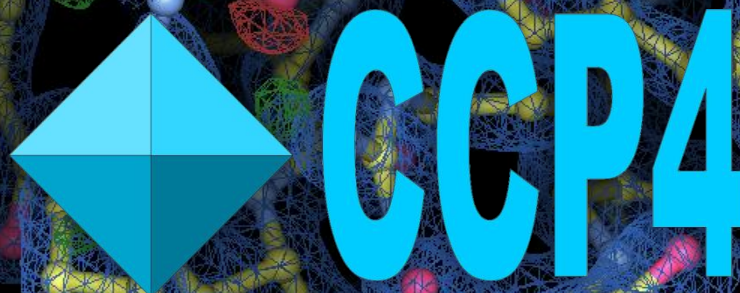


Photon Factory 2014

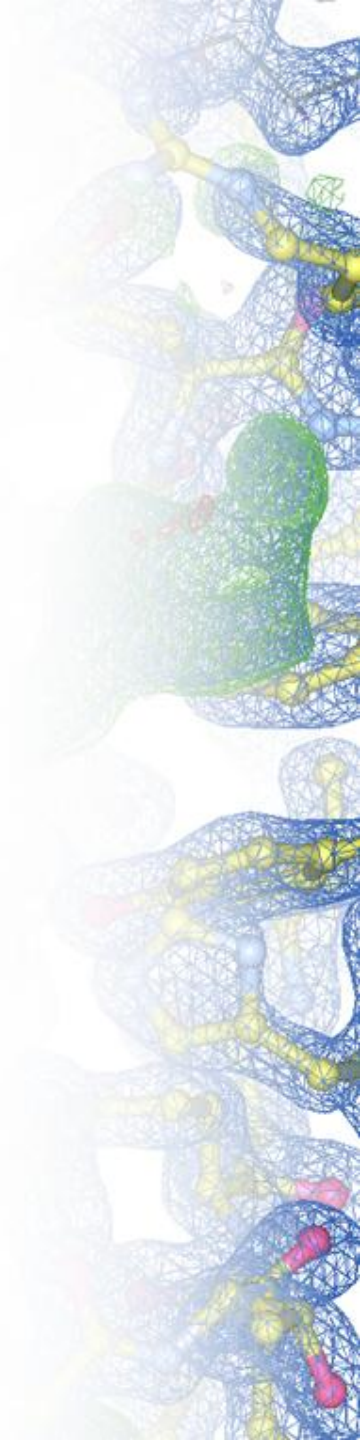
*A brief tour of ~~CCP4~~  
the workshop*

Charles Ballard, CCP4,  
Research Complex at Harwell



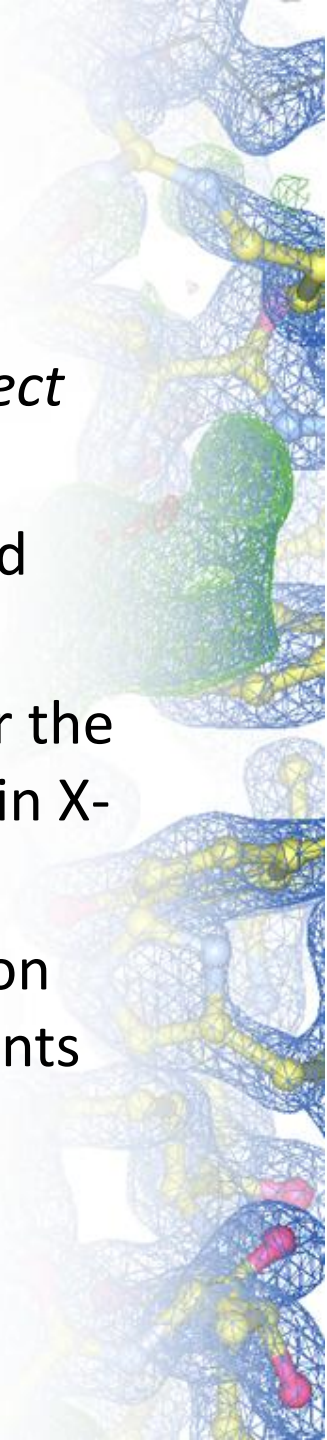
# Outline

- What is CCP4?
- Brief tour of current status of suite
- This workshop



# What is CCP4?

- CCP4 stands for “*Collaborative Computational Project Number 4*”
- One of several CCPs set up in the UK to advance and support scientific software development
- CCP4 was set up in the late 1970’s to bring together the leading developers of software in the field of protein X-ray crystallography in the UK
- The aim was to assemble a comprehensive collection of software to satisfy the computational requirements of the relevant UK groups



- Core Group (Oxford):
  - Maintain and support software suite
  - Application and infrastructural software developments
  - Collaborate with Diamond on software deployment on the beamline
  - Educational outreach
  - Maintaining CCP4 resources such as the CCP4 bulletin board
- Cambridge:
  - Laboratory of Medical Biology
    - Data processing software - Mosflm, Aimless
    - Refinement software – Refmac
    - Model building - Coot
  - University of Cambridge
    - Phaser group
- University of York:
  - Software development – CCP4mg, Buccaneer, Nautilus
  - CCP4 GUI2 development
- Others include the Crank group at Leiden in the Netherlands and AMPLE developers at the University of Liverpool

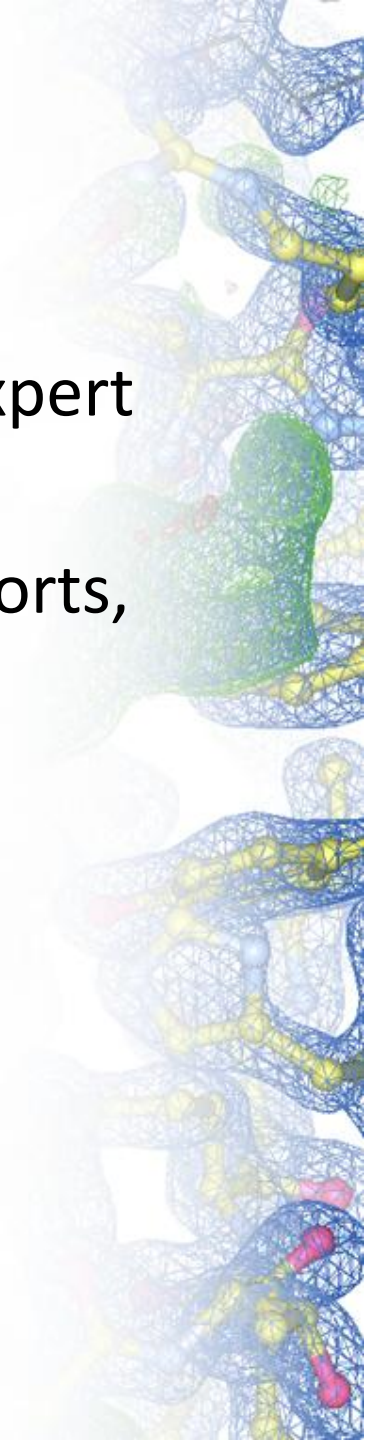


# CCP4 Usage



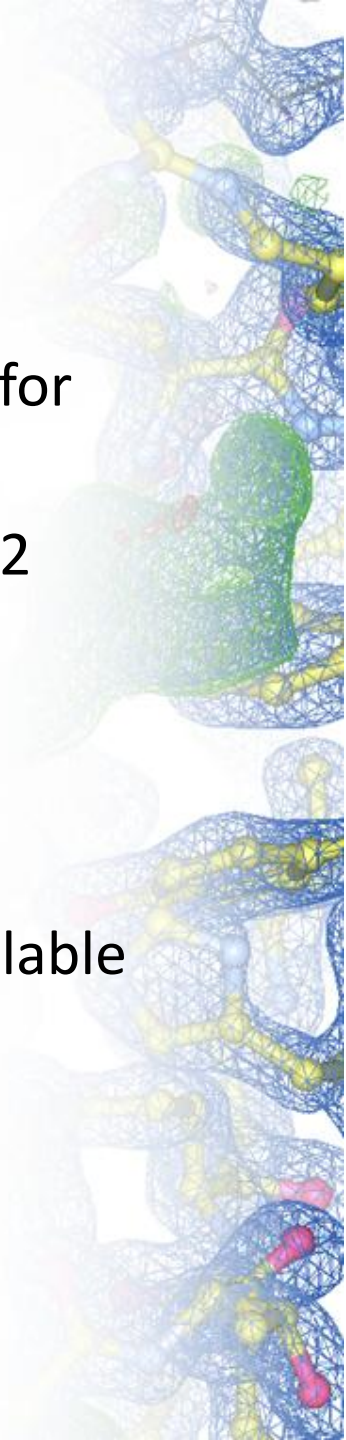
# Other Resources

- CCP4BB mailing list – bulletin board, news, expert help and advice
- CCP4 help desk – [ccp4@ccp4.ac.uk](mailto:ccp4@ccp4.ac.uk) – bug reports, problems with software
- CCP4 wiki – <http://ccp4wiki.org> – program documentation and tutorials
- CCP4 web page – <http://www.ccp4.ac.uk>

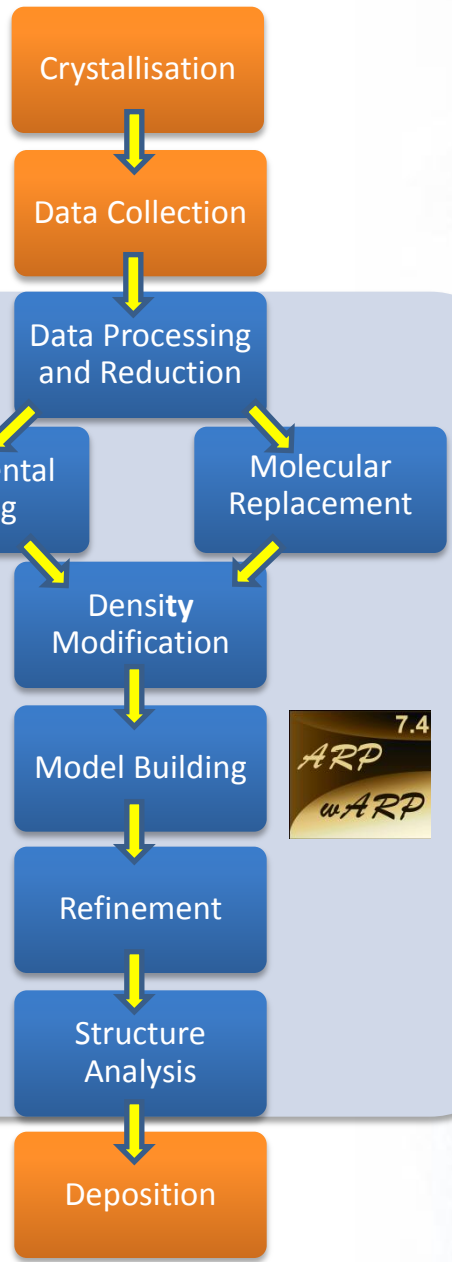
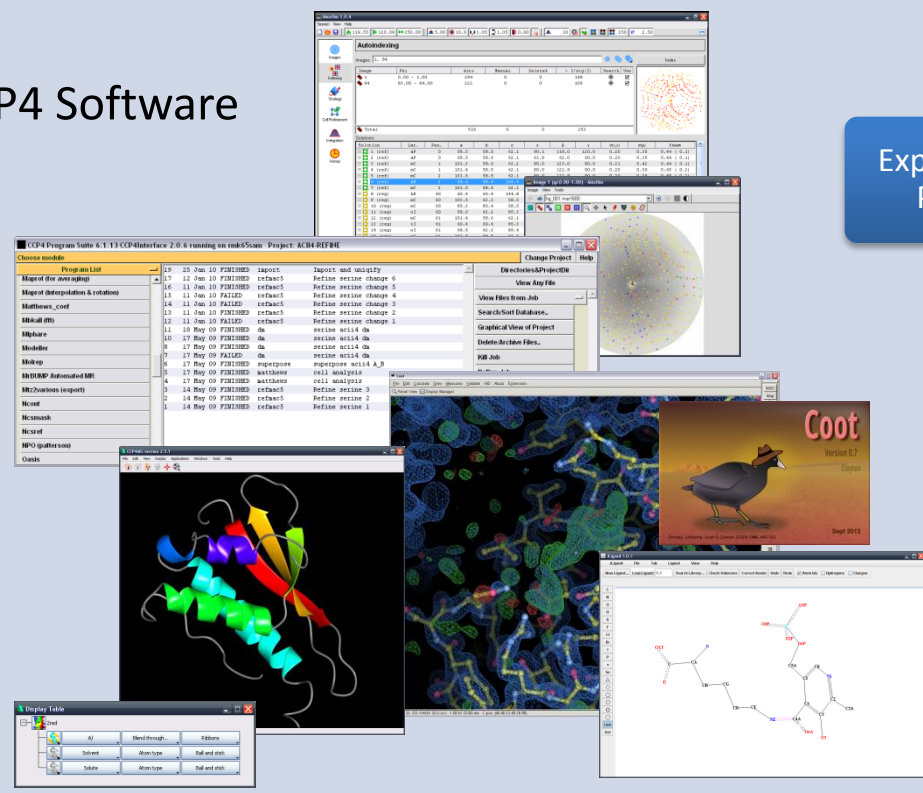


# The CCP4 software suite

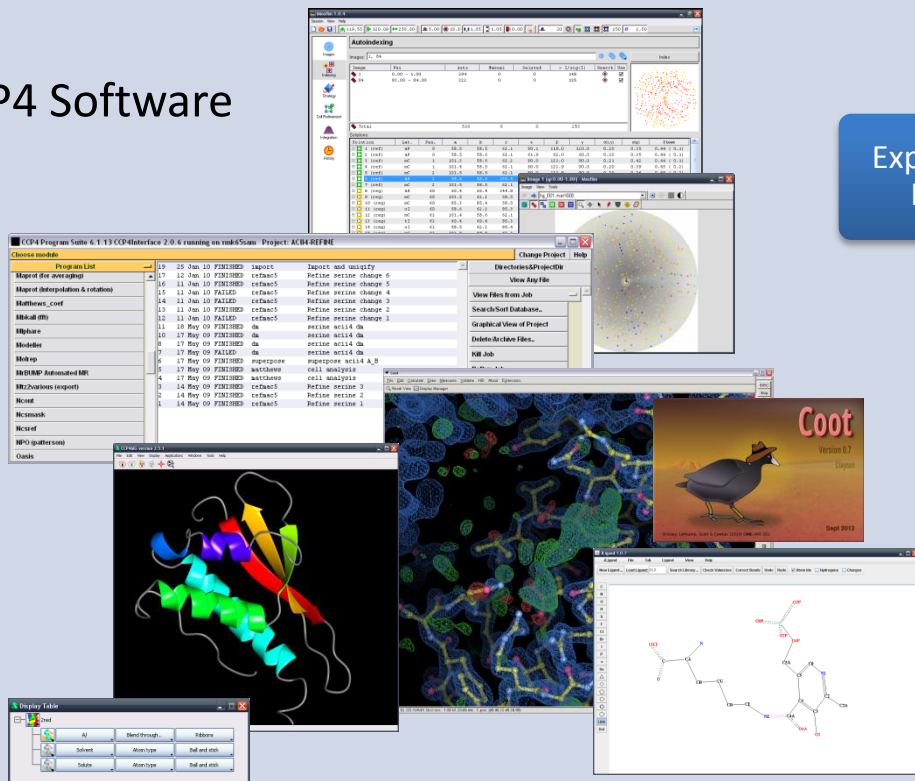
- The CCP4 suite is a comprehensive suite of software for protein crystallography
- New versions of the suite are released about every 12 months
  - New programs
  - Major updates to existing programs
  - Other new features such as changes to CCP4i interface
- **New** - Revisions to the current release are made available through the CCP4 updates manager
- Releases now checkpoints in this process



CCP4 Software



# CCP4 Software



Crystallisation

Data Collection

Data Processing and Reduction

Experimental Phasing

Molecular Replacement

Density Modification

Model Building

Refinement

Structure Analysis

Deposition

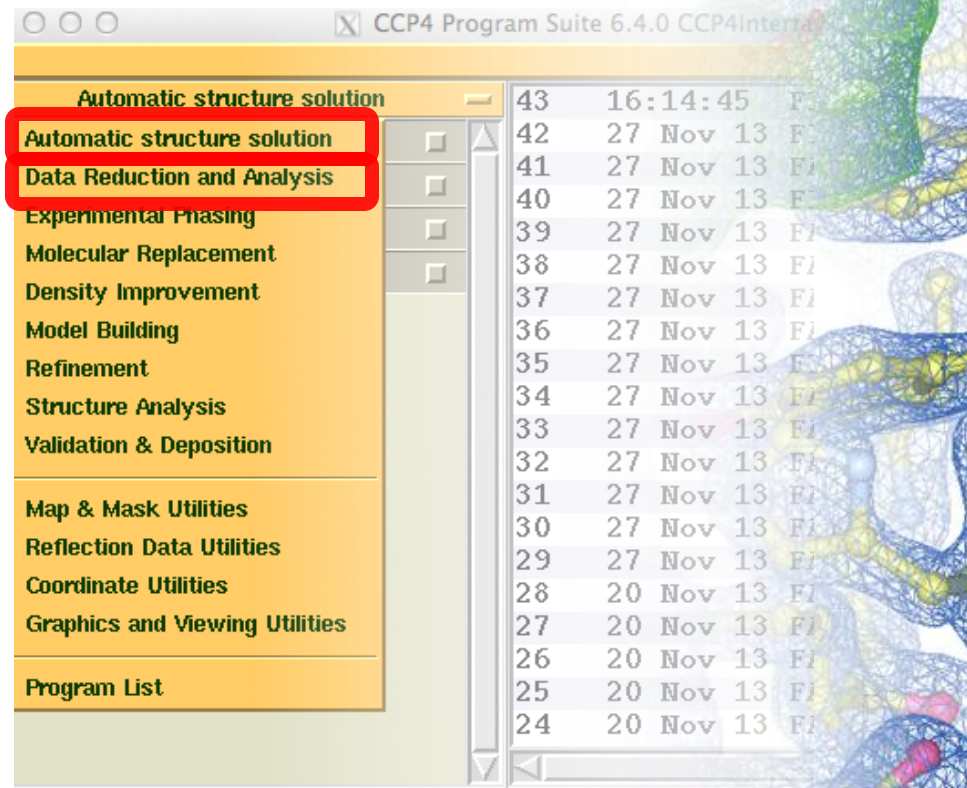


# xia2

Graeme Winter (DLS)

- Automated data-reduction – from diffraction images to merged reflection file
- Wraps xds, mosflm, elements of CCP4 and cctbx (and phenix)
- Coming later this year:  
Xia2 with DIALS integration  
Scoring for multi-crystal

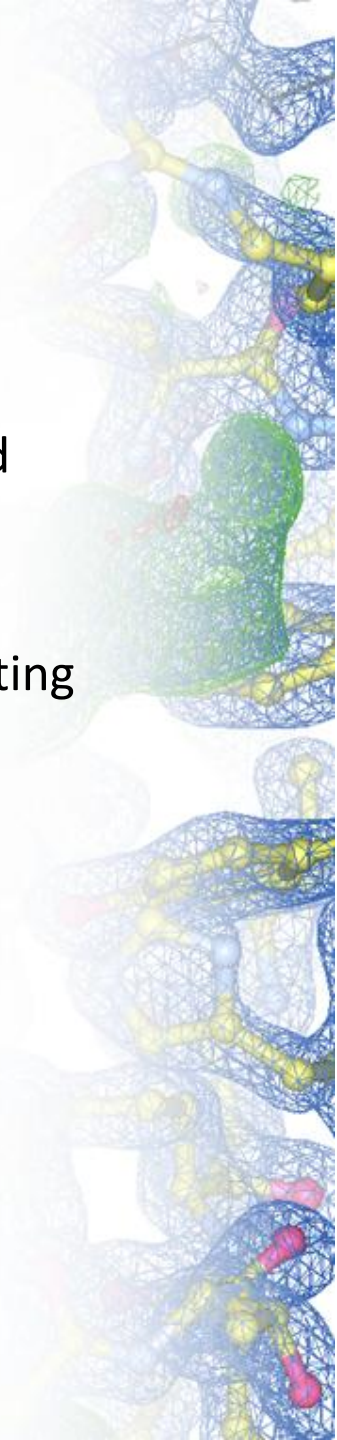
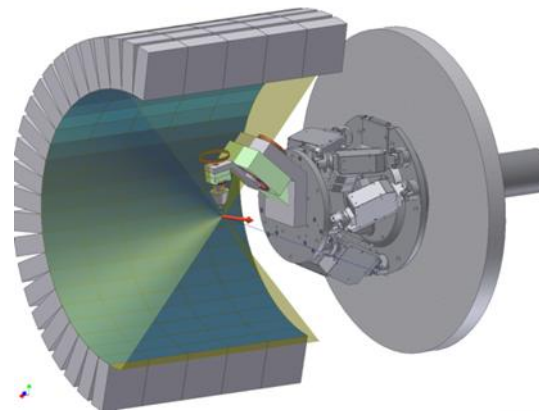
## Automatic Data Processing and Reduction



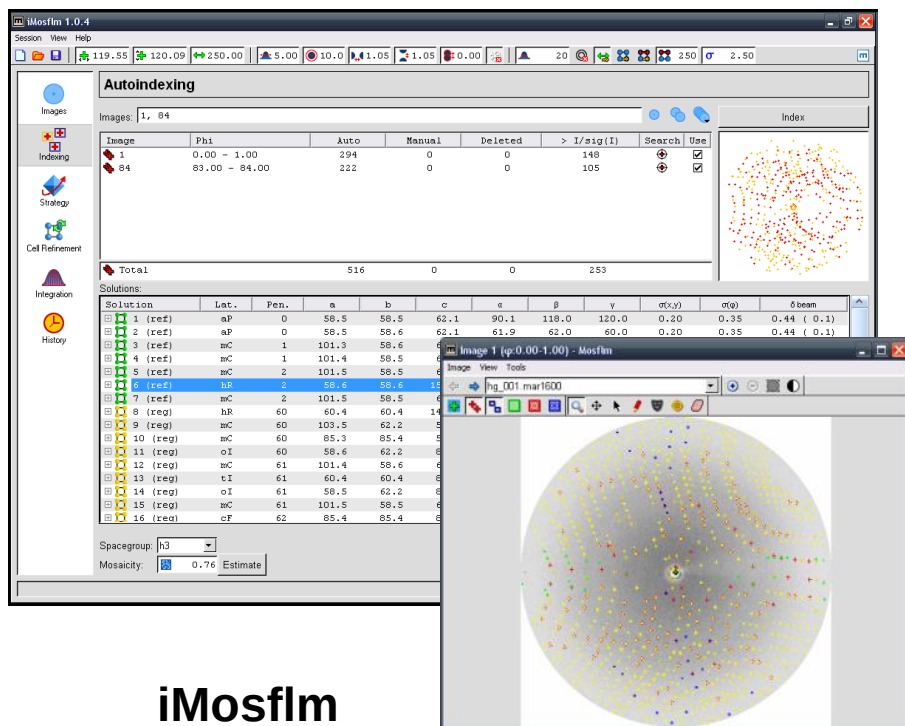
# *Data processing - DIALS*

- **D**iffraction **I**ntegration for **A**dvanced **L**ight **S**ources
- New data processing software designed for: speed, flexibility and accuracy, esp. with challenging data
- Will cater for both synchrotron and XFEL experiments
- Main philosophy: build a comprehensive toolkit. Thus implementing both 3D and 1D FFT indexing methods, 2D and 3D integration methods, multiple optimisation engines and allowing extension through an algorithm plug-in system
- First alpha release in September

<http://dials.sourceforge.net/>



# Data Processing and Reduction



## iMosflm

New:

Multiple lattice processing  
Parallelisation – quickens processing

## Pointless

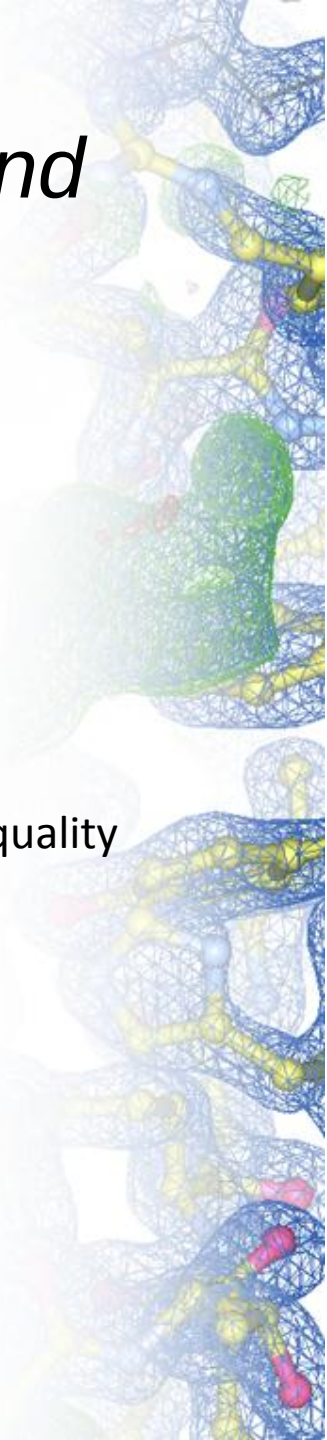
Determine point-group (& space group)

## Aimless

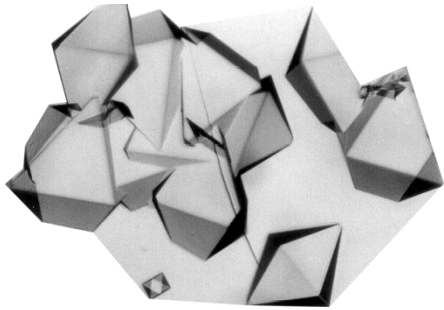
Scale-symmetry-related intensities together  
Produce statistics on data quality

## Feckless

Multi-crystal merging



# *Multi-crystal - BLEND*

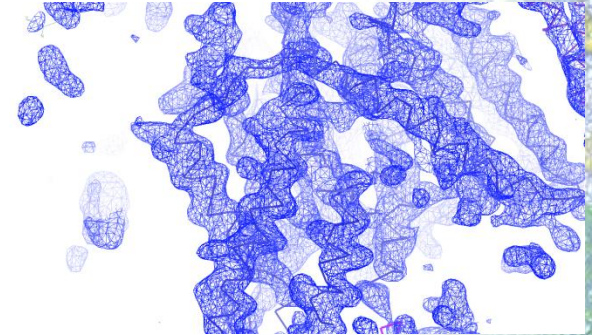


Multiple Crystals

New Methods and Software



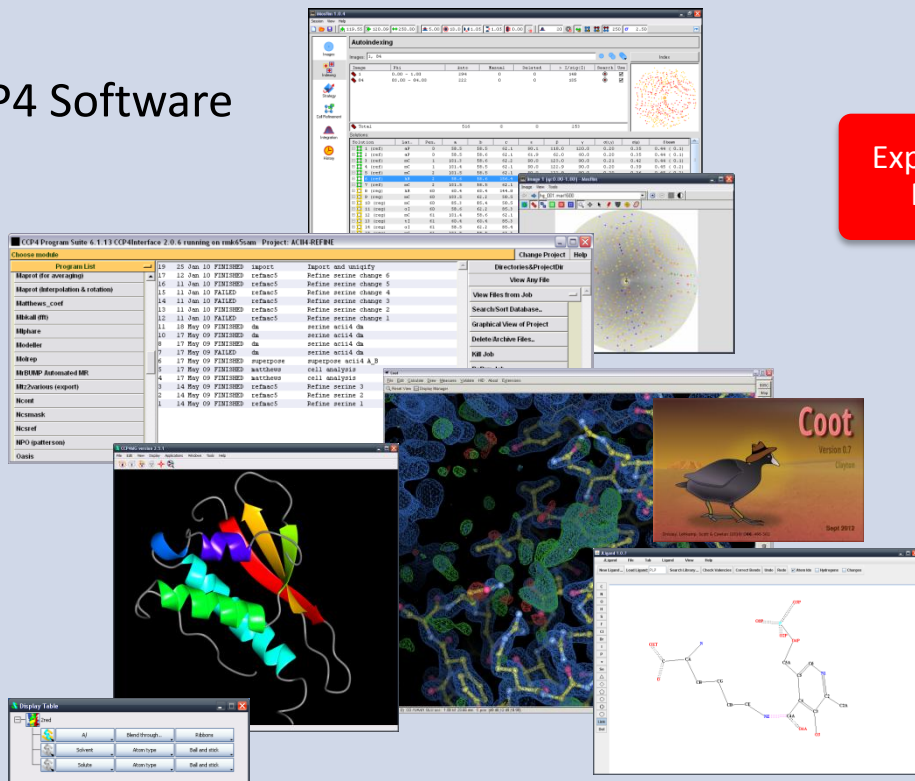
***BLEND***



- Merging from multiple crystals can
  - Aid in structural solution for radiation sensitive samples
  - Lead to anomalous signal increase (Hendrickson)
- Use clustering analysis and linear decomposition

J. Foadi, P. Aller, Y. Alguel, A. Cameron, D. Axford, R. L. Owen, W. Armour, D. G. Waterman, S. Iwata and G. Evans  
*Clustering procedures for the optimal selection of data sets from multiple crystals in macromolecular crystallography*  
*Acta Cryst.* (2013), **D69**, 1617-1632

# CCP4 Software



Crystallisation

Data Collection

Data Processing  
and Reduction

Experimental  
Phasing

Molecular  
Replacement

Density  
Modification

Model Building

Refinement

Structure  
Analysis

Deposition

*PDB\_REDO*



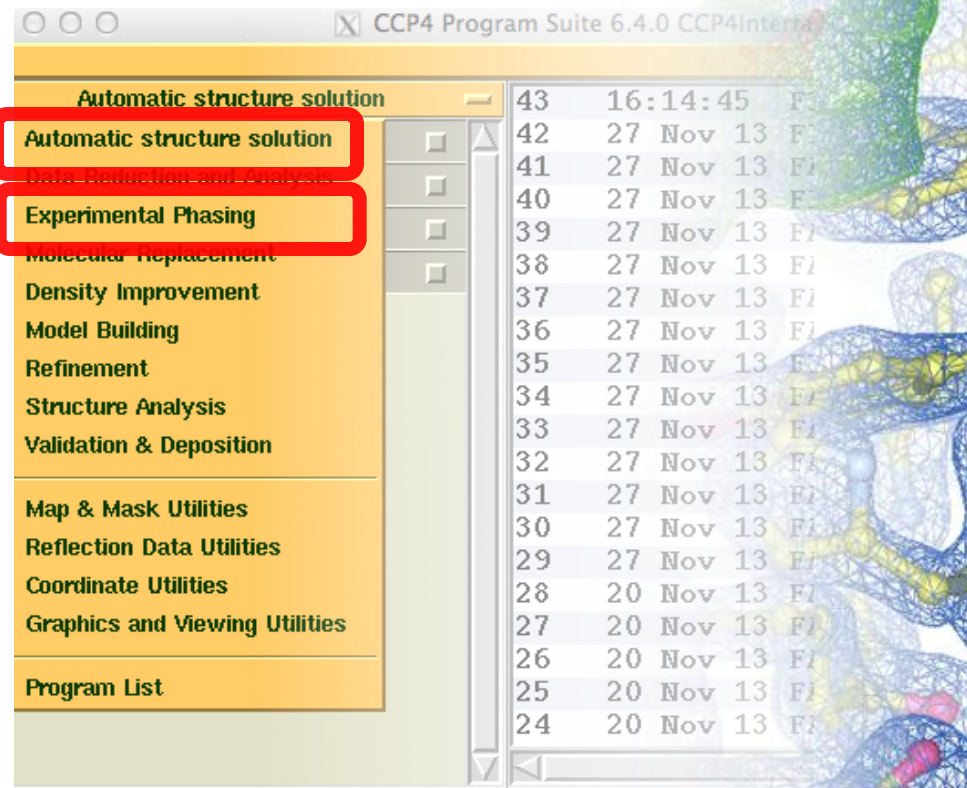
# Phasing - Experimental Phasing

## Crank/Crank2

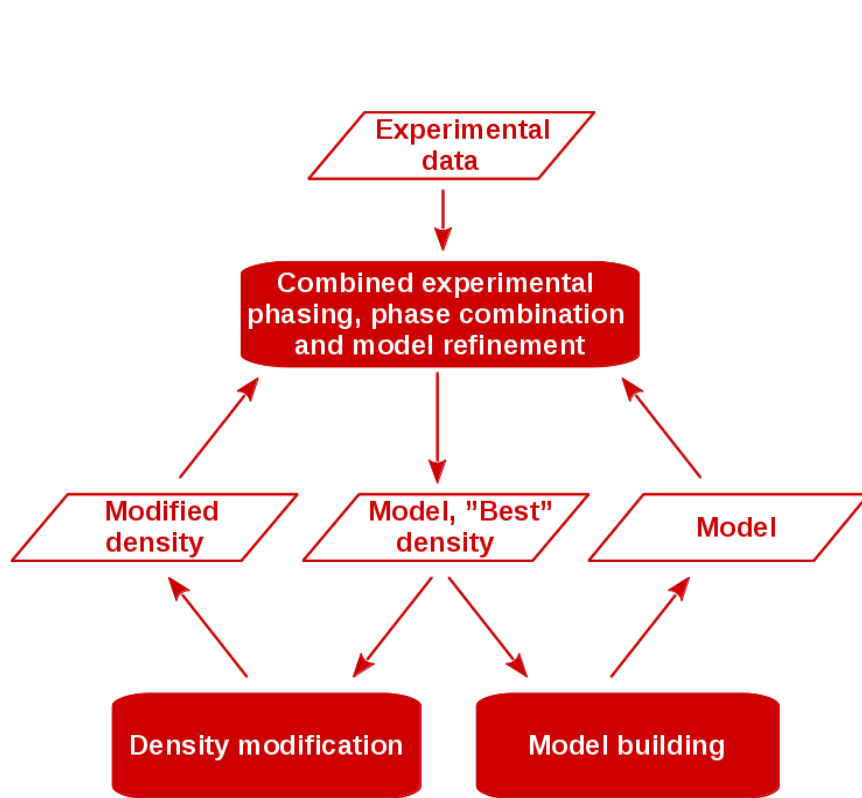
- Automated experimental phasing pipeline
- Heavy atom location through to Model Building

## Phaser EP

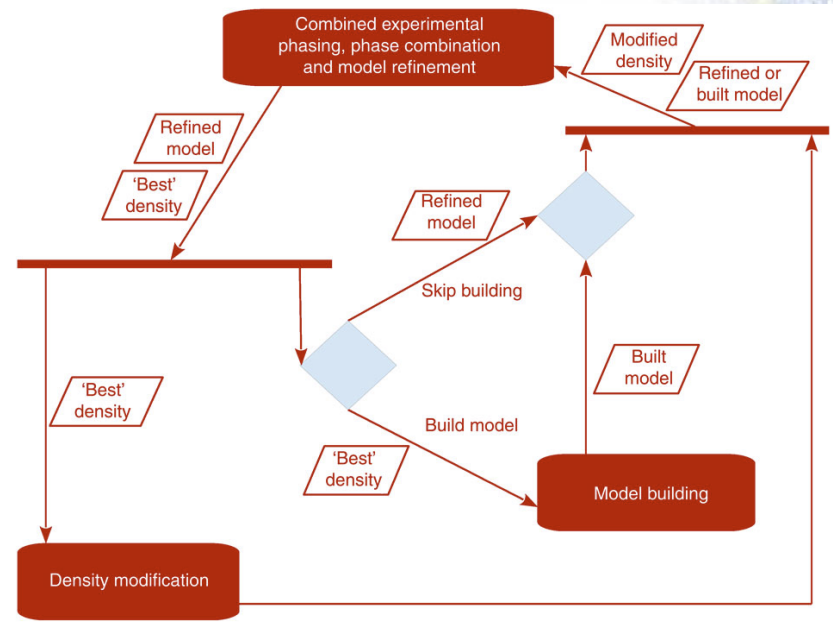
- Automated experimental phasing
- SAD phasing
- Combined MR and SAD Phasing



# Experimental Phasing – CRANK2



Combined SAD workflow



P. Skubak and N.S. Pannu  
*Automatic protein structure  
 solution for weak X-ray data*  
 Nature Comm. (2013), Aug

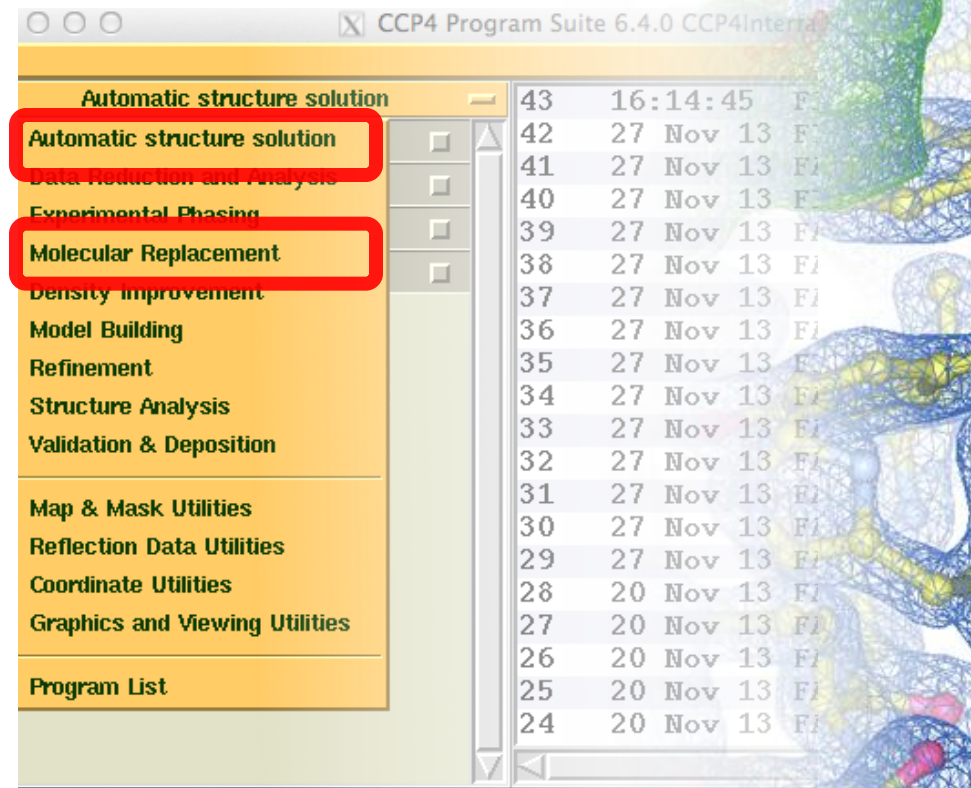
# Automatic Molecular Replacement

## MrBUMP

- Automated molecular replacement
- From model search and preparation through to initial refinement
- Uses Phaser and Molrep
- Now includes Model building options

## BALBES

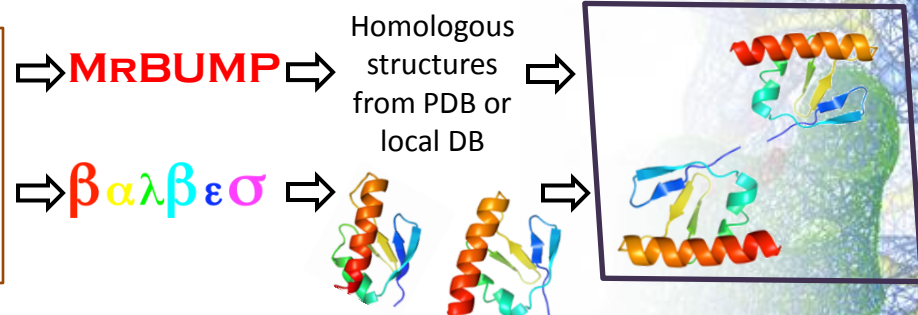
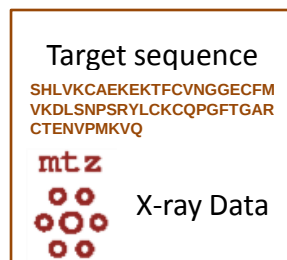
- Automated molecular replacement using Molrep and Refmac
- Custom DB with search models monomer, domain and multimeric form



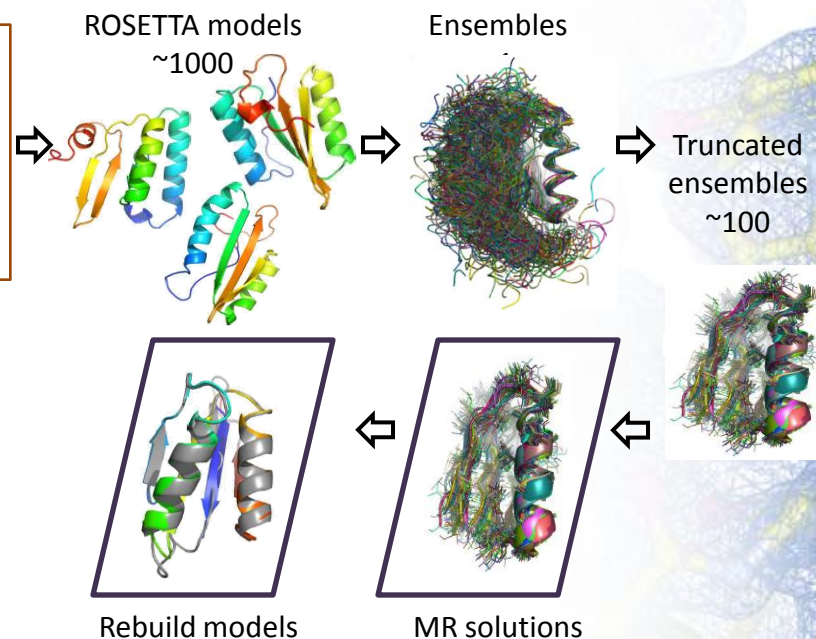
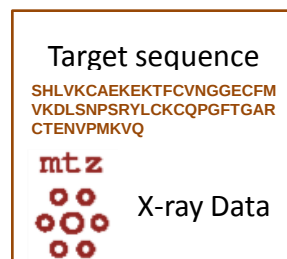
# Molecular Replacement – *Ab initio* search models

## AMPLE (Keegan)

- Generate and prepare *ab initio* or *de novo* models using Rosetta and other software for use as search models in molecular replacement
- Works well for smaller,  $\alpha$ -helical proteins (<120 residues) but has been known to work for larger targets



## AMPLE



## Phaser MR

- Automated Maximum Likelihood method for molecular replacement
  - Twinning
  - Translational NCS
  - $\sigma$ ms refinement



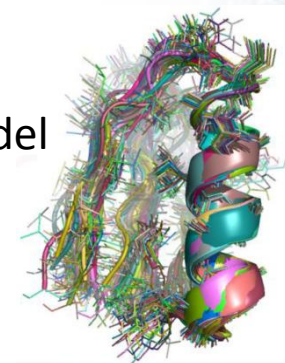
# *Molecular Replacement*

## Sculptor

- Multi-protocol search model preparation
  - weighted sequence similarity
  - accessible surface area

## Ensembler

- Ensemble search model generation tool from the developers of Phaser



# CCP4 Online Webservice



**Collaborative Computational Project  
No. 4**  
**Software for Macromolecular X-Ray  
Crystallography**



Home

**Welcome to CCP4 online**  
[Login](#)  
 Other Options - [Register](#), [Forgotten Password](#), [Change Password](#)  
**Runnable programs**  
 The following programs are available:
 

|        |                                                                                                                                                                                                                                                                                                                           |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Balbes | An automated Molecular Replacement (MR) pipeline - Balbes integrates into one system all the components necessary for solving a crystal structure by Molecular Replacement                                                                                                                                                |
| MrBUMP | An automated Molecular Replacement (MR) pipeline - Given a target sequence and experimental structure factors, it will search for homologous structures, create a set of suitable search models from the template structures, do molecular replacement, and test the solutions with some rounds of restrained refinement. |
| Zanuda | Space group and crystallographic origin validation                                                                                                                                                                                                                                                                        |
| jsPISA | Calculation and analysis of macromolecular surfaces and interfaces                                                                                                                                                                                                                                                        |


CCP4 STFC Rutherford Appleton Laboratory, Harwell Campus  
 Didcot Oxon OX11 0FA  
 Tel: (+44) 1235 567726 | Fax: +44 (+44) 1235 567720 | Email: ccp4@ccp4.ac.uk

About | Legal Statements



**Collaborative Computational Project  
No. 4**  
**Software for Macromolecular X-Ray  
Crystallography**



[Logout](#) > [Login](#) > [Programs](#) > [Balbes](#) > New Balbes Run
 Username: **ccbn63**

**as Run**  
 Data accepted for input are **mtz** and **cif** (structure factors) and **FASTA** (sequence target). **Note:** checking the checkbox will send Balbes's results to the **ARP/wARP** server (it is assumed that you agree to the **ARP/wARP** [license conditions](#))

actors:  no file selected  
 target:  no file selected  
 Instead of entering a Sequence Target file you can paste your **FASTA** sequence below:  
 (Note that a comment line beginning with a ';' character must precede each sequence)

Spacegroup: ☐  
 ARP solution: ☐ Dissemination Level:

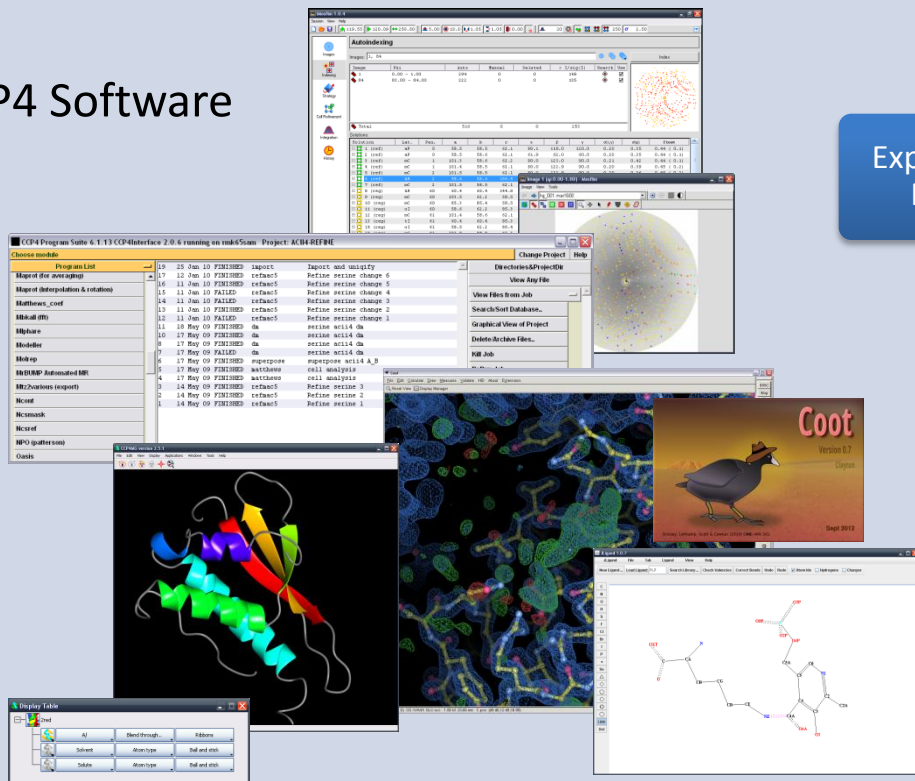
After clicking submit, **PLEASE WAIT** for your files to upload - this may take some time

CCP4 STFC Rutherford Appleton Laboratory, Harwell Campus  
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About | Legal Statements

<http://www.ccp4.ac.uk/ccp4online>  
 or Google for "ccp4online"  
 coming soon CRANK2

# CCP4 Software



Crystallisation

Data Collection

Data Processing  
and Reduction

Experimental  
Phasing

Molecular  
Replacement

Density  
Modification

Model Building

Refinement

Structure  
Analysis

Deposition

PDB\_REDO



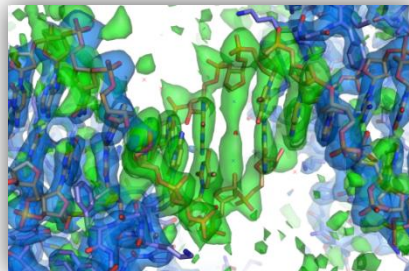
# Model Building And Refinement

## Buccaneer

- Chain tracing by identifying connected alpha-carbon positions using a likelihood-based density target
- Low resolution model building

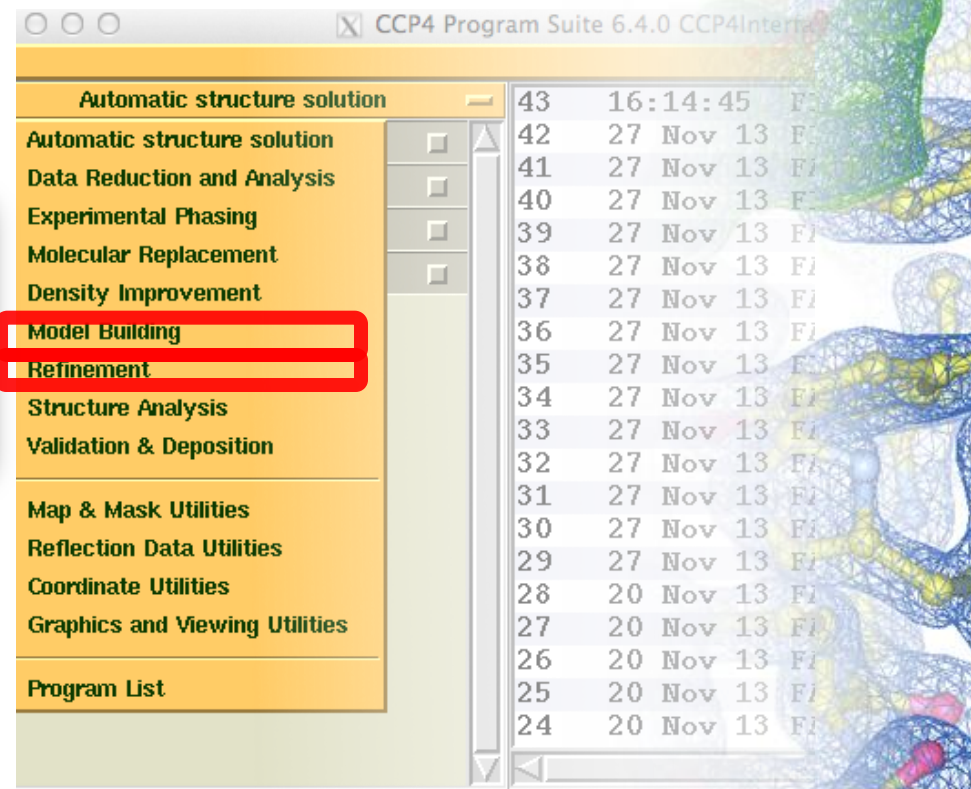
## Nautilus

- Automatic model building of nucleotide structures in electron density maps



## Sloop

- Loop building by finding gaps in the chain and using fragments from the Richardson's Top500 library of structures to fill the gaps

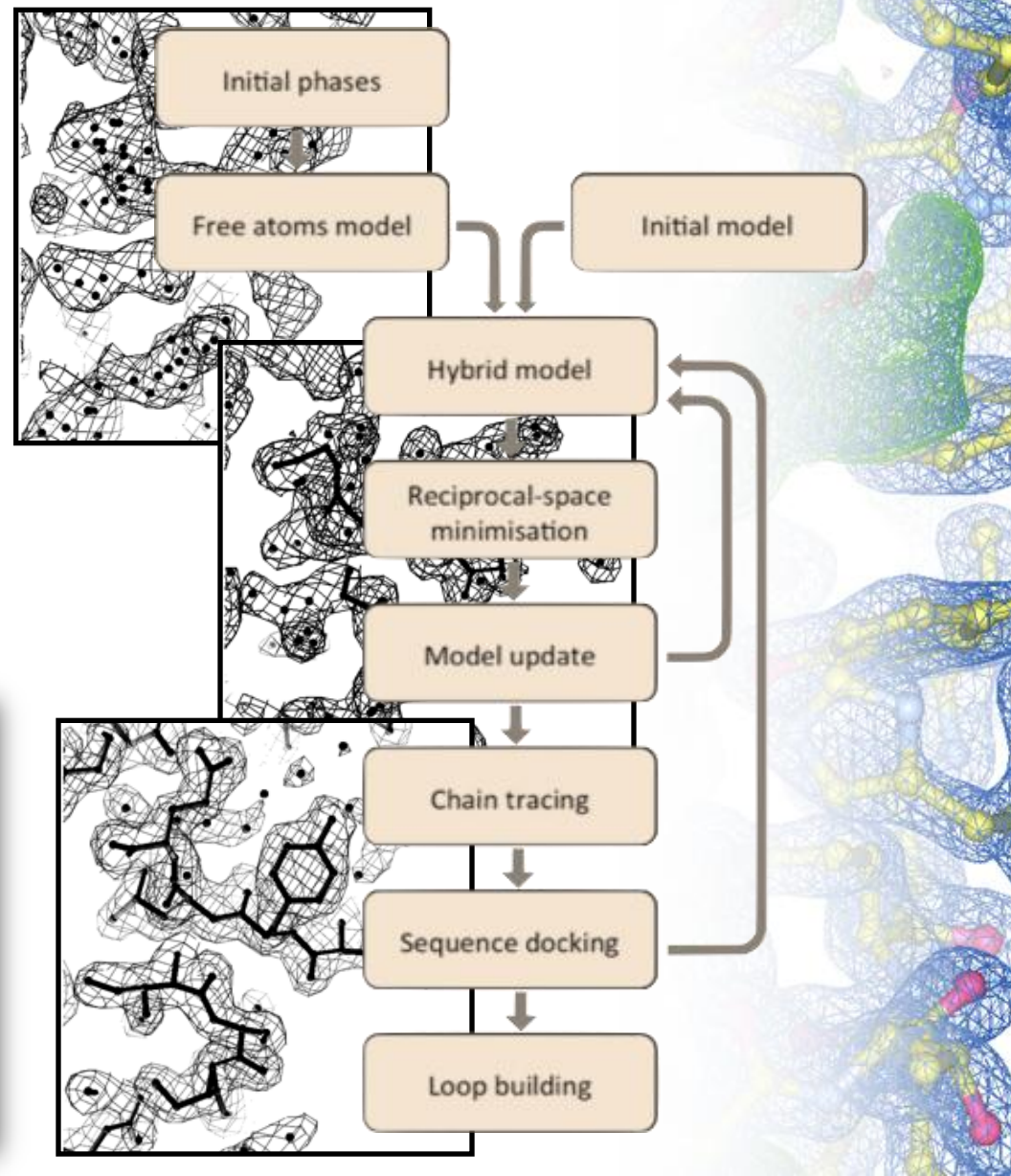
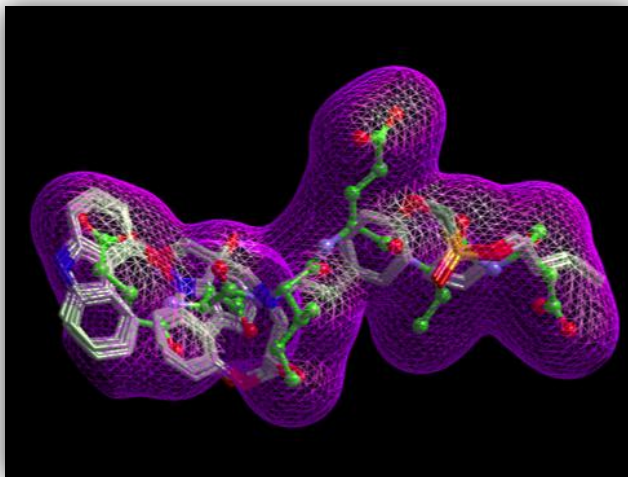


# Model Building

7.4  
ARP  
wARP

## ARP/wARP 7.4

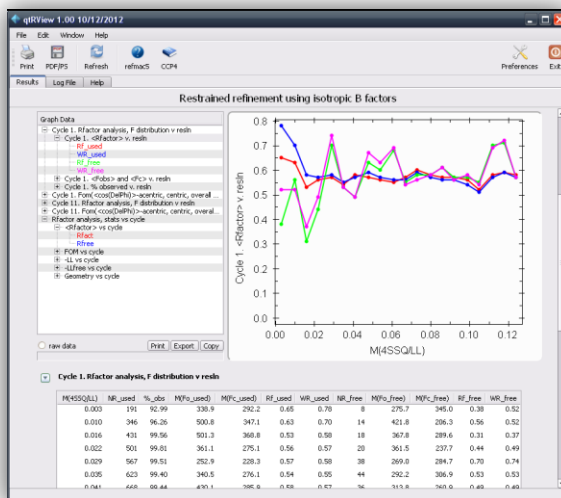
- Automated building of
  - proteins
  - RNA/DNA
  - secondary structure
  - side chains
  - loops
  - solvent
  - ligands
- Now jointly distributed by CCP4



## Refinement

## Refmac 5.8

- Simultaneous density modification and refinement (initially SAD, more general later)
- Better anomalous difference maps
- Multi-imputation and averaging of structure factors to desired resolution
- Sampling of conformational space – Gibbs sampling
- Estimation of individual errors of atoms – less biased electron density calculation

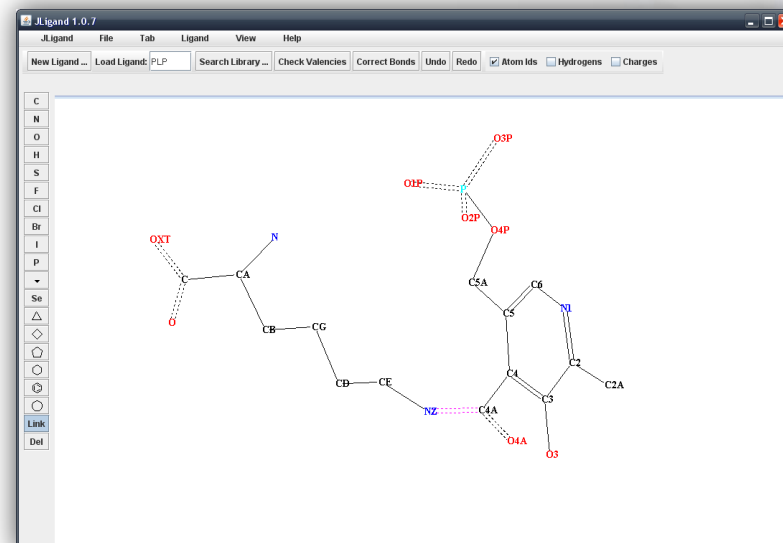


# ProSMART

- Structure alignment
- Generation of external restraints for use in refinement

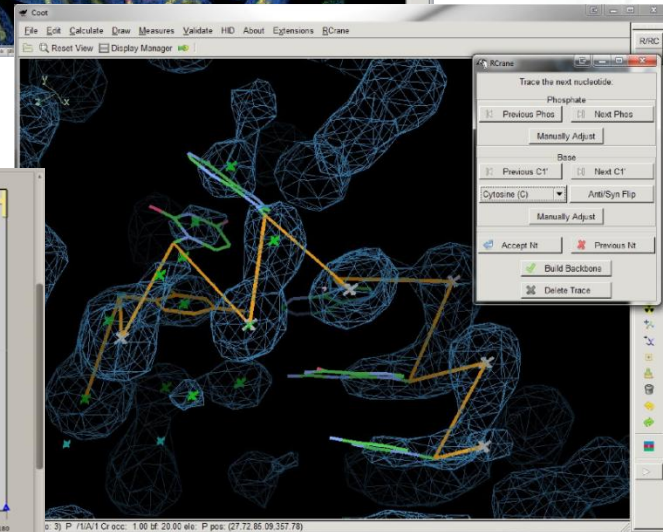
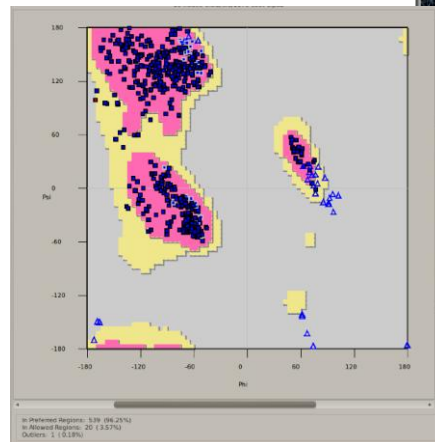
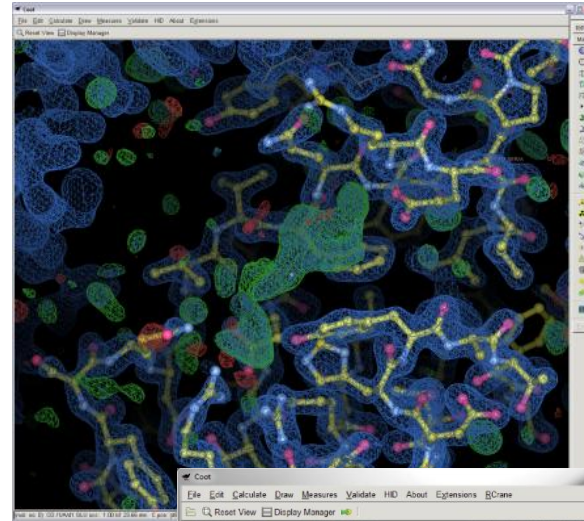
## AceDrg

- Generate restraints for ligands for use in refinement and model building

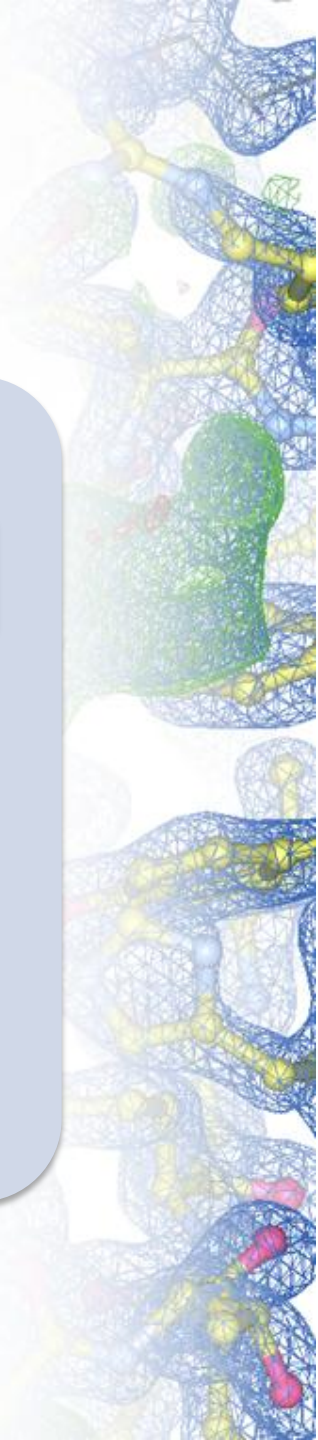
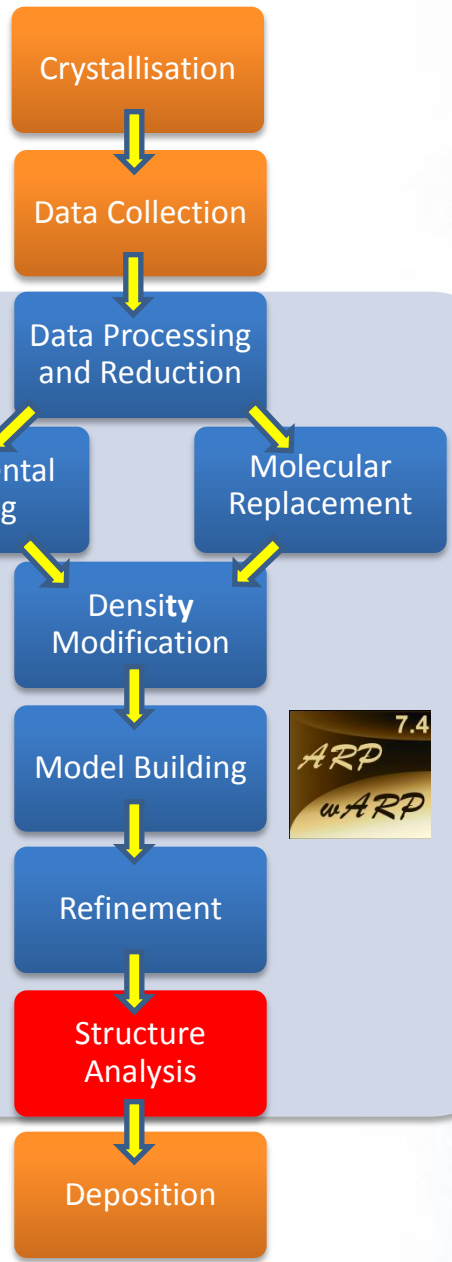
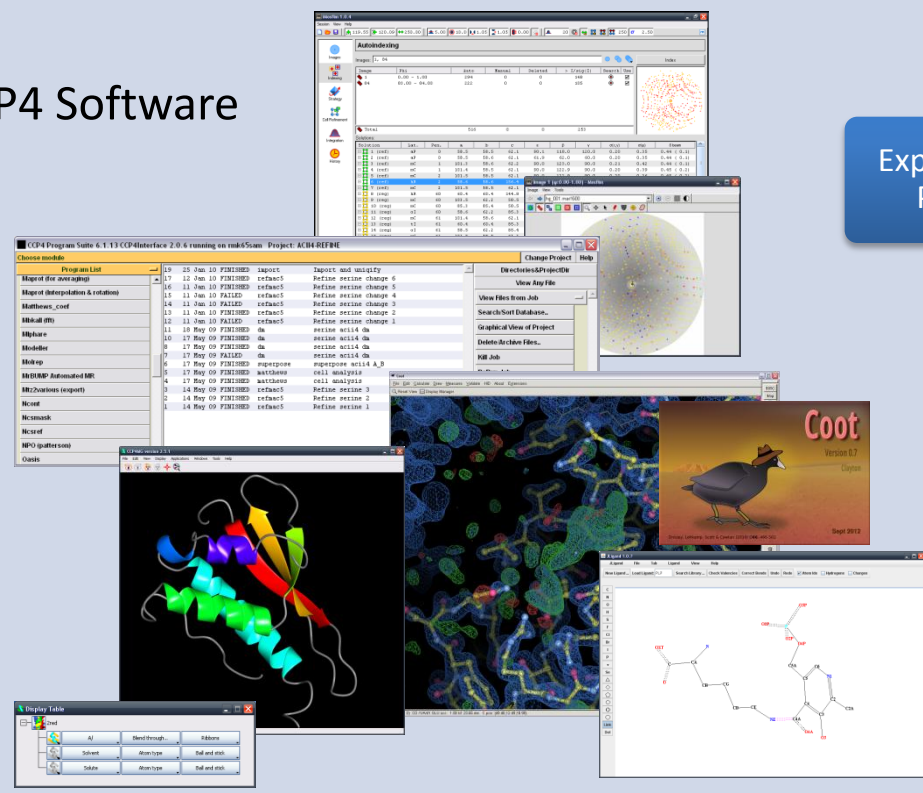


# Manual Model Building - Coot

- **Coot**
  - RCrane and Cootaneer for RNA building
  - Real space refinement
  - EM maps
  - Pyrophen restraints
  - External restraints
    - CCDC mogul
  - Morphing
  - Validation
  - Ligand docking
  - updates



CCP4 Software



## QtPISA

- Automatic inference on multimeric states from crystal packing
- Analysis of macromolecular interfaces and macromolecular interactions

QtPISA 1.12 25/06/2012 [PDB => 1nio]

File View Help

# Structure Analysis

CCP4 Program Suite 6.4.0 CCP4

Automatic structure solution

Automatic structure solution

Data Reduction and Analysis

Experimental Phasing

Molecular Replacement

Density Improvement

Model Building

Refinement

**Structure Analysis**

Validation & Deposition

Map & Mask Utilities

Reflection Data Utilities

Coordinate Utilities

Graphics and Viewing Utilities

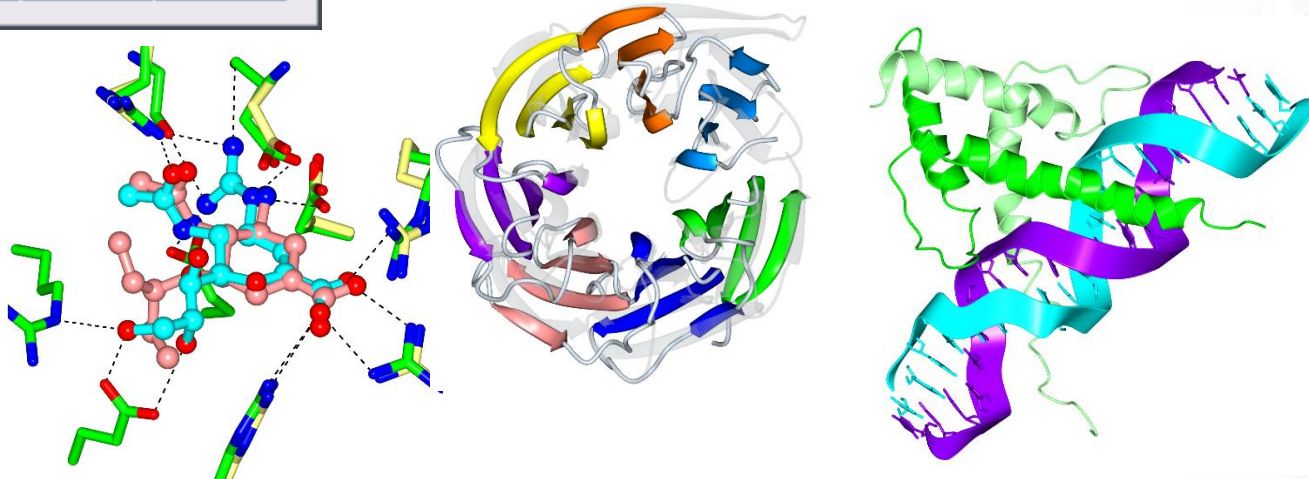
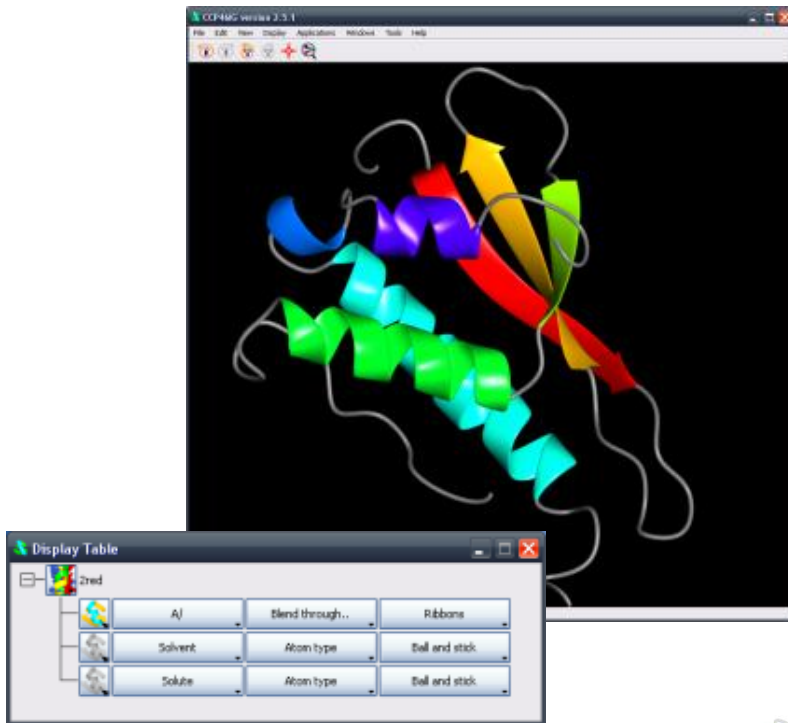
Program List

|    |           |
|----|-----------|
| 43 | 16:14:45  |
| 42 | 27 Nov 13 |
| 41 | 27 Nov 13 |
| 40 | 27 Nov 13 |
| 39 | 27 Nov 13 |
| 38 | 27 Nov 13 |
| 37 | 27 Nov 13 |
| 36 | 27 Nov 13 |
| 35 | 27 Nov 13 |
| 34 | 27 Nov 13 |
| 33 | 27 Nov 13 |
| 32 | 27 Nov 13 |
| 31 | 27 Nov 13 |
| 30 | 27 Nov 13 |
| 29 | 27 Nov 13 |
| 28 | 20 Nov 13 |
| 27 | 20 Nov 13 |
| 26 | 20 Nov 13 |
| 25 | 20 Nov 13 |
| 24 | 20 Nov 13 |

# Structure Analysis

## CCP4MG 2.9.0

- Picture wizard
- Movies
- EM maps
- Sequence viewer
- Prosmart and pisa interfaces
- Biological assemblies
- Normal modes
- Basis for CCP4 GUI2



# Acknowledgements

- **CCP4 Core group:** Andrey Lebedev, Eugene Krissinel, Charles Ballard, David Waterman, Marcin Wojdyr, Ville Uski, Karen McIntyre, Carol Malpass, Martyn Winn
- **LMB/MRC:** Andrew Leslie, Phil Evans, Garib Murshudov, Rob Nicholls, Harry Powell, Owen Johnson, Fei Long, Paul Emsley, Andrea Thorn
- **Phaser Group:** Airlie McCoy, Randy Read, Rob Oeffner, Gabor Bunkoczi
- **YSBL York:** Keith Wilson, Kevin Cowtan, Liz Potterton, Stuart McNicholas, Eleanor Dodson
- **Diamond:** Gwyndaf Evans, Graeme Winter
- **Leiden:** Raj Pannu, Pavol Skubak
- **Others:** Bernhard Lohkamp, Clemens Vonnrhein, Ruslan Sanishvili, Frank Von Delft, Martin Noble, Jaclyn Bibby, Daniel Rigden, Jens Thomas, Alun Ashton, David Brown, Arwen Pearson, Tim Gruene, George Sheldrick and many more..



# Workshop outline

Mornings – lectures on theory and programs

Afternoons/Evenings – tutorials and problem solving

Day 1 – data processing

Day 2 – experimental phasing

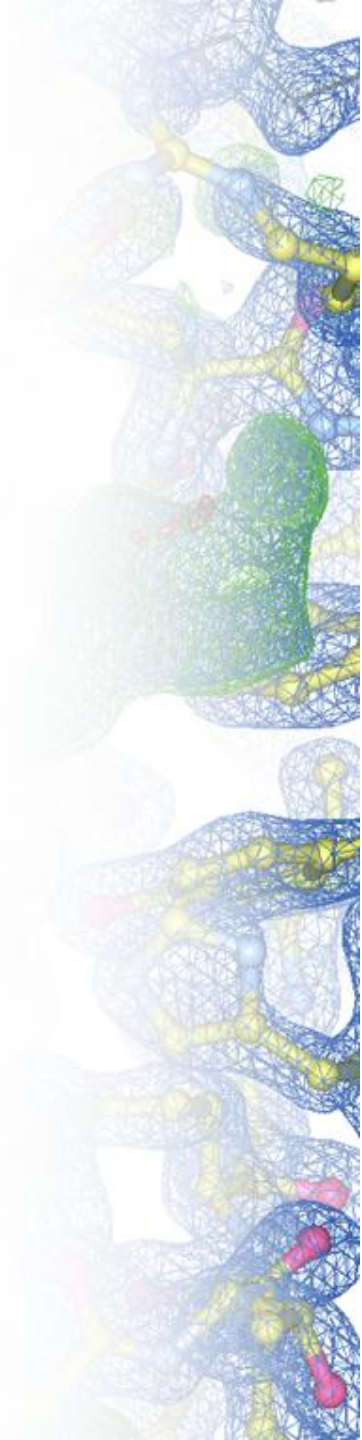
Day 3 – molecular replacement

Day 4 – model building and refinement

Day 5 – ligands, validation

If you have your own data, questions problems, don't be shy, grab an expert.

Talks and tutorials, with data should appear on your desktop.



# People



Phil Evans – MRC-LMB, UK.  
-Data scaling. Aimless, pointless etc

Garib Murshudov – MRC-LMB, UK.  
-refinement, maximum likelihood. Refmac, BALBES



Raj Pannu – Leiden, NL.  
-experimental phasing. CRANK2, bp3

Bernhard Lohkamp – Karolinska Institutet, SW.  
- winCOOT, Glasgow Rangers fan.



# More People



Eugene Krissinel - CCP4, UK.  
PISA, structure analysis.



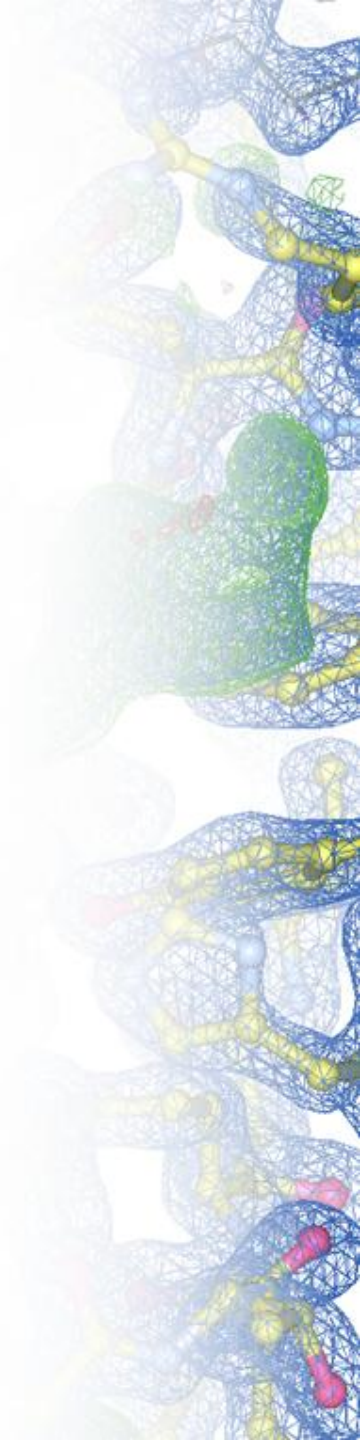
Andrey Lebedev - CCP4, UK.  
ZANUDA, Molecular replacement



Andrea Thorn – MRC-LMB, UK.  
Experimental phasing



Joana Pereria – EMBL, DE.  
ARP/wARP



# Command line – terminal.app

File commands:

**ls** - directory listing

**cd <dir>** - change to <dir>

**cd** - goto home

**pwd** - show current dictory

**mkdir <name>** - make directory <name>

**rm <file>** - remove <file>

**rm -r <dir>** - recursively remove <dir>

**rm -rf \*** - delete everything

**cp <f1> <f2>** - copy file <f1> to <f2>

**cp -r <d1> <d2>** - copy dir <d1> to <d2>

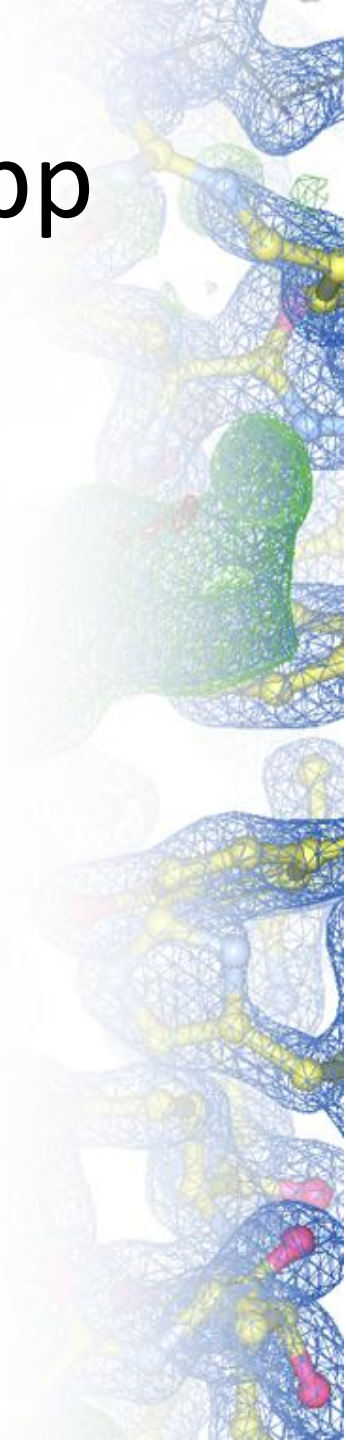
**mv <f1> <f2>** - move file or directory <f1> to <f2>

Compression commands:

**tar -xf tmp.tar <file>** - extract <file> from tmp.tar

**tar -xzf tmp.tar.gz** – extract everything from gzipped tar

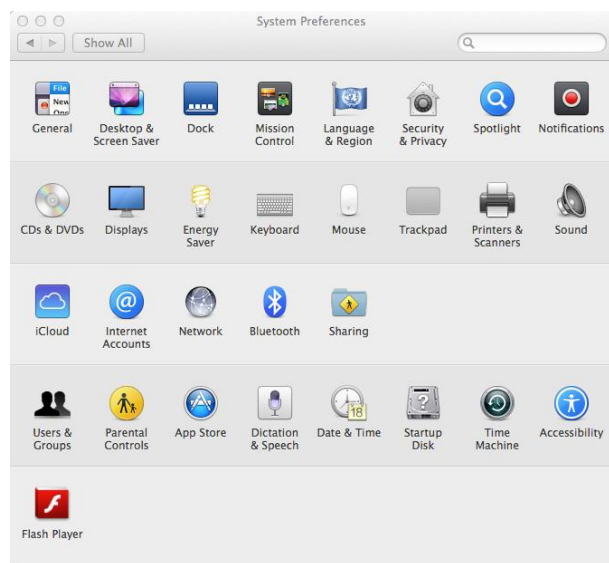
**tar -czf tmp.tar.gz <file>** - create tar archive from <File>



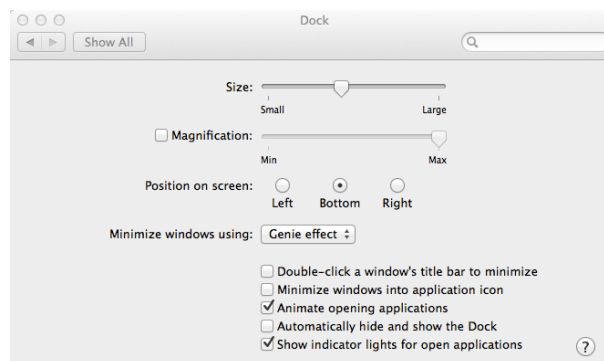
# OS X – the dock



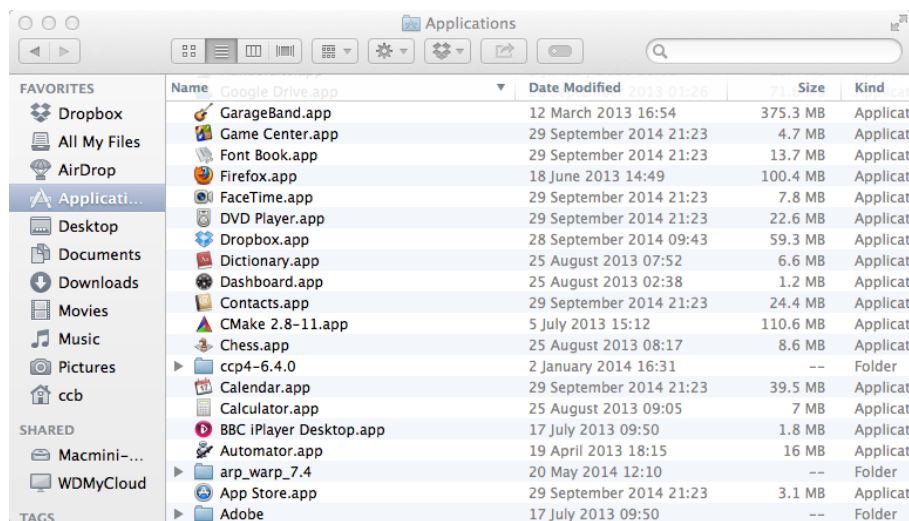
Dock – shows running mac applications, open documents, launches applications. Can add applications to dock by dragging and dropping.



System Preferences – on the dock, control look and feel. eg. Move the dock using Dock (see bottom of imosflm window)



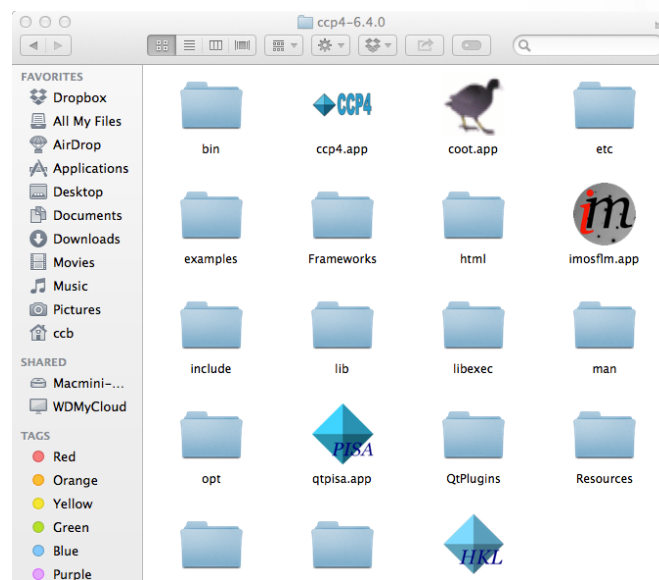
# OS X – the finder



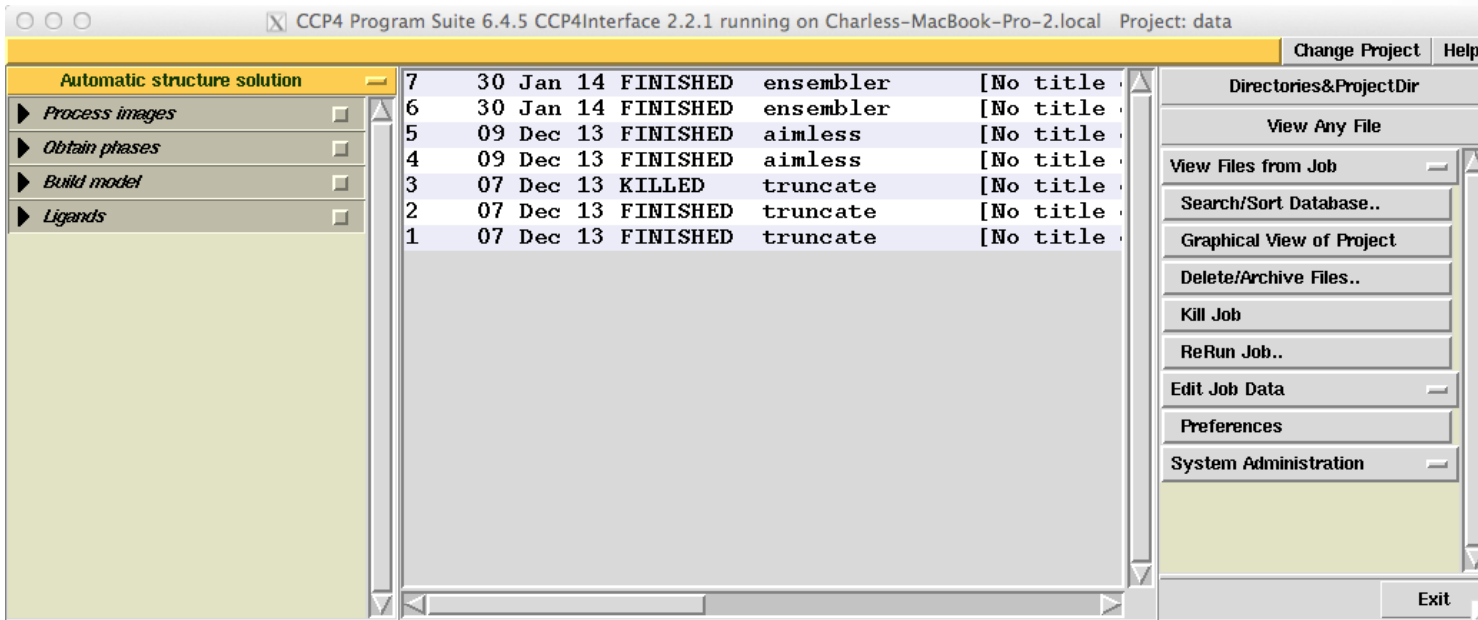
Finder – default environment on mac. Finder accessible from dock. Finder window launched via toolbar File -> New Finder Window. Here applications, showing ccp4-6.4.0. Terminal.app under utilities (why???)

View of the ccp4-6.4.0 in the Finder window, icon view.

Launch CCP4 by double clicking on ccp4.app.

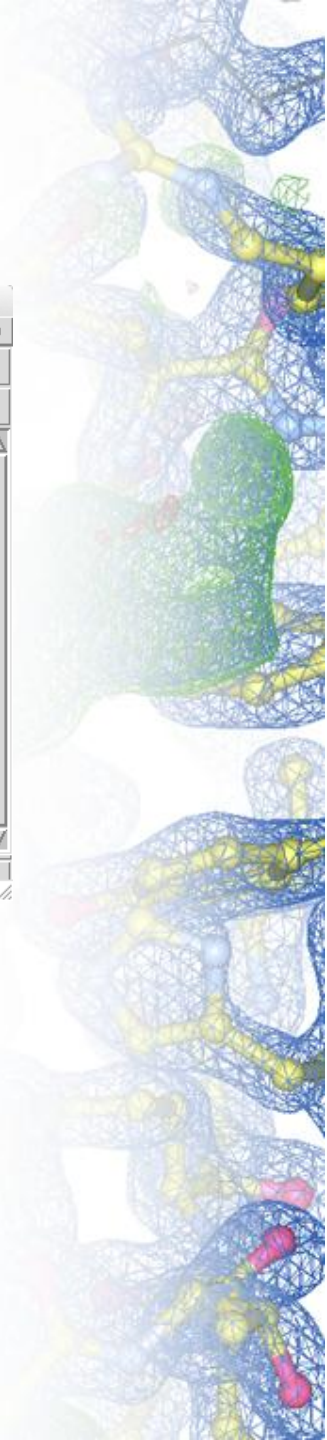


# Launch ccp4i

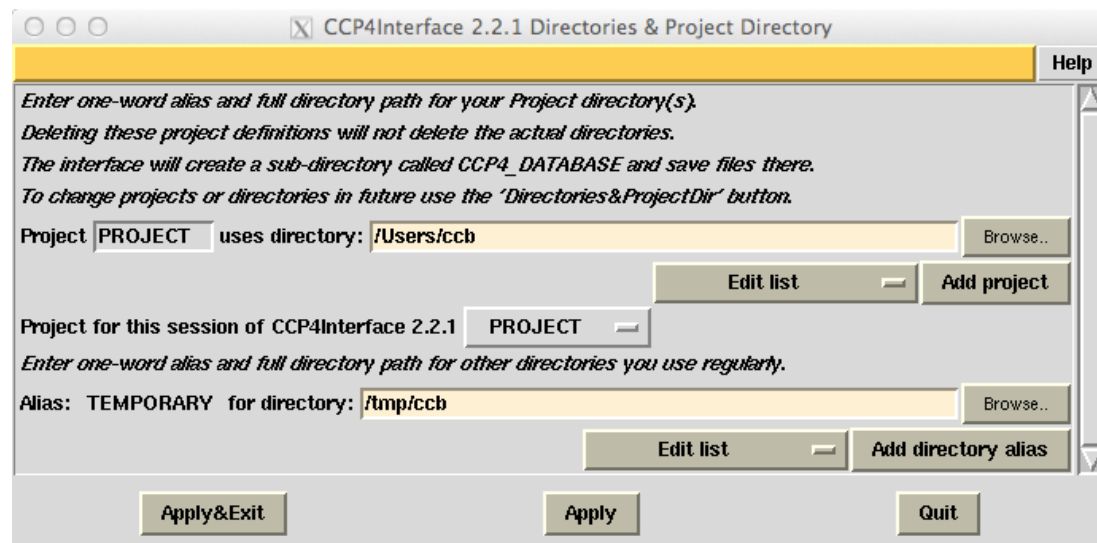


```
Charless-MacBook-Pro-2:~ ccb$ source /Applications/ccp4-6.4.0/bin/ccp4.setup-sh
Charless-MacBook-Pro-2:~ ccb$ ccp4i
Top level CCP4 directory is /Applications/ccp4-6.4.0
Using CCP4 programs from /Applications/ccp4-6.4.0/bin
```

Linux style command line  
launch of ccp4i from the  
folder /Applications/ccp4-  
6.4.0/bin

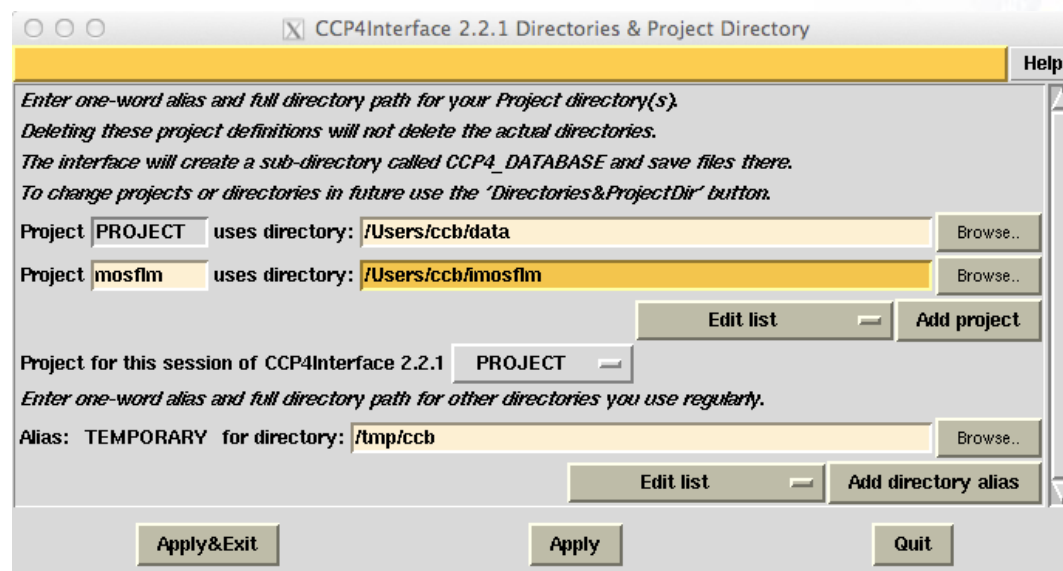


# Set project



You must set a project. At first launch ccp4i will set a default project in your home directory, don't Apply as that way lies madness – a cluttered home directory.

Set a sensible project. If the directory does not exist it will be created. First layer of organisation in ccp4i.



# Ccp4i - tour

CCP4 Program Suite 6.4.5 CCP4Interface 2.2.1 running on Charless-MacBook-Pro-2.local Project: data

Change Project Help

**Automatic structure solution**

- Process images ☐
- Obtain phases ☐
- Build model ☐
- Ligands ☐

|   |           |          |           |            |
|---|-----------|----------|-----------|------------|
| 7 | 30 Jan 14 | FINISHED | ensampler | [No title] |
| 6 | 30 Jan 14 | FINISHED | ensampler | [No title] |
| 5 | 09 Dec 13 | FINISHED | aimless   | [No title] |
| 4 | 09 Dec 13 | FINISHED | aimless   | [No title] |
| 3 | 07 Dec 13 | KILLED   | truncate  | [No title] |
| 2 | 07 Dec 13 | FINISHED | truncate  | [No title] |
| 1 | 07 Dec 13 | FINISHED | truncate  | [No title] |

Directories&ProjectDir

View Any File

View Files from Job

Search/Sort Database..

Graphical View of Project

Delete/Archive Files..

Kill Job

ReRun Job..

Edit Job Data

Preferences

System Administration

Exit

Automatic structure solution

Data Reduction and Analysis

Experimental Phasing

Molecular Replacement

Density Improvement

Model Building

Refinement

Structure Analysis

Validation & Deposition

Map & Mask Utilities

Reflection Data Utilities

Coordinate Utilities

Graphics and Viewing Utilities

Program List

Modules list and task list (Automatic structure solution)

- Arranges jobs by type
- Task list to launch jobs

# Anatomy of a Task interface

Chain tracing/refinement using Buccaneer/Refmac

Job title

Perform model building/reginement starting from  phases.

Data for (unsolved) work structure: (Note: perform phase improvement/density modification first)

☐ Specify an initial model to be extended.

Work SEQ in

Work MTZ in

FP

HLA

HLC

Free R flag

Use Free-R flag: ☒ Use map coefficients: ☐ Use PHI/FOM instead of HL coefficients: ☐

Work PDB out

Options ☒

☒ Apply anisotropy correction to input data.

☐ Build Selenomethionine (MSE instead of MET).

Calculation options:

Number of cycles of building/refinement to run:

☐ Use  CPUs for calculation. ☒ Use fastest methods.

Model building parameters ☐

Refinement parameters ☐

Advanced ☐

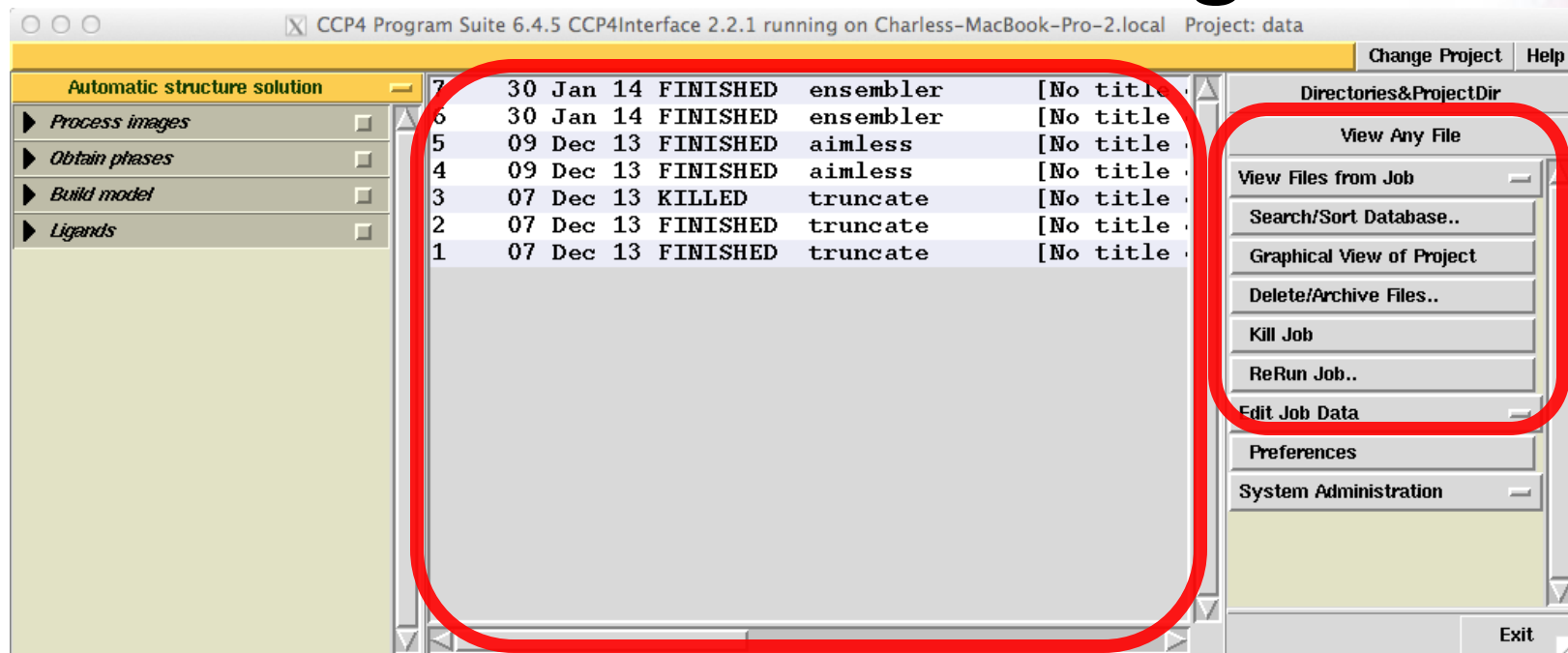
Use title input to track what you have done.

Job type selection at top.

Compulsary input in yellow.

Run menu to launch job, or view input.

# Jobs list and viewing files



The screenshot shows the CCP4 Program Suite 6.4.5 CCP4Interface 2.2.1 running on Charless-MacBook-Pro-2.local. The interface includes a sidebar with a tree view of the project structure, a central Jobs list, and a right-hand menu.

**Jobs List:**

| Job ID | Date      | Status   | Program   | Title      |
|--------|-----------|----------|-----------|------------|
| 7      | 30 Jan 14 | FINISHED | ensampler | [No title] |
| 6      | 30 Jan 14 | FINISHED | ensampler | [No title] |
| 5      | 09 Dec 13 | FINISHED | aimless   | [No title] |
| 4      | 09 Dec 13 | FINISHED | aimless   | [No title] |
| 3      | 07 Dec 13 | KILLED   | truncate  | [No title] |
| 2      | 07 Dec 13 | FINISHED | truncate  | [No title] |
| 1      | 07 Dec 13 | FINISHED | truncate  | [No title] |

**View Any File Menu:**

- View Files from Job
- Search/Sort Database..
- Graphical View of Project
- Delete/Archive Files..
- Kill Job
- ReRun Job..
- Edit Job Data
- Preferences
- System Administration

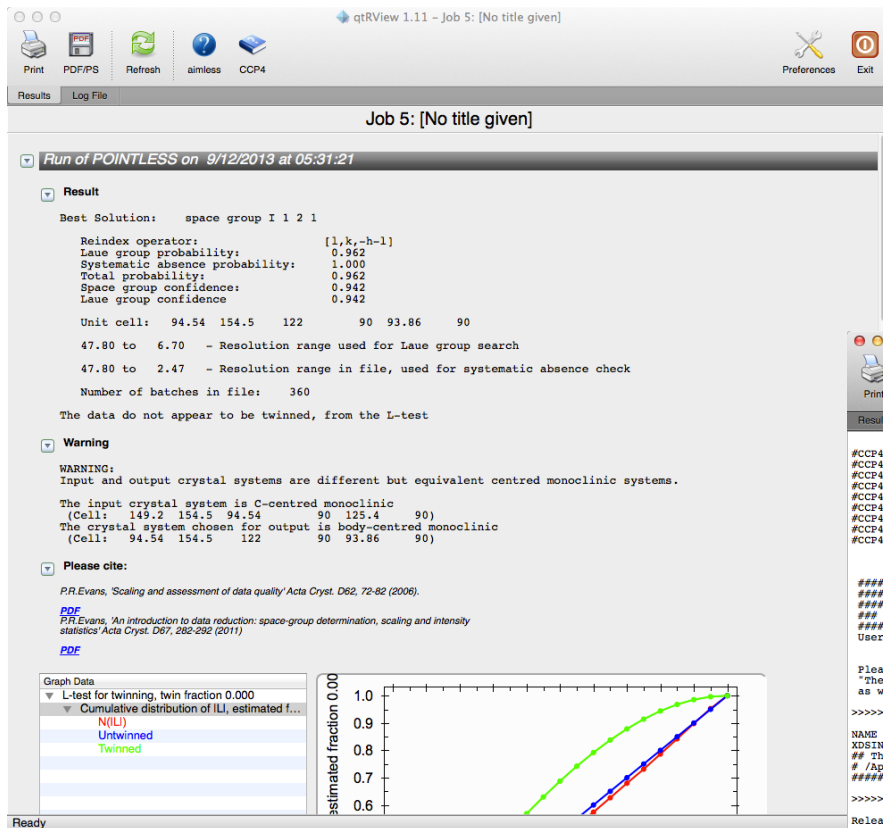
Jobs list is a chronological list of tasks run for a project.

Without titles, this is not very instructive.

“Edit Job Data” leads to a notebook (not used to the best of my knowledge).

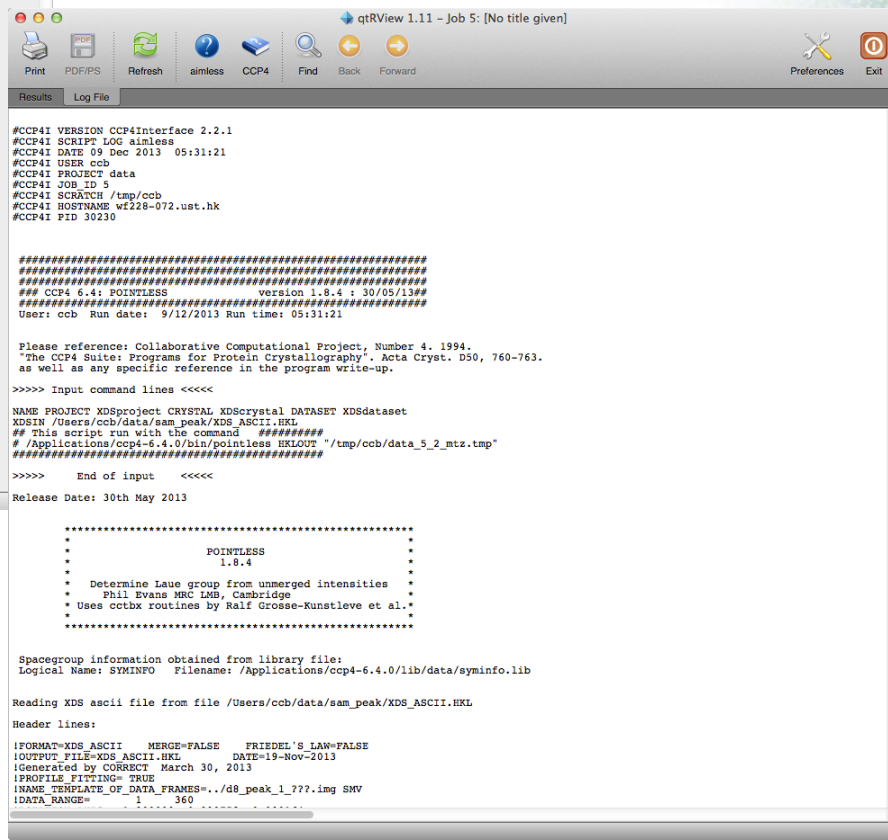
“View Any File”, “View Files from Job” or double-click on Jobs list gives access to output. For refinement (refmac) and model building should be able to view in coot.

# File output

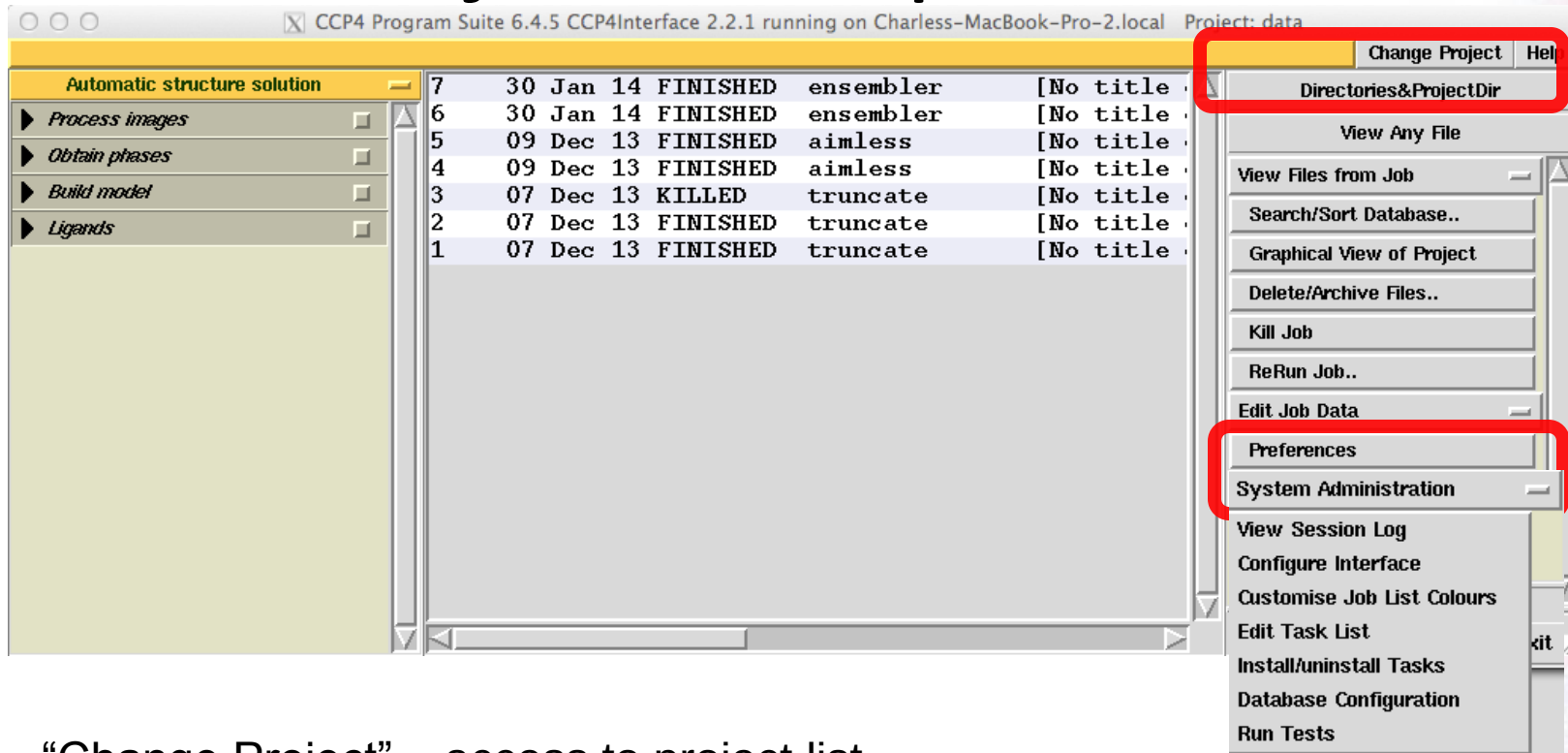


Same job in plain version.

ccp4i logfile view (qtrview), parsed version that includes loggraph output – L-test from aimless



# Projects and preferences



CCP4 Program Suite 6.4.5 CCP4Interface 2.2.1 running on Charless-MacBook-Pro-2.local Project: data

| 7 | 30 | Jan | 14 | FINISHED | ensampler | [No title] |
|---|----|-----|----|----------|-----------|------------|
| 6 | 30 | Jan | 14 | FINISHED | ensampler | [No title] |
| 5 | 09 | Dec | 13 | FINISHED | aimless   | [No title] |
| 4 | 09 | Dec | 13 | FINISHED | aimless   | [No title] |
| 3 | 07 | Dec | 13 | KILLED   | truncate  | [No title] |
| 2 | 07 | Dec | 13 | FINISHED | truncate  | [No title] |
| 1 | 07 | Dec | 13 | FINISHED | truncate  | [No title] |

Automatic structure solution

- Process images
- Obtain phases
- Build model
- Ligands

Change Project Help

Directories&ProjectDir

View Any File

View Files from Job

Search/Sort Database..

Graphical View of Project

Delete/Archive Files..

Kill Job

ReRun Job..

Edit Job Data

Preferences

System Administration

View Session Log

Configure Interface

Customise Job List Colours

Edit Task List

Install/uninstall Tasks

Database Configuration

Run Tests

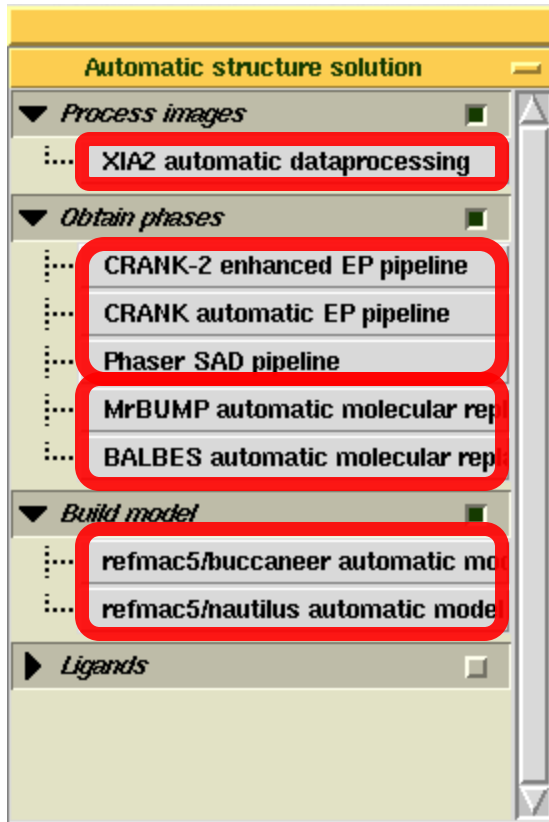
“Change Project” – access to project list

“Directories&ProjectDir” – add new projects (as on first launch)

“Preferences” – control interface behaviour

“System Administration” -> “Configure Interface” controls default programs

# Simple structure solution



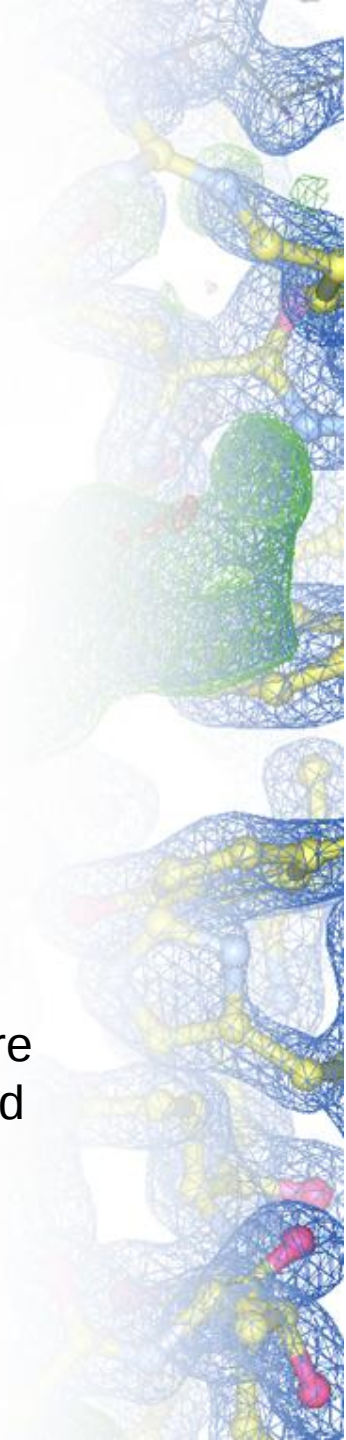
Process data with XIA2

Experimental phasing and model building.

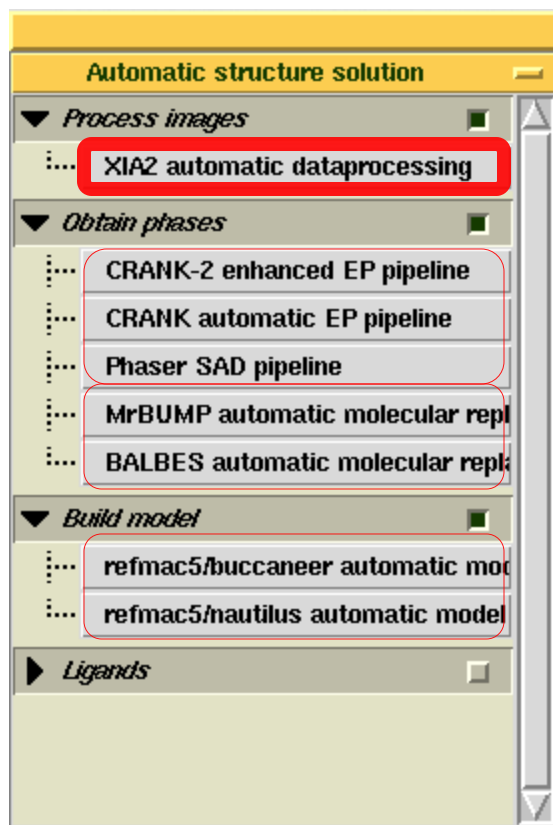
MR with custom database (BALBES) or homology models (MrBUMP), and model building.

Automatic model building and refinement, complete with coot

Also available through modules, where AMPLE and ARP/wARP may be found



# Simple Structure Solution



Use interface to generate xia2 xinfo file.

Process data with XIA2

Experimental phasing and model building

MR with

or hom

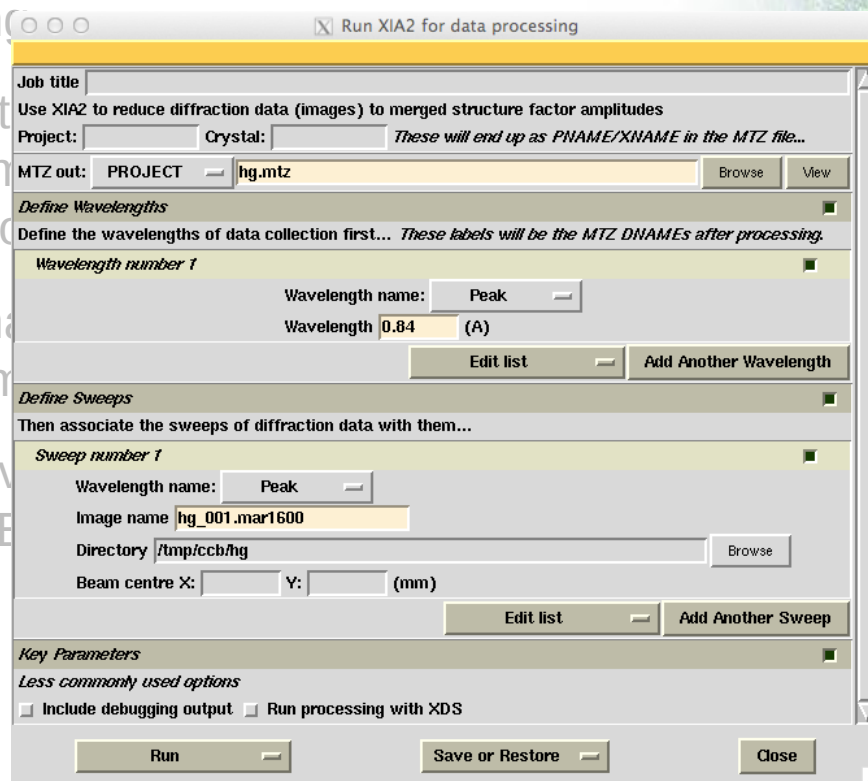
and mo

Autom

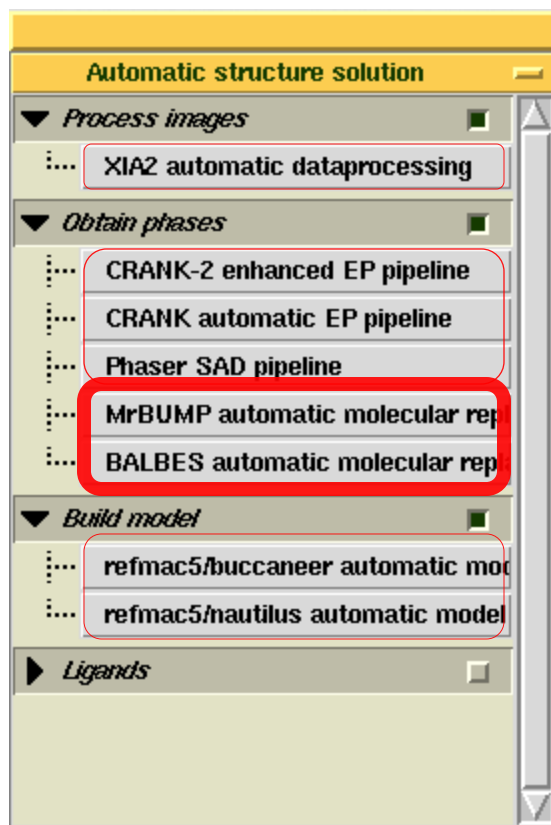
refinem

Also av

AMPLE



# Simple structure solution



Homology model search, MR and model building using MrBUMP

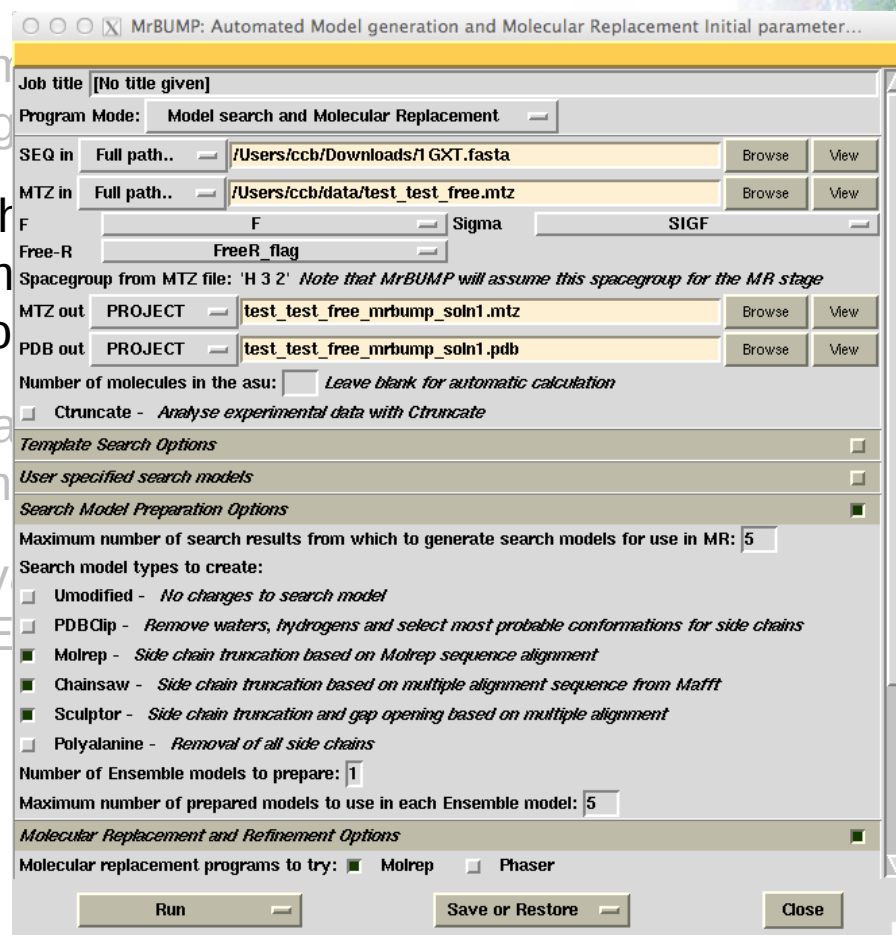
Process data with XIA2

Experiment building

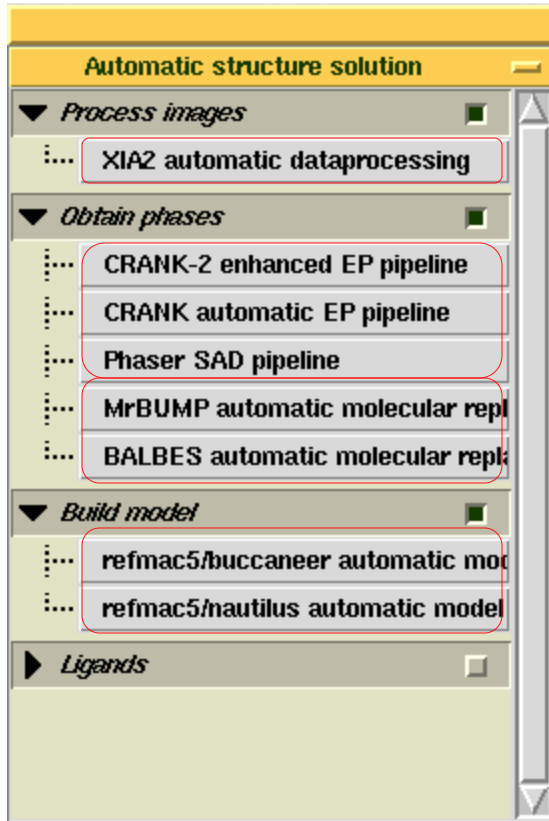
MR with or homology and model building

Automatic refinement

Also available in CCP4



# Simple structure solution



Check solution and rebuild in coot

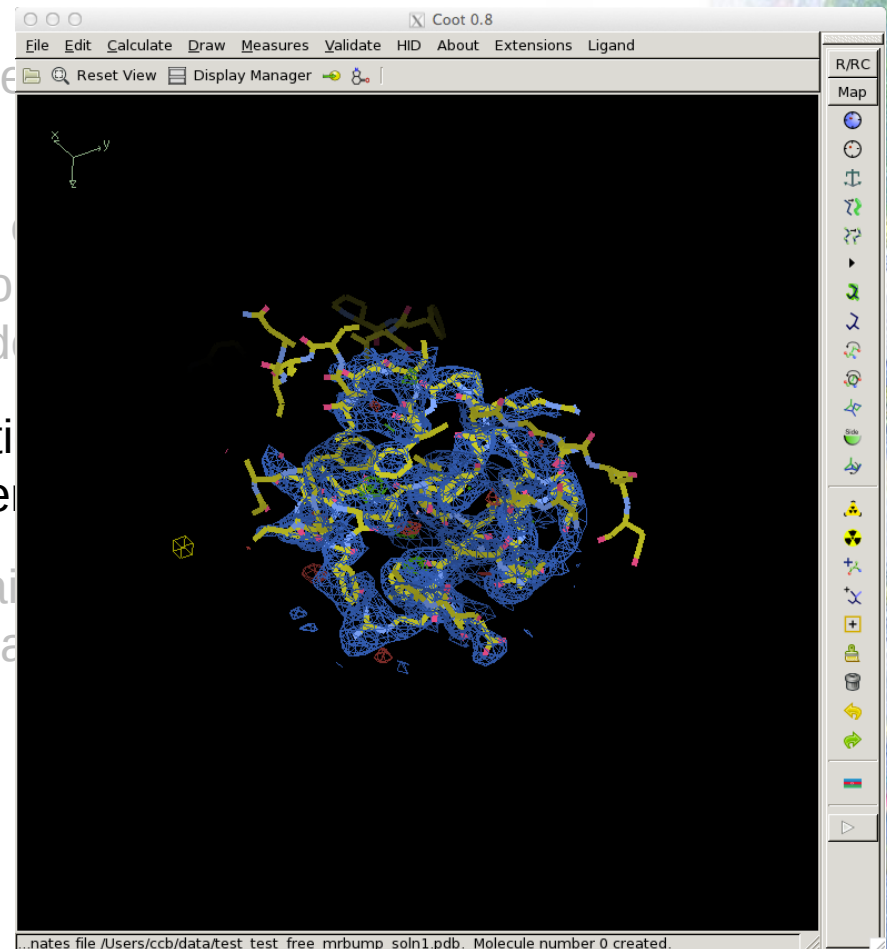
Process data with XIA2

Experimental data  
building.

MR with  
or homo  
and model

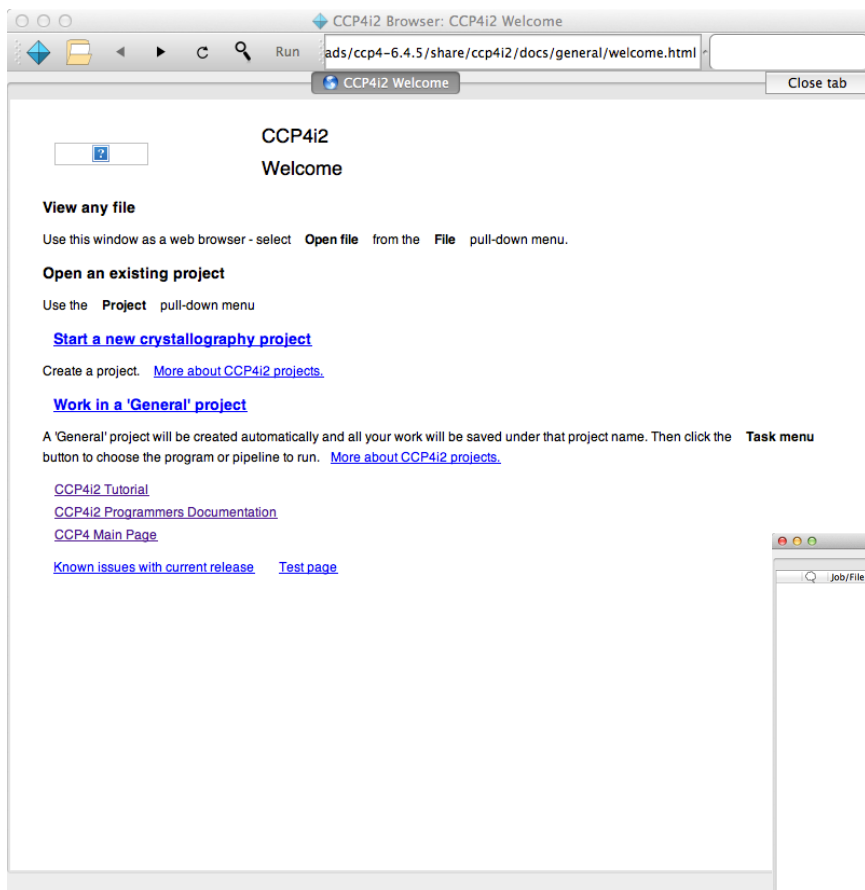
Automatic  
refinement

Also available  
AMPLE and



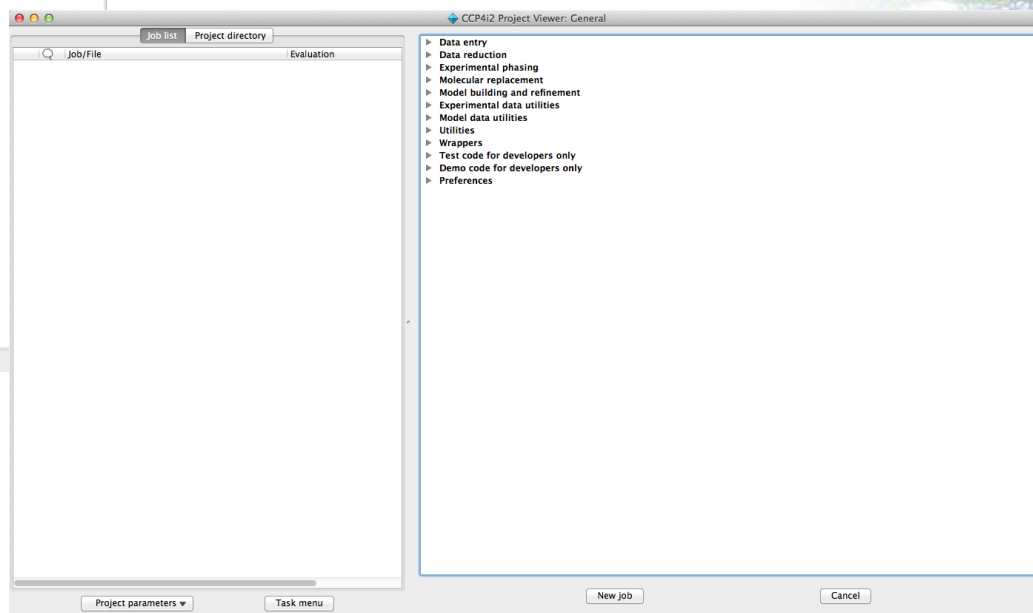


# ccp4i2



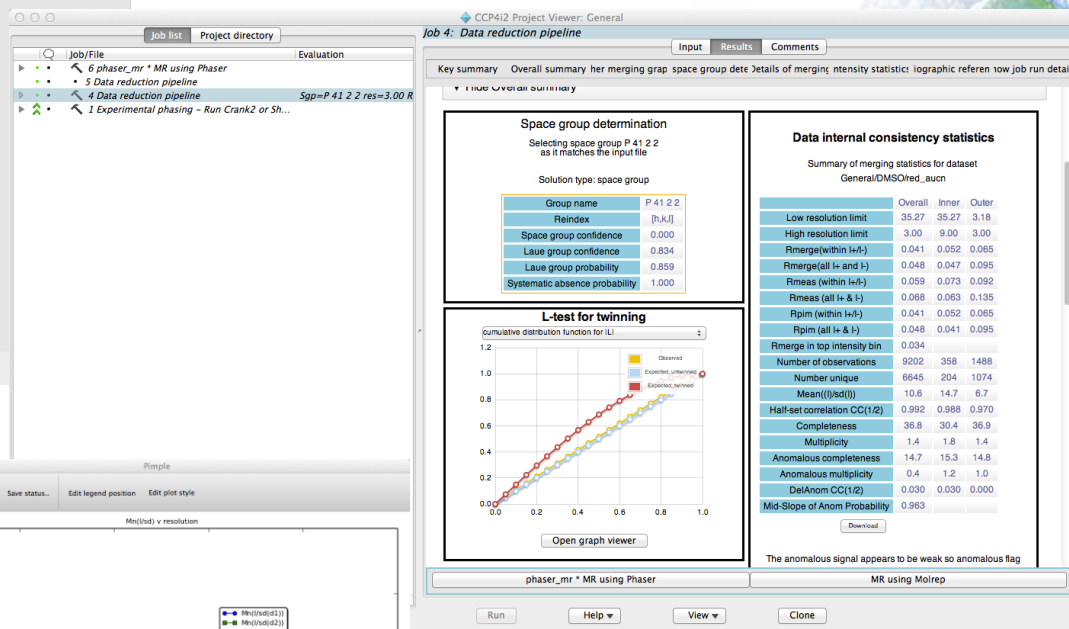
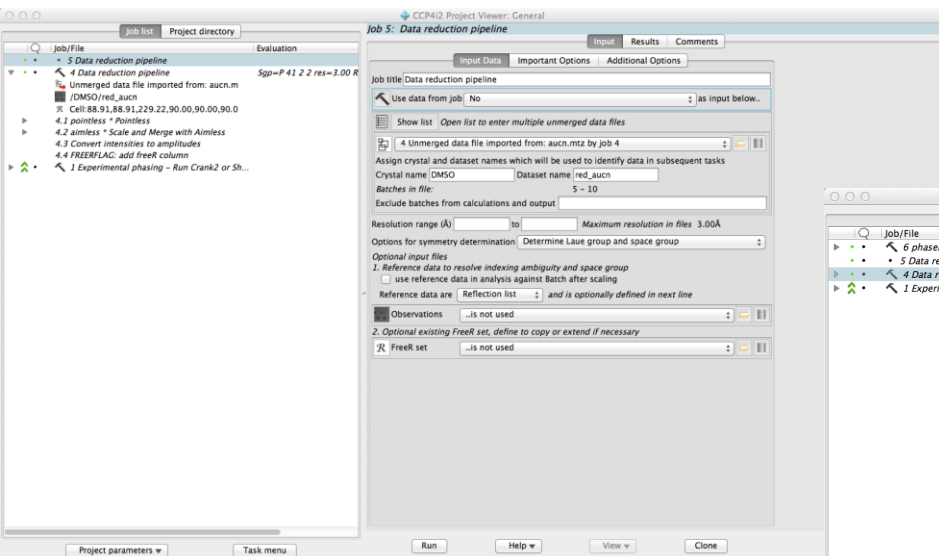
What happens if I double click on the ccp4i2.app icon?

You get the rapidly evolving alpha version of the new CCP4 GUI.



If you have time give it a go.  
Note: it is not fully featured  
and has some tidying to be  
done.

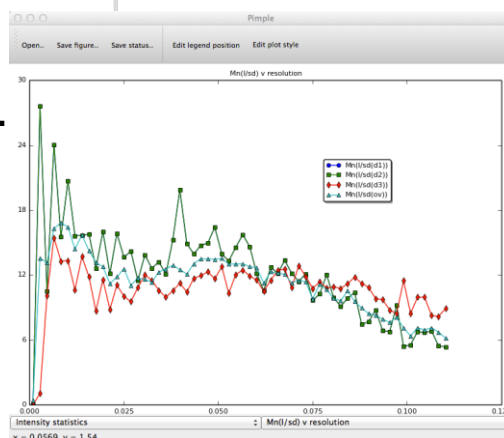
# Unmerged data import



Experimental data scaling and quality checking pipeline.

Table 1 produced.

Suggested next steps.



# Experimental phasing in GUI2

Crank2 experimental interface.

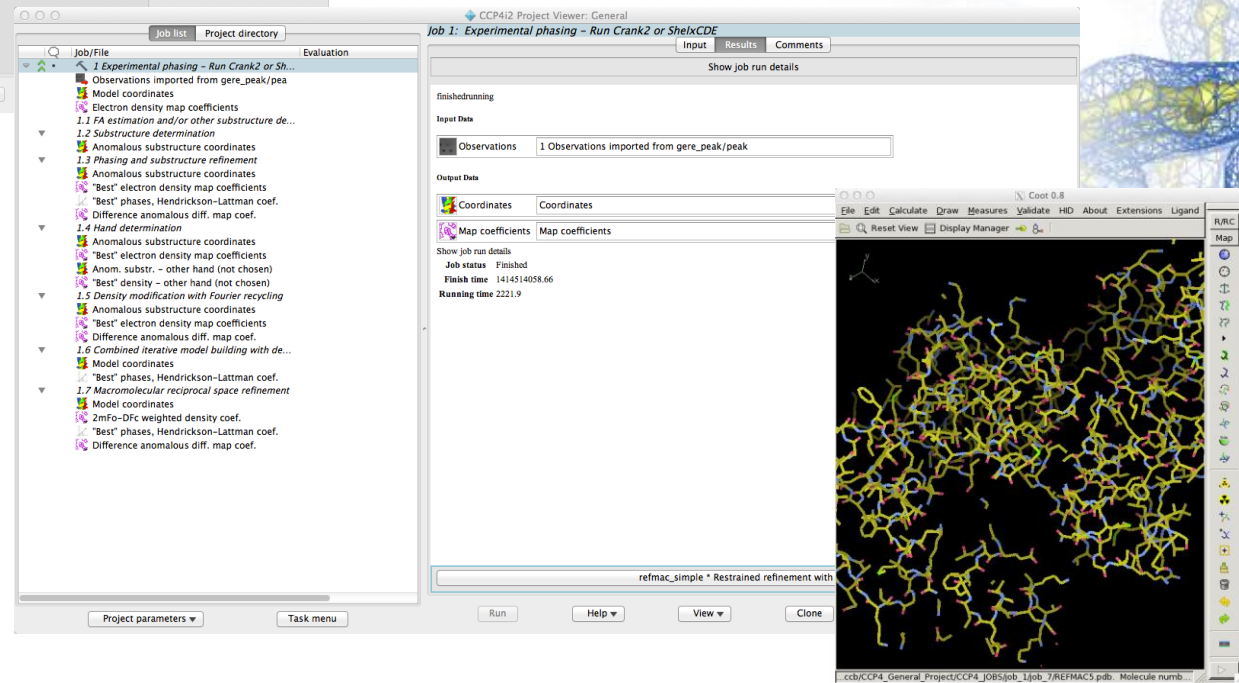
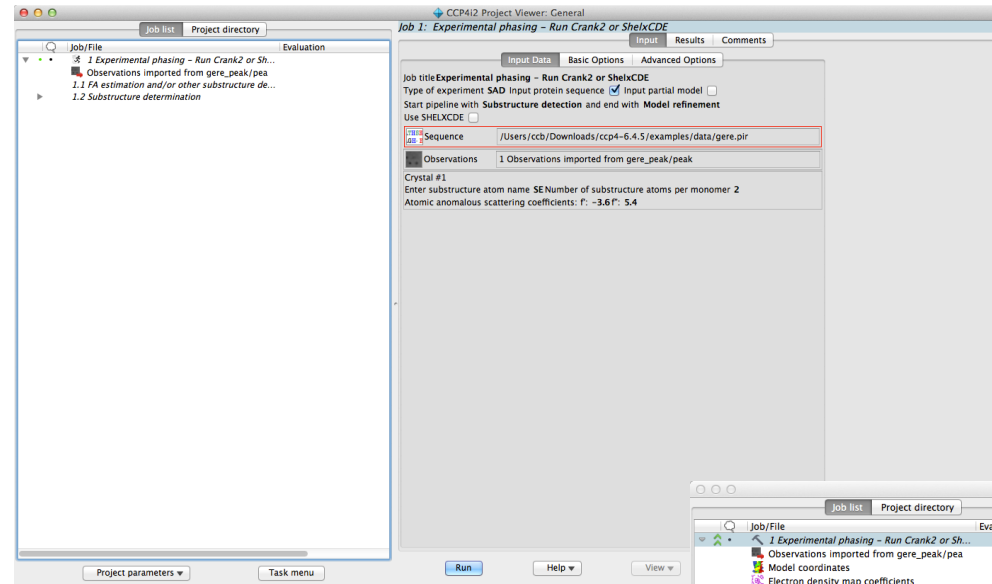
Simplified input.

Simplified output.

Nested subjobs.

Edit result in coot, save  
editing back to GUI2.

More refmac?



# And Finally...

Ask questions. If you don't understand, probably other people do not either.

Otherwise we will start asking you questions...

Above all. Enjoy yourselves !

