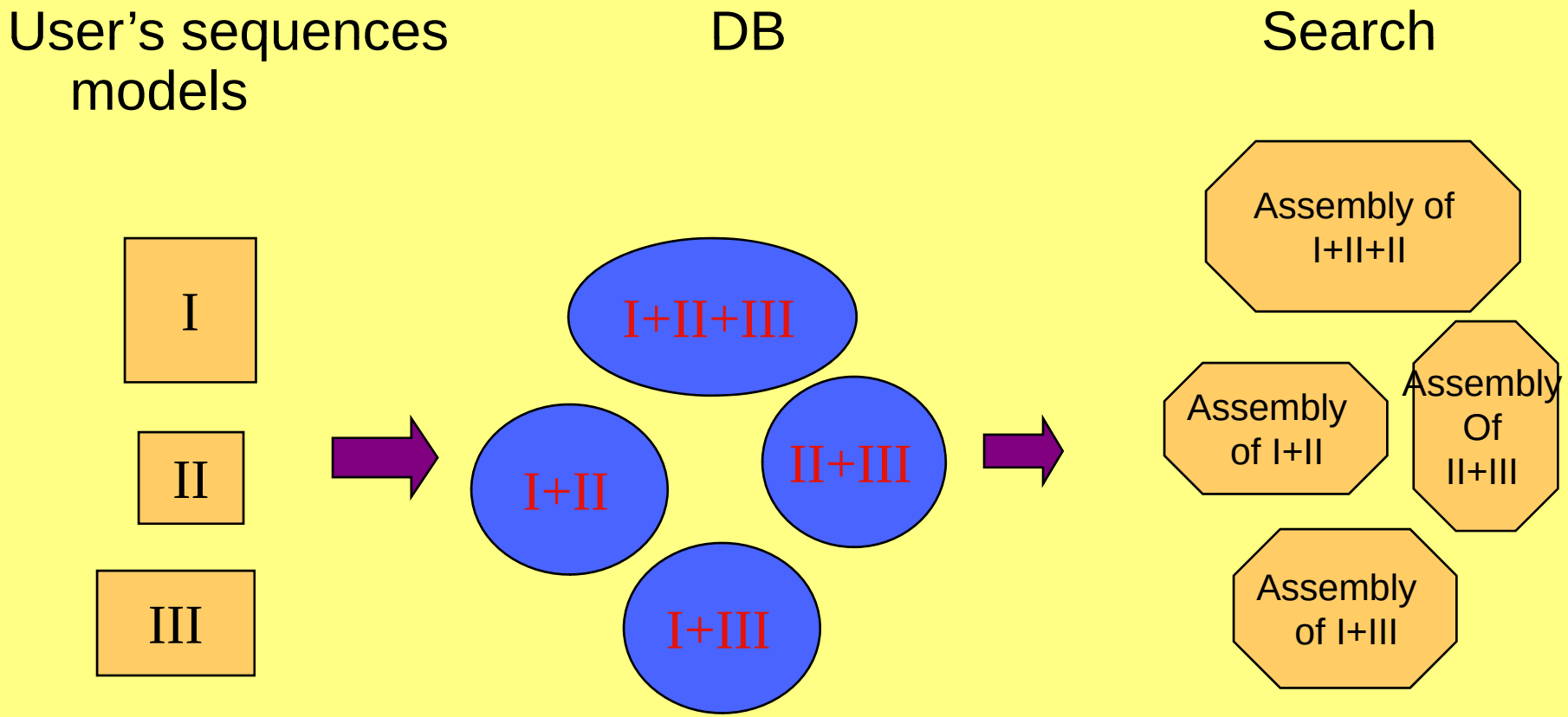
# Model preparation

All models are corrected by sequence alignment
and by atomic accessible surface area



# Heterogeneous Search Models

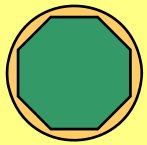
If a user provide several sequences, BALBES will search the database for complexes of models containing all or most of the sequences.



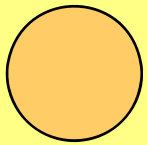
# Example 1: 2dwr

## Homologues

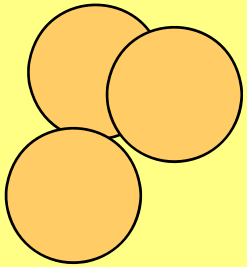
2aen: monomer and one domain definition associated with it.
Identity = 82%



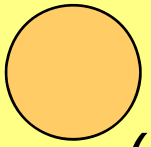
1kqr: monomer, no domain definitions
Identity = 45%



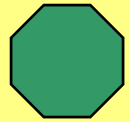
1z0m: dimer, no domain definitions
Identity = 25%



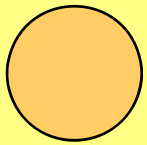
## Derived search models (and their priority)



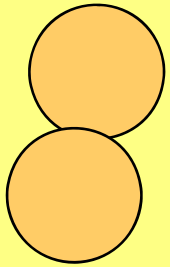
(1)



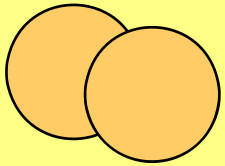
(2)



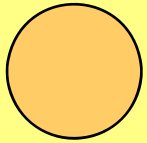
(3)



(4)



(5)

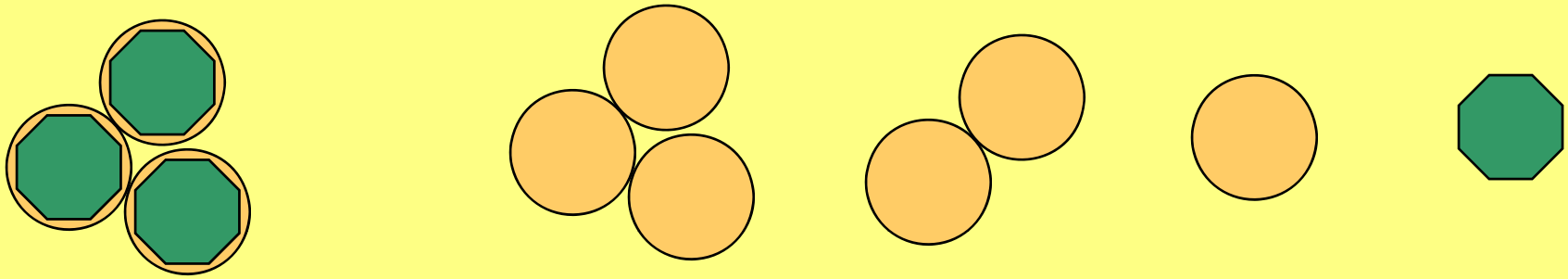


(6)

# Example 2: 2chp

Homologues

Derived search models
(and their priority)



1ji5: trimer
Identity = 50%

(1) (2) (3) (4)

1jig: trimer
Identity = 49%

(5) (6) (7) (8)

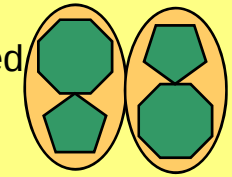
1n1q: trimer
Identity = 48%

(9) (10) (11) (12)

# Example 3: 2gi7

## Homologues

1p7q: homo-dimer;
each monomers is formed by two domains.
Identity = 45%

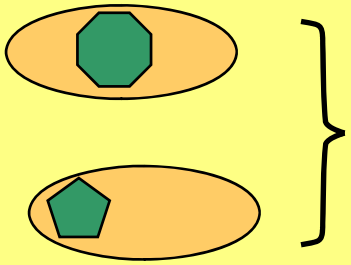


1ufu: monomer
formed by two domains.
Identity = 45%

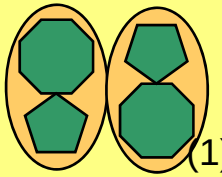
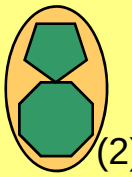
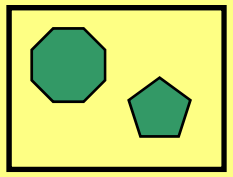
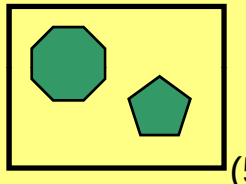
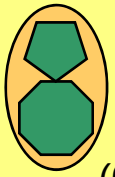
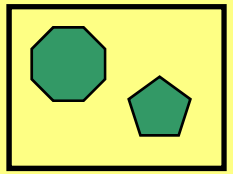
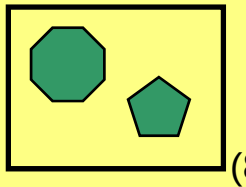
2d3v: monomer
formed by two domains.
Identity = 46%

xxxx: contains domain 1
Identity = 42%

yyyy: contains domain 2
Identity = 56%



## Derived search models (and their priority)

| dimeric | monomeric | "multi-domain" |
|---|---|---|
|  (1) |  (2) |  (3) |
| |  (4) |  (5) |
| |  (6) |  (7) |
| | |  (8) |

"Multi-domain" models:
placing domains one by one and attempting to maintain proper composition of the asymmetric unit

# Example 4: assembly (two sequences are submitted)

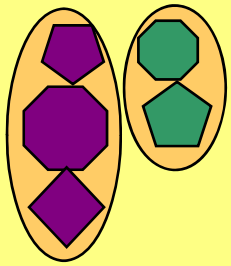
Assembly models

In case when two or more sequences are submitted attempt will be made to find hetero-oligomer matching all or some of these sequences.

If found, such hetero-oligomers will be first models to try.

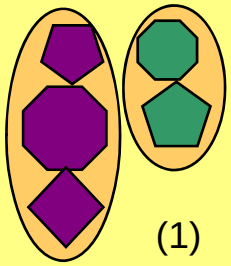
Homologues structure:

2b3t: hetero-dimer; monomers are formed by two and three domains.



Derived search models (and their priority):

assembly monomeric “multi-domain”



(1)

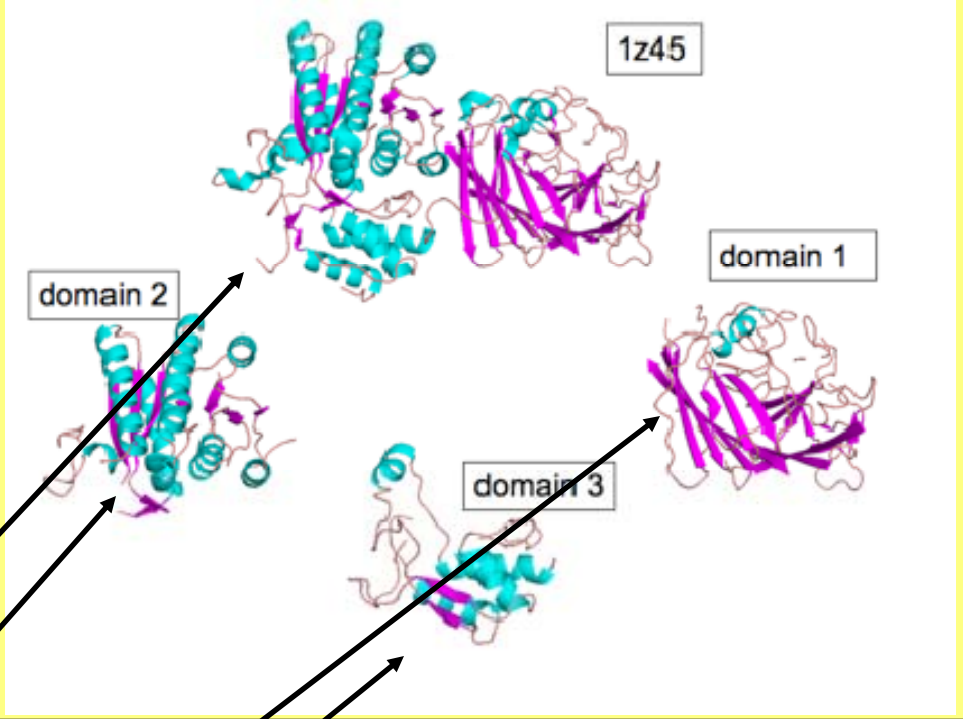
Other homologues (1t43, 1nv8, 1zbt, 1rq0) are matching only one of two sequences. Priority rules applied to them are as in previous examples.

Note: If the system cannot find a good solution from assembly then it tries to solve using individual molecules (domains) and combine them. Individual models (domains) may come from different proteins.

# Example of search: Multi-domain protein

This structure can be solved with multi-domain model.

PDB entry 1z45 has three major domains. One of the domains has also two subdomains. Domain 1 is similar to 1ek6 (seq id 55%). Domain 2 similar to 1yga (seq id 51%) and domain 3 is similar to 1udc (seq id 49%)




1z45 - isomerase
1ek6 - two domains of isomerase
1yga - another domain of isomerase
1udc - two domains of isomerase

All these proteins are although isomerases they have slightly different activities

# Updating and Calibrating the System

Only non- redundant structures are stored

All structures newly deposited to the PDB are tested

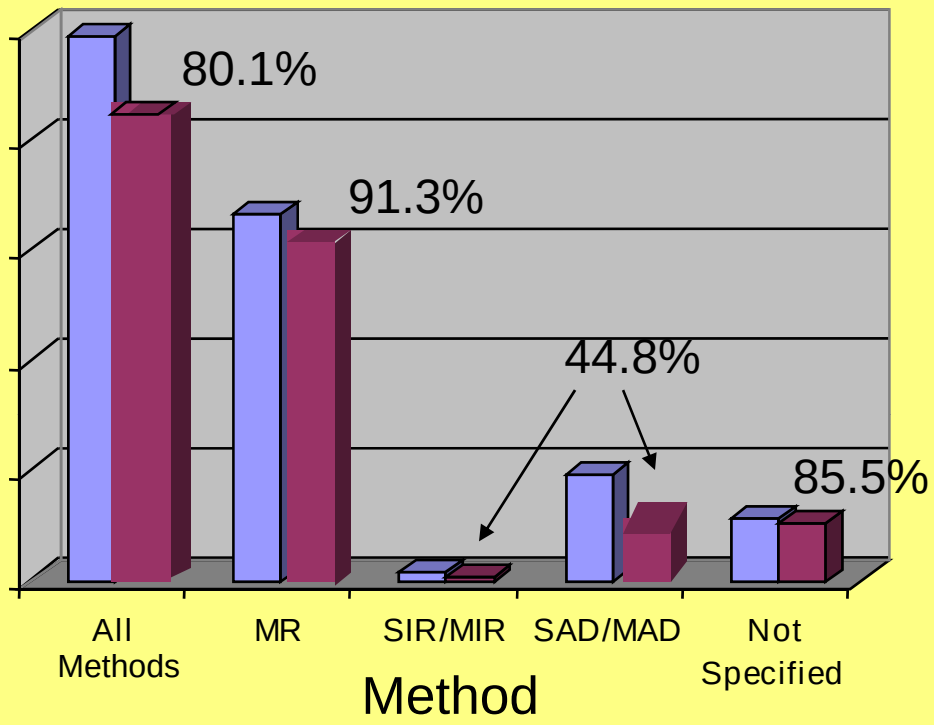
against the old internal database by using BALBES.

Only after that the DB is updated.

Updating and test are carried out every half a month.

automatically generated domains are checked manually
to make sure that automatic domain-definition transfer
does not introduce errors.

# The success rate of the latest tests (Jan - Feb 2008)




N structures = 950

Blue: the number of structures originally solved by a given method

Magenta: the number of structures BALBES was able to solve

Note: the fraction of structures solved by MR = 67%

The success rate of our latest tests was more than 80%

Note that some of the structures solved by experimental phasing could be actually solved by MR!

# Space group uncertainty

When we added a new option: To check all potential space groups with full automatic molecular replacement. It was used more than we expected. We had to turn this option off temporarily. We have already updated the system and it is now available.

# How to run BALBES:

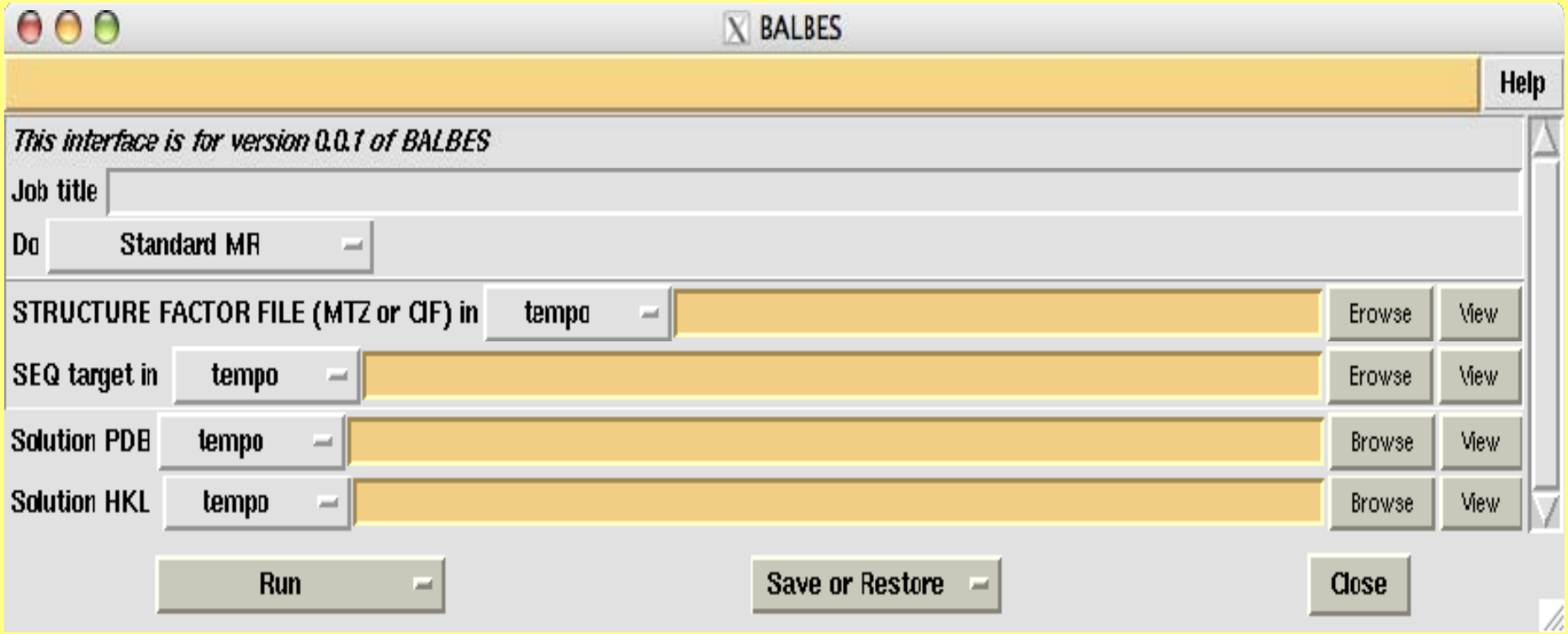
As an automated pipeline, BALBES tries to minimise users' intervention. The only thing a user needs to do is to provide two input files (a structure factor and a sequence file)

Running BALBES from the command line:

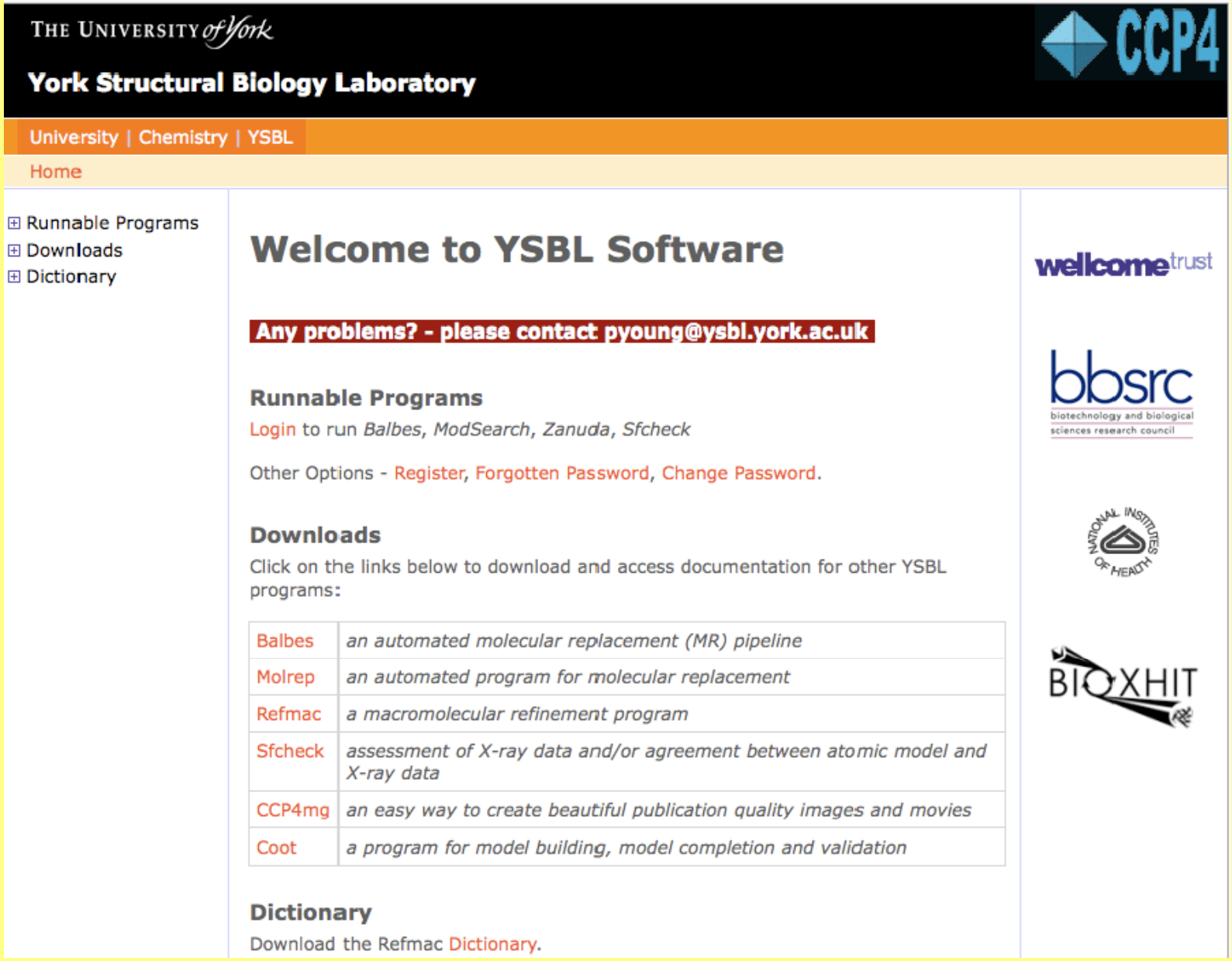
balbes –f structure_factors_file -s sequence_file –o output_directory

-f required
-s required
-o optional

# BALBES CCP4i interface



# BALBES Interface in Our Web Server
# (running using our Linux cluster) designed by P.Young



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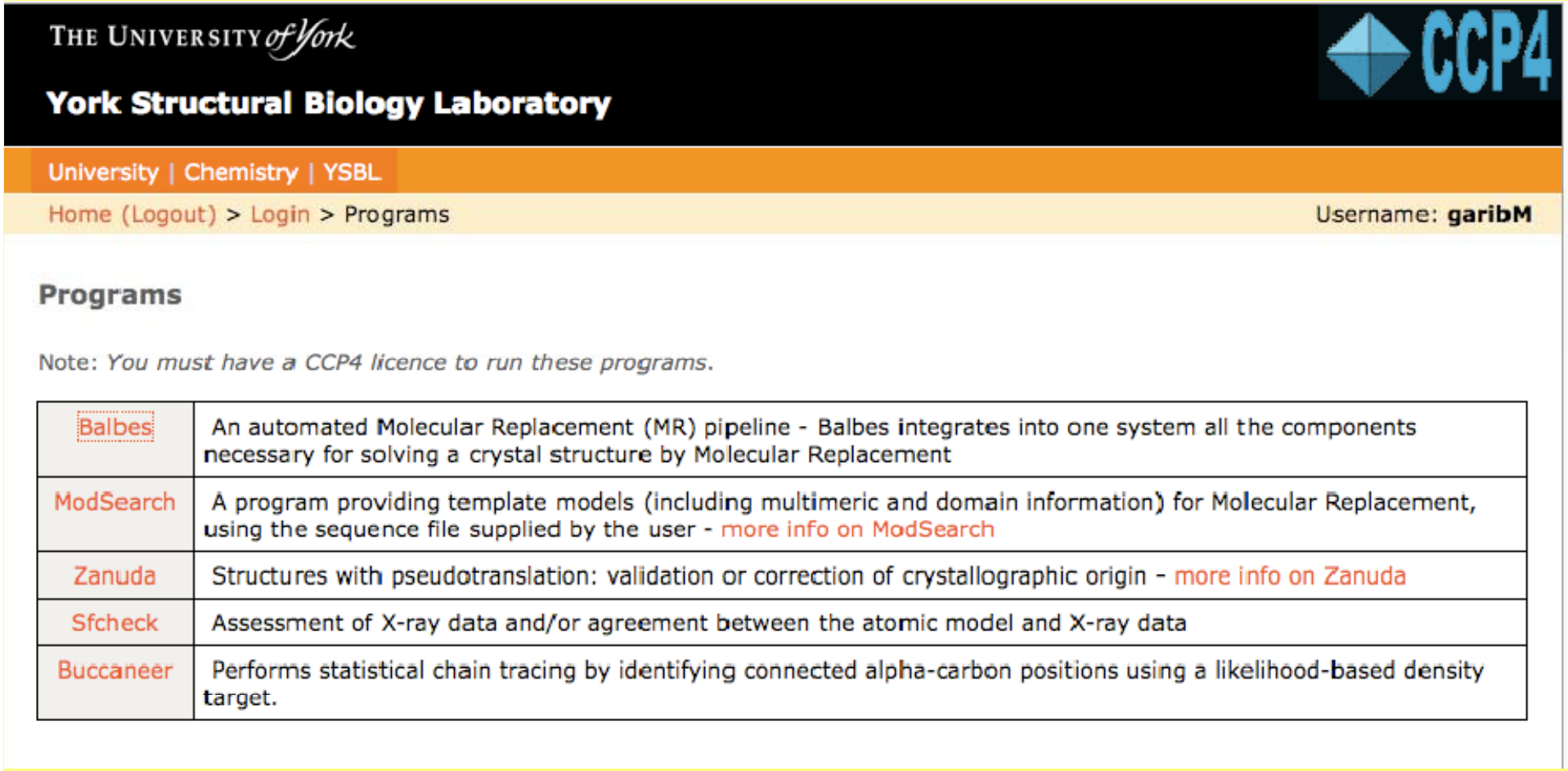

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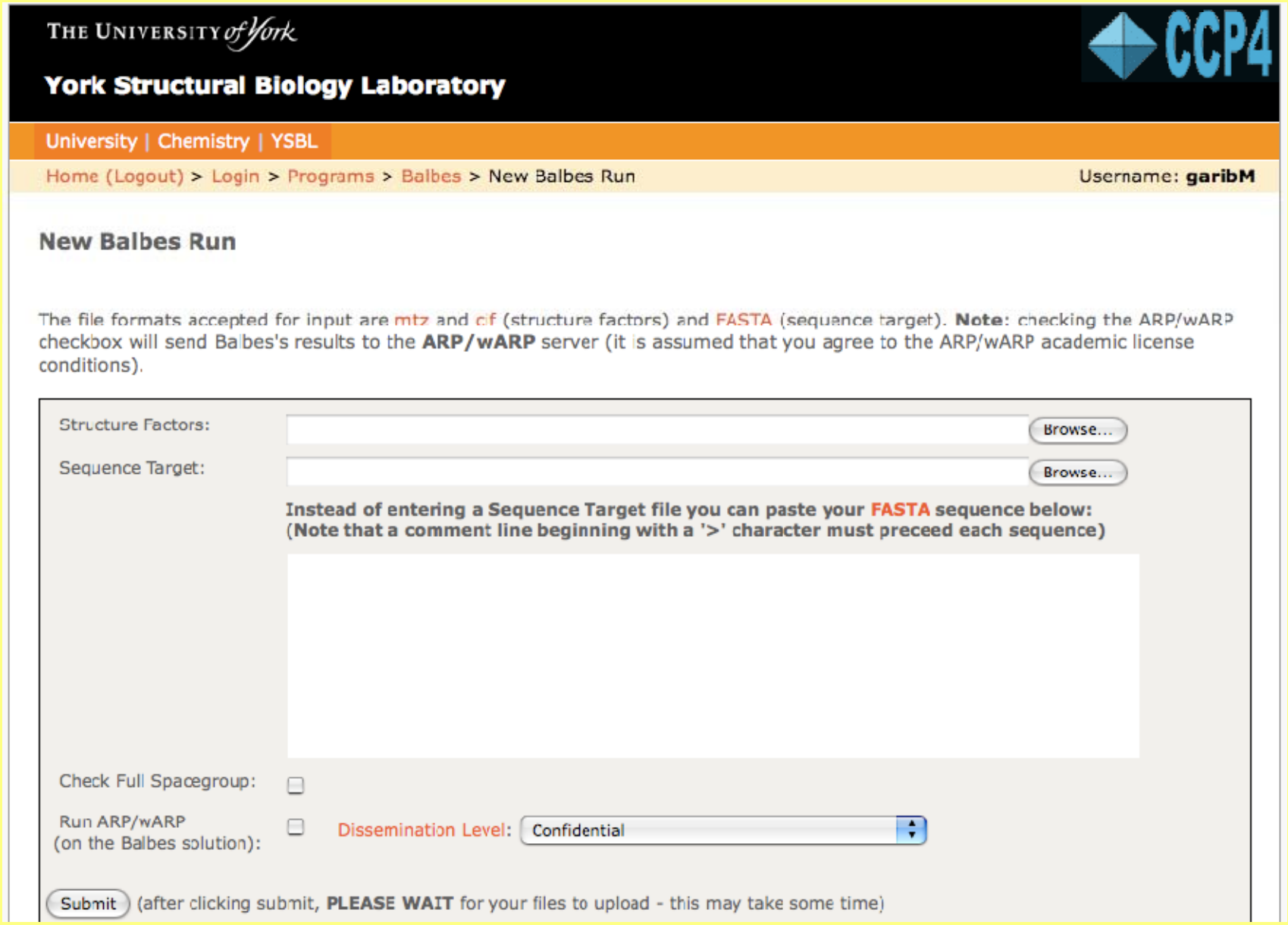


New version generates psuedo-NMR models automatically and uses them

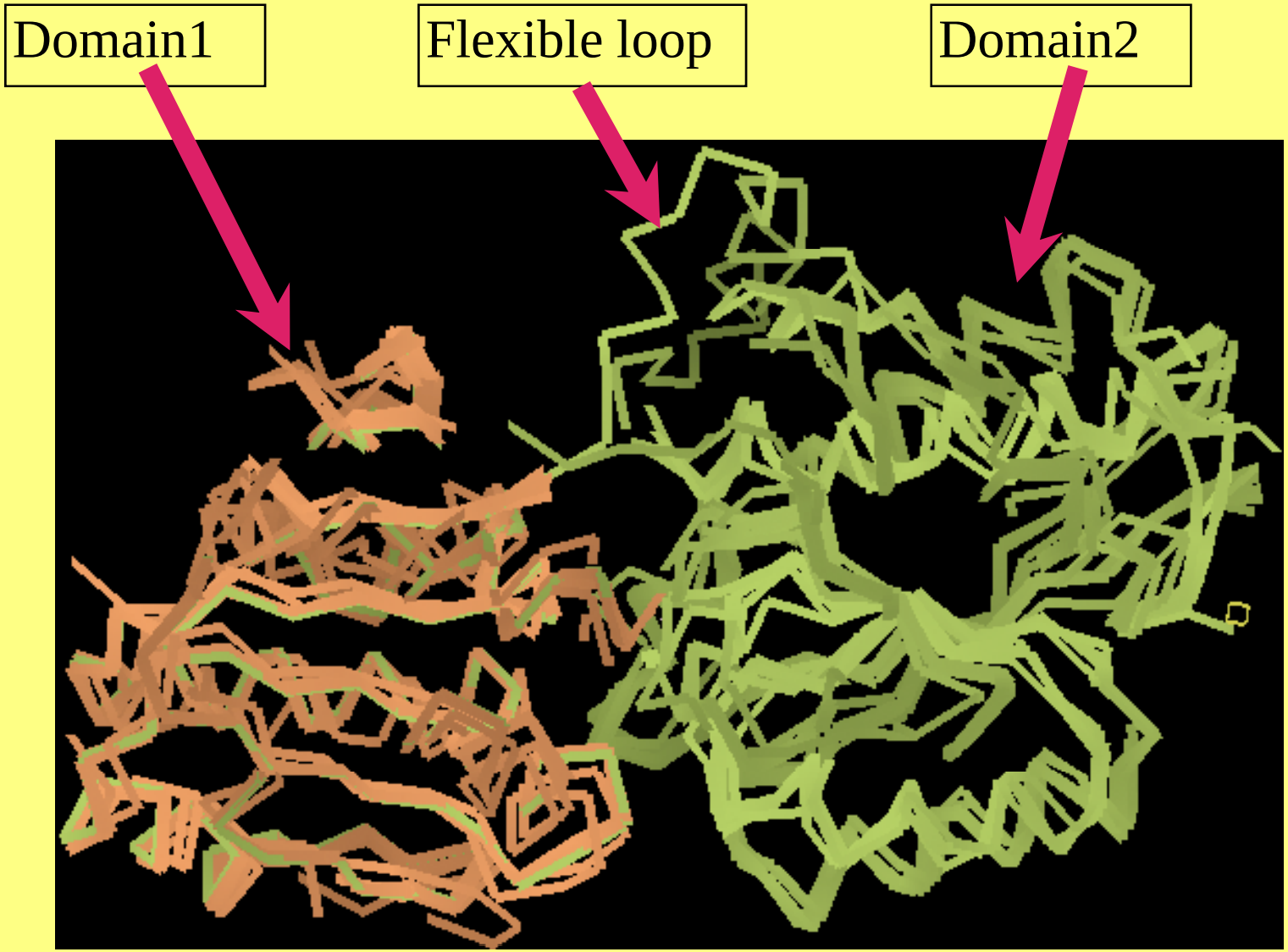
In the new version the program first identifies domains for each sequence using alignment. Then for each domain it creates ensemble of molecules using internal domain database. Then using profile of sequence generated from these ensembles it realigns sequences to improve reliability.
Then for each ensemble it tries moleculare replacement and refinement. Then takes the best “solution”, fixes it and tries to find more. When the score cannot be improved or maximume number of molecules expected is reached the program stops and gives (hopefully) solution with it quality factor.

# Complexes

In cases of complexes (more than one sequence) the system first tries assemblies (if available). If it can find solution it stops. If it cannot find solution then it switches to individual sequence (with and without ensembles). For each sequence best solution is stored. The best among the best is fixed and program continues to search for second, third etc proteins. Again with and without ensembles.

Moreover if space group is uncertain then the program will do all calculation for each potential space group candidate. Decision about space group is made at the very end of all runs (It may take some time).

# Complexes: Two domain example

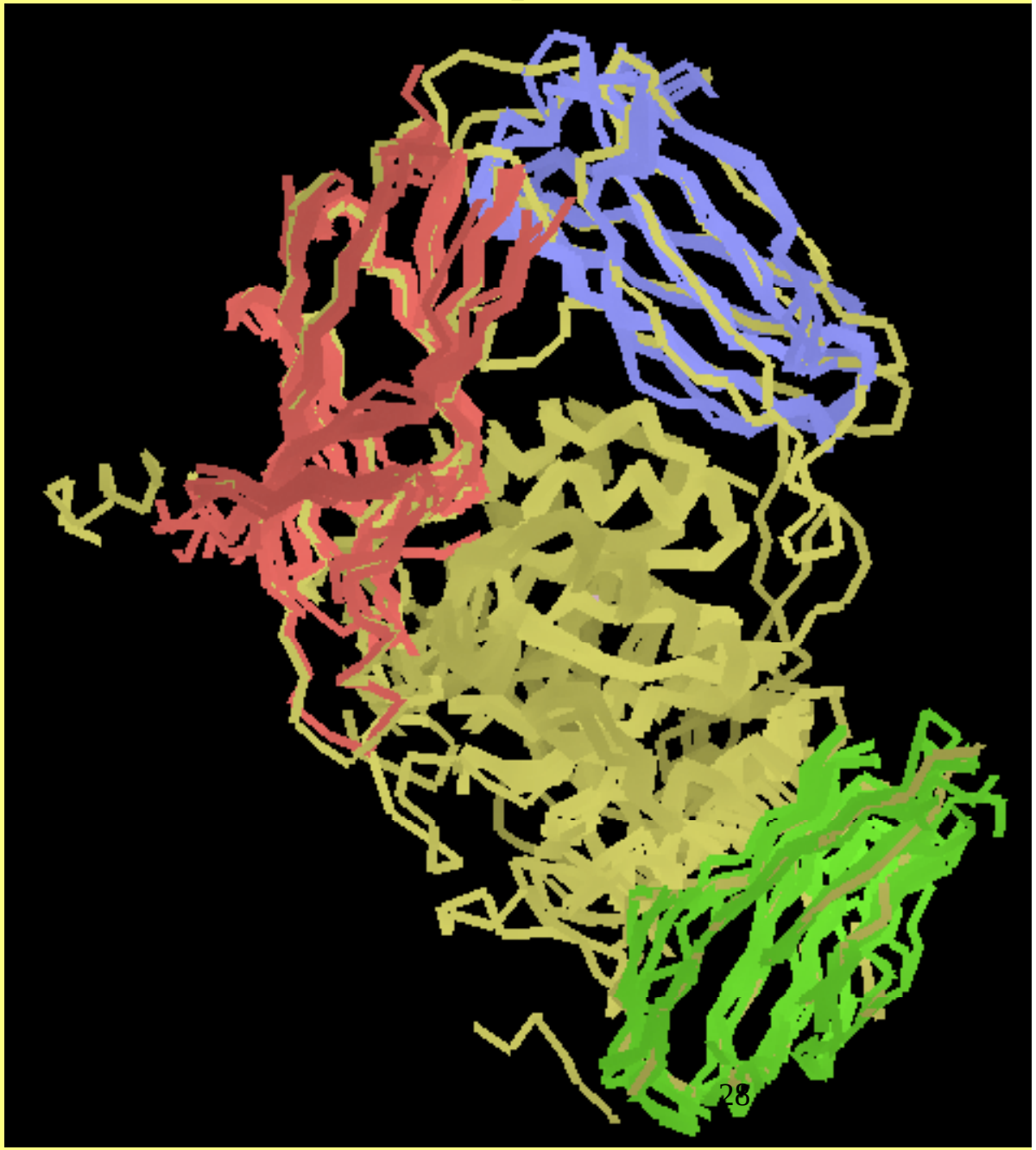



Domain1 and domain2 are used for MR. Flexible loops are not used if they are too small

# Ensembles: Four domain example

Four domain protein with different domains. For each domain there are number of similar structures taken from BALBES's domain database.

During MR ensemble for each domain is tried and then solutions are combined to give final solution.



# Conclusions

1. Internal database is an essential ingredient of efficient automation

2. With relatively simple protocols, BALBES is able to solve around 80% of structures automatically

3. Interplay of different protocols is very promising

4. Huge number of tests help to prioritise developments and generate ideas

## Team (YSBL, York)

Alexei Vagin
Fei Long
Paul Young
Andrey Lebedev

## Acknowledgements

E.Krissinel for PISA  MSD/EBI, Cambridge

All CCP4 and YSBL people for support

ARP/wARP development team

Wellcome Trust, BBSRC, EU BIOXHIT, NIH for support

# The End

The site to download BALBES:
http://www.ysbl.york.ac.uk/~fei/balbes/

Webserver:
http://www.ysbl.york.ac.uk/YSBLPrograms/index.jsp

This and other talks:
http://www.ysbl.york.ac.uk/refmac/presentations/