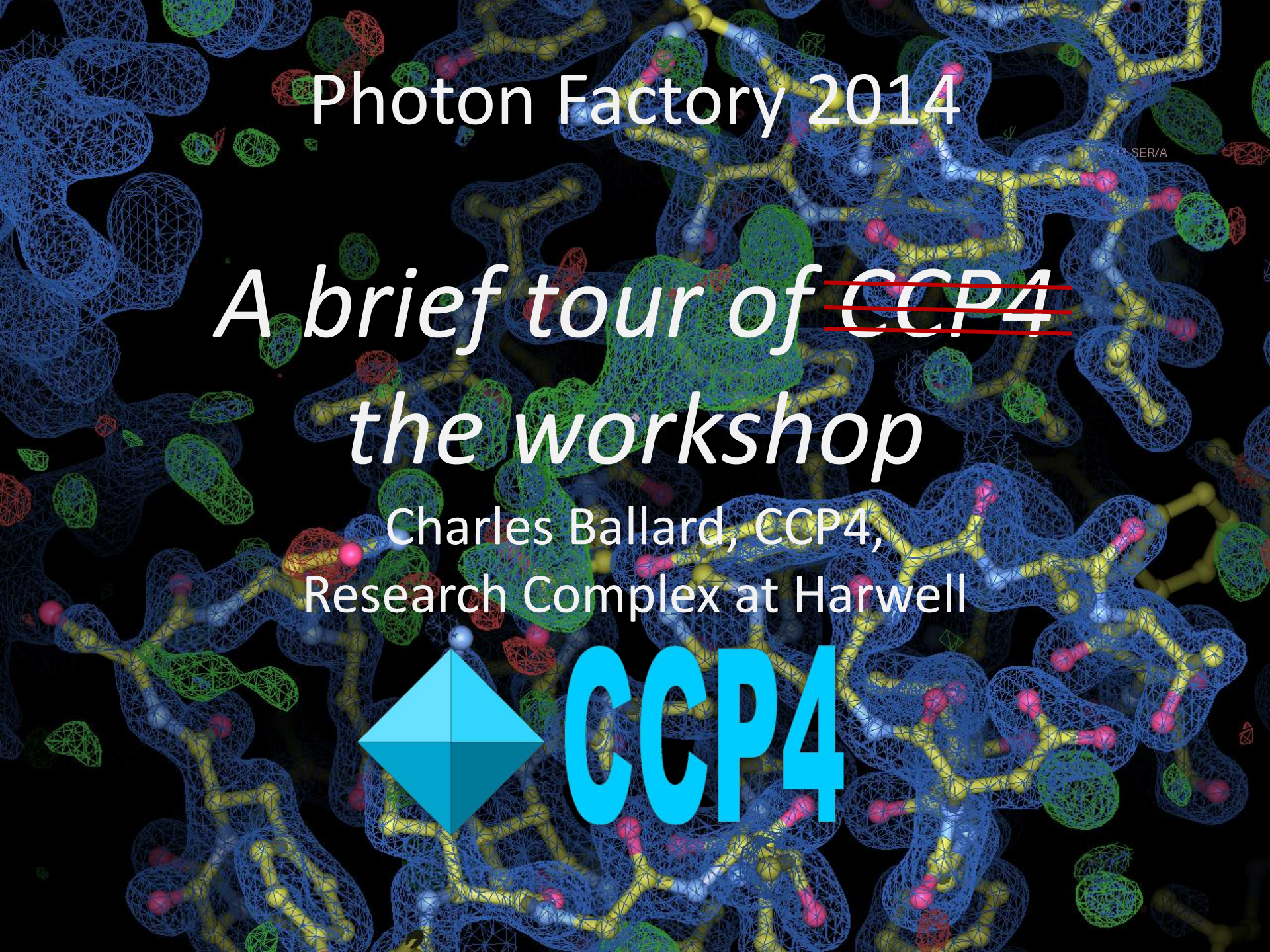


Photon Factory 2014

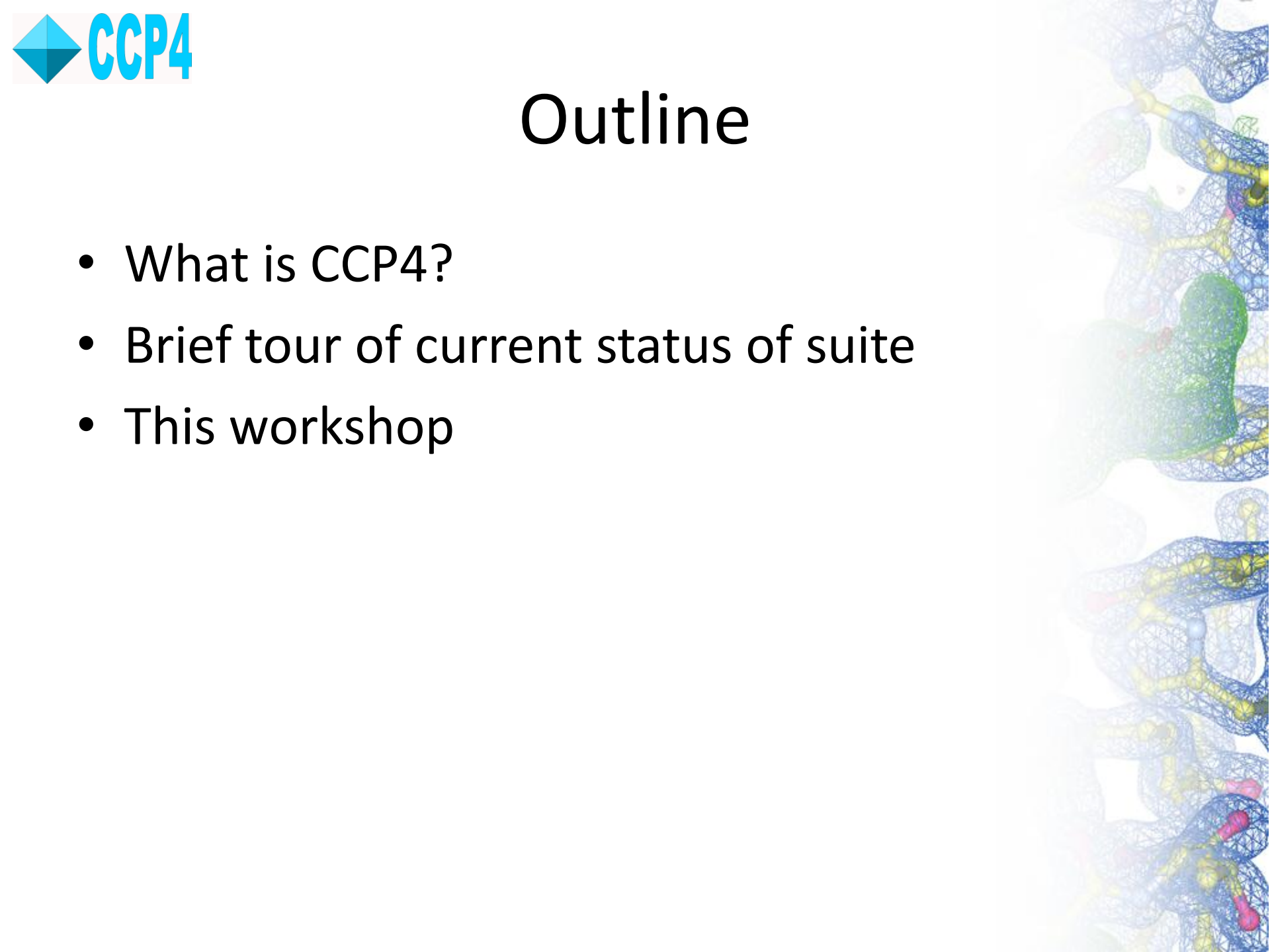
A brief tour of ~~CCP4~~ the workshop

Charles Ballard, CCP4,
Research Complex at Harwell

CCP4

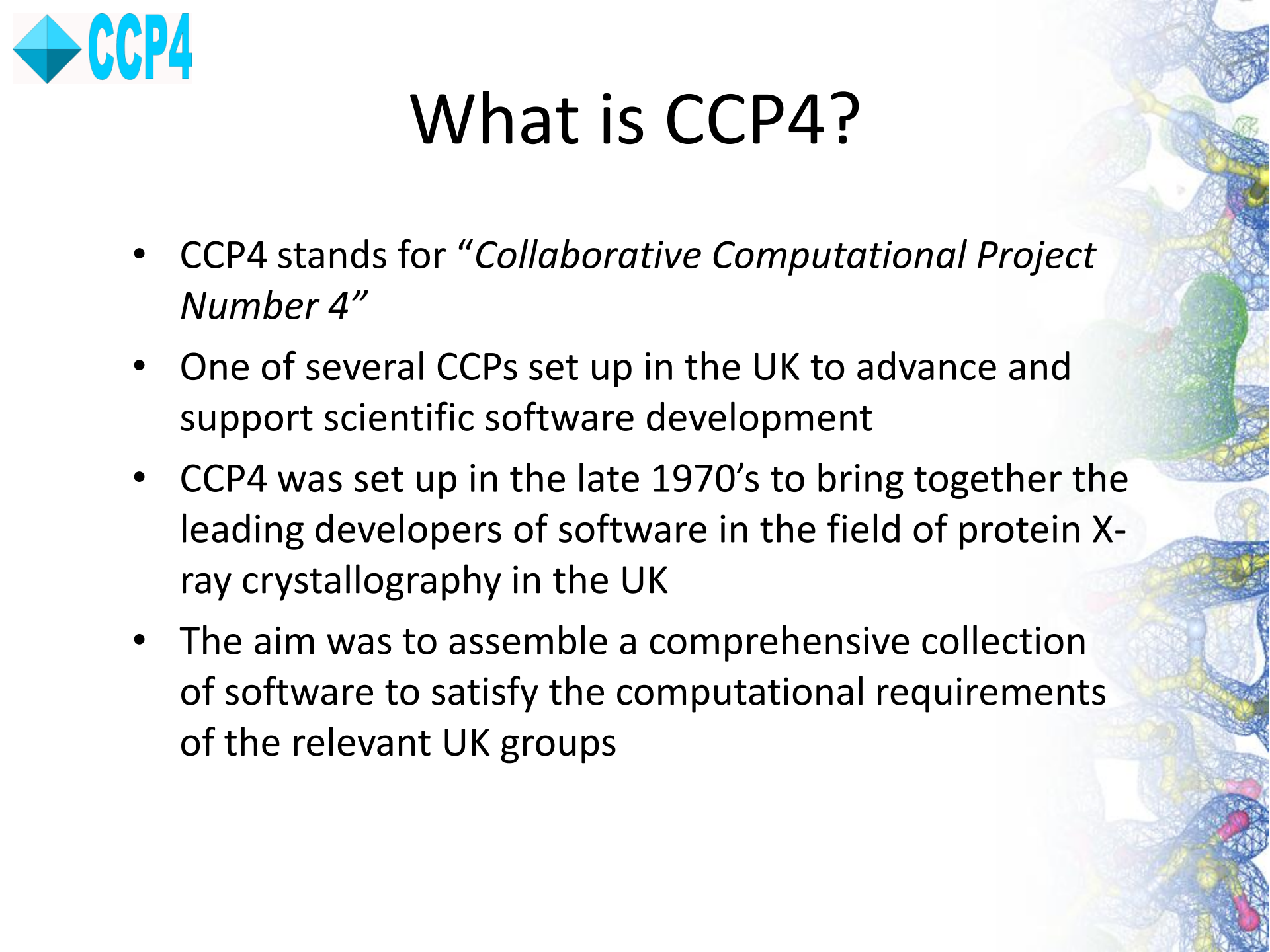
Outline

- 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


Greenland
Iceland
Canada
United States
Mexico
Pacific Ocean
North Atlantic Ocean
South Pacific Ocean
South Atlantic Ocean
Indian Ocean
Suomi Finland
Sverige Sweden
Norge Norway
Россия Russia
Україна Ukraine
Kazakhstan
Mongolia
中国 China
S Korea
Iraq
Iran
Afghanistan
Pakistan
India
Saudi Arabia
Egypt
Libya
Algeria
Mauritania
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
Kenya
DR Congo
Tanzania
Angola
Namibia
Botswana
Madagascar
Venezuela
Colombia
Brasil Brazil
Perú
Bolivia
Chile
Argentina
Indonesia
Papua New Guinea
Australia
New Zealand

Other Resources

- CCP4BB mailing list – bulletin board, news, expert help and advice
- CCP4 help desk – *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

PiMS

DIALS

Crystallisation

Data Collection

Data Processing and Reduction

Experimental Phasing

Molecular Replacement

Density Modification

Model Building

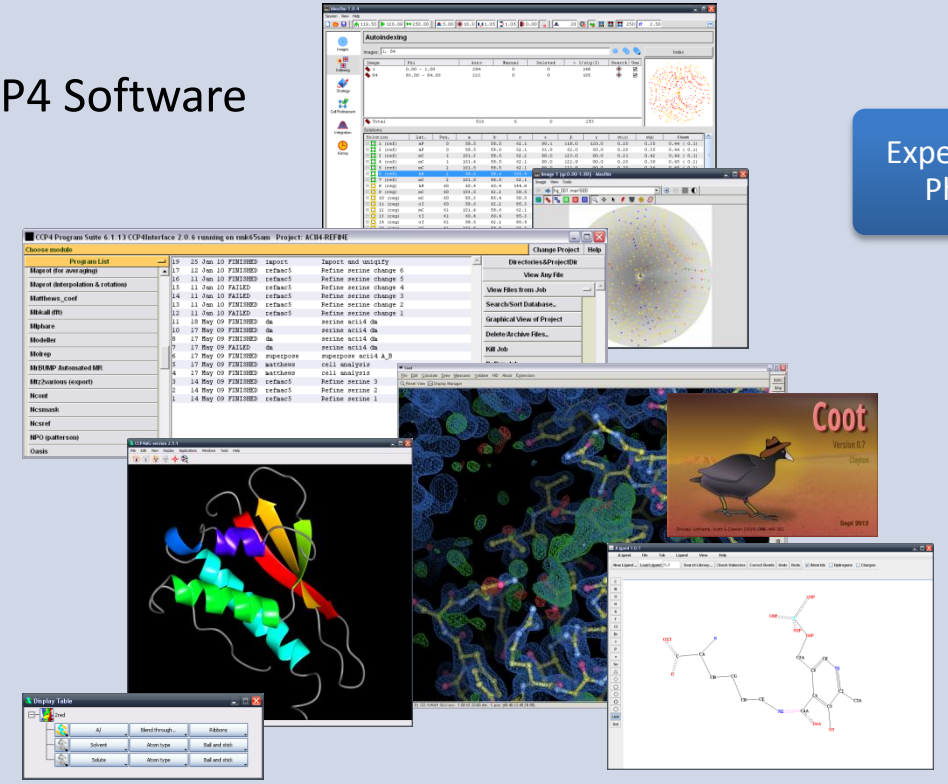
Refinement

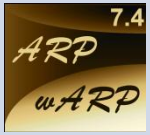
Structure Analysis

Deposition

PDB_REDO

CCP4 Software





CCP4

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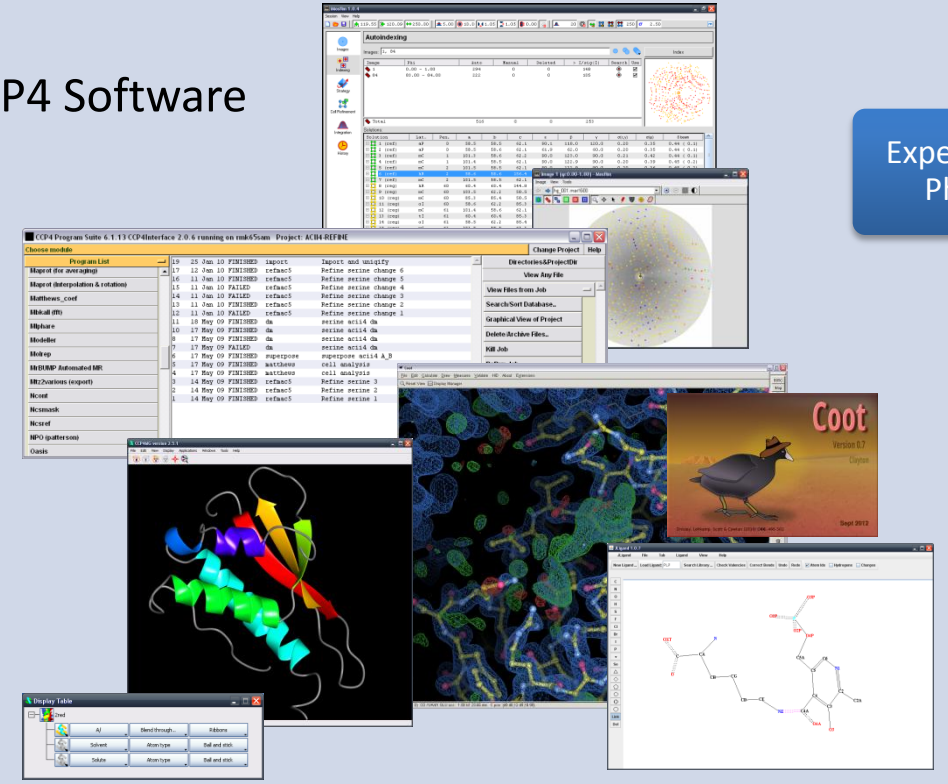
ARP wARP 7.4

CCP4 Software



Automatic Data Processing and Reduction

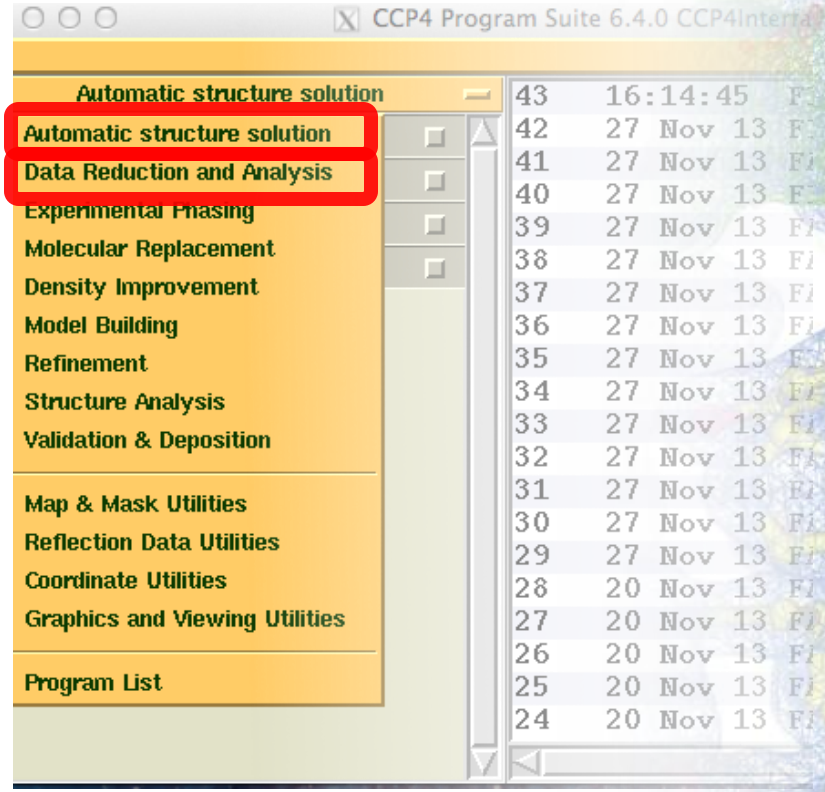
xia2

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

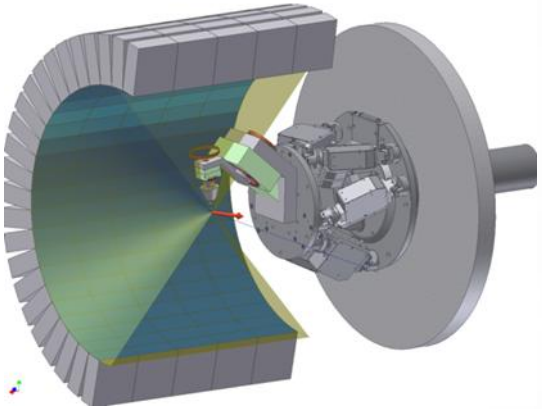
  Scoring for multi-crystal



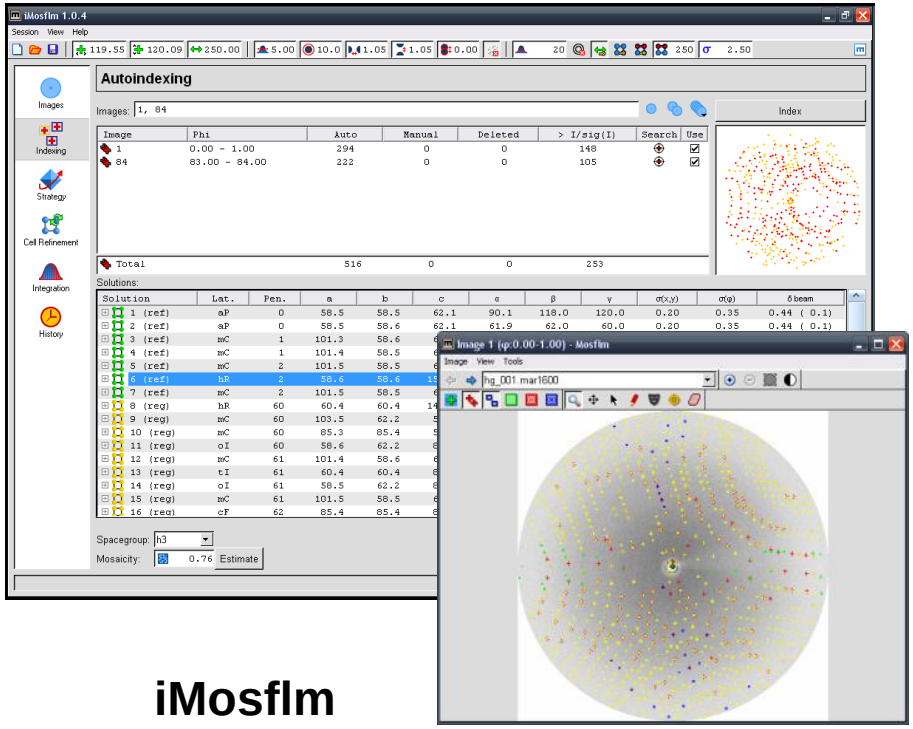
Data processing - DIALS

- ***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

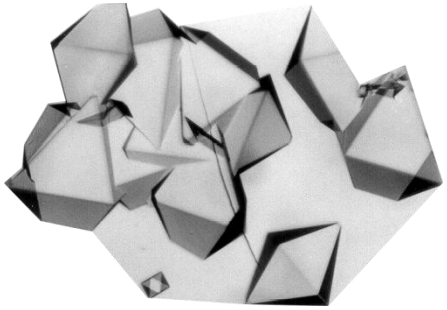
Scale-symmetry-related intensities together

Produce statistics on data quality

Feckless

Multi-crystal merging

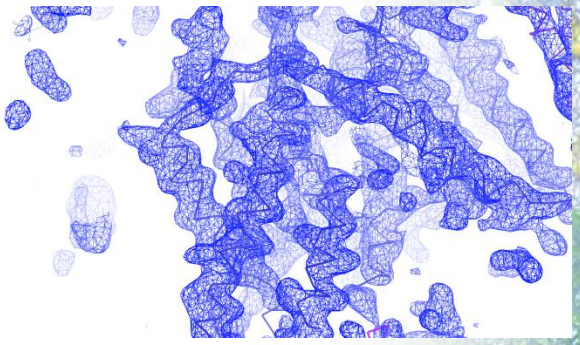
Multi-crystal - BLEND



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), D**69**, 1617-1632

CCP4

PiMS

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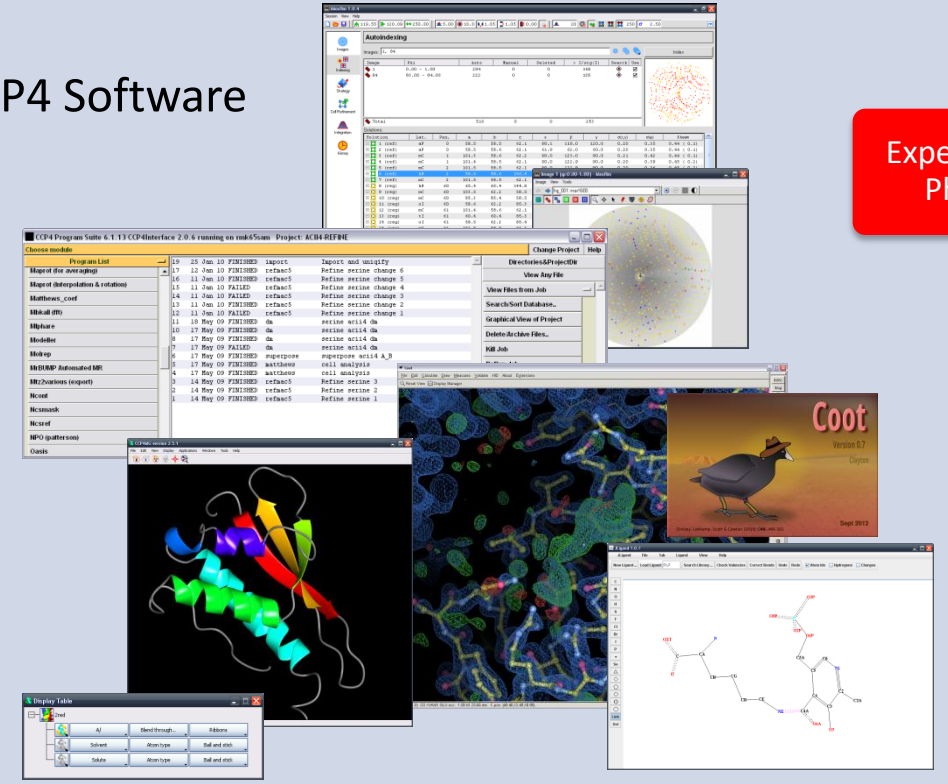
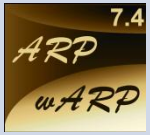




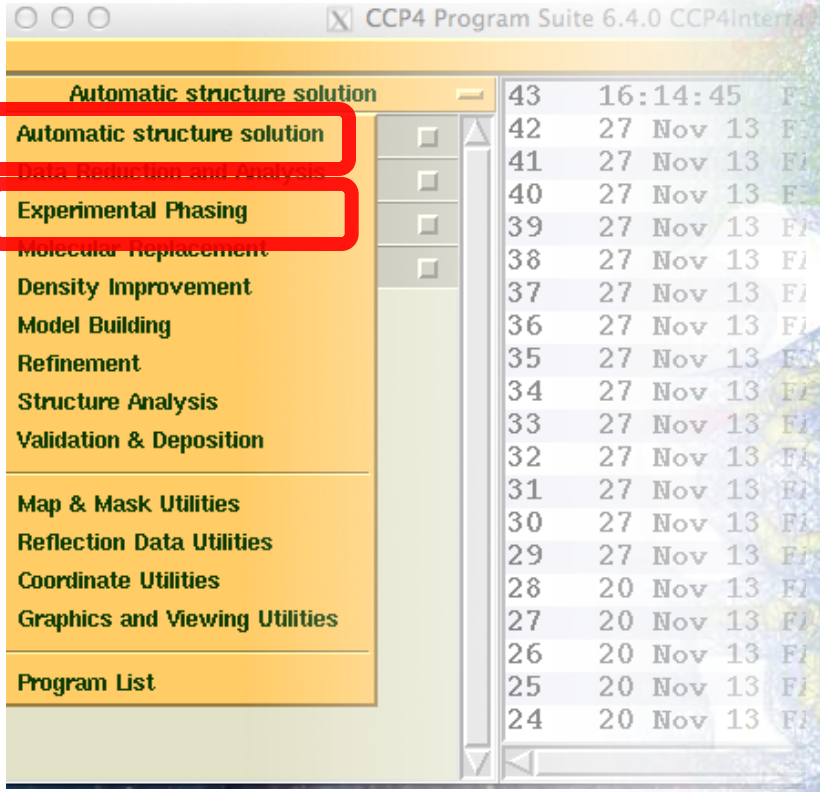
Phasing - Experimental Phasing

Crank/Crank2

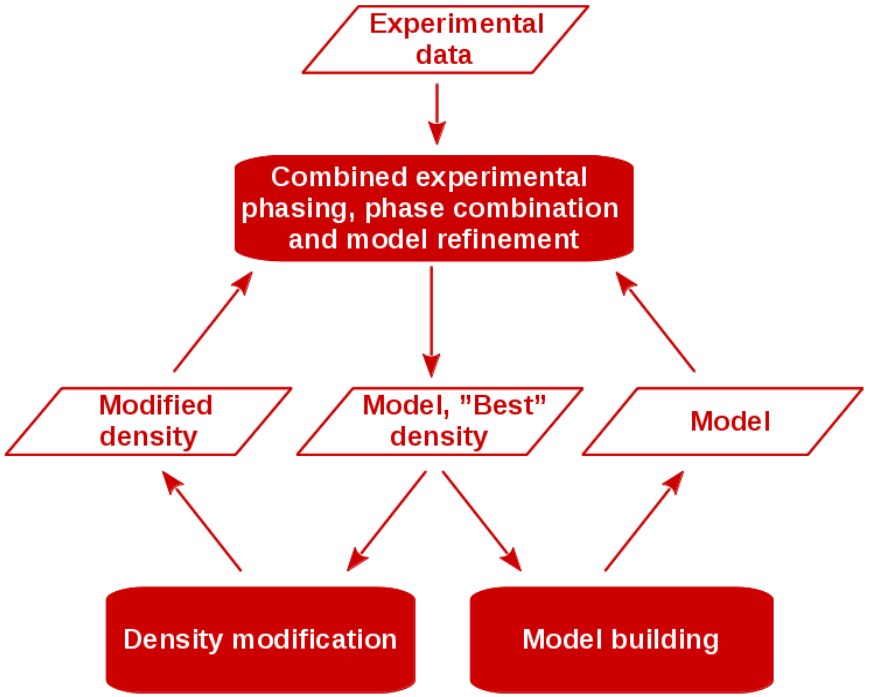
- Automated experimental phasing pipeline
- Heavy atom location through to Model Building

Phaser EP

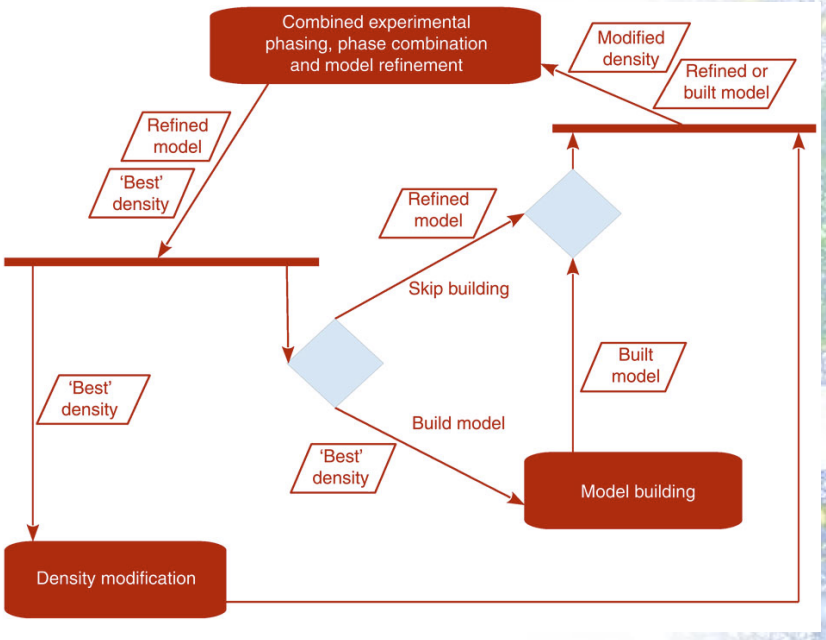
- Automated experimental phasing
- SAD phasing
- Combined MR and SAD Phasing



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

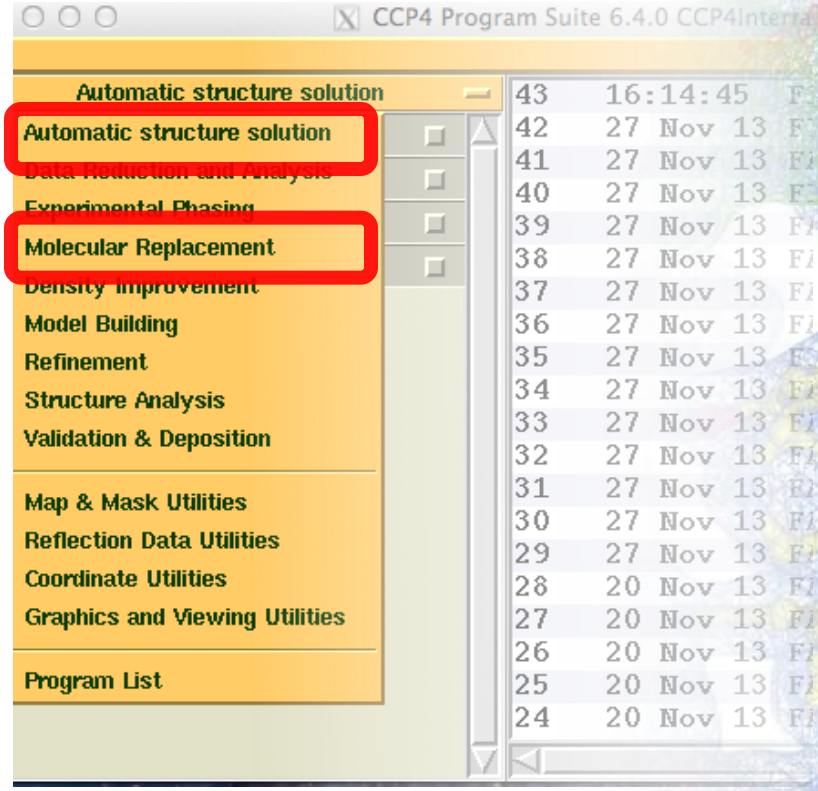
Automatic Molecular Replacement

MrBUMP

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

BALBES

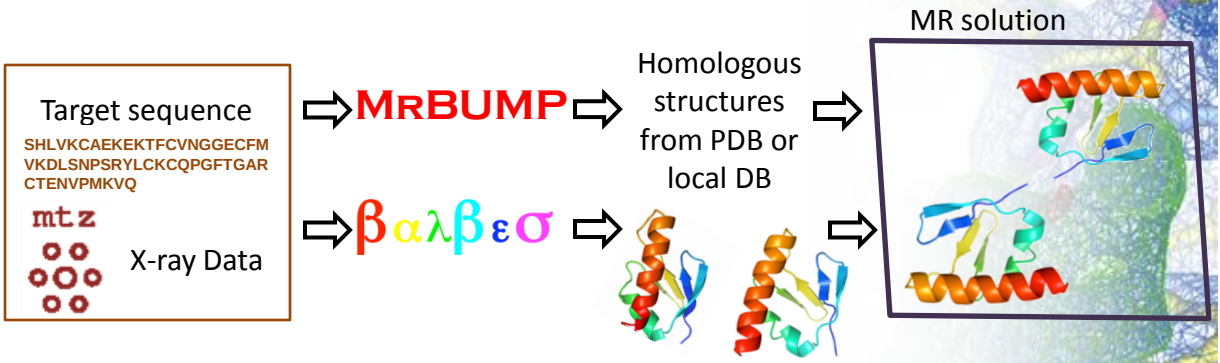
- Automated molecular replacement using Molrep and Refmac
- Custom DB with search models monomer, domain and multimeric form

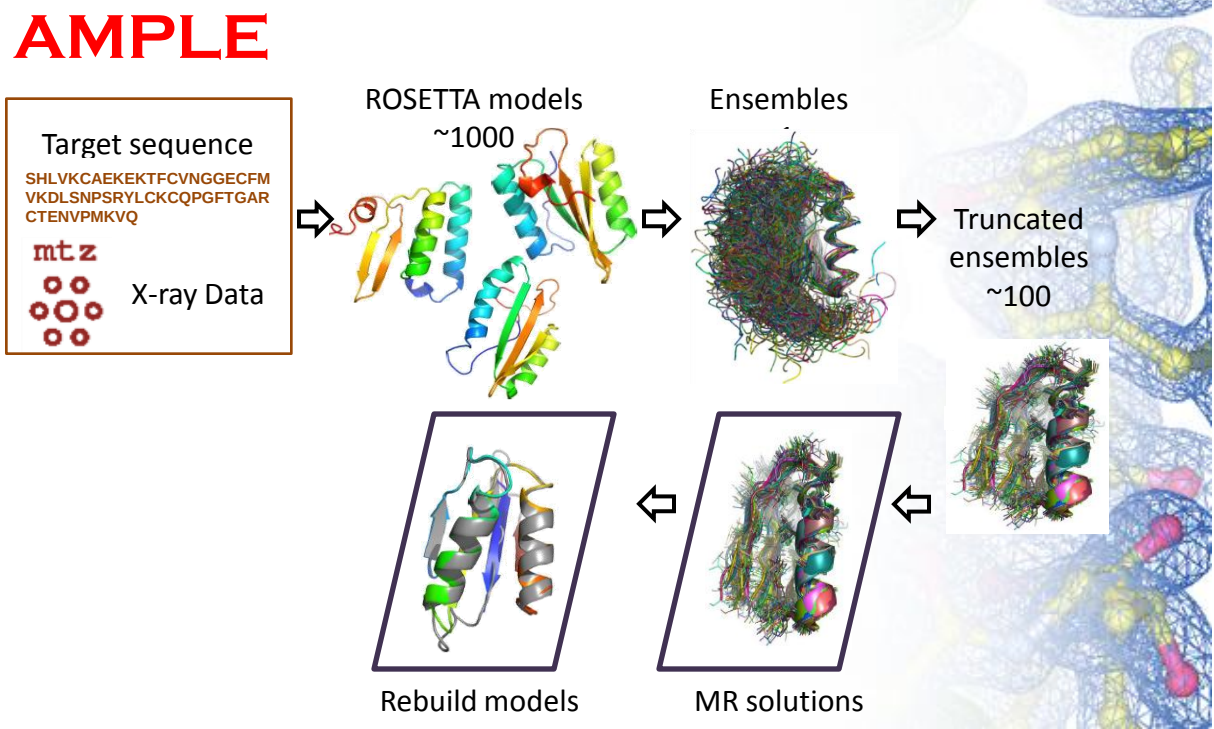


Molecular Replacement – Ab initio search models

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, α-helical proteins (<120 residues) but has been known to work for larger targets





Molecular Replacement

Phaser MR

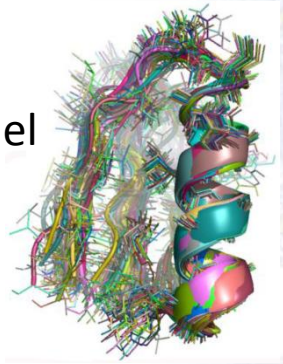
- Automated Maximum Likelihood method for molecular replacement
  - Twinning
  - Translational NCS
  - σrms refinement

Sculptor

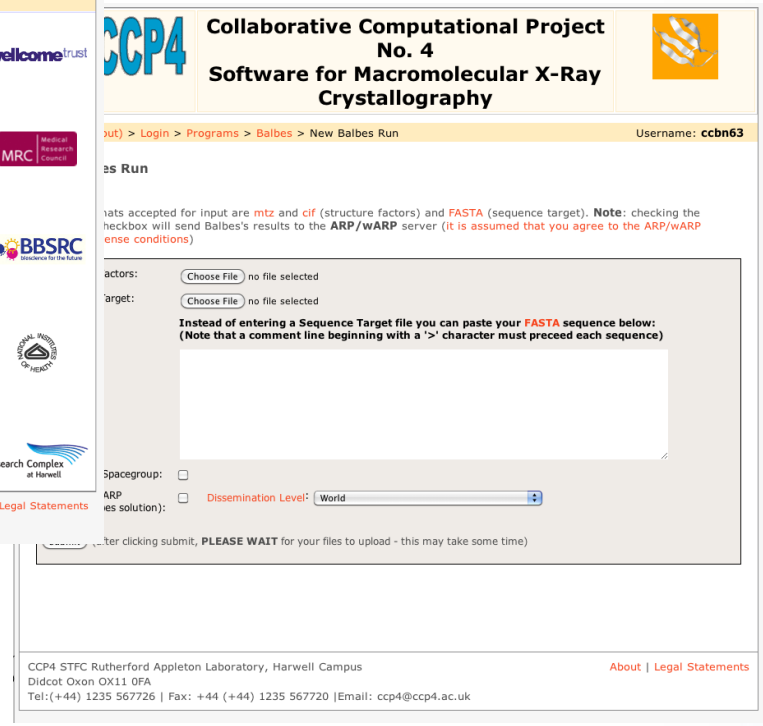
- Multi-protocol search model preparation
  - weighted sequence similarity
  - accessible surface area

Ensembler

- Ensemble search model generation tool from the developers of Phaser



CCP4 Online Webservice



http://www.ccp4.ac.uk/ccp4online

or Google for"ccp4online"

coming soon CRANK2

CCP4

PiMS

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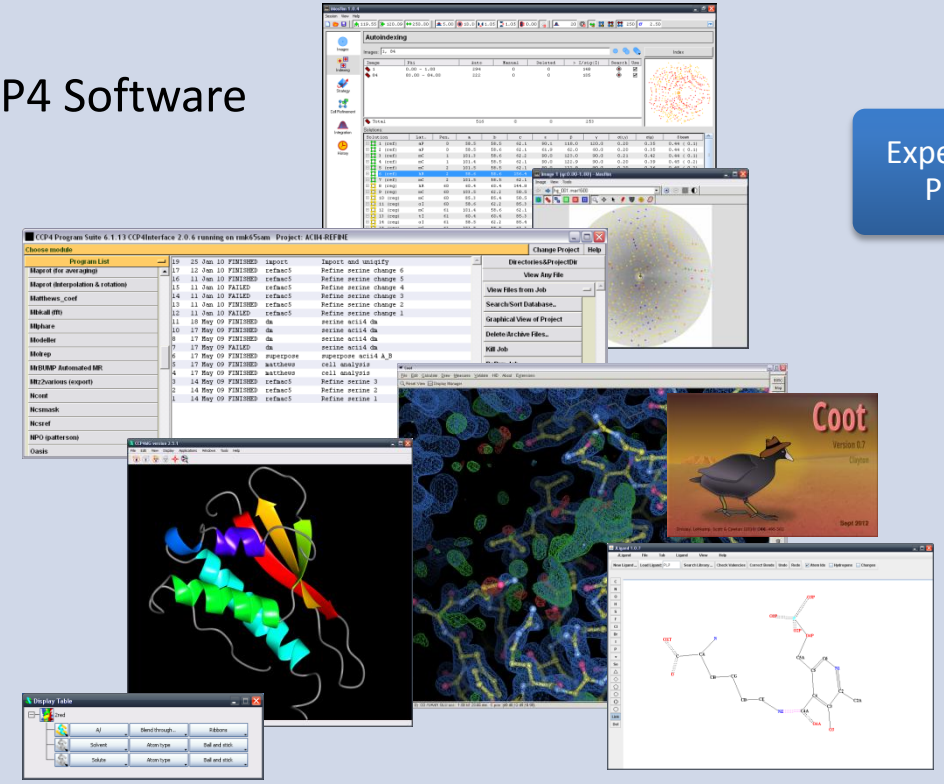


7.4
ARP
wARP

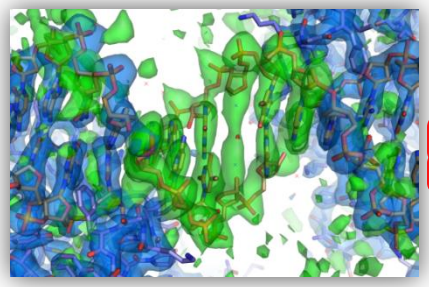
Model Building And Refinement

Buccaneer

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

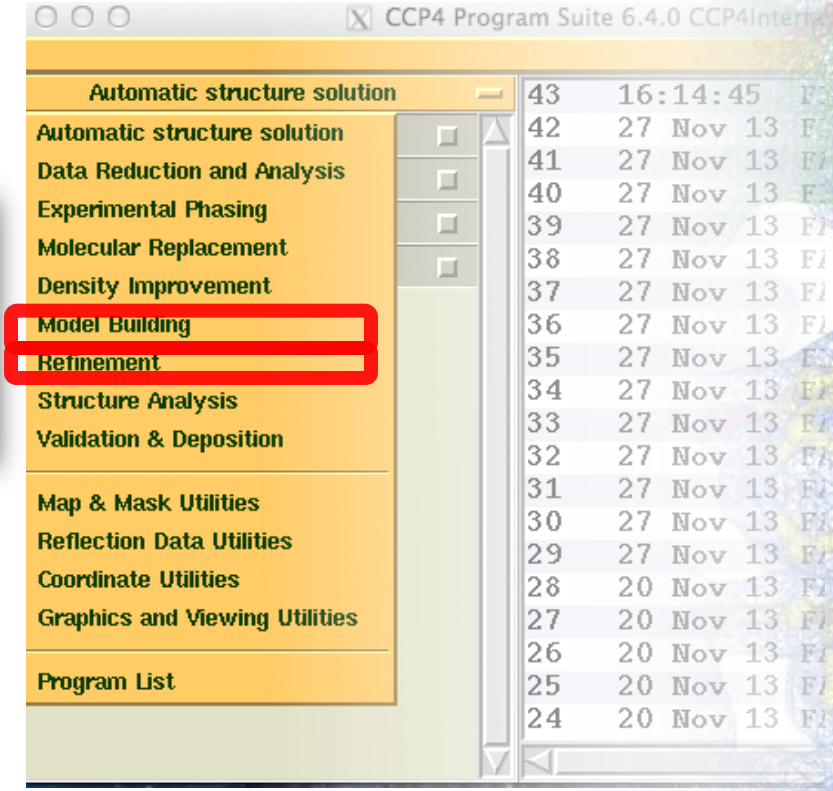
Nautilus

- Automatic model building of nucleotide structures in electron density maps



Sloop

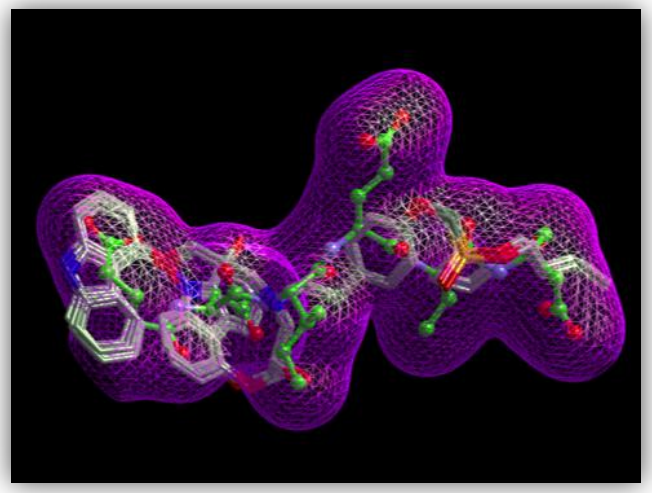
- Loop building by finding gaps in the chain and using fragments from the Richardson's Top500 library of structures to fill the gaps

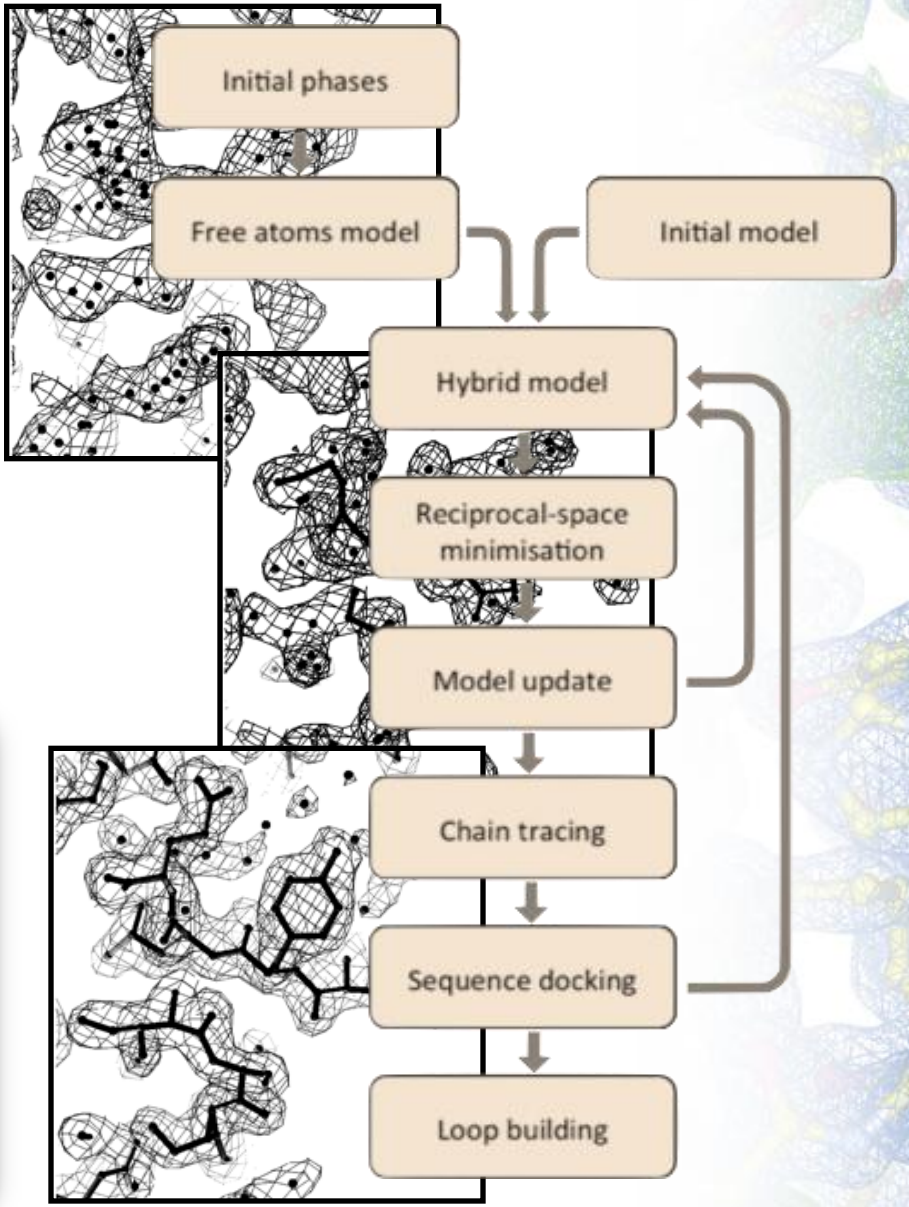


Model Building

ARP/wARP 7.4

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

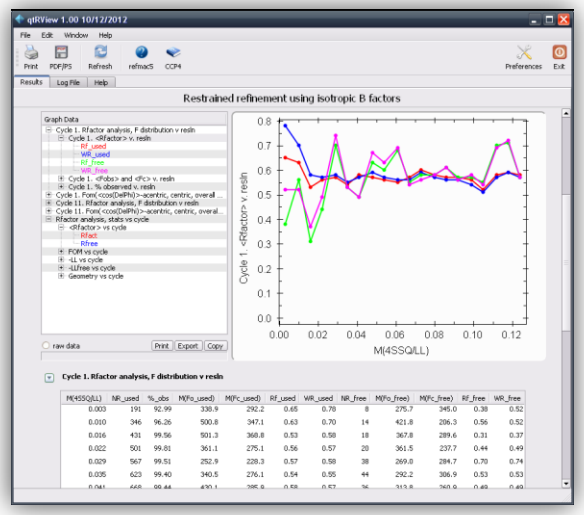




Refinement

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

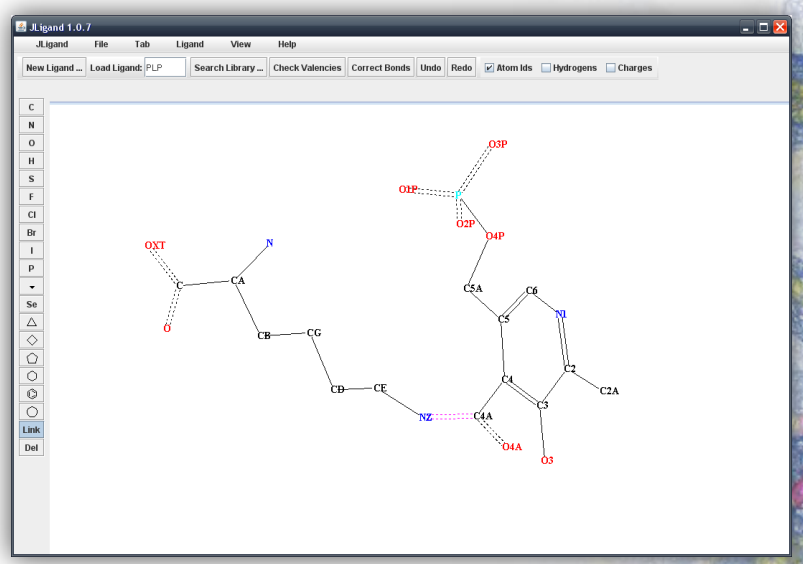


ProSMART

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

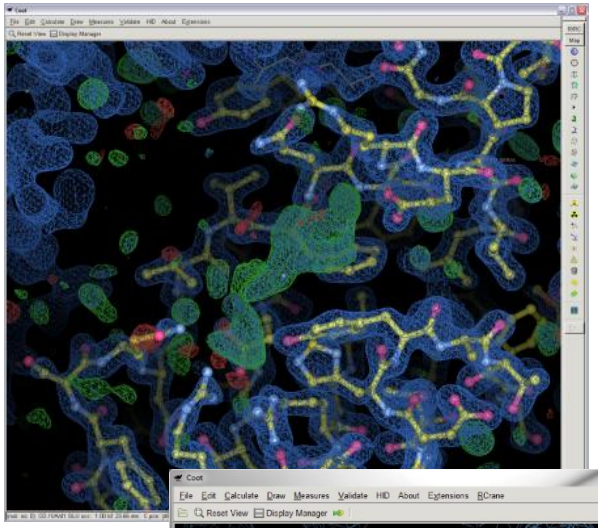
AceDrg

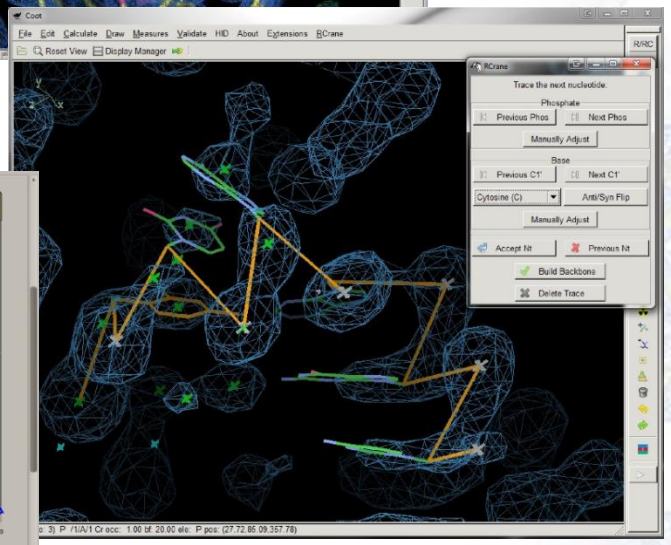
- Generate restraints for ligands for use in refinement and model building

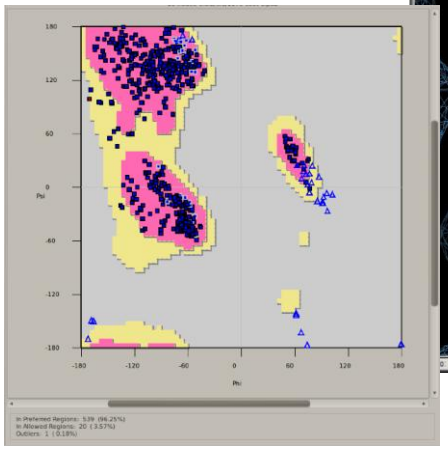


Manual Model Building - Coot

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







CCP4

PiMS

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Diffraction Integration for Advanced Light Sources

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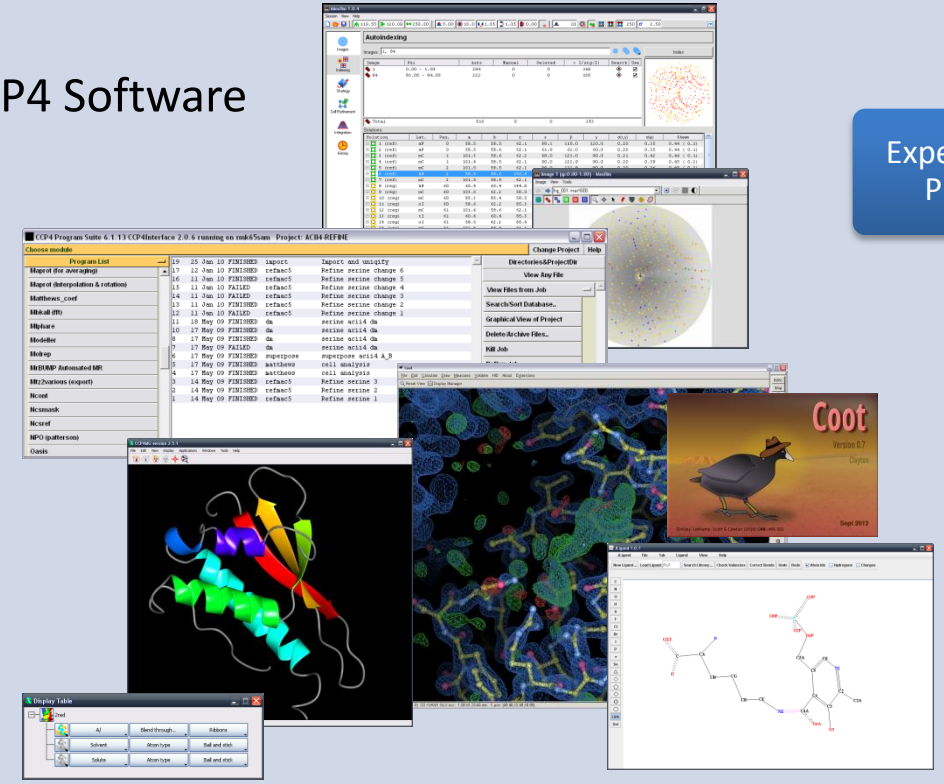
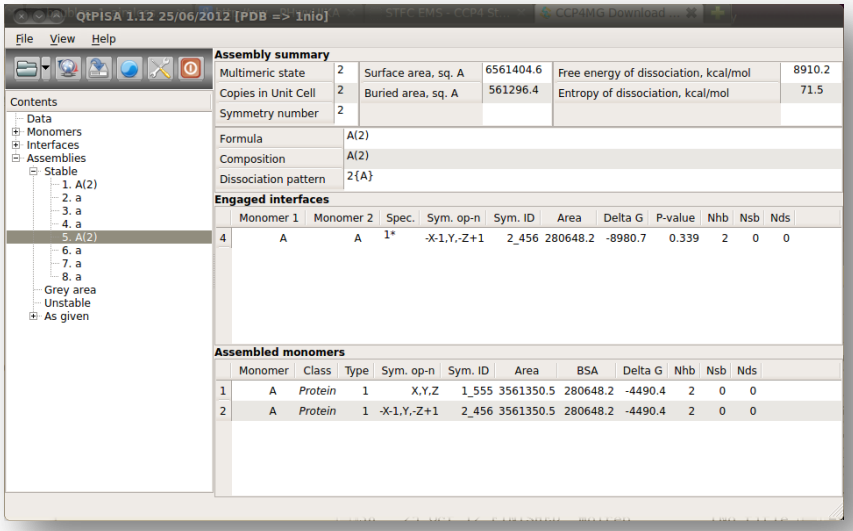


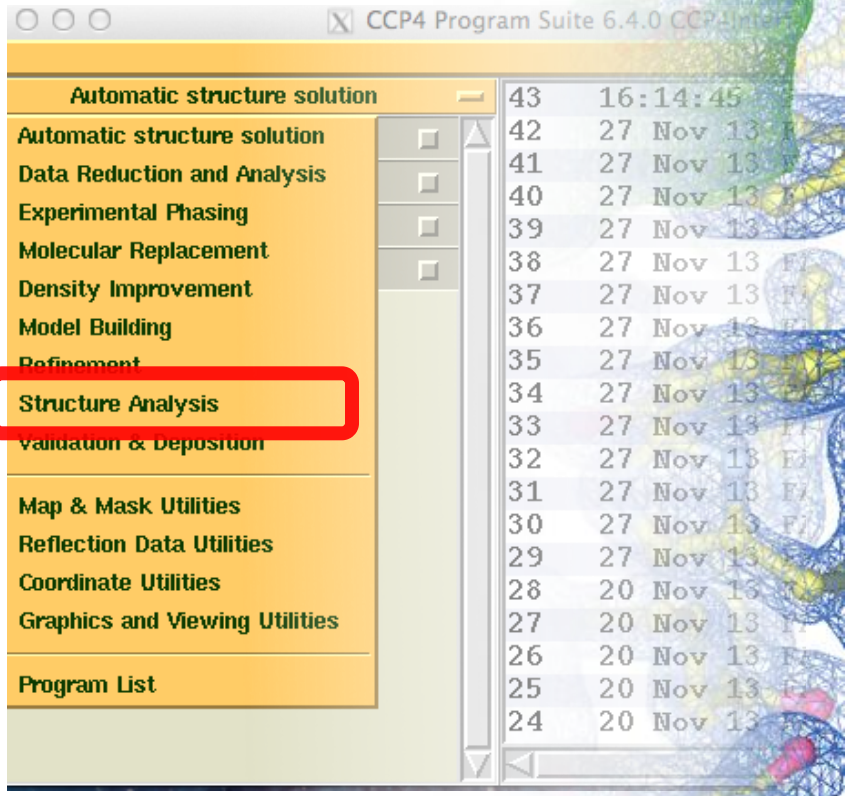
7.4
ARP
wARP

Structure Analysis

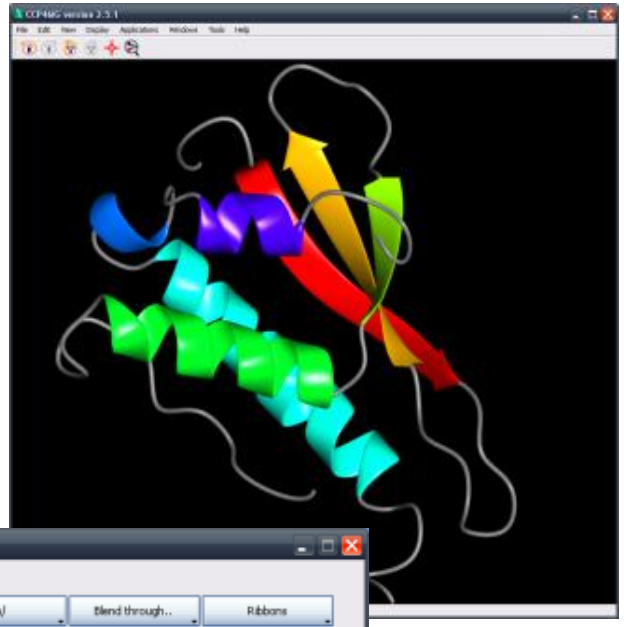
QtPISA

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



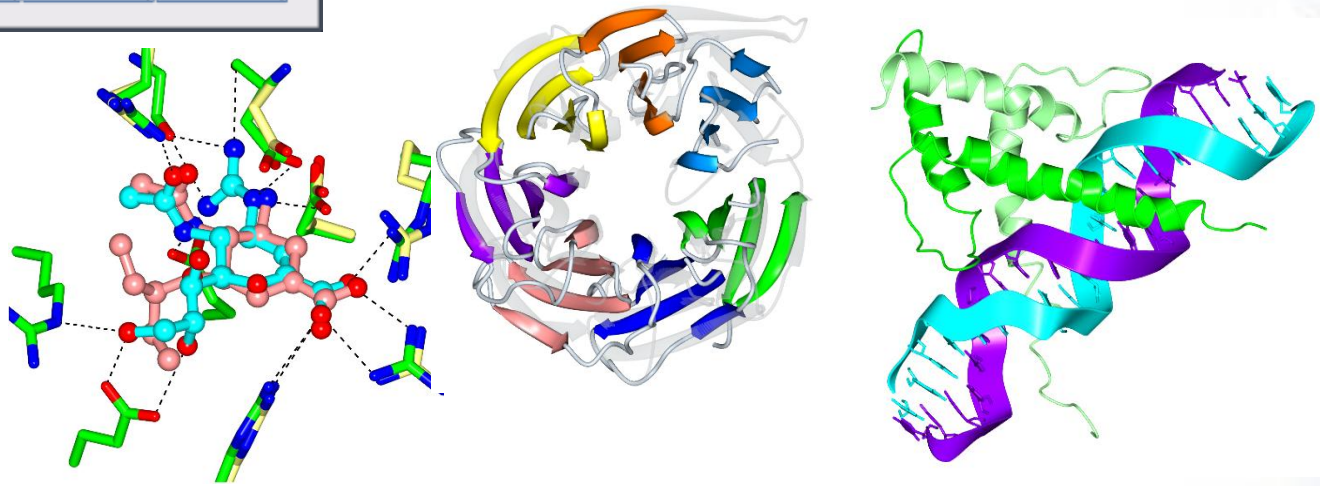


Structure Analysis



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 Vonrhein, 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..

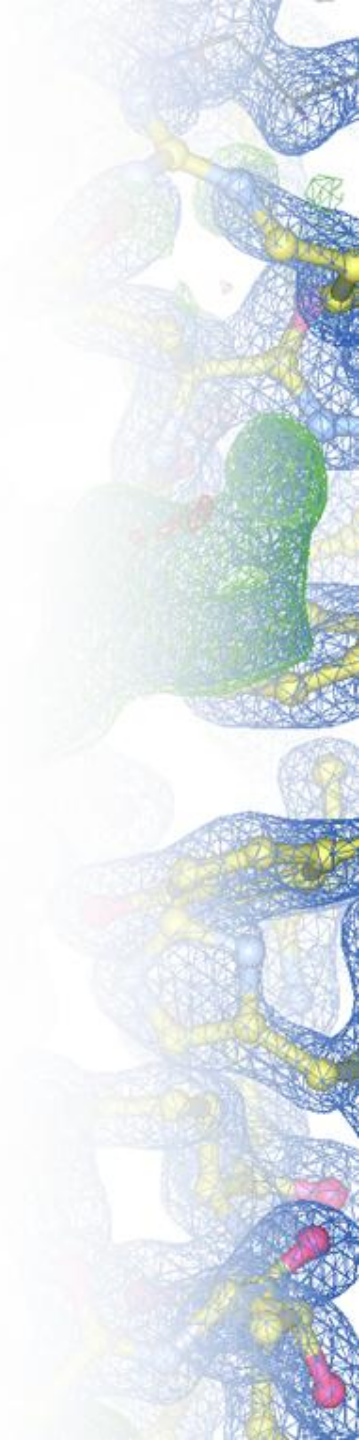
Workshop outline

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

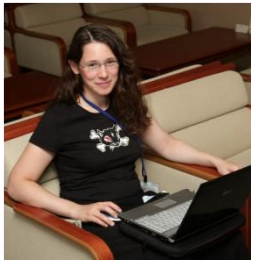
Raj Pannu – Leiden, NL.
-experimental phasing. CRANK2, bp3

Bernhard Lohkamp – Karolinska Institut, 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

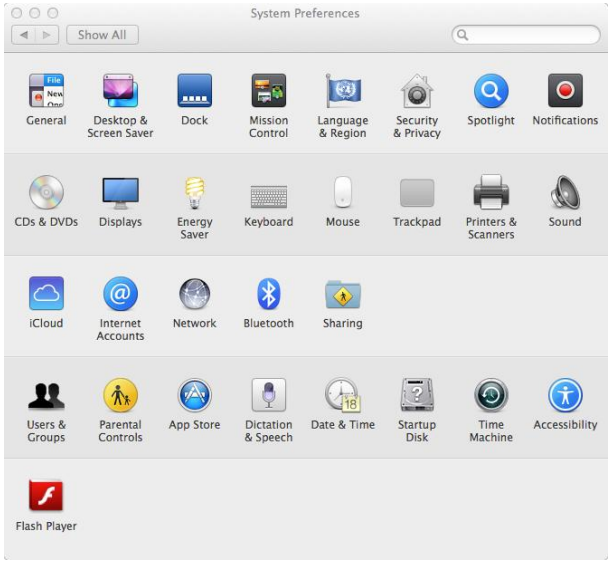
Command line – terminal.app

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>

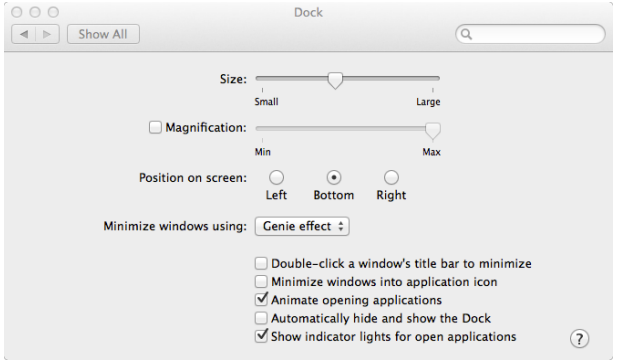
Compression commands:
tar –xf tmp.tar <file> - extract <file> from tmp.tar
tar –xzf tmp.tar.gz – extract everything from gzipped tar
tar –czf tmp.tar.gz <file> - create tar archive from <File>

OS X – the dock

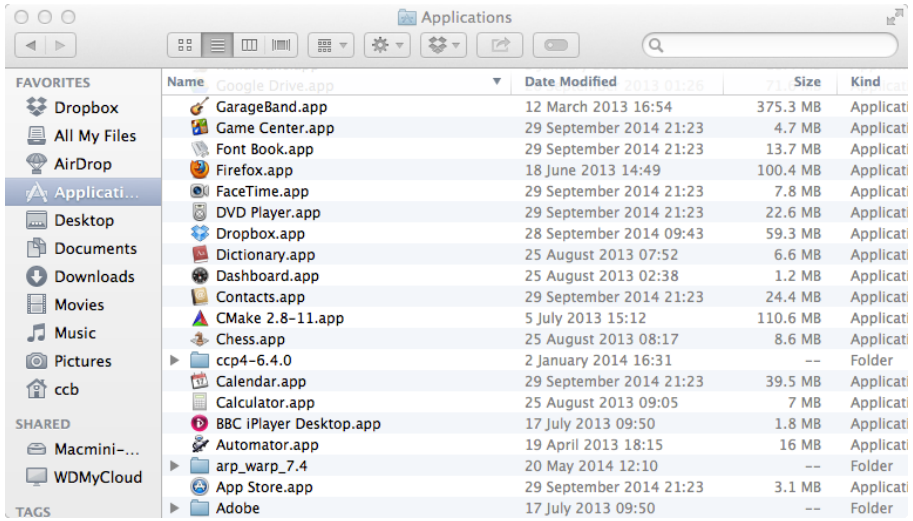
Dock – shows running mac applications, open documents, launches applications. Can add applications to dock by dragging and dropping.



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

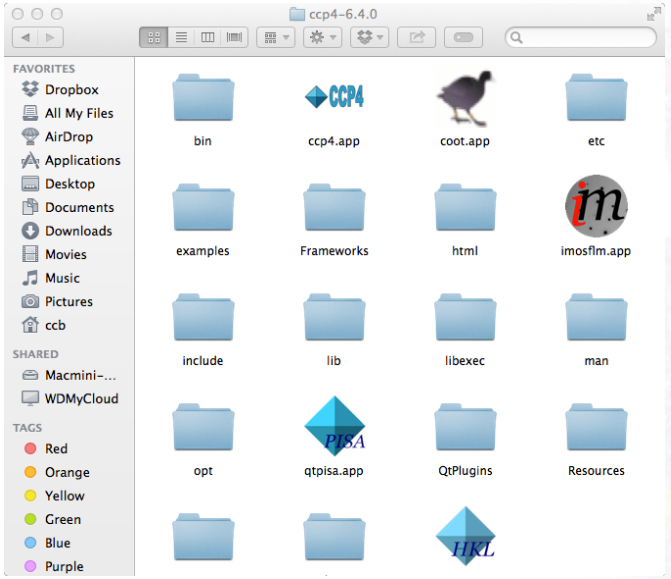


OS X – the finder

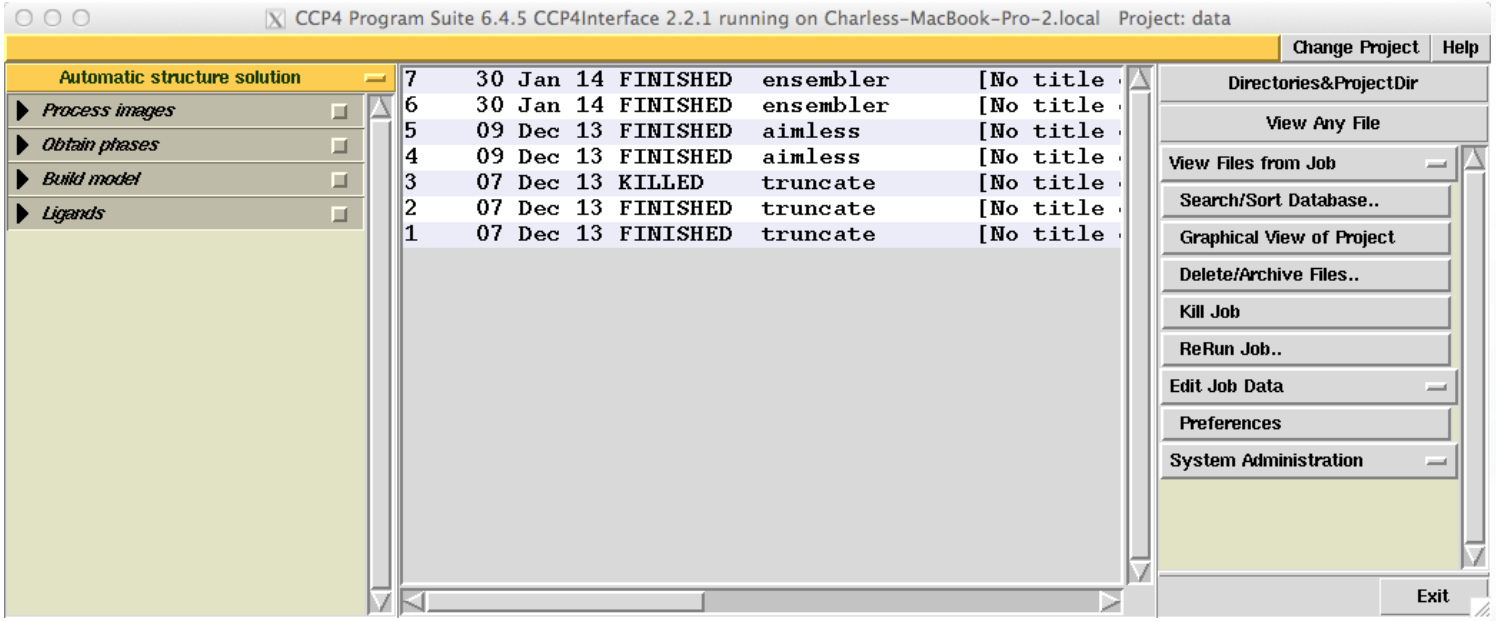


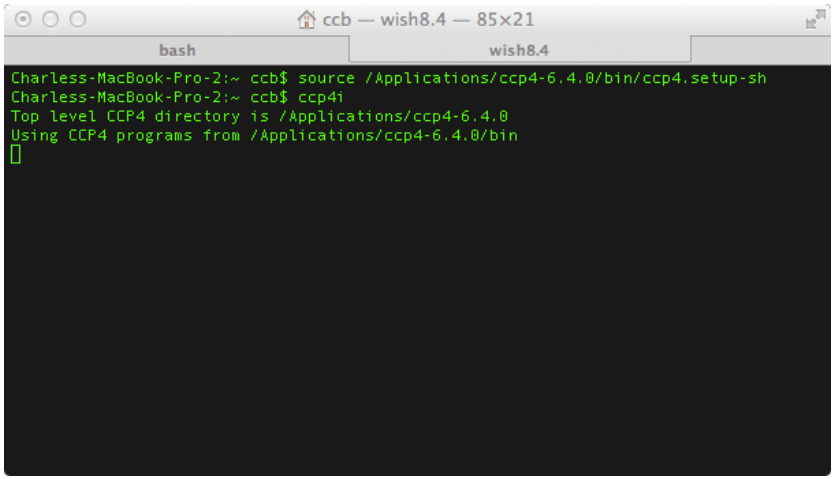
Finder – default environment on mac. Finder accessible from dock. Finder window launched via toolbar File -> New Finder Window.
Here applications, showing ccp4-6.4.0. Terminal.app under utilities (why???)

View of the ccp4-6.4.0 in the Finder window, icon view.
Launch CCP4 by double clicking on ccp4.app.



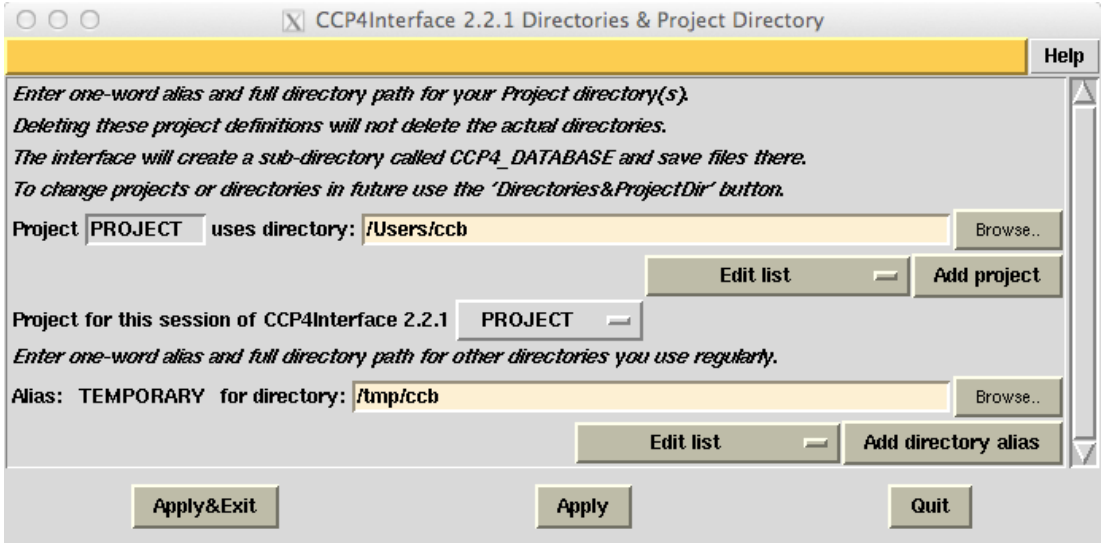
Launch ccp4i





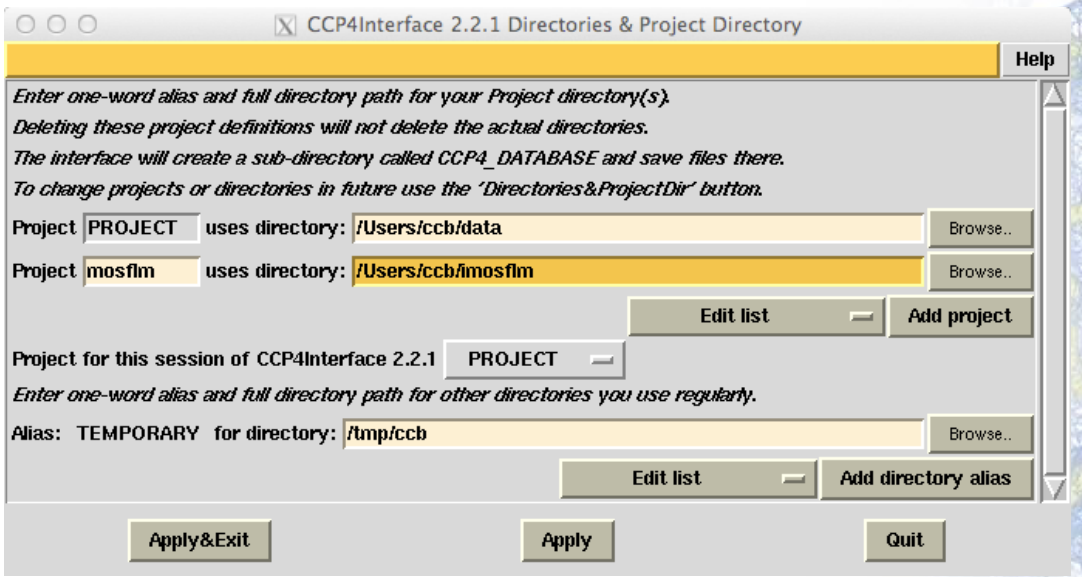
Linux style command line launch of ccp4i from the folder /Applications/ccp4-6.4.0/bin

Set project

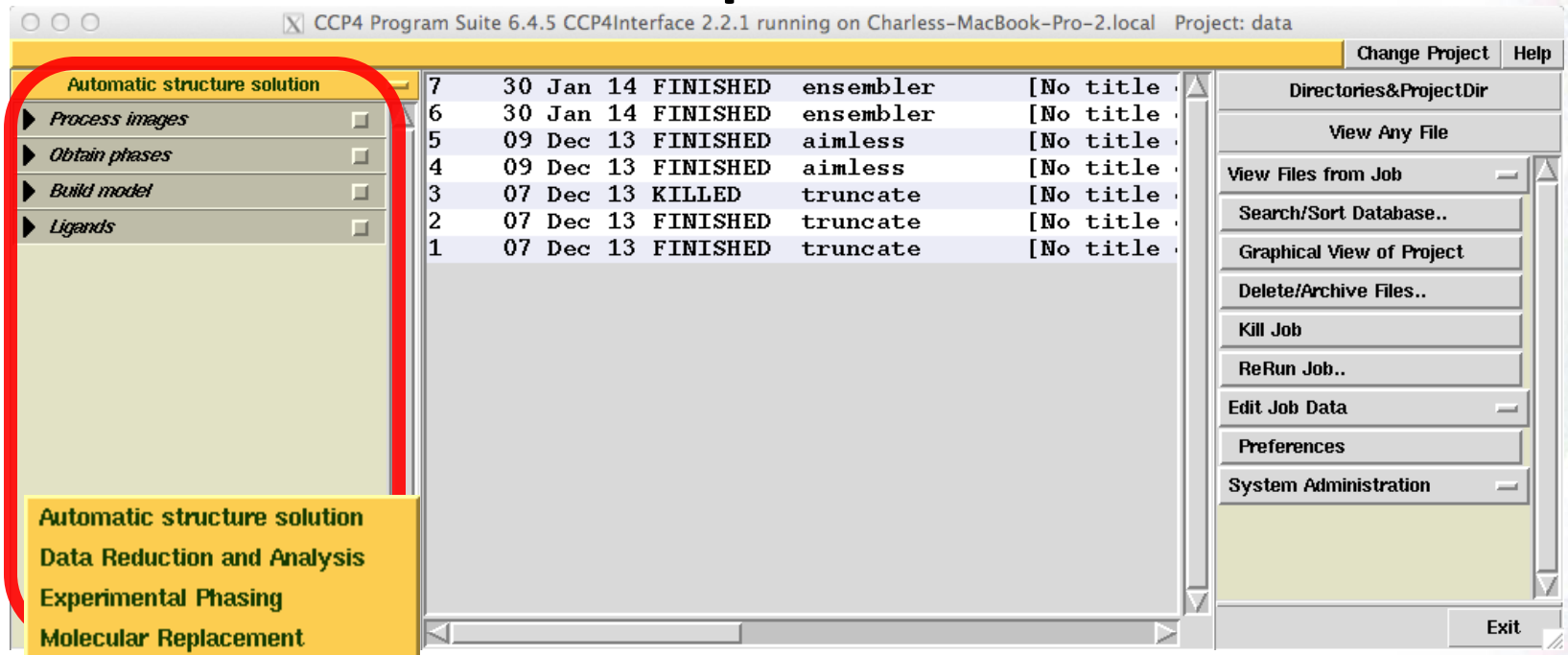


You must set a project. At first launch ccp4i will set a default project in your home directory, don't Apply as that way lies madness – a cluttered home directory.

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

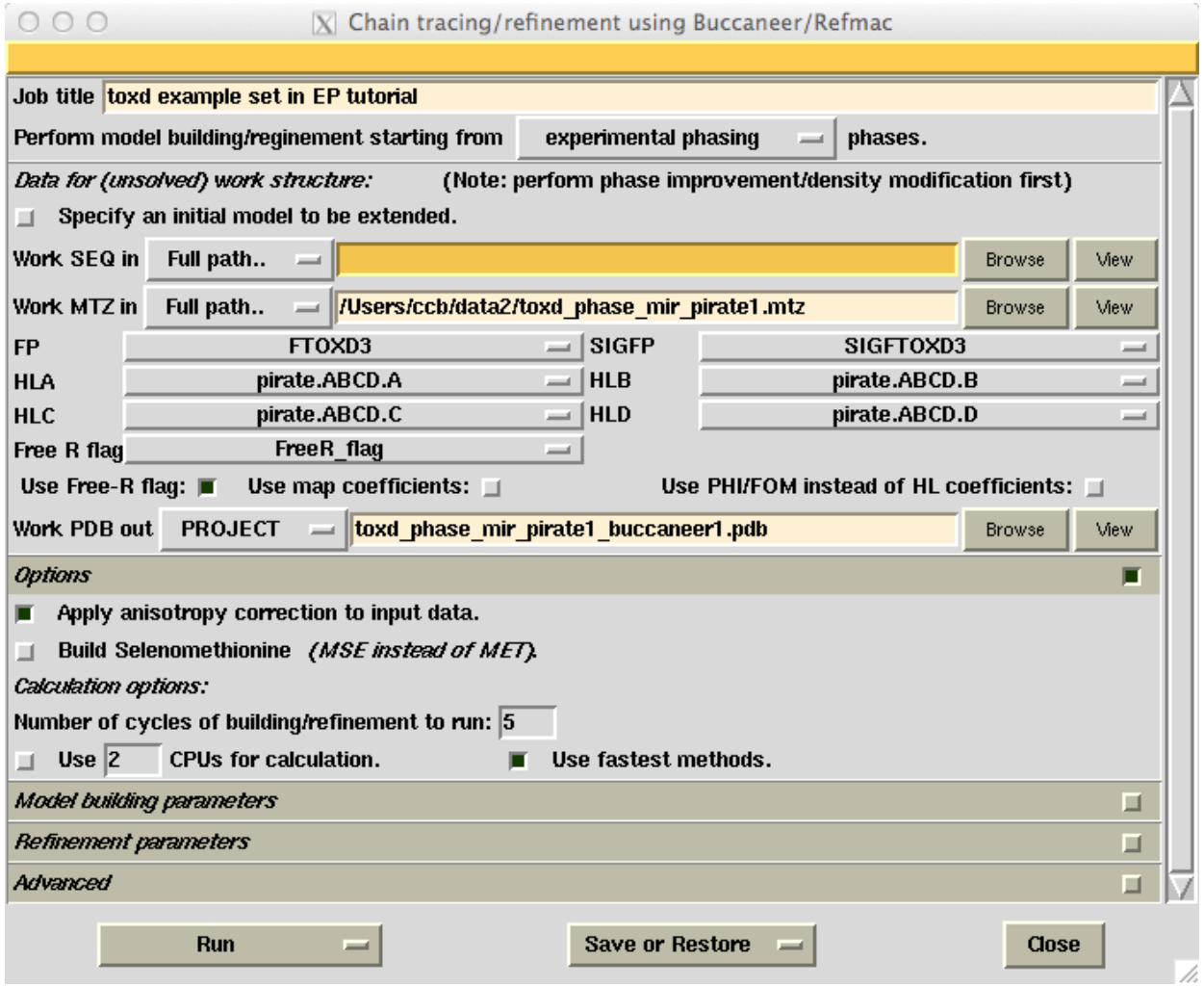


Ccp4i - tour



Modules list and task list (Automatic structure solution)
- Arranges jobs by type
- Task list to launch jobs

Anatomy of a Task interface



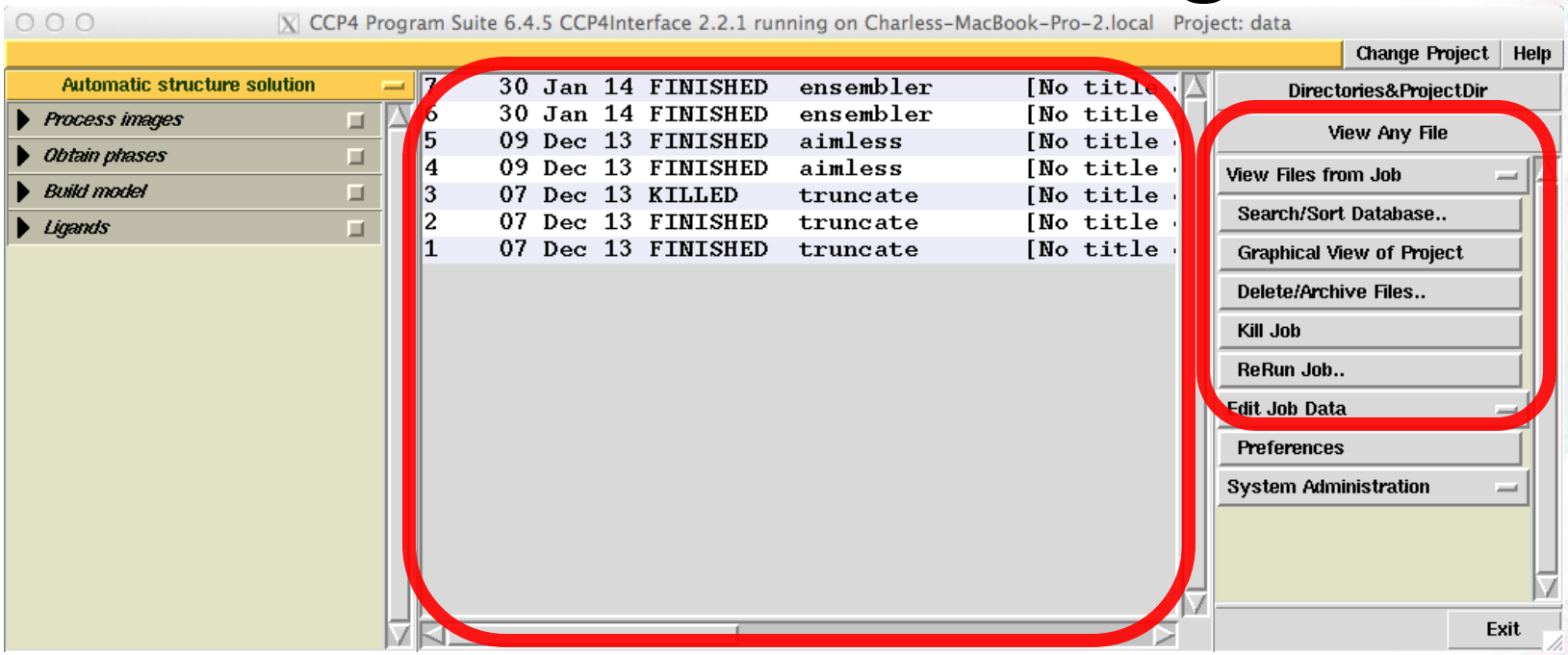
Use title input to track what you have done.

Job type selection at top.

Compulsary input in yellow.

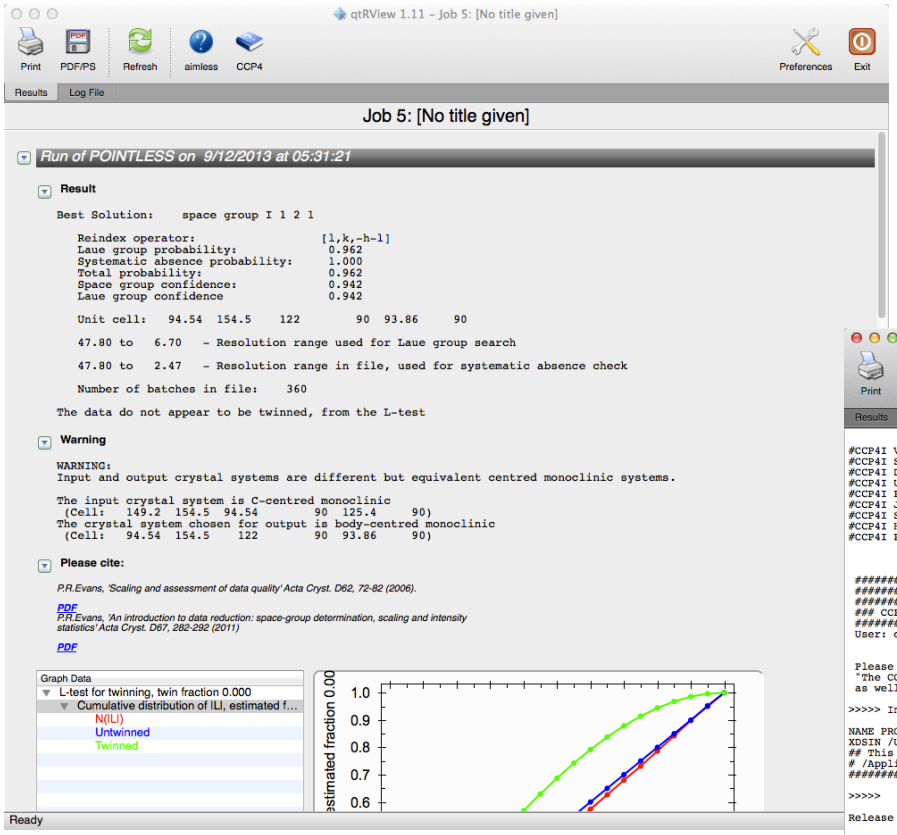
Run menu to launch job, or view input.

Jobs list and viewing files

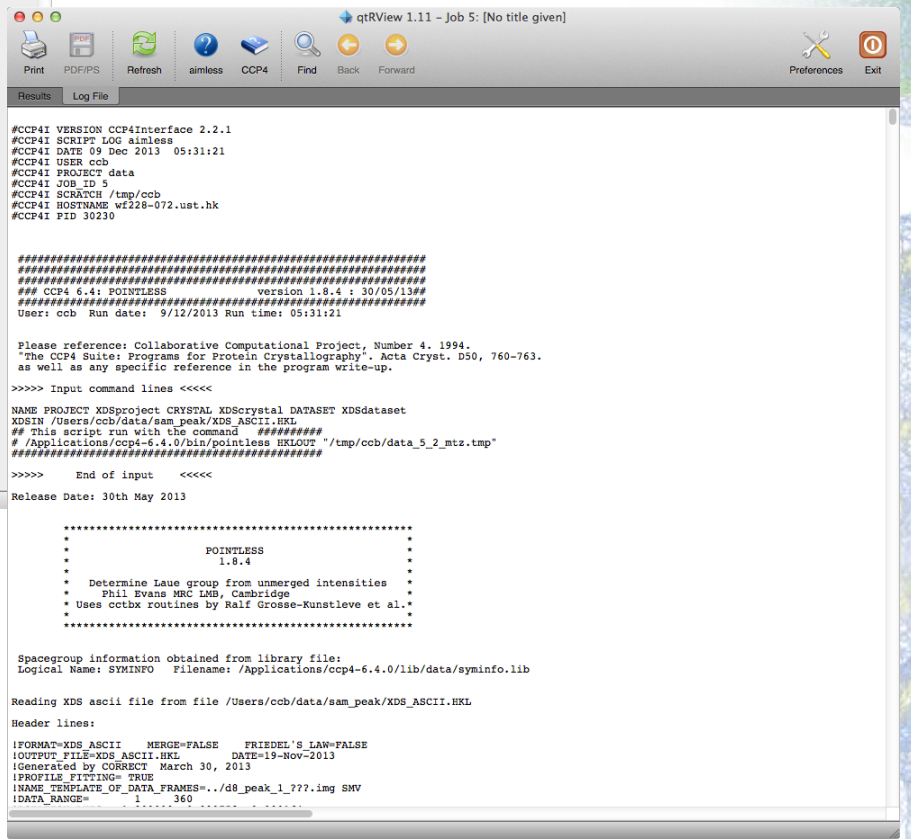


Jobs list is a chronological list of tasks run for a project.
Without titles, this is not very instructive.
“Edit Job Data” leads to a notebook (not used to the best of my knowledge).
“View Any File”, “View Files from Job” or double-click on Jobs list gives access to output. For refinement (refmac) and model building should be able to view in coot.

File output

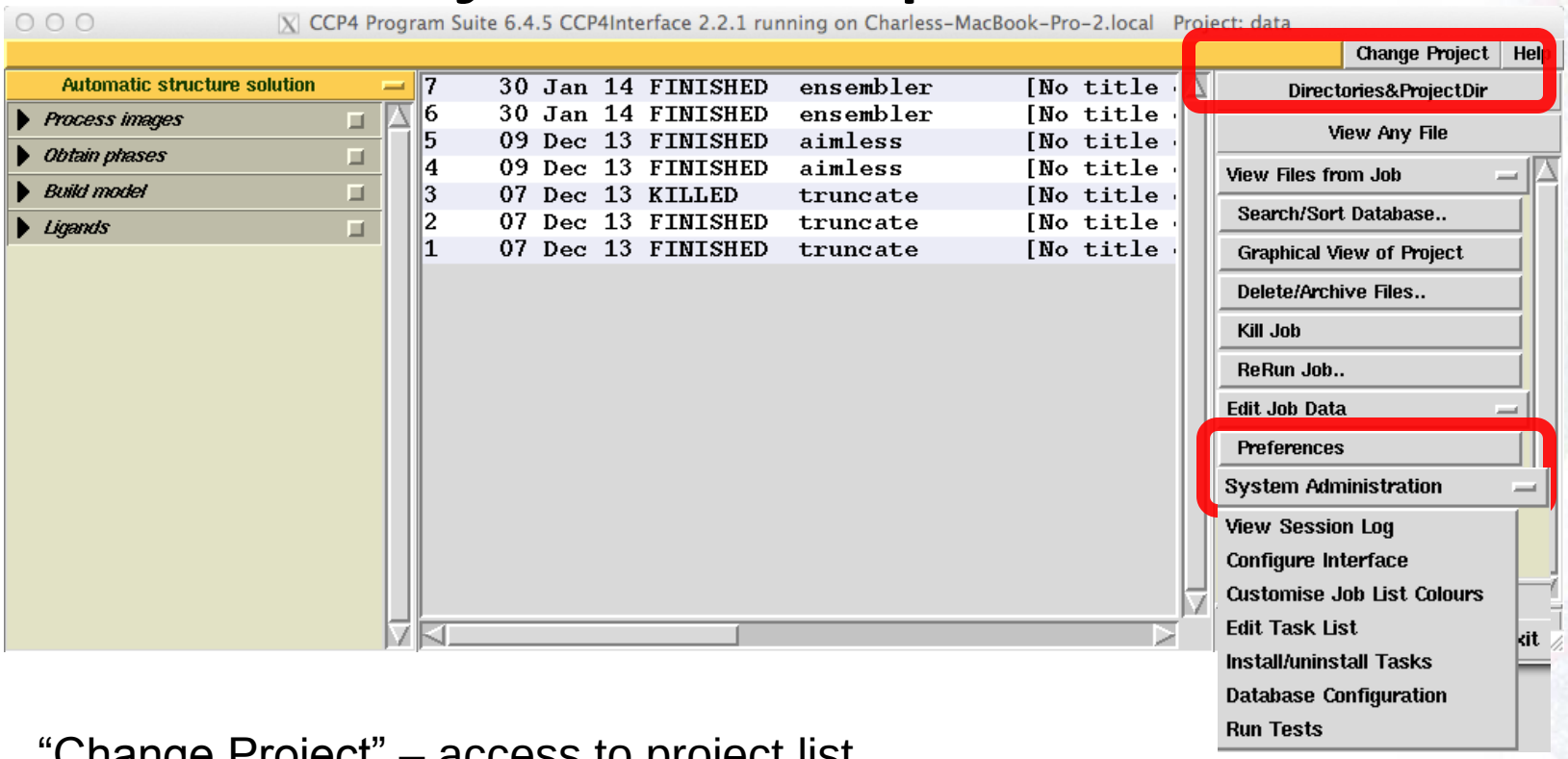


ccp4i logfile view (qtrview), parsed version that includes loggraph output – L-test from aimless



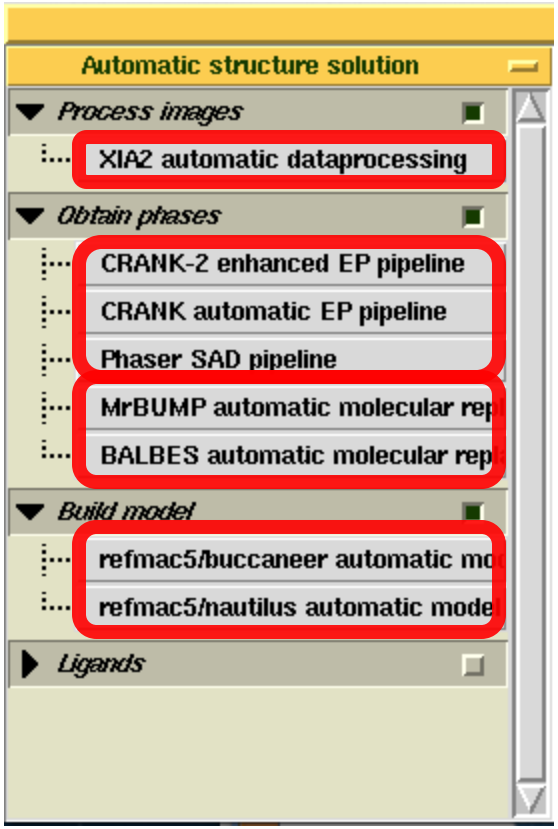
Same job in plain version.

Projects and preferences



"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

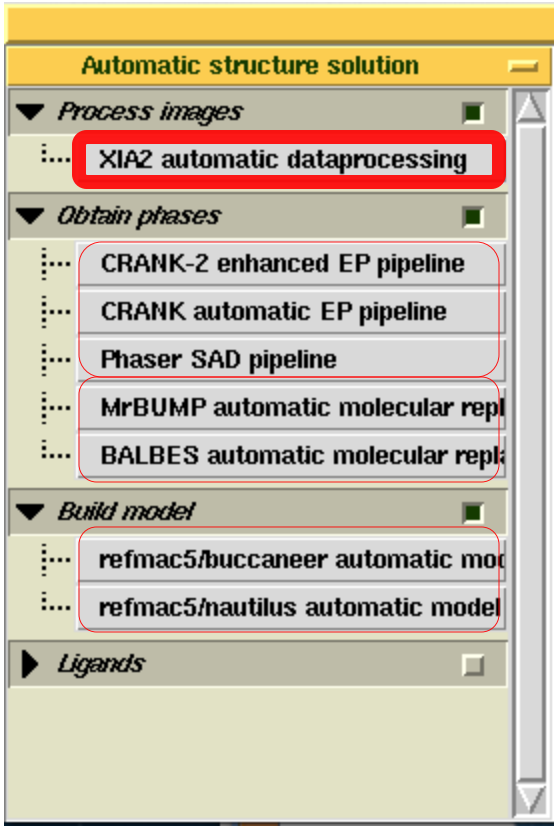
Experimental phasing and model building.

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 buildin

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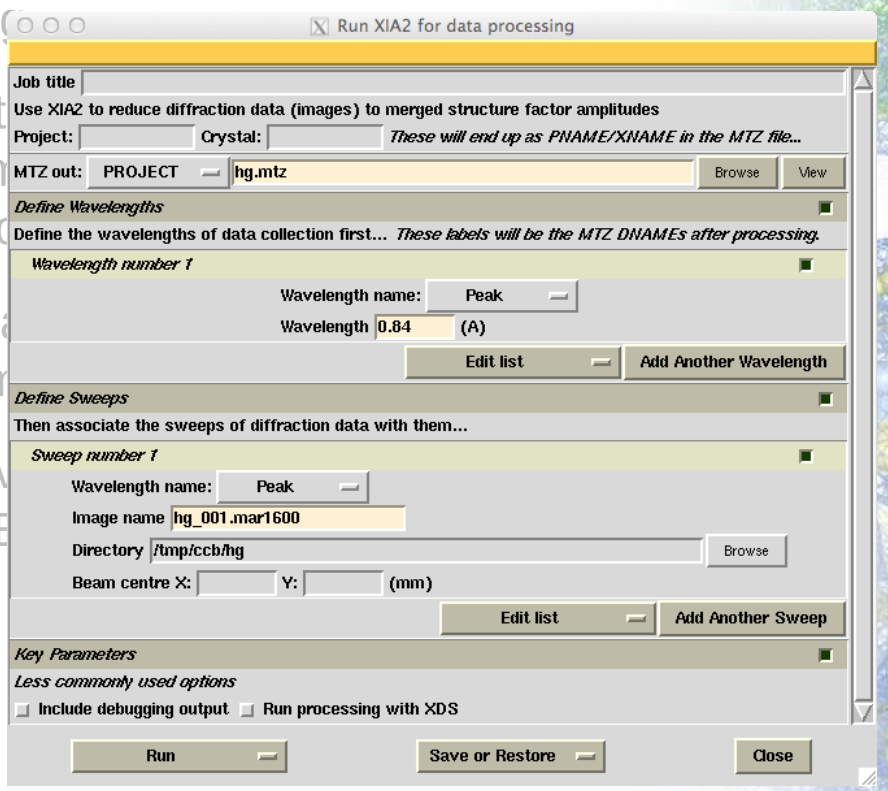
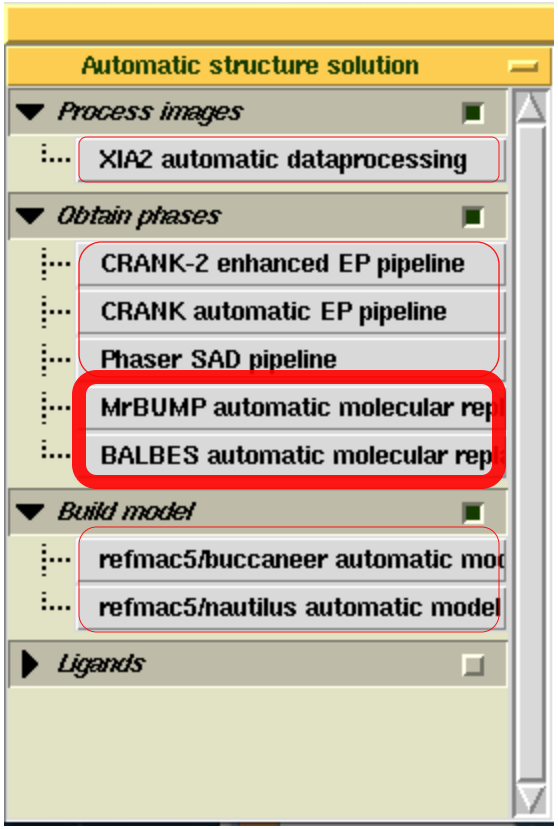


Simple structure solution



Process data with XIA2

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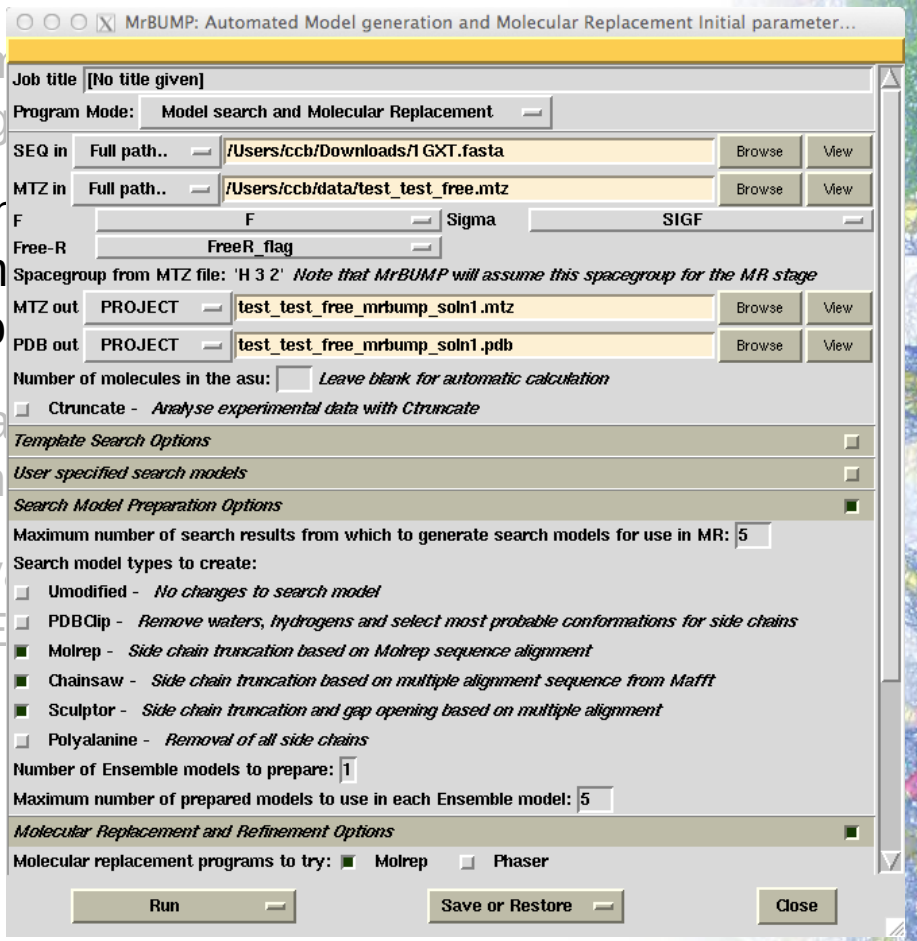
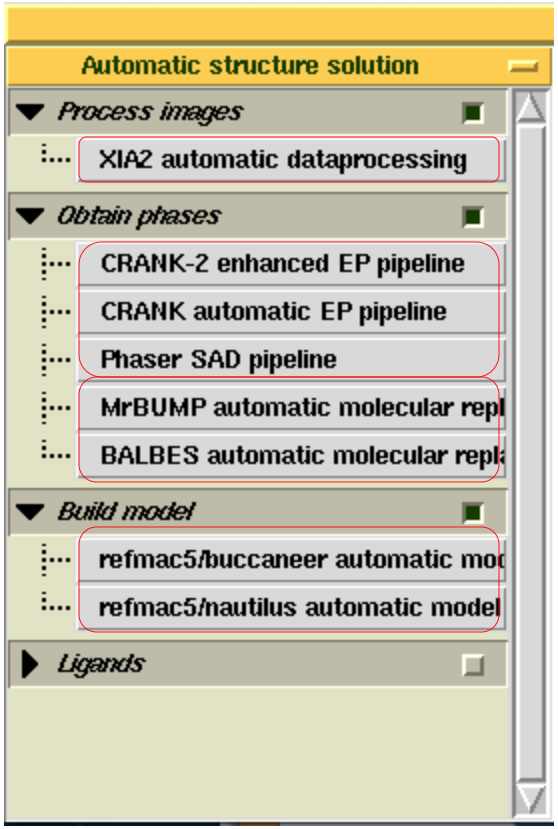


Homology model search, MR and model building using MrBUMP

Simple structure solution



Process data with XIA2

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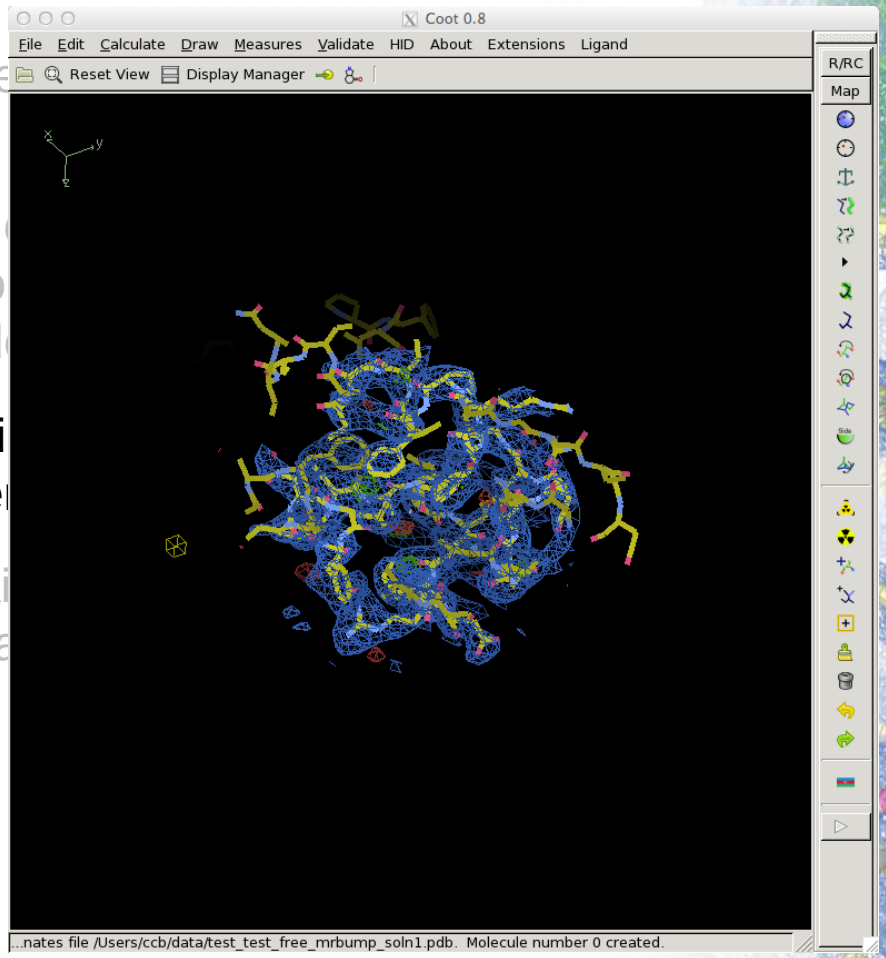
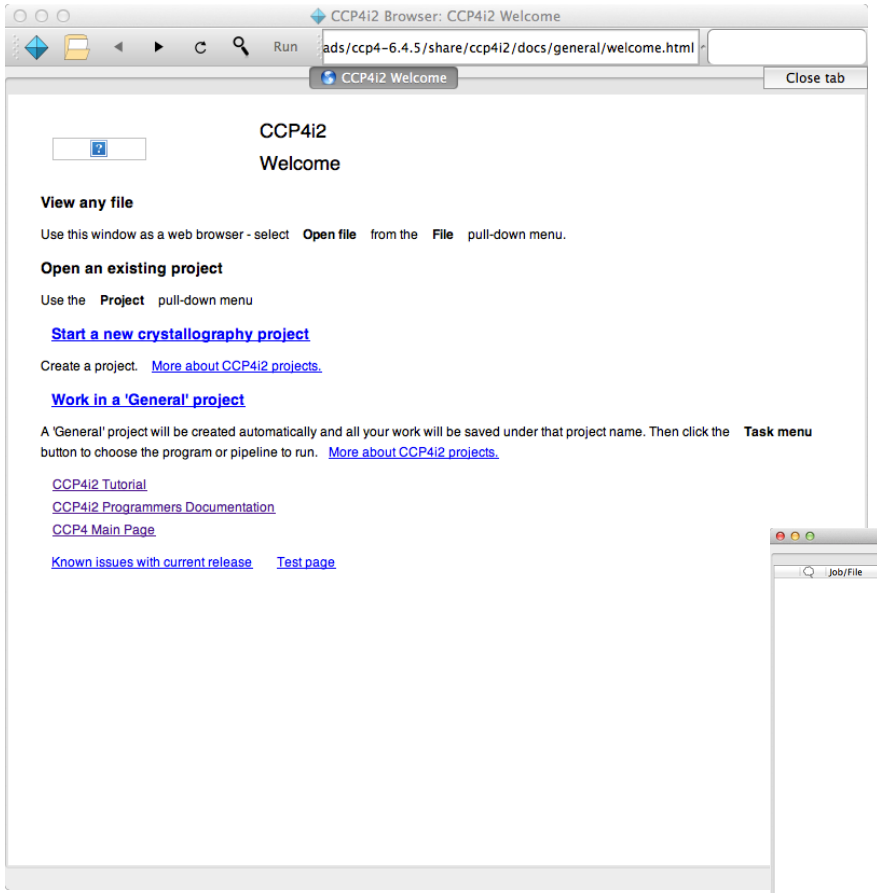


Check solution and rebuild in coot

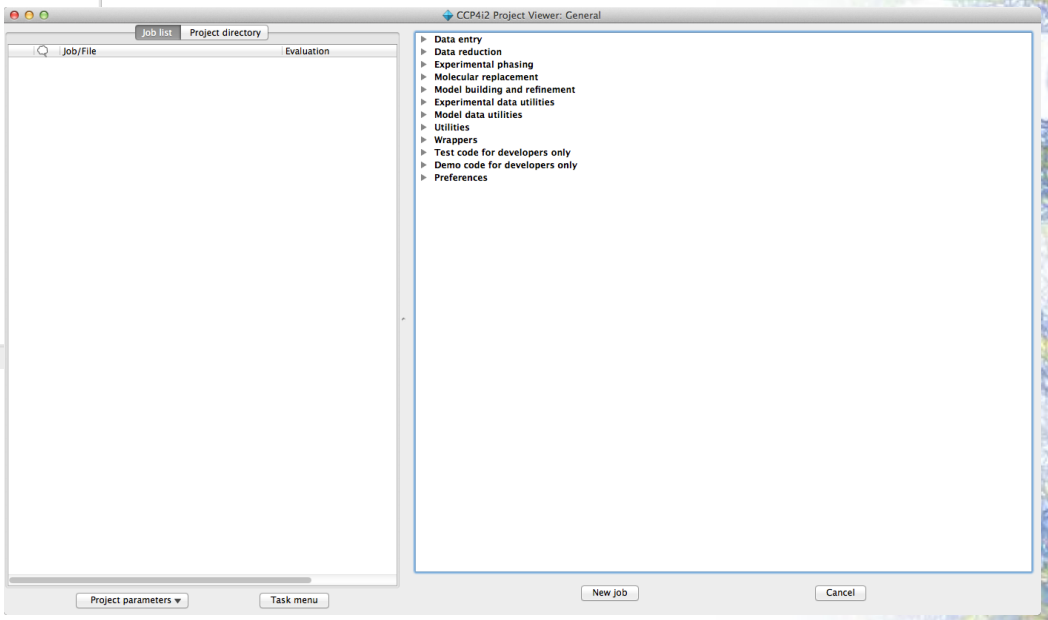
ccp4i2



ccp4i2.app

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

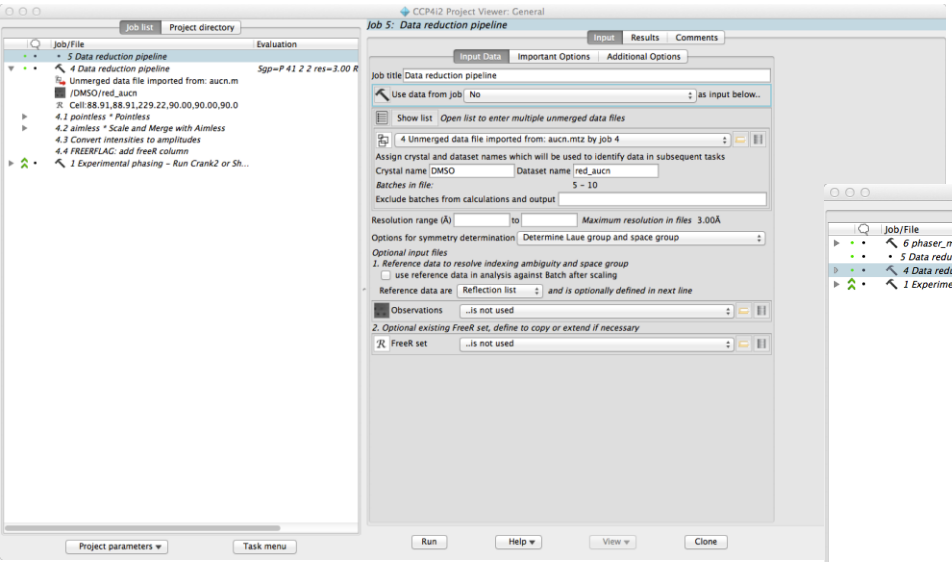
You get the rapidly evolving alpha version of the new CCP4 GUI.

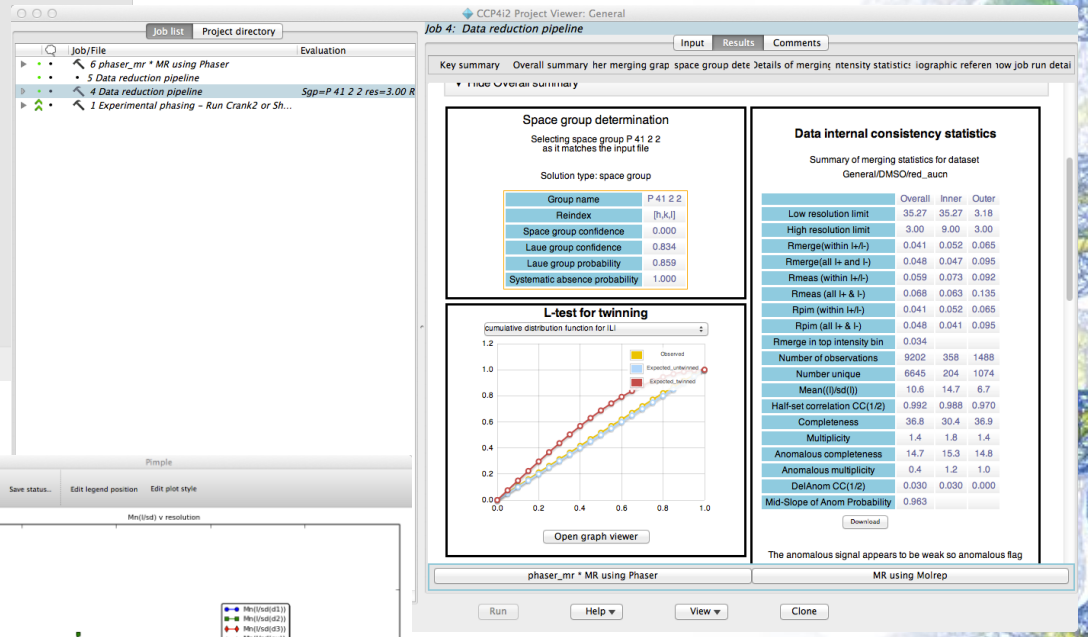


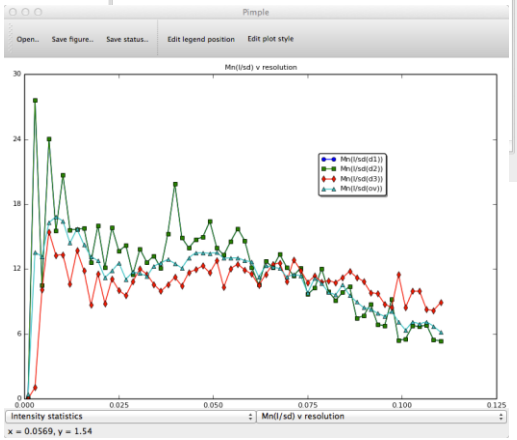
If you have time give it a go.
Note: it is not fully featured and has some tidying to be done.

Unmerged data import

ccp4i2.app







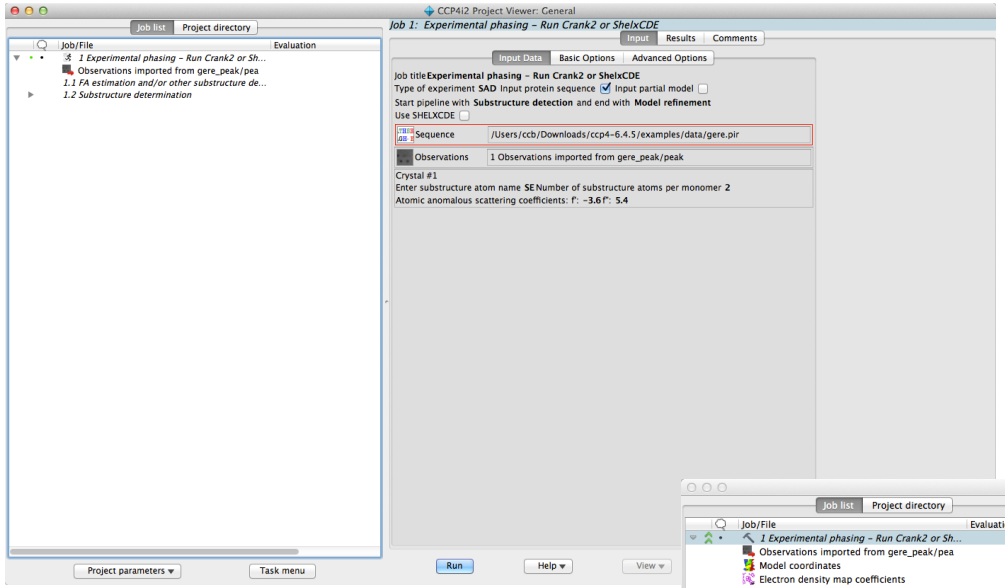
Experimental data scaling and quality checking pipeline.

Table 1 produced.

Suggested next steps.

Experimental phasing in GUI2

ccp4i2.app



Crank2 experimental interface.

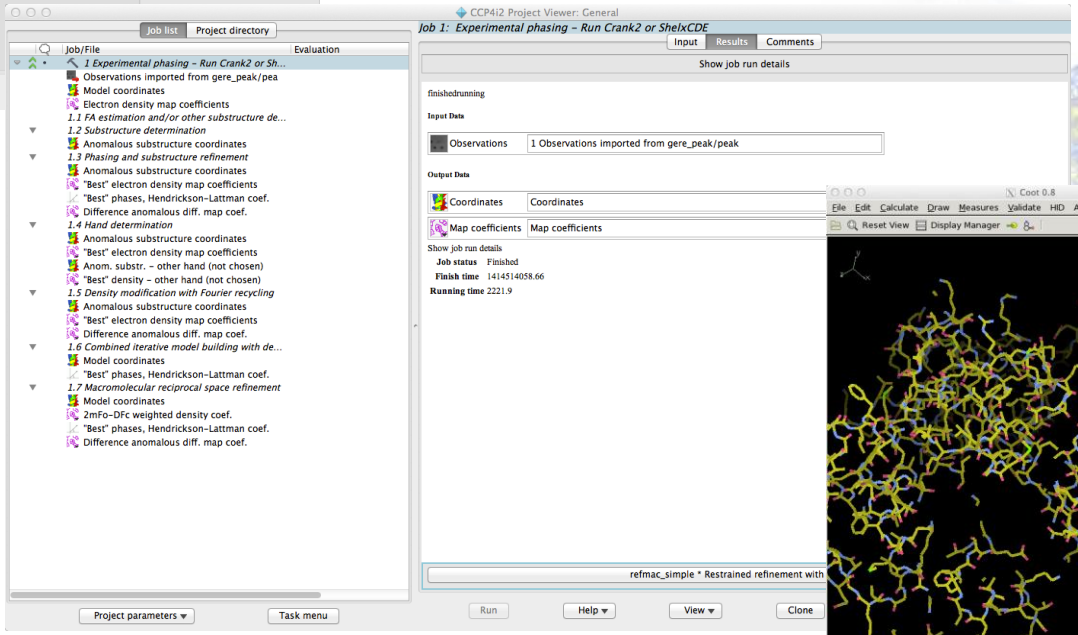
Simplified input.

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 !