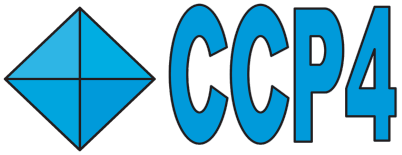
Modelling Ligands with CCP4

DLS-CCP4 Data Collection and Structure Solution Workshop

1st December 2024

Rob Nicholls

robert.nicholls@stfc.ac.uk



Research Complex at Harwell

MRC Laboratory of Molecular Biology

Science and Technology Facilities Council

Introduction

(1) Protein–ligand complexes are important

E.g. used for structure-based drug design

(2) Production of accurate models is vital

Inference of biological conclusions

Calculation of interaction energies & protein-induced strain

(3) Poor quality of many ligands in the PDB is concerning

Acknowledged in the literature in past years (e.g. Cooper et al., 2011)

Introduction

Journal
Expert Opinion on Drug Discovery ›
Volume 6, 2011 - Issue 8

X-ray crystallography: assessment and validation of protein–small molecule complexes for drug discovery

David R Cooper, Przemyslaw J Porebski, Maksymilian Chruszcz & Wladek Minor

Pages 771-782 | Published online: 21 May 2011

Expert opinion: The quality of structures of small molecules in the PDB varies so widely that the databank should not be considered a reliable repository of structural information about these molecules. This is due to the difficulty in identifying and positioning ligands in medium or low resolution macromolecular crystal structures and the immaturity of the available validation tools. The poor quality of small molecule structures in the PDB hinders the derivation of general principles that govern small molecule-protein interactions.

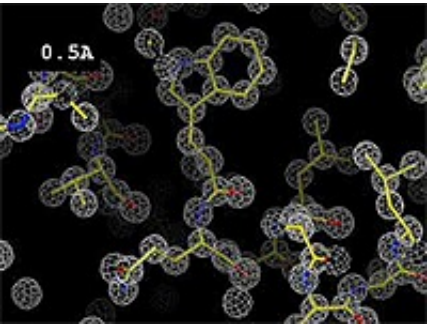
Introduction

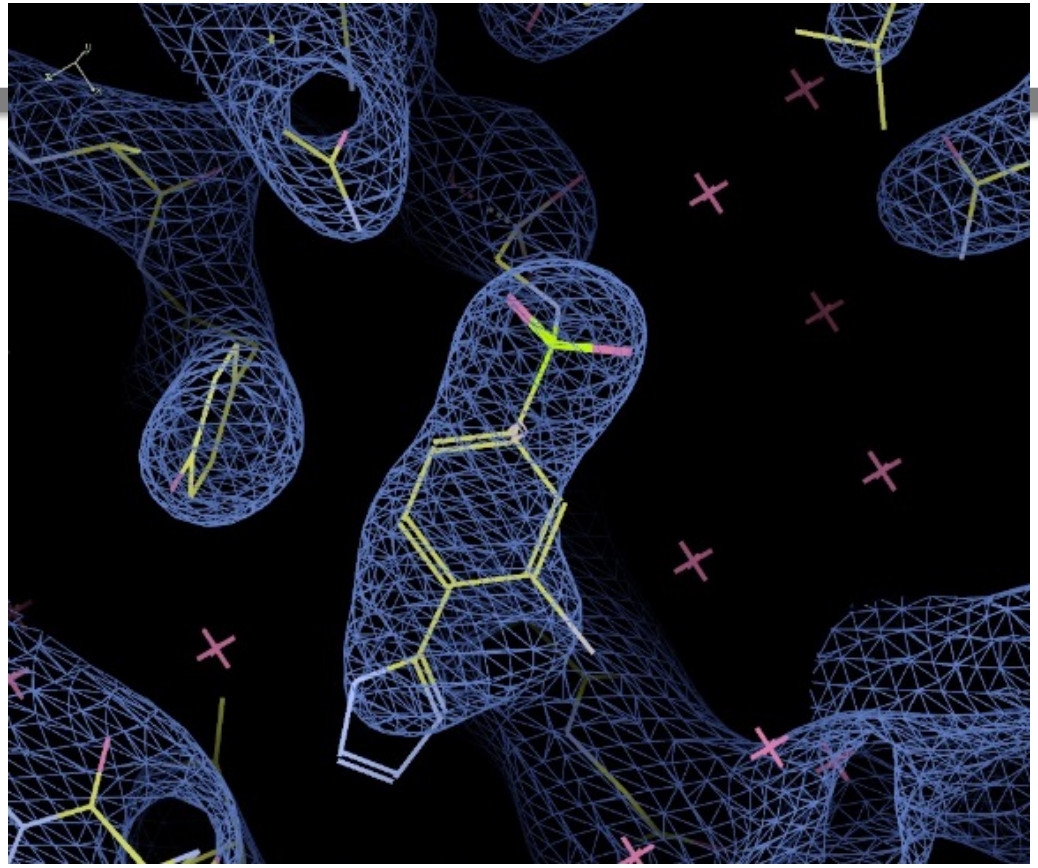
Lead to development of improved software tools & features:

- Ligand description
- Fitting
- Analysis & validation
- Dealing with covalent linkages

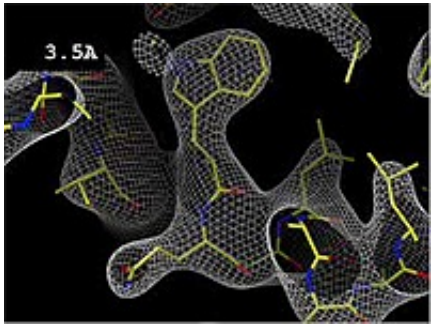
Introduction

1.0Å





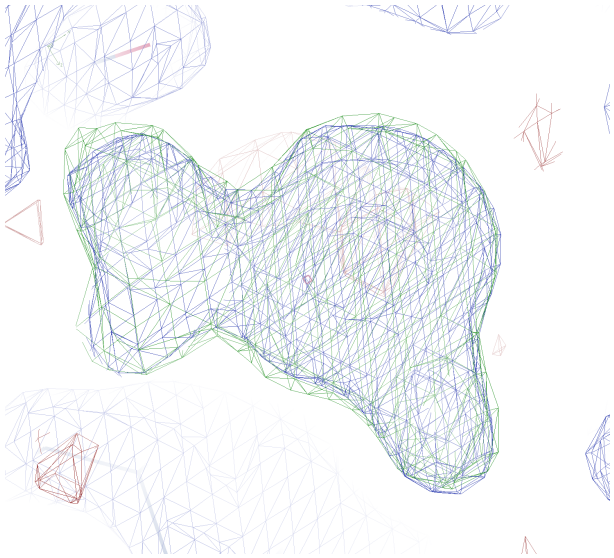
4.0Å



Protein-ligand complex models are often a result of subjective interpretation

Ligand Fitting

1. **Density interpretation**
2. Generation of Ligand Description
3. Conformer generation
4. Ligand Fitting
5. Full-model refinement
6. Validation, analysis & visualisation



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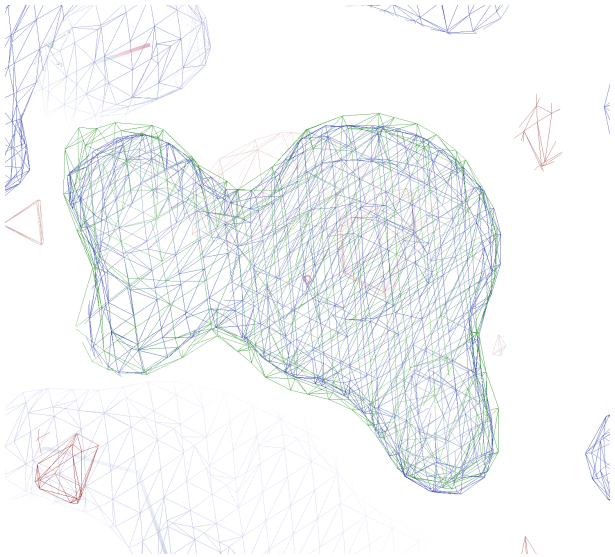
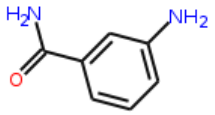


c1cc(cc(c1)N)C(=O)N



Restraints (bonds, angles, torsions, etc.)

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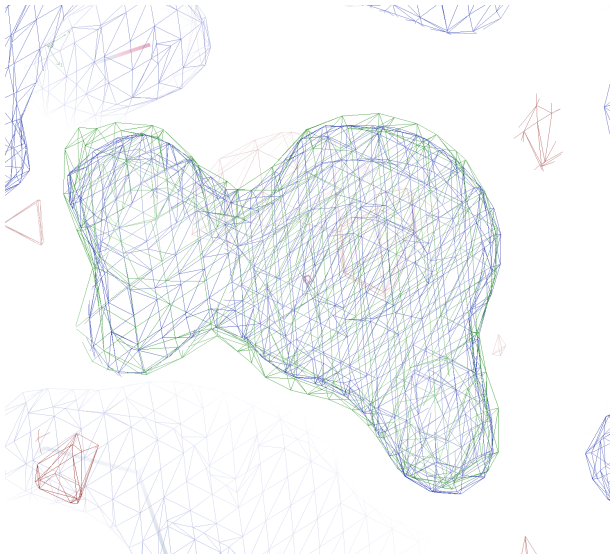

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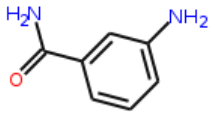

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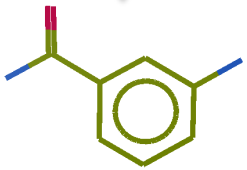

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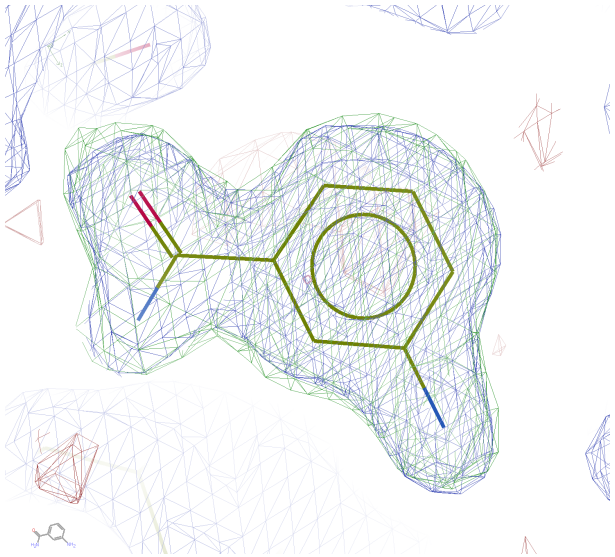

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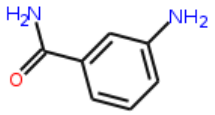

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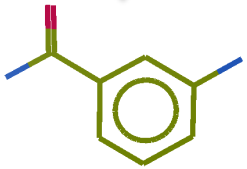

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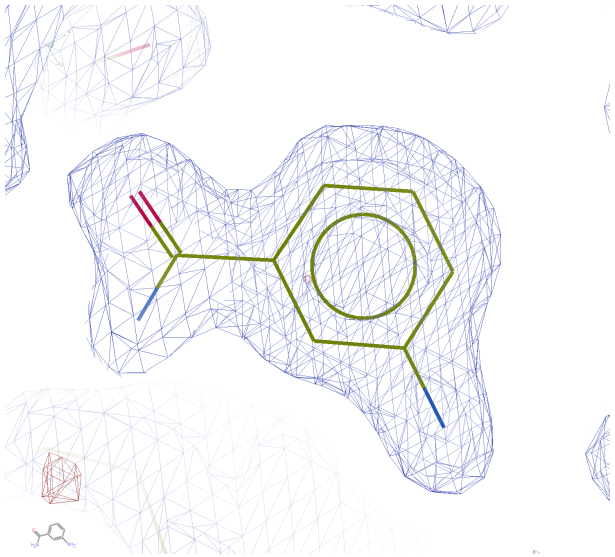

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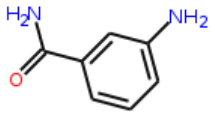

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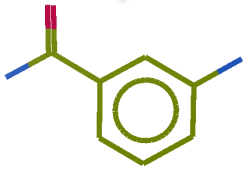

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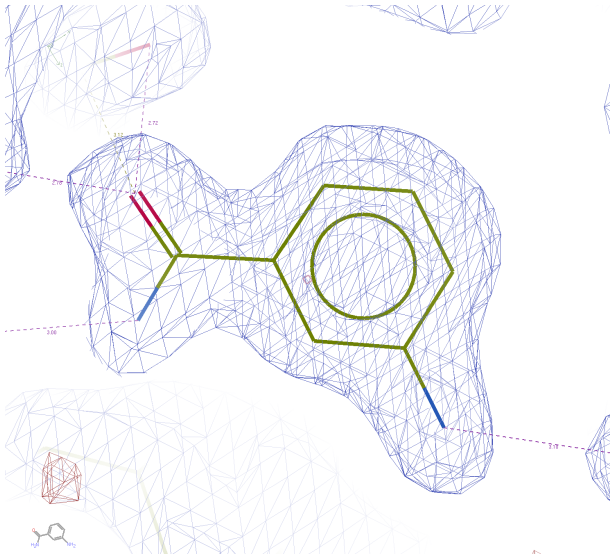

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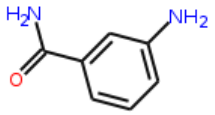

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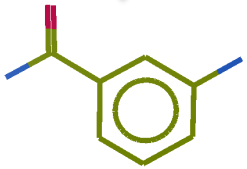

Ligand Fitting in CCP4

Ligand description & conformer generation:

Common ligand: 3-letter code —auto→ CCP4 Monomer Library

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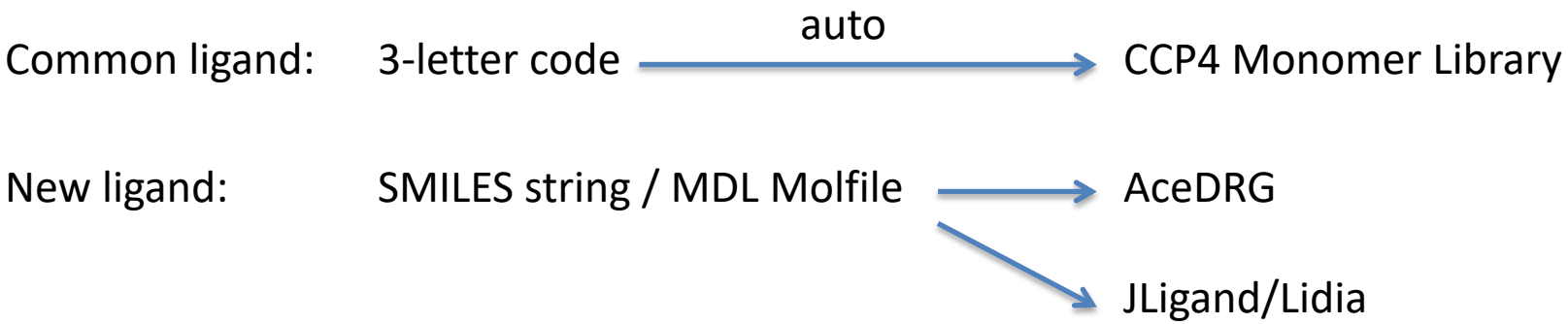
New ligand: SMILES string / MDL Molfile → AceDRG

ACEDRG:

- Generates ligand description
- Generates conformer (RDKit, REFMAC5)

Ligand Fitting in CCP4

Ligand description & conformer generation:



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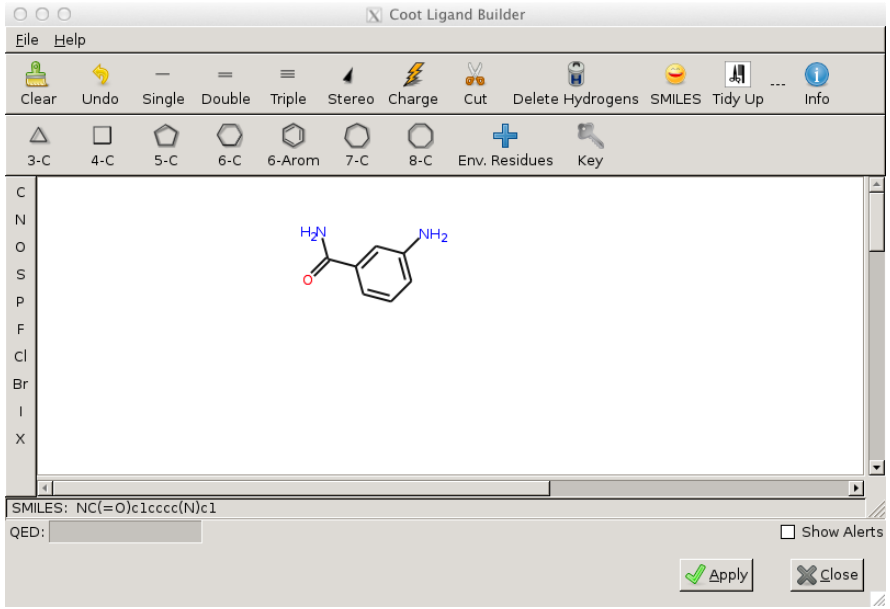
→ JLigand/Lidia

Lidia (Coot Ligand Builder):

- 2D sketcher
- Sbase search
- Coot integration



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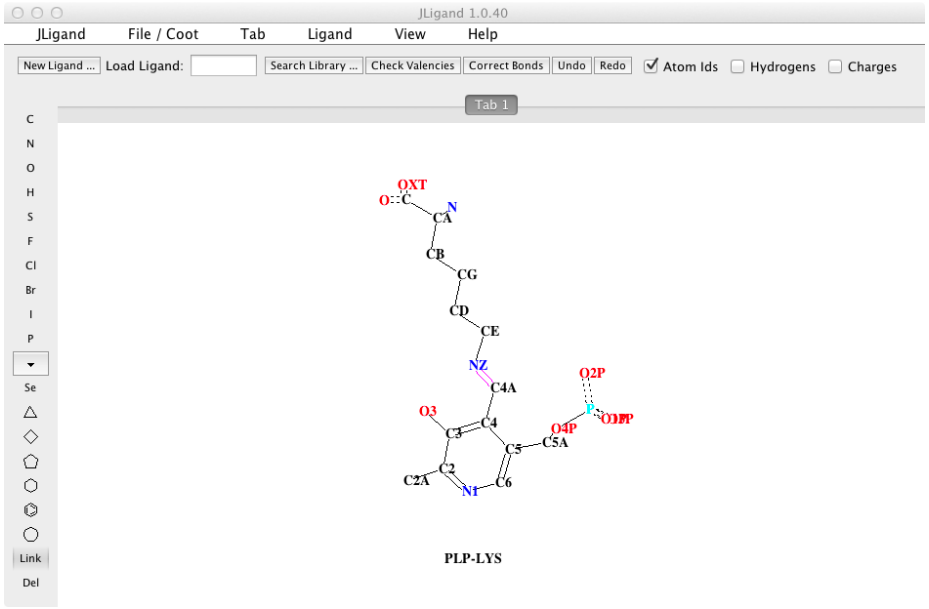
JLigand:

- 3D sketcher
- Regularisation
- Links



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Output: (1) Restraint dictionary (CIF)
(2) Initial coordinates (PDB/mmCIF)

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Ligand fitting: Coot

- Finding the position & pose
- Fitting into the density & local refinement

Full refinement: REFMAC5

Also worth mentioning:

- ARP/wARP – automated model building
- PANDDAs – ligand detection in high-throughput crystallography / drug discovery
- Privateer – automated detection, building & validation of carbohydrates

Ligand Dictionary Generation

Geometric restraints for protein / nucleic acids are pre-tabulated

Ligands are more complicated

Need a source of prior information

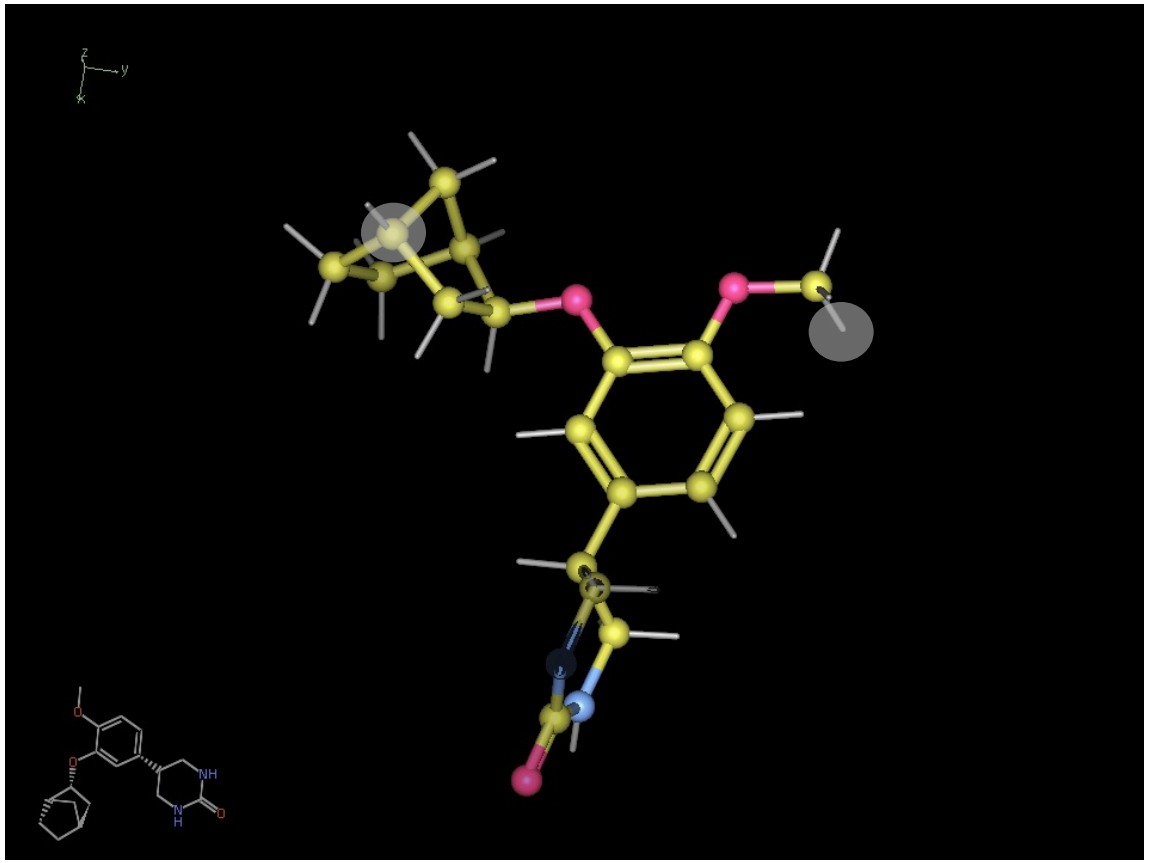
- Common/known structures are dealt with automatically
  - CCP4 Monomer Library has many pre-computed descriptions
- New ligands require a new description (CIF file)
  - AceDRG

How Does AceDRG Work?

Detailed atom types

Full 2nd order neighbour-based atom description

In some cases, 3rd order information is encapsulated also…



H1B: H(CHHO)
C9: C[5,5,6](C[5,5]CHH)(C[5,6]CHH)(C[5,6]CHO)(H)

AceDRG derives atom types from small molecule databases
These are tabulated, distributed as part of CCP4

What does AceDRG Do?

Functionalities:

(1) Restraint Dictionary Generator
- Uses restraint tables to generate restraints for given molecule
- Output – CIF – bond lengths, angles, torsions, planes, chiralities

(2) Conformer Generator
- Generates coordinates from graph-based molecule description
- Generates one of the lower-energy conformations
- Output – PDB/mmCIF

(3) Link Creation
- Creates a description of a link between two components
  - The link itself – bonds, angles, torsions, planes, etc.
  - Modifications to both components
- These may be from the CCP4 monomer library, or custom CIFs
- Output: CIF

Acta Cryst D STRUCTURAL BIOLOGY
ISSN 2059-7983

AceDRG: a stereochemical description generator for ligands

Fei Long,[a] Robert A. Nicholls,[a] Paul Emsley,[a] Saulius Gražulis,[b] Andrius Merkys,[b] Antanas Vaitkus[b] and Garib N. Murshudov[a]*

[a]Structural Studies, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, England, and [b]Institute of Biotechnology, Sauletekio al. 7, LT-10257 Vilnius, Lithuania. *Correspondence e-mail: garib@mrc-lmb.cam.ac.uk

Received 29 September 2016
Accepted 3 January 2017

Other Dictionary Generators?

Acta Cryst D STRUCTURAL BIOLOGY
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Keep it together: restraints in crystallographic refinement of macromolecule–ligand complexes

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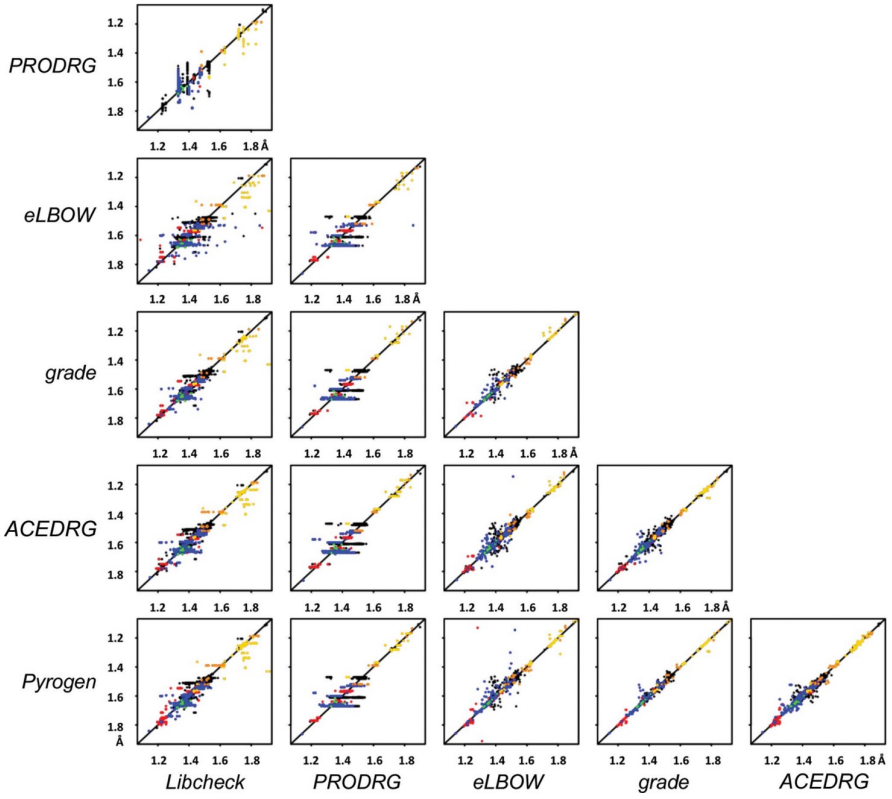
[a]Randall Division of Cell and Molecular Biophysics, King's College London, London SE1 1UL, England, and [b]Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Newcastle University, Framlington Place, Newcastle-upon-Tyne NE2 4HH, England. *Correspondence e-mail: julie.tucker@newcastle.ac.uk, roberto.steiner@kcl.ac.uk

Received 30 September 2016
Accepted 8 November 2016

- Overview of the use of geometry restraints in refinement
- General description of the dictionary generation process
- Comparative analysis of many dictionary generation programs (mainly qualitative)



Other Dictionary Generators?

Two main classes:

Small-molecule database

- AceDRG – COD
- GRADE – CSD
- eLBOW-Mogul – CSD
- Pyrogen – COD/CSD
- …

Quantum Mechanical calculations (semi-empirical – use CSD)

- eLBOW (multiple QM forcefield plugins)
- GRADE
- Corina
- Prodrg2
- …

Other Dictionary Generators?

Current Medicinal Chemistry, 2022, *29*, 1193-1207

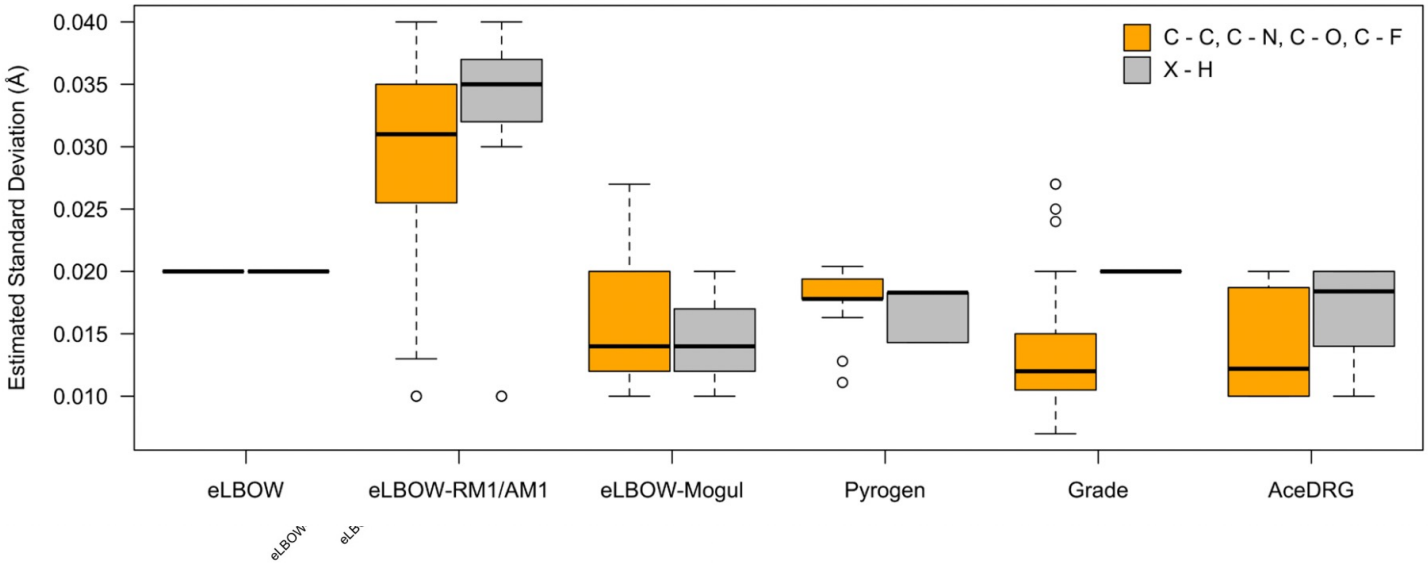
REVIEW ARTICLE

BENTHAM SCIENCE

Towards Consistency in Geometry Restraints for Carbohydrates in the Pyranose form: Modern Dictionary Generators Reviewed

Robbie P. Joosten[1,#], Robert A. Nicholls[2,#] and Jon Agirre[3,*,#]

[1]*Oncode Institute and Division of Biochemistry, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands;* [2]*Structural Studies, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, England;* [3]*York Structural Biology Laboratory, Department of Chemistry, University of York, YO10 5DD, England*



Take away messages:

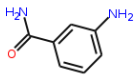
- Small-molecule database methods generally considered the current gold standard
- QM-based methods are a good fallback for unsupported cases (metals)
- Different programs produce qualitatively different dictionaries – take care!

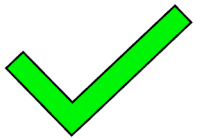
Ligand Fitting with Coot

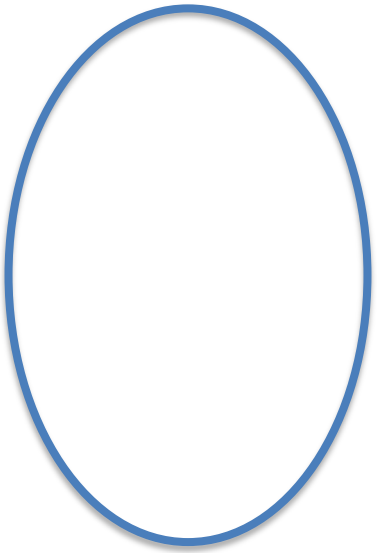
Ligand Fitting Scenarios:

Position known

Ligand type known



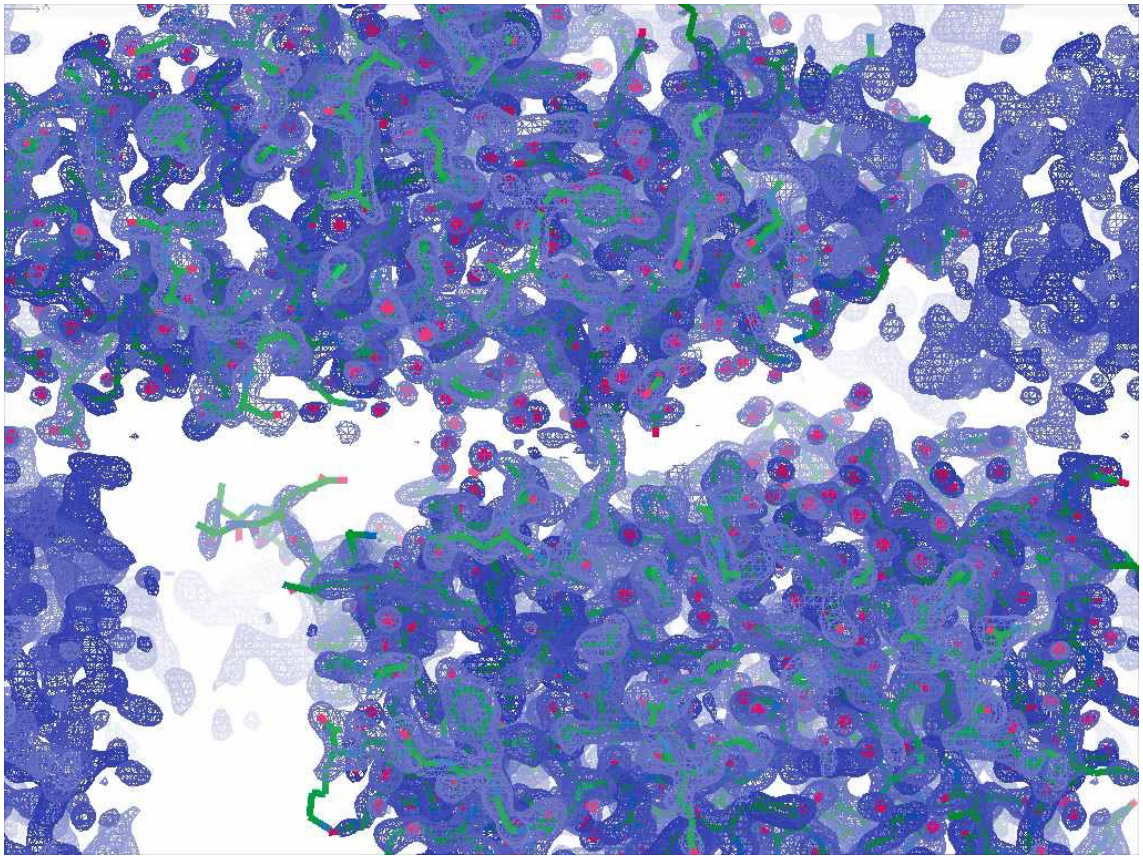




Ligand Fitting with Coot

Finding the ligand position

1. Mask map



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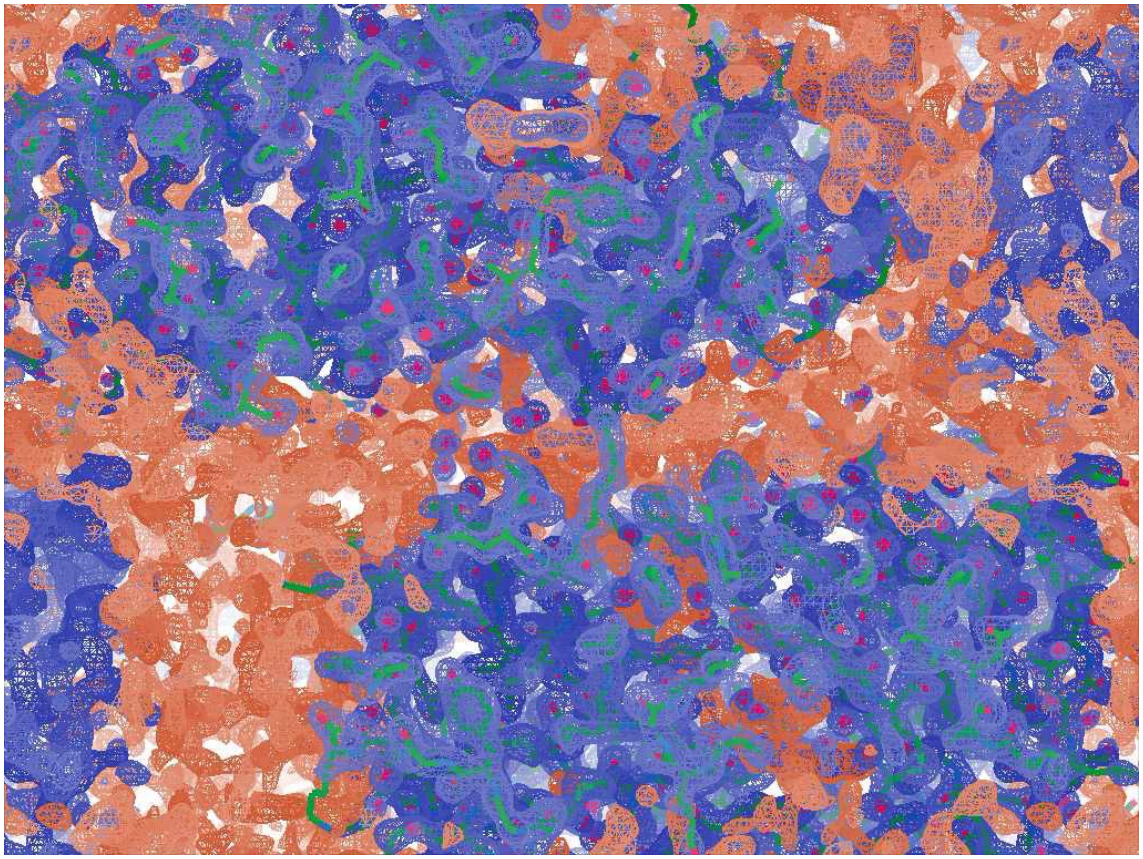

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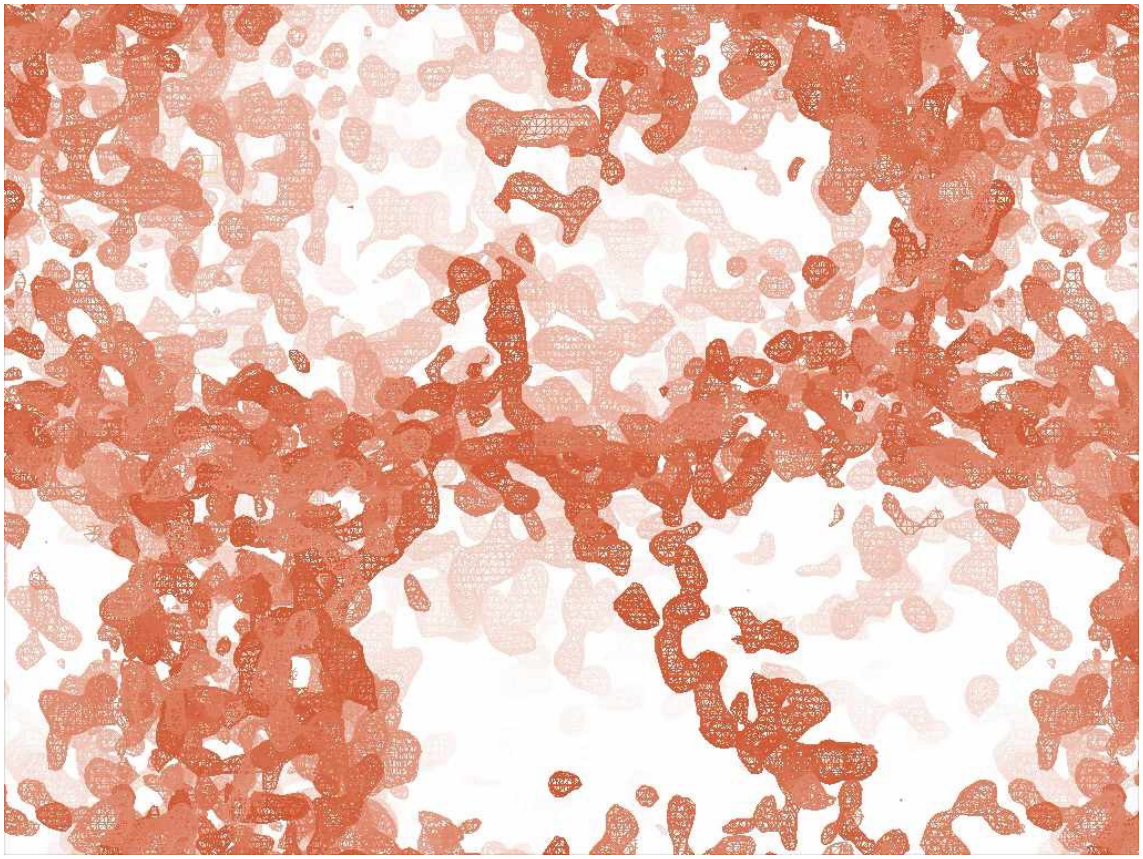
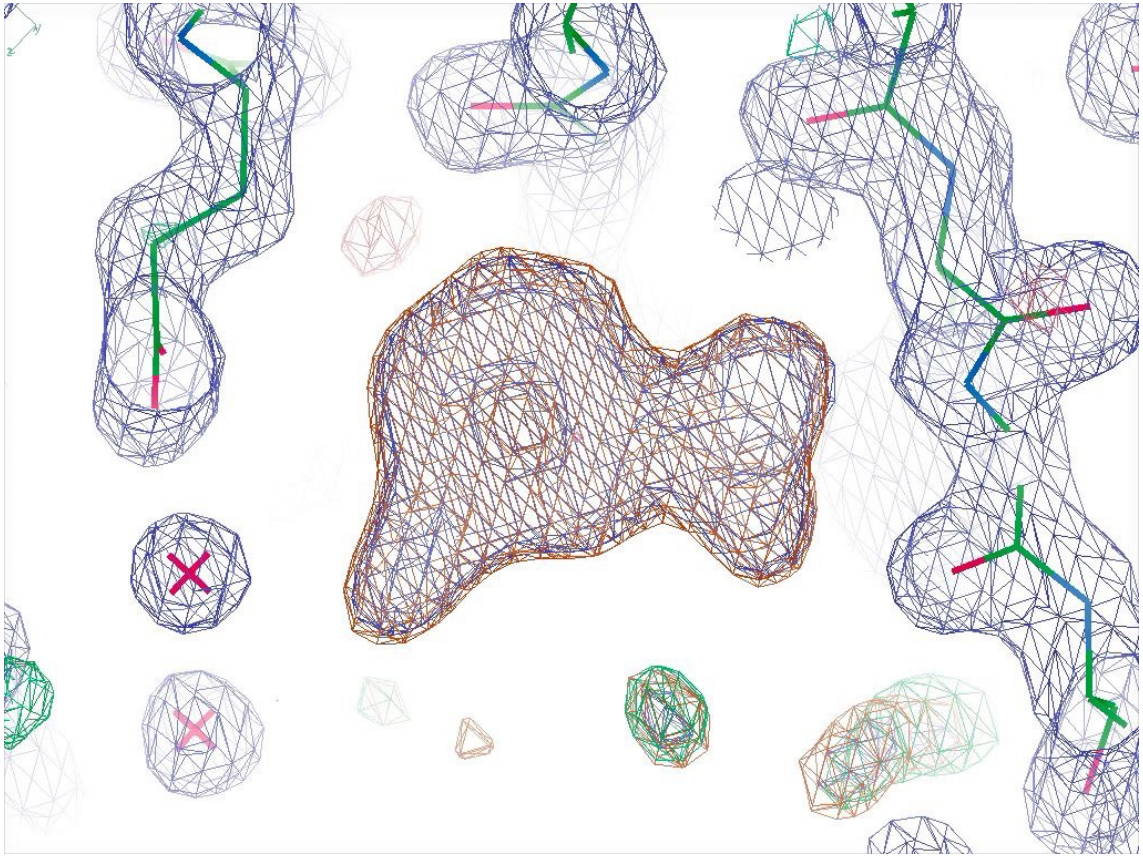


Ligand Fitting with Coot

Finding the ligand position

1. Mask map
2. Find blobs (above threshold)



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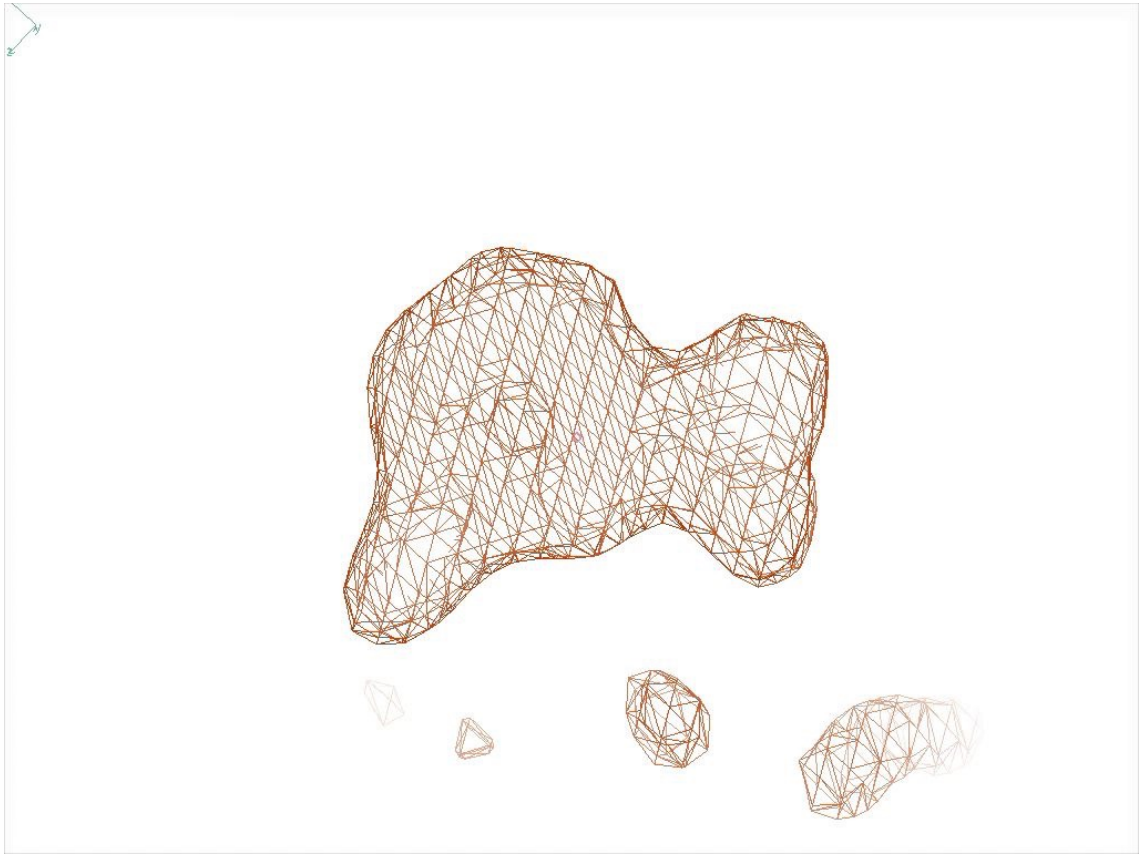
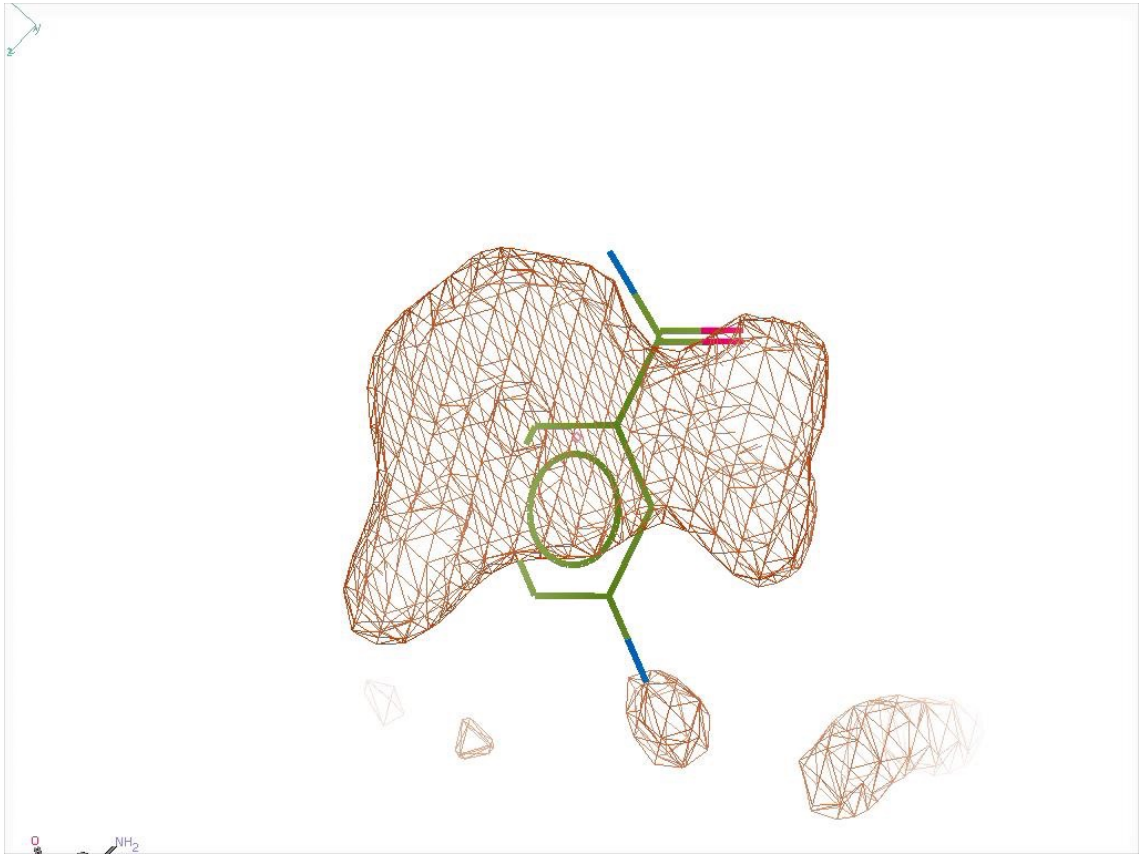


Ligand Fitting with Coot

Finding the ligand position

1. Mask map
2. Find blobs (above threshold)
3. Compare blobs to ligands
    - Match centres



Ligand Fitting with Coot

Finding the ligand position

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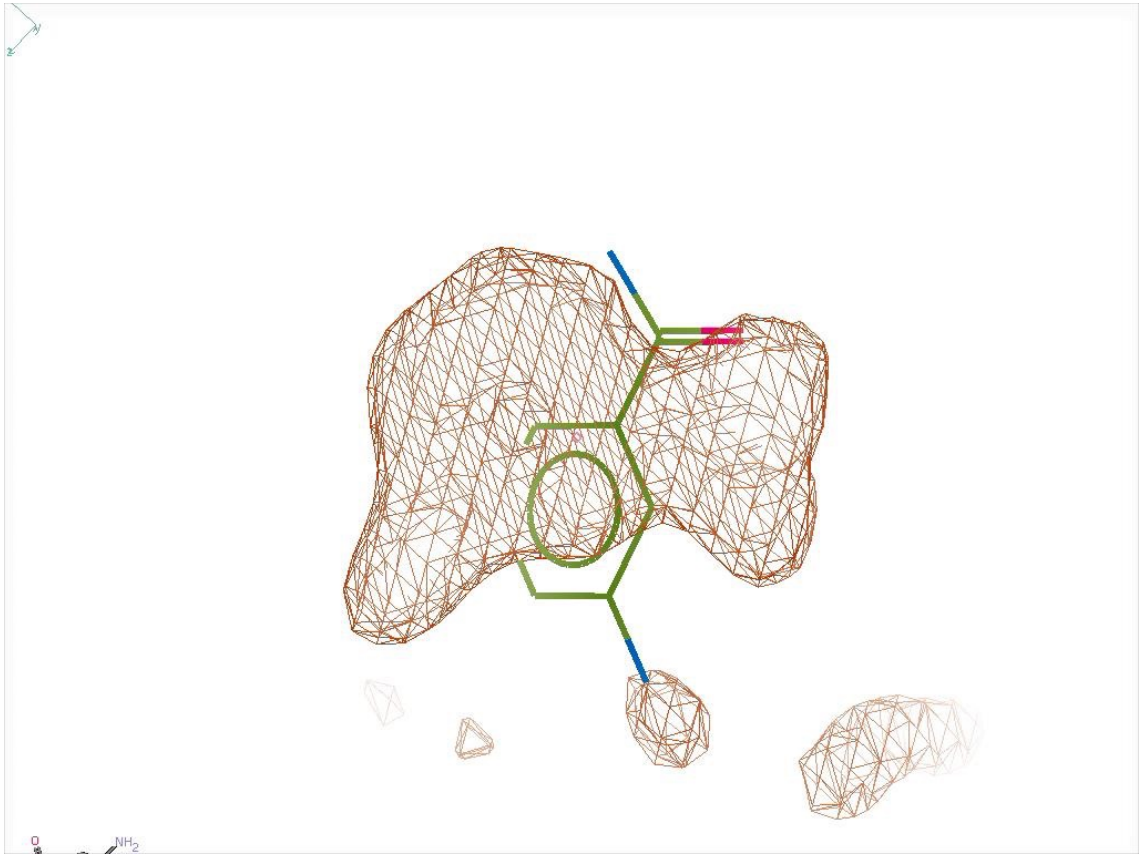
2. Find blobs (above threshold)
3. Compare blobs to ligands
    - Match centres
    - Orient optimally



Ligand Fitting with Coot

Finding the ligand position

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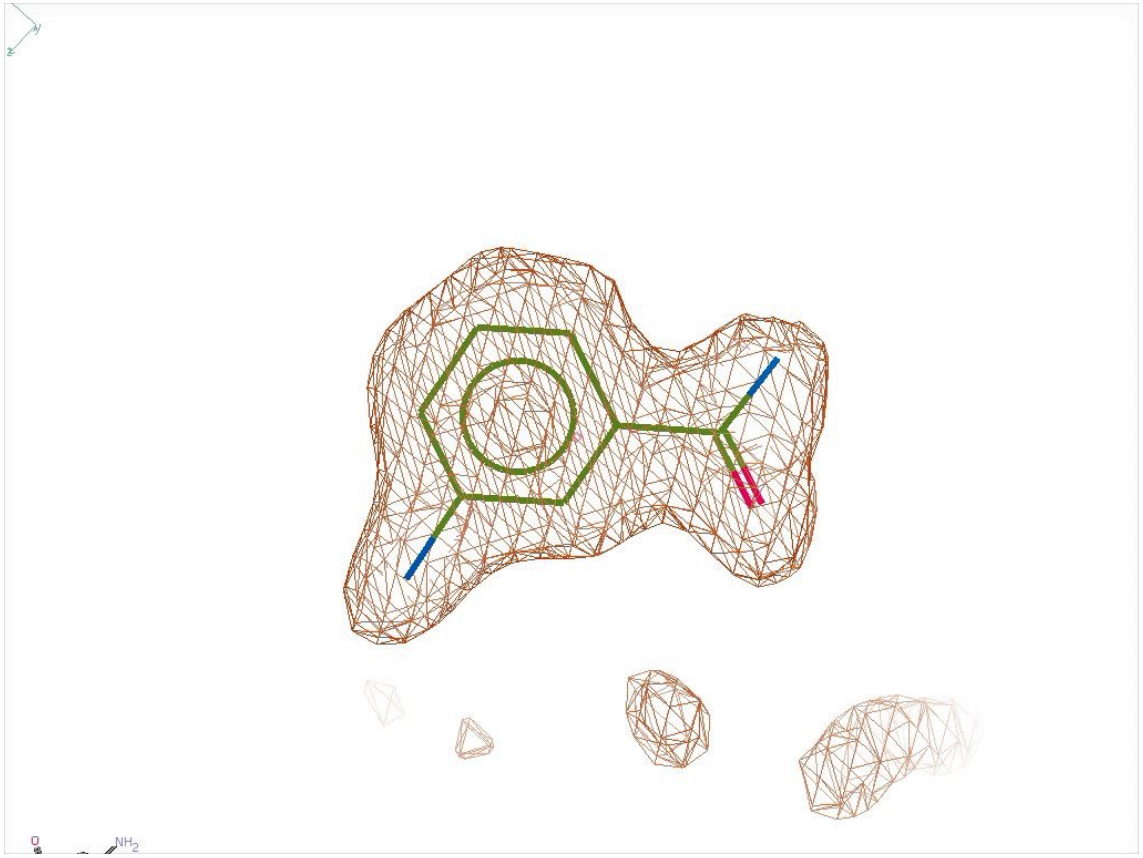
    - Match centres
    - Orient optimally
    - Rigid-body refine



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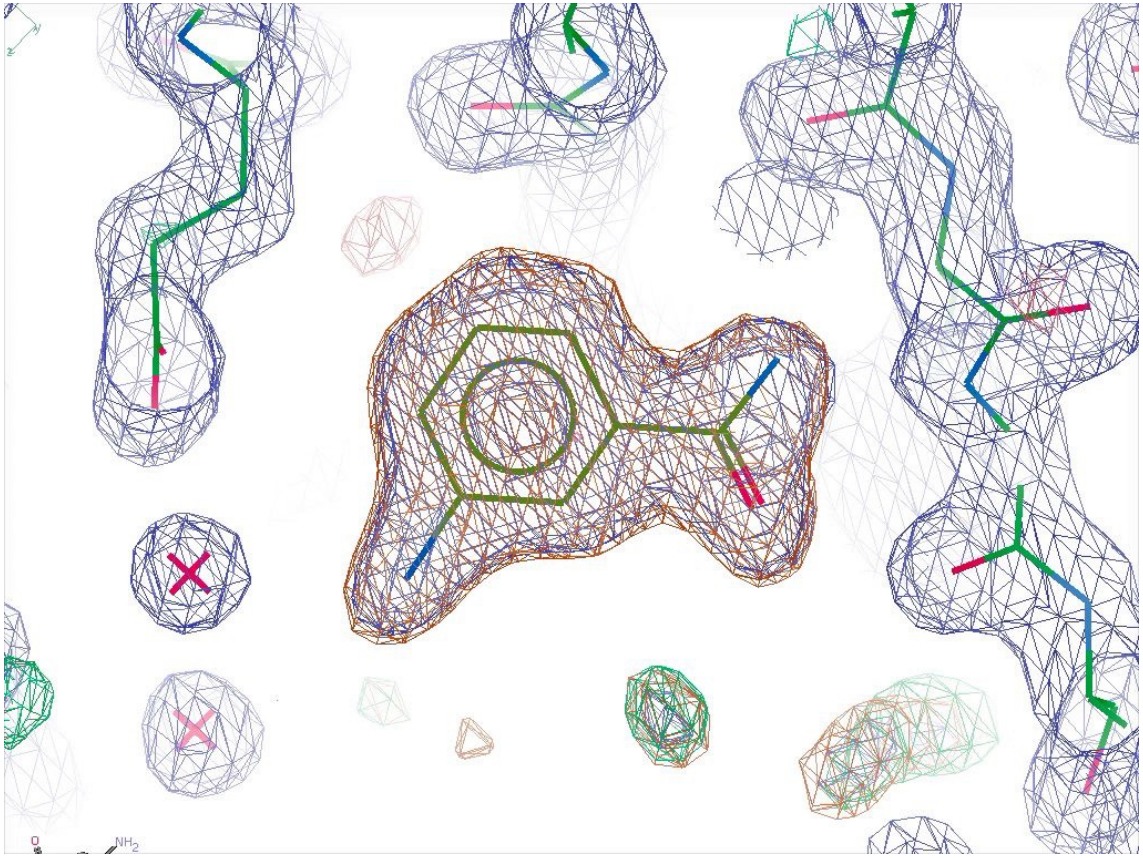
    - Orient optimally
    - Rigid-body refine



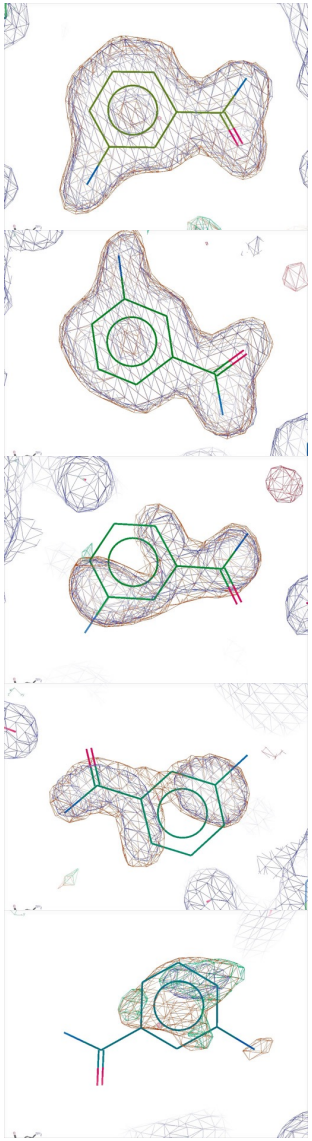
Ligand Fitting with Coot

Finding the ligand position

1. Mask map
2. Find blobs (above threshold)
3. Compare blobs to ligands
    - Match centres
    - Orient optimally
    - Rigid-body refine
4. Score & rank

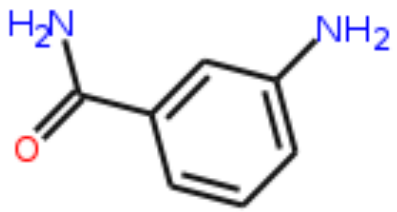
Ligands with rotatable bonds

are sampled in many conformations



Ligand Fitting with CCP4

Example: 3-amino-benzamide



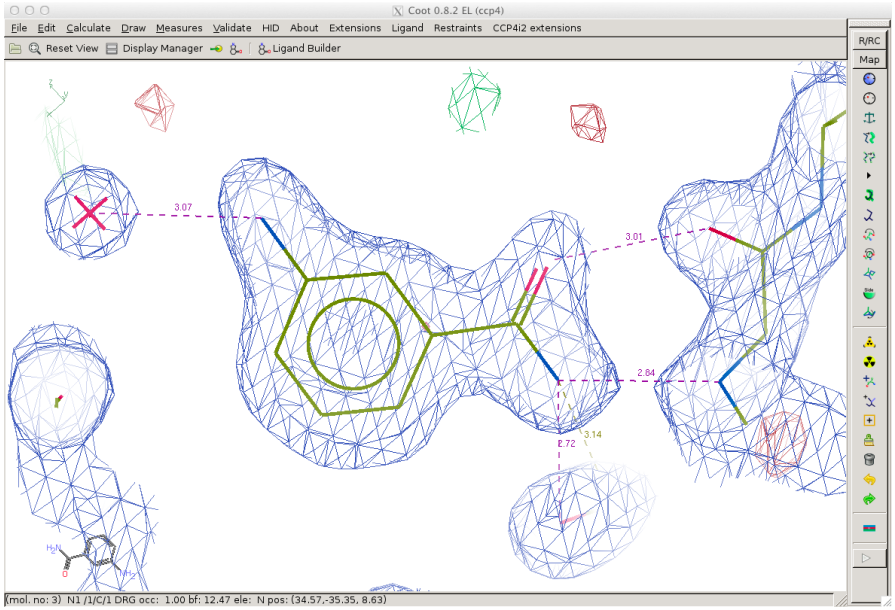
We have:

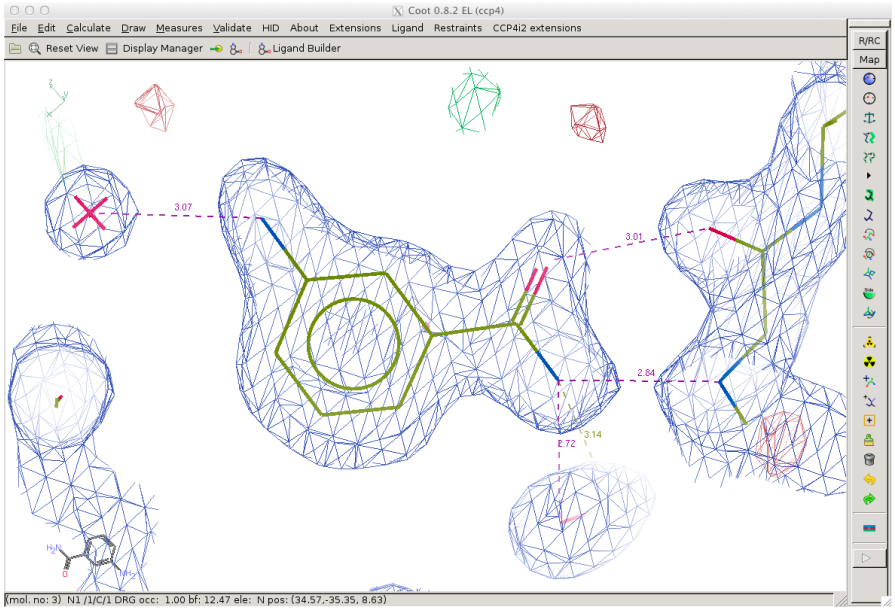
- Refined model & data (PDB & MTZ)
- Ligand SMILES string: c1cc(cc(c1)N)C(=O)N

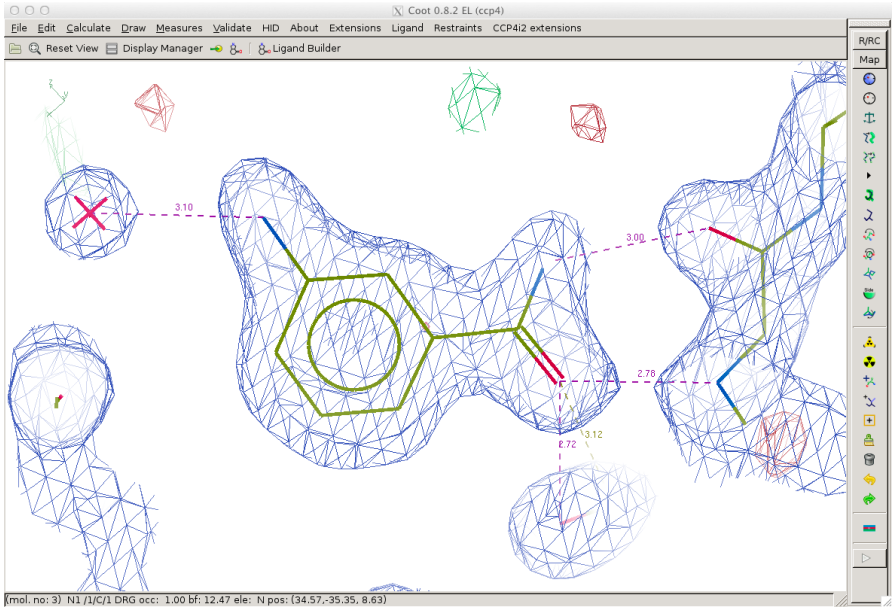
We want to:

- Use AceDRG to generate ligand dictionary & initial conformer
- Find sites for the ligand using Coot
- Fit & refine ligand using Coot & REFMAC5

PDB code: 3kcz (2Å)


Coot 0.8.2 EL (ccp4)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints CCP4i2 extensions
Reset View
Display Manager
Ligand Builder
R/RC
Map
3.07
3.01
2.84
2.72
3.14
(mol. no: 3) N1 /1/C/1 DRG occ: 1.00 bf: 12.47 ele: N pos: (34.57,-35.35, 8.63)


Coot 0.8.2 EL (ccp4)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints CCP4i2 extensions
Reset View
Display Manager
Ligand Builder
R/RC
Map
3.07
3.01
2.84
2.72
3.14
(mol. no: 3) N1 /1/C/1 DRG occ: 1.00 bf: 12.47 ele: N pos: (34.57,-35.35, 8.63)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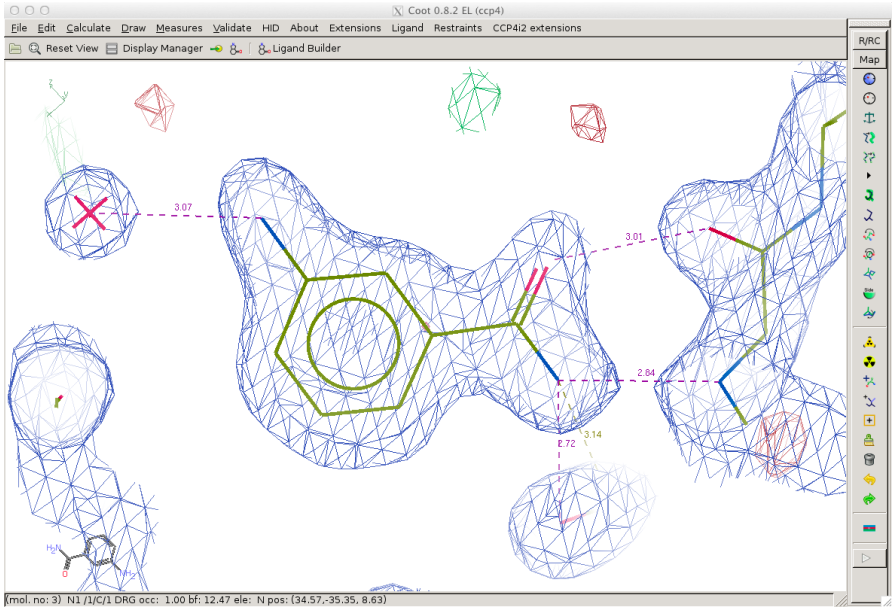
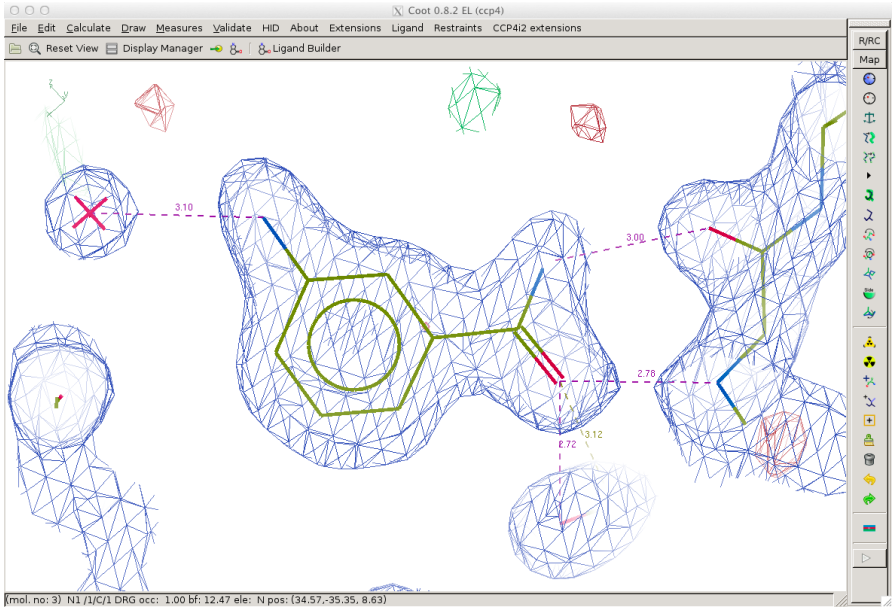
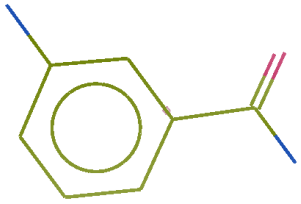
Reset View
Display Manager
Ligand Builder
R/RC
Map
3.10
3.00
2.78
2.72
3.12
(mol. no: 3) N1 /1/C/1 DRG occ: 1.00 bf: 12.47 ele: N pos: (34.57,-35.35, 8.63)

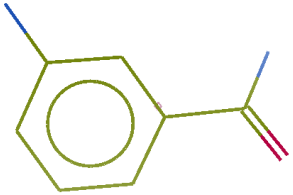






B=17.7

B=12.5



B=15.2

B=13.8

Ligand Fitting with CCP4

research papers

STRUCTURAL BIOLOGY

ISSN 2059-7983

Ligand fitting with *CCP*4

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Received 30 September 2016
Accepted 19 December 2016

Keywords: *CCP*4; *Coot*; ligand fitting; model building.

Crystal structures of protein–ligand complexes are often used to infer biology and inform structure-based drug discovery. Hence, it is important to build accurate, reliable models of ligands that give confidence in the interpretation of the respective protein–ligand complex. This paper discusses key stages in the ligand-fitting process, including ligand binding-site identification, ligand description and conformer generation, ligand fitting, refinement and subsequent validation. The *CCP*4 suite contains a number of software tools that facilitate

Ligand Validation in Coot

research papers

Acta Cryst D STRUCTURAL BIOLOGY

ISSN 2059-7983

Tools for ligand validation in *Coot*

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Received 30 September 2016
Accepted 1 March 2017

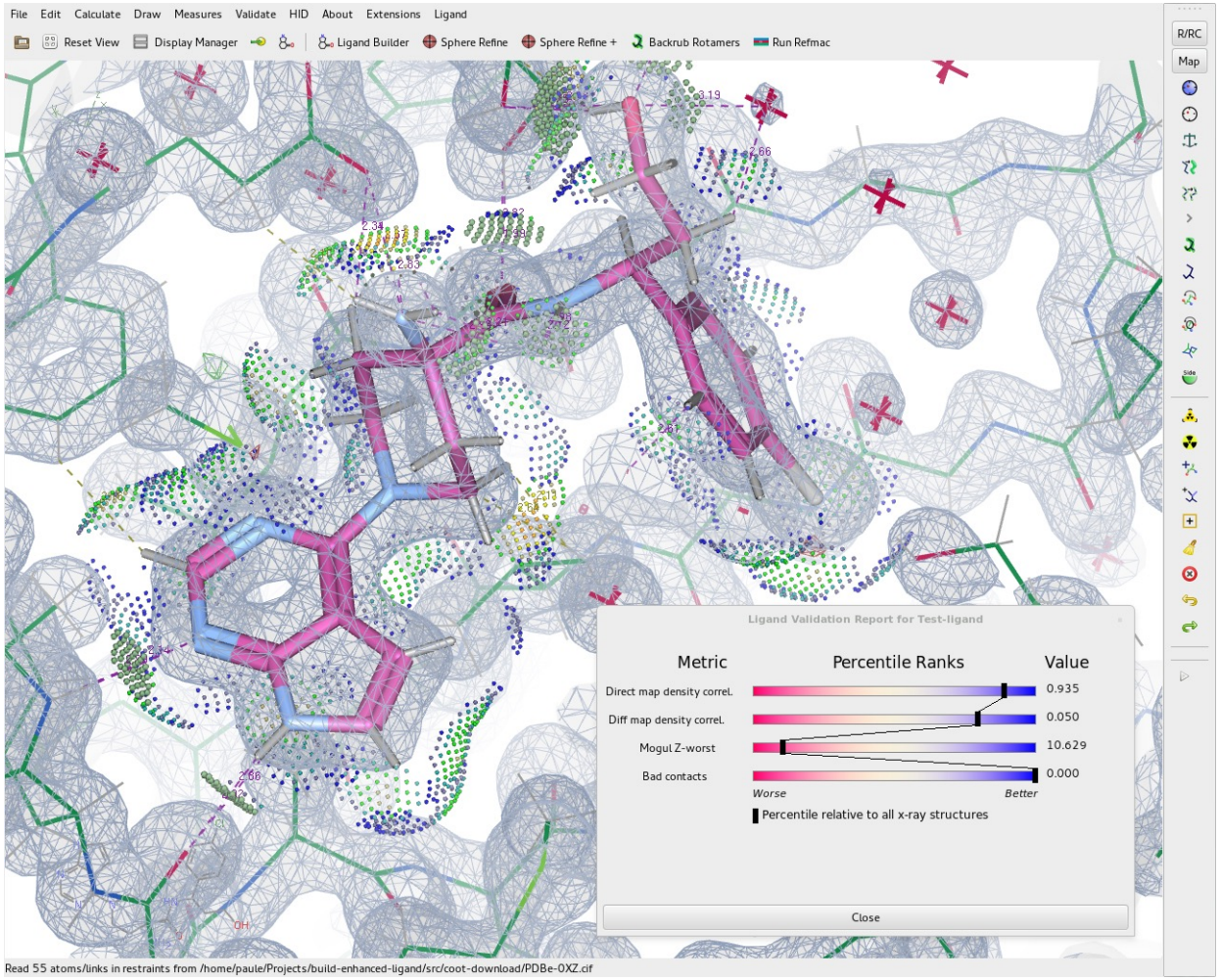
Keywords: *Coot*; ligand validation; model building; ligand representation; ligand manipulation.

Coot is a molecular-graphics program primarily aimed at model building using X-ray data. Recently, tools for the manipulation and representation of ligands have been introduced. Here, these new tools for ligand validation and comparison are described. Ligands in the wwPDB have been scored by density-fit, distortion and atom-clash metrics. The distributions of these scores can be used to assess the relative merits of the particular ligand in the protein–ligand complex of interest by means of 'sliders' akin to those now available for each accession code on the wwPDB websites.

- Density correlation
- Difference map correlation
- Worst Mogul Z-score
- Ligand clashes (MolProbity Probe / Coot dots)

Ligand Validation in Coot

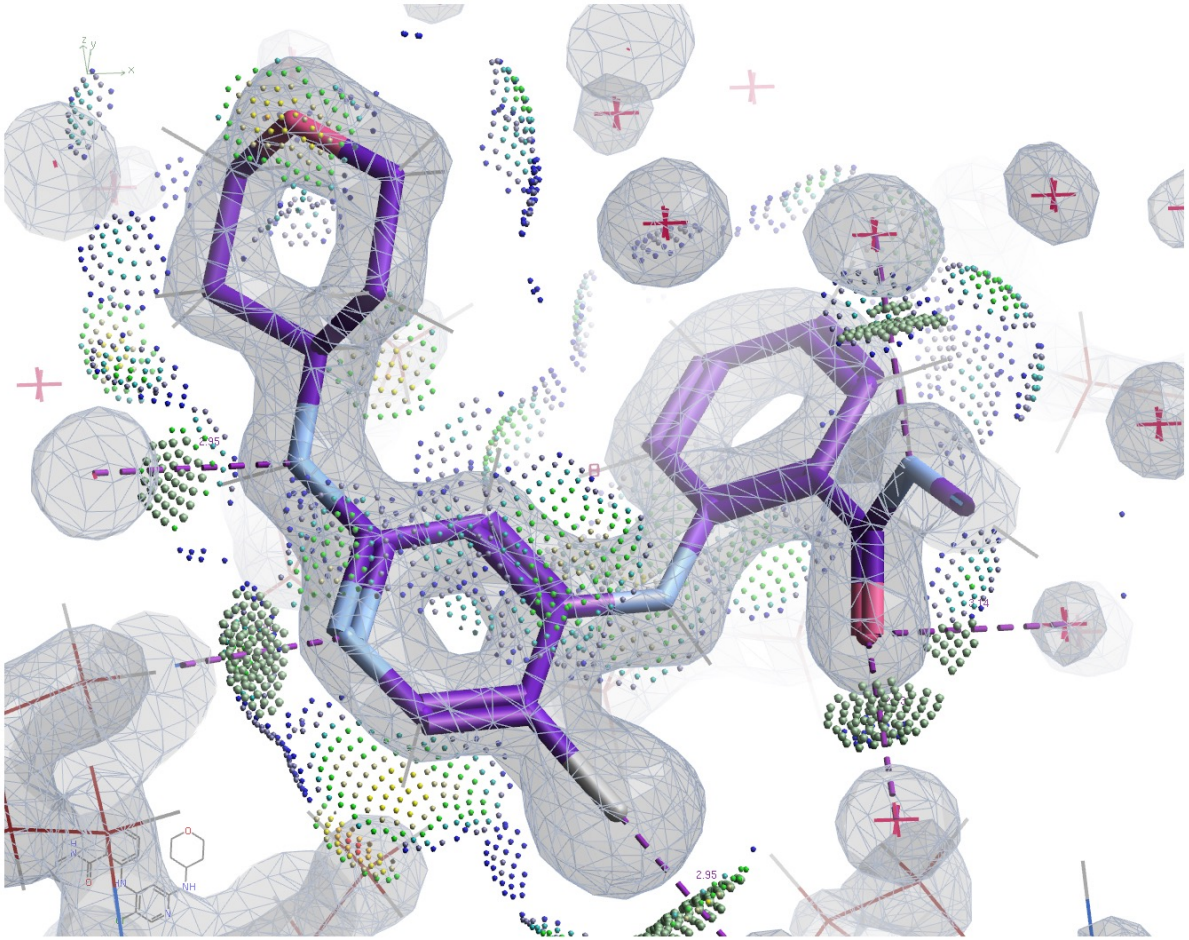
Ligand menu -> Ligand validation sliders



Ligand Validation in Coot

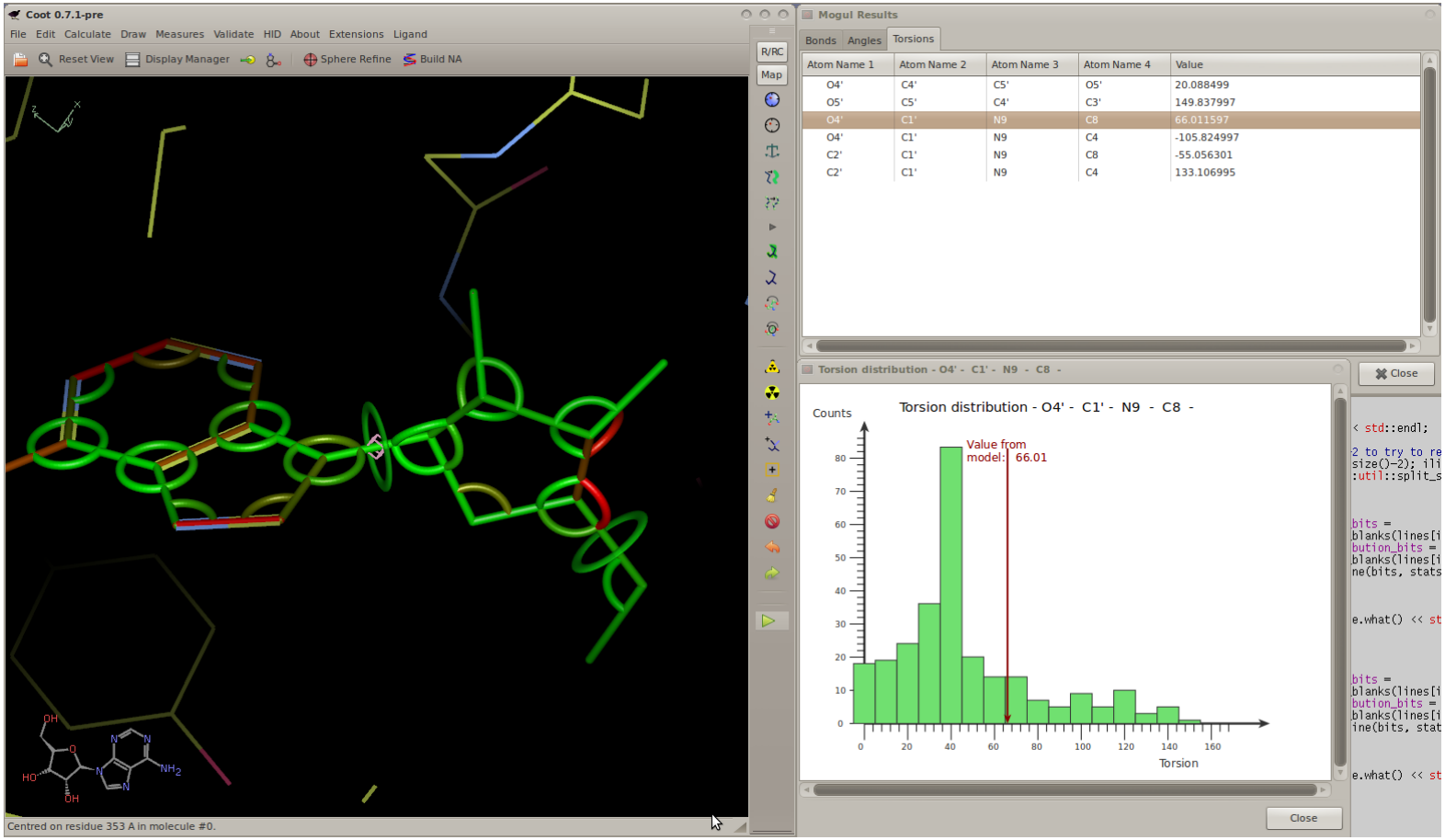
Ligand menu -> Isolated MolProbity dots for this ligand

-> Contact dots for this ligand



Ligand Validation in Coot

Ligand menu -> Display ligand distortions

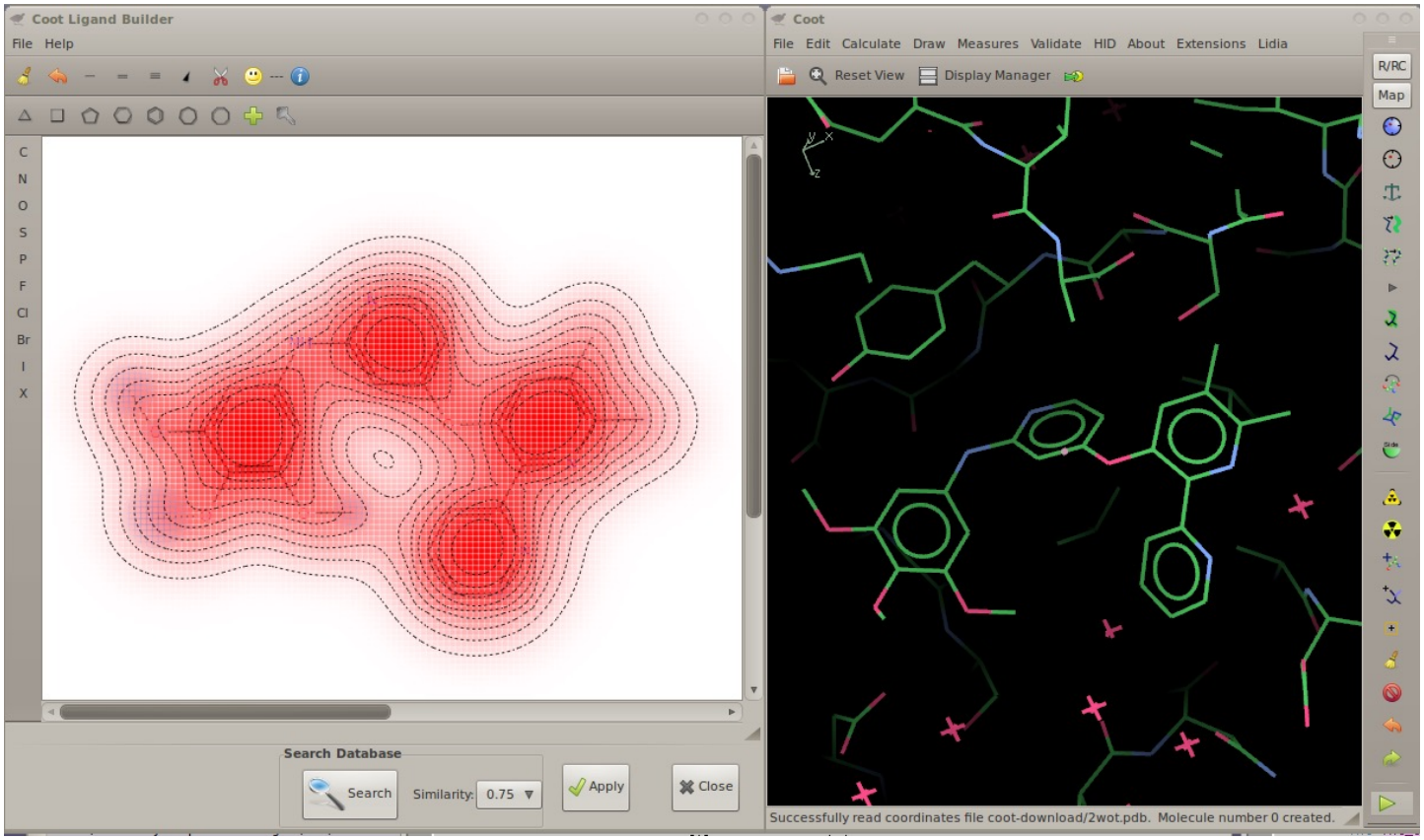


(the full-featured histogram requires a local Mogul installation)

Ligand Validation in Coot

Flatland Ligand Environment View

Ligand menu -> FLEV this residue

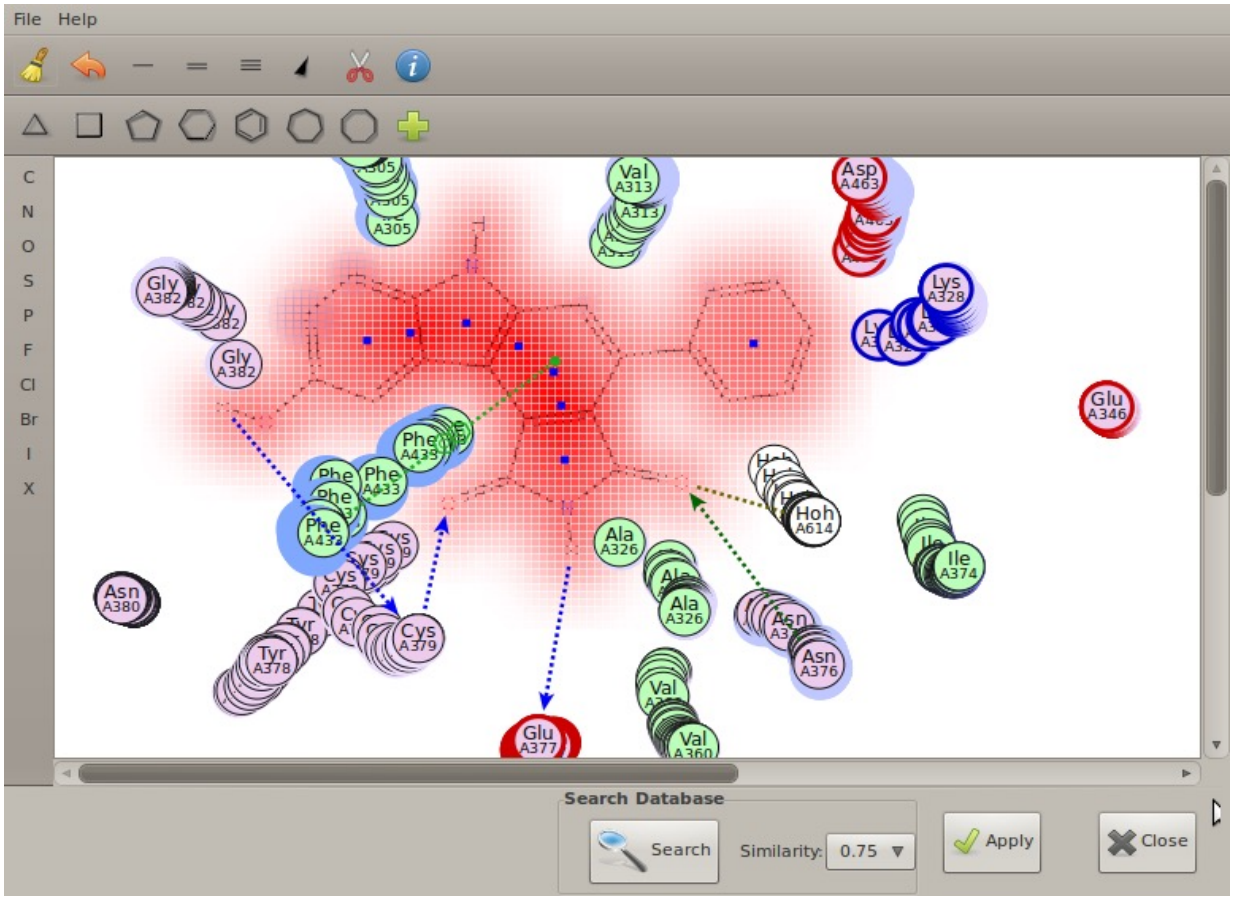


Don't overlap the ligand!!!

Ligand Validation in Coot

Flatland Ligand Environment View

Ligand menu -> FLEV this residue

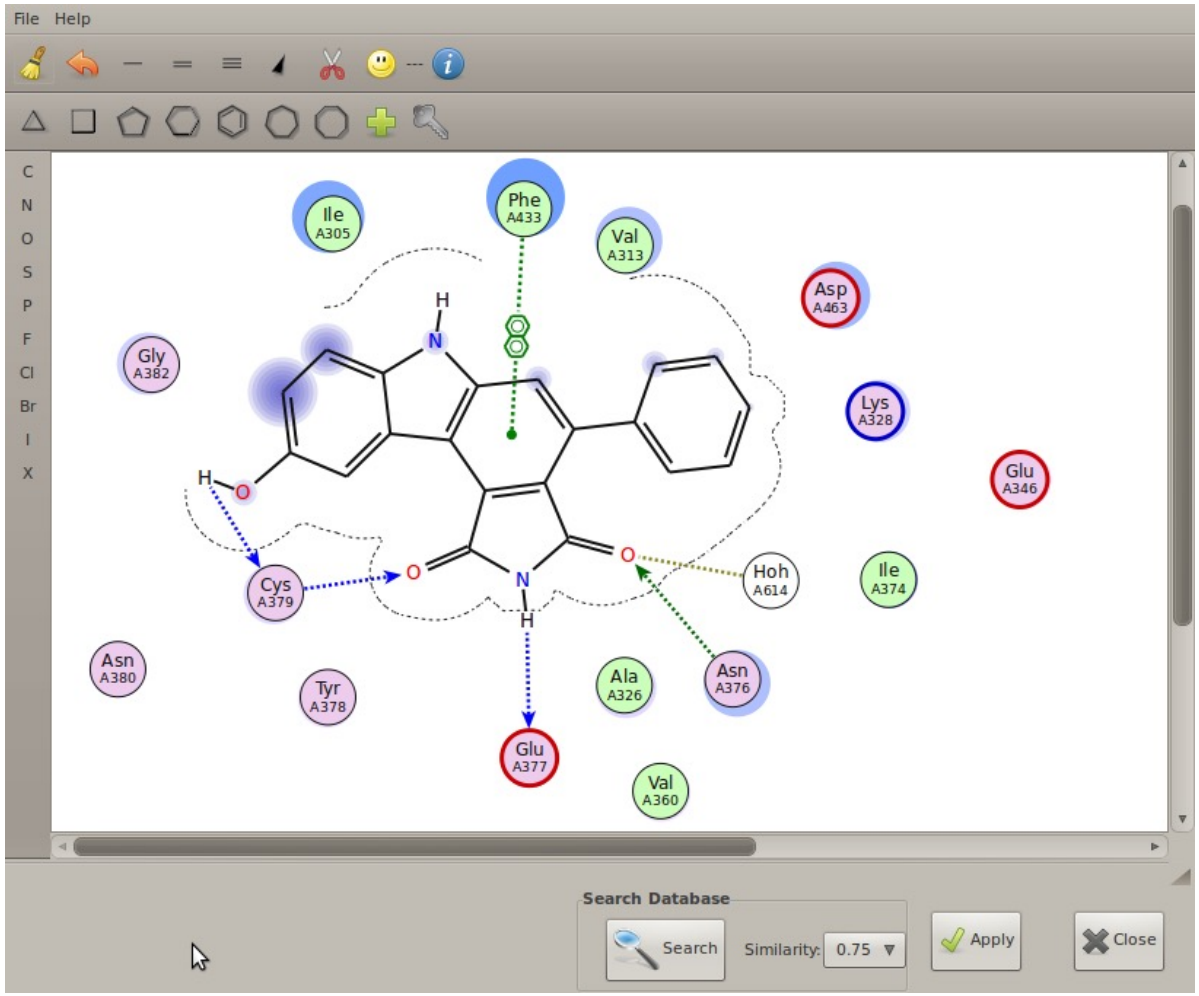


Residue position minimisation

Ligand Validation in Coot

Flatland Ligand Environment View

Ligand menu -> FLEV this residue



Covalent Linkages

We're talking about:

- Post-translational modifications
  - Glycosylations, phosphorylations, methylations
  - Disulphide bridges
- Polymeric links – protein, nucleic acid, carbohydrate
- Metals are complicated…

Dealt with using:

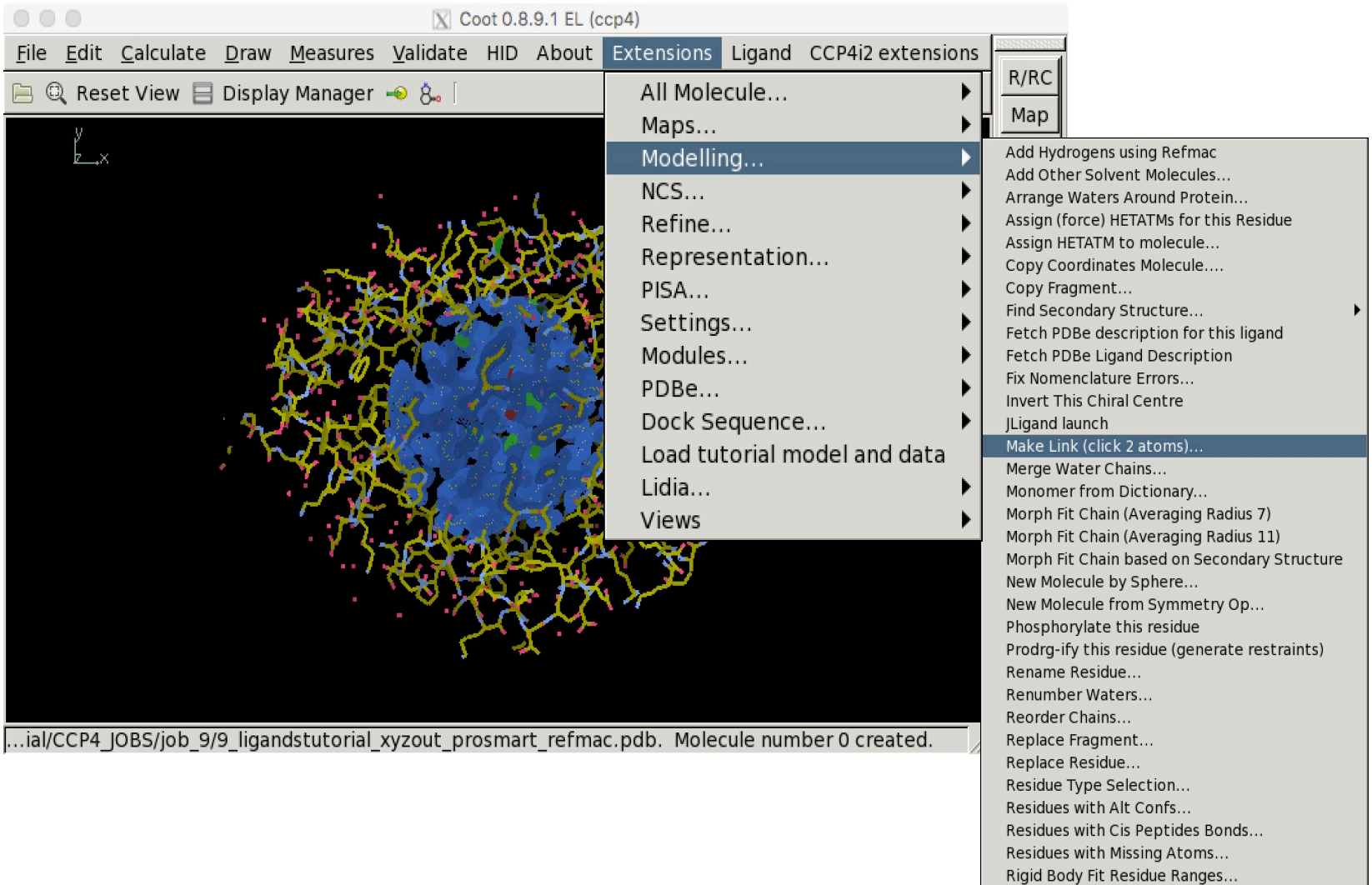
- A restraint dictionary – CIF file
- Link records to specify instances within the model
  - LINKR records – in a PDB file
  - Struct Conn records – in an mmCIF file

Some link types already have descriptions in the Monomer Library

You can make links in Coot…

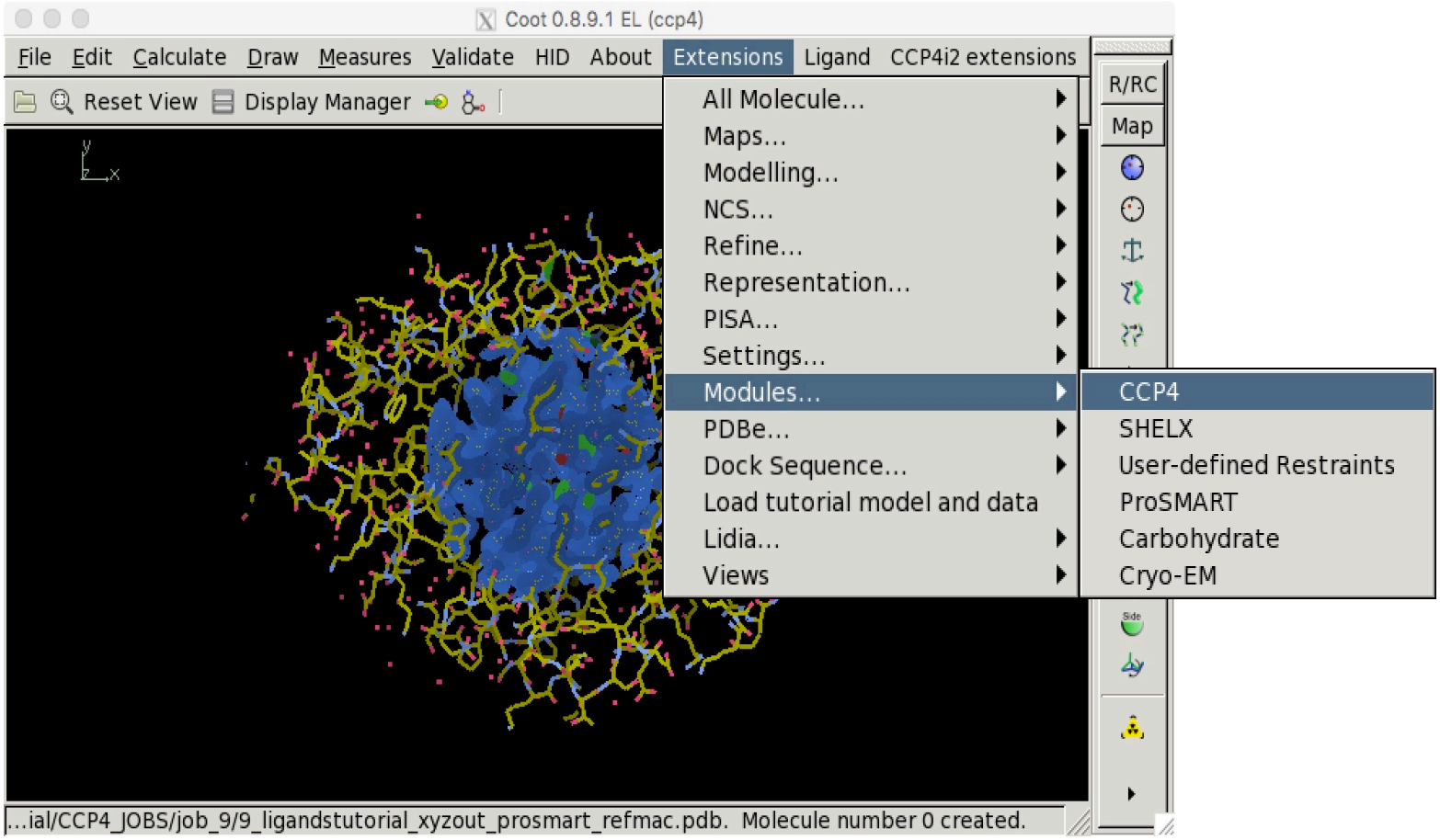
Covalent Linkages

You can make links in Coot… (this is not recommended)

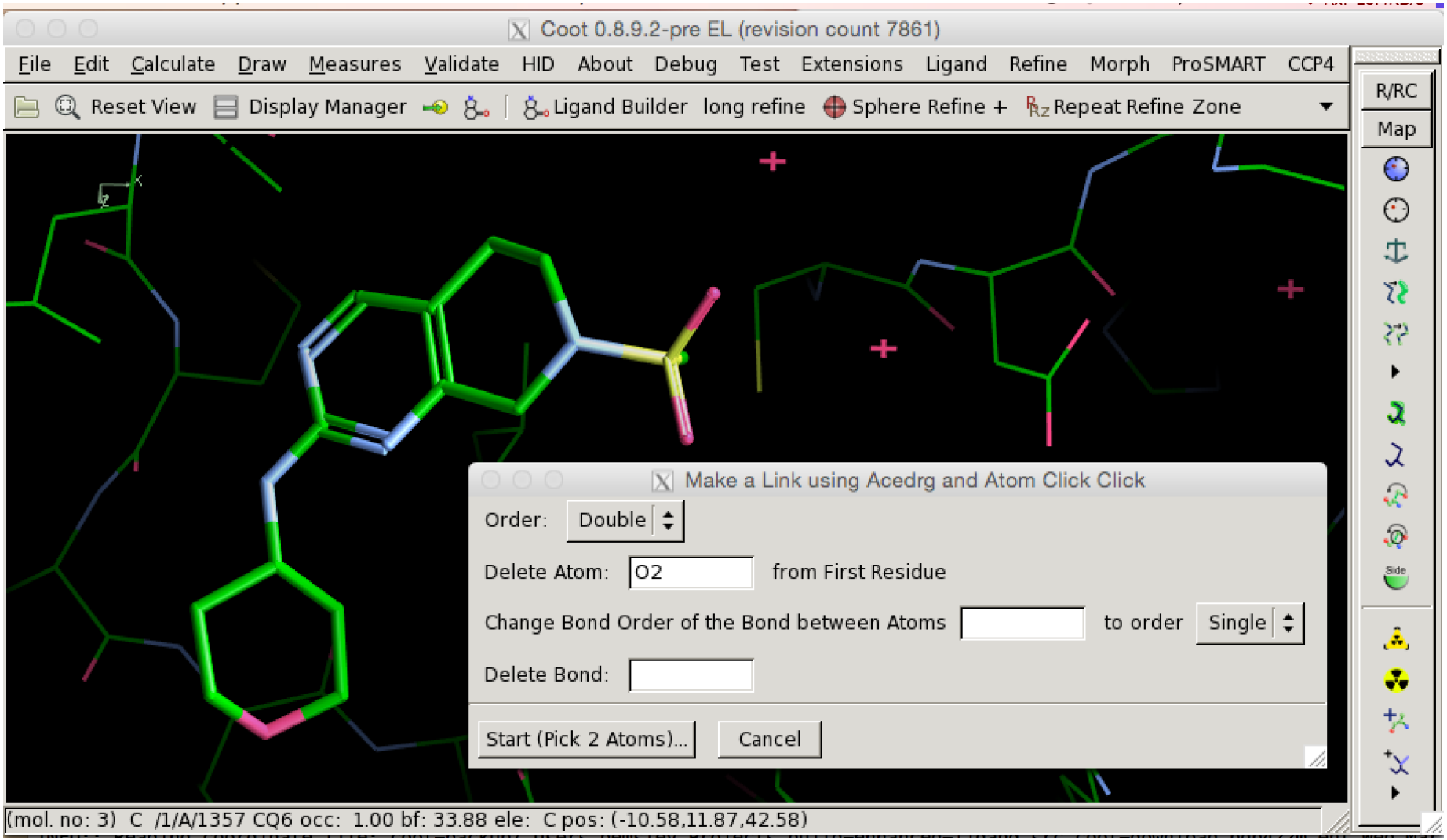


Covalent Linkages

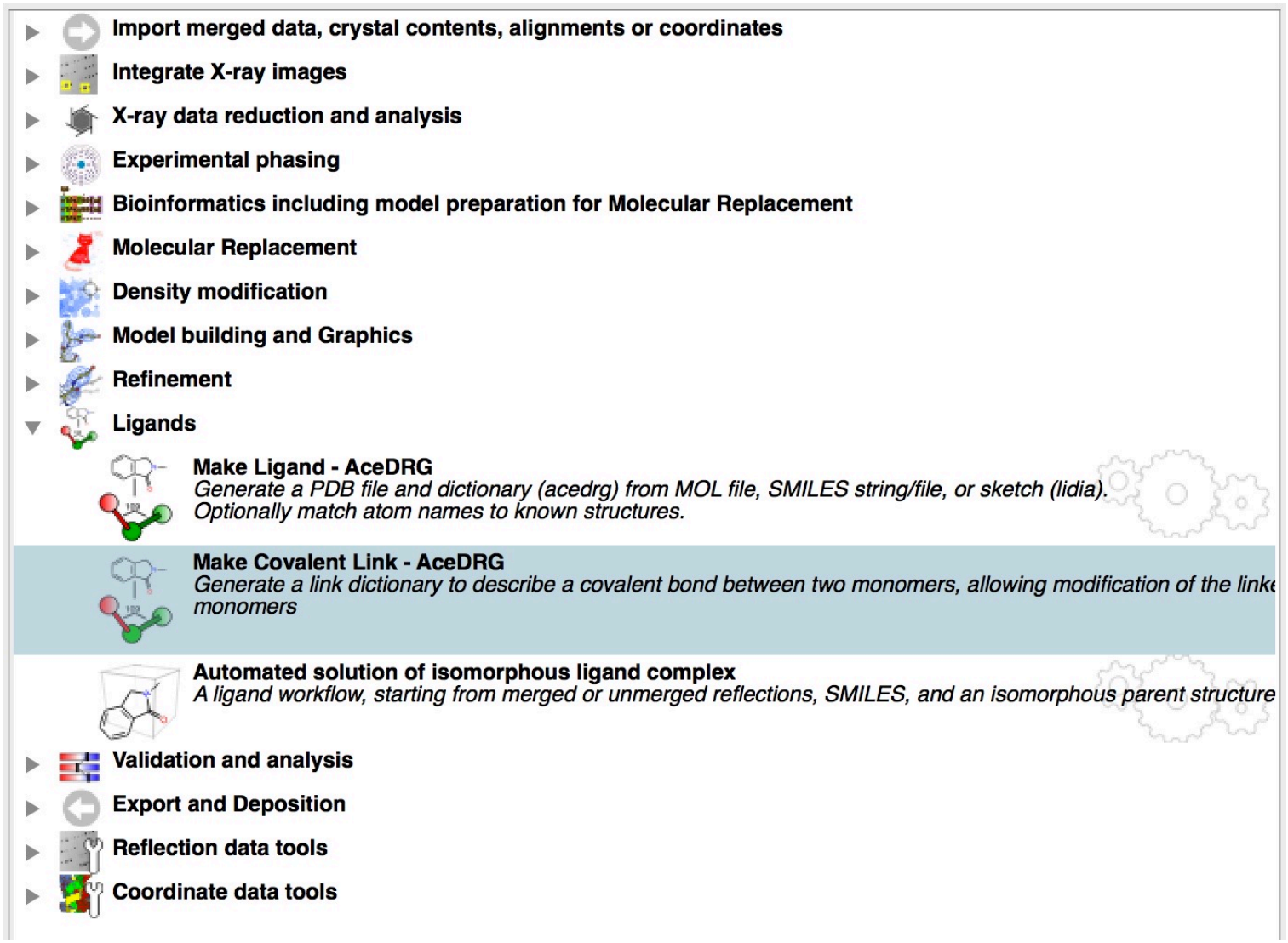
You can make links in Coot… using AceDRG!



AceDRG Links in Coot



AceDRG Links in CCP4i2



AceDRG Link Dictionary Generation

Includes the ability to:

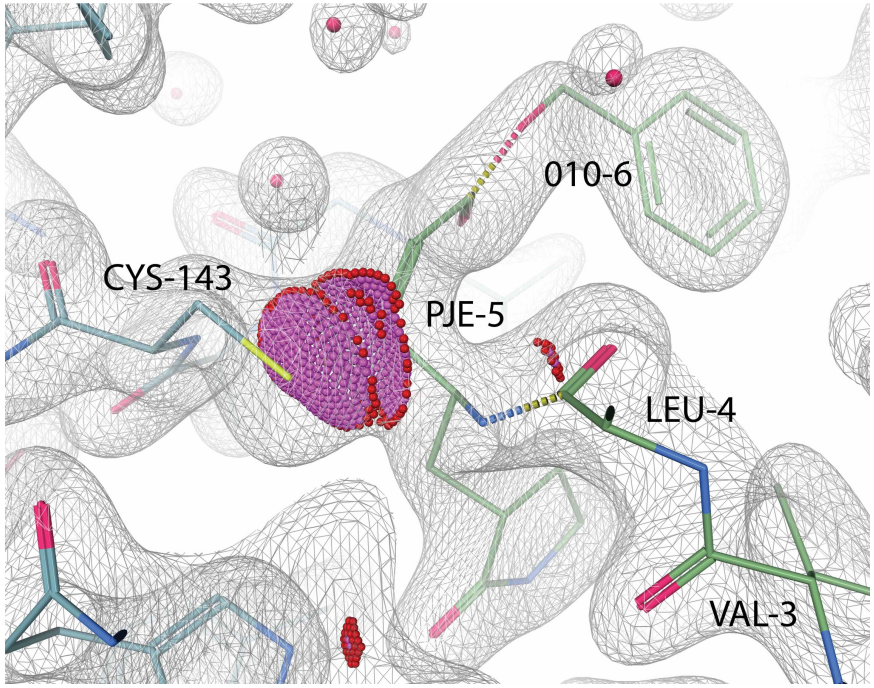
- Change bond order of the link
- Change bond order of other bonds in the components
- Delete non-H atoms (H atoms are added/deleted automatically)
- Change formal charge of atoms

In CCP4i2 – automatically apply link/struct_conn records to model

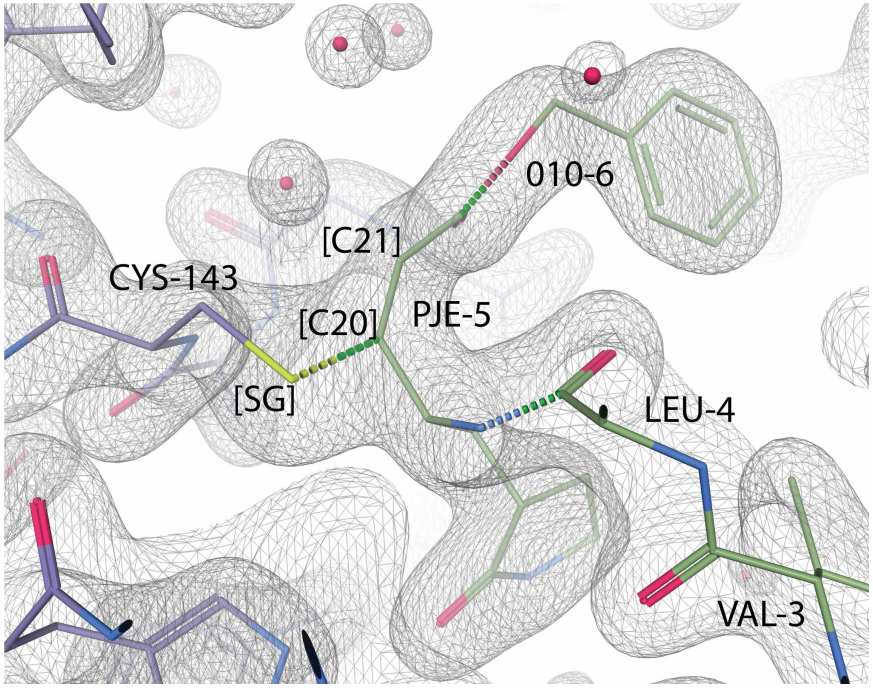
Wil be available in CCP4 Cloud in future…

N3 Inhibitor linkage to SARS-CoV-2 M^{Pro}

PDB ID: 2q6f

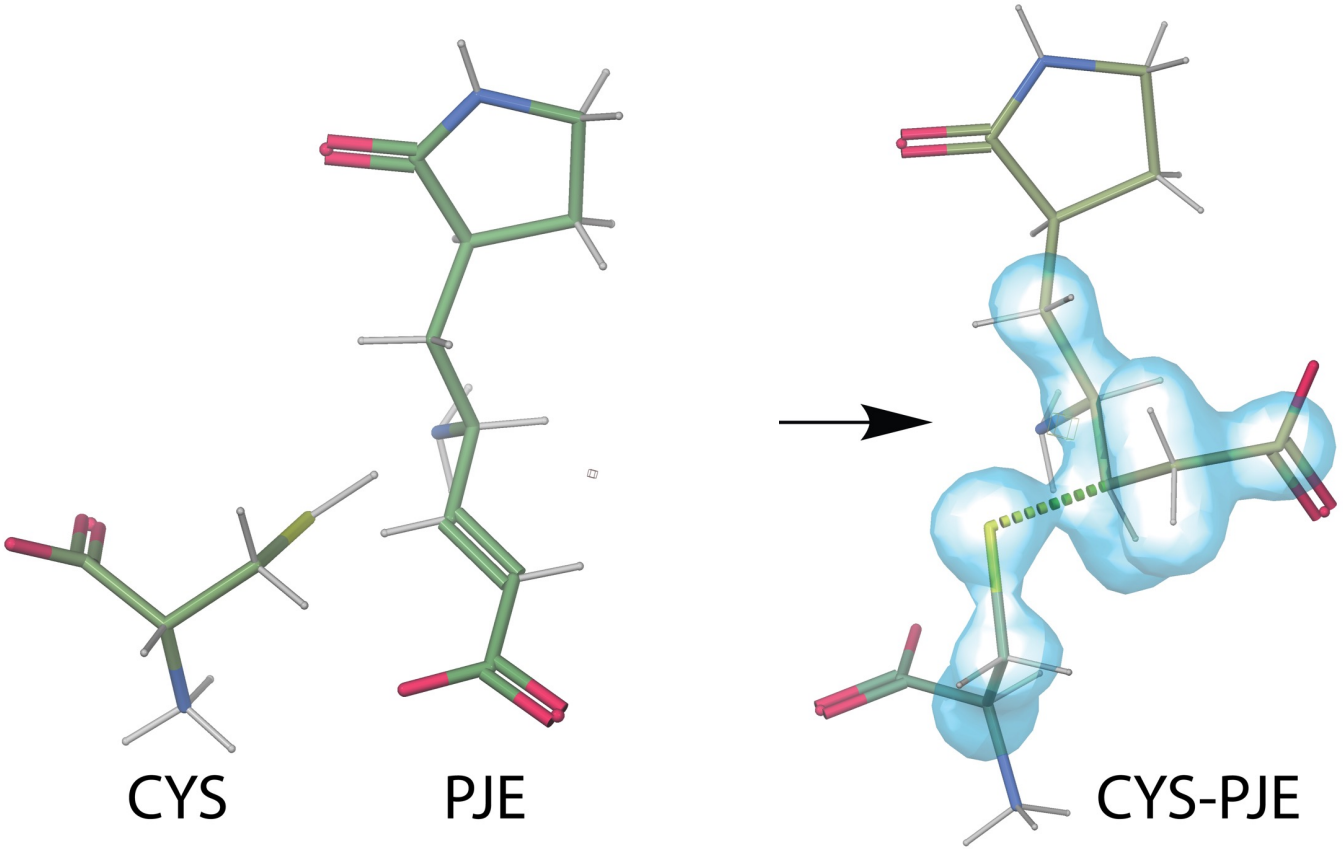


With AceDRG link dictionary:



- Link between Cys[SG] and PJE[C20]
- PJE[C20]–[C21] bond order changed from double to single
- Various restraints in the local environment are changed – modifications

N3 Inhibitor linkage to SARS-CoV-2 M^{Pro}



- Link between Cys[SG] and PJE[C20]
- PJE[C20]–[C21] bond order changed from double to single
- Various restraints in the local environment are changed – modifications

N3 Inhibitor linkage to SARS-CoV-2 M^{Pro}

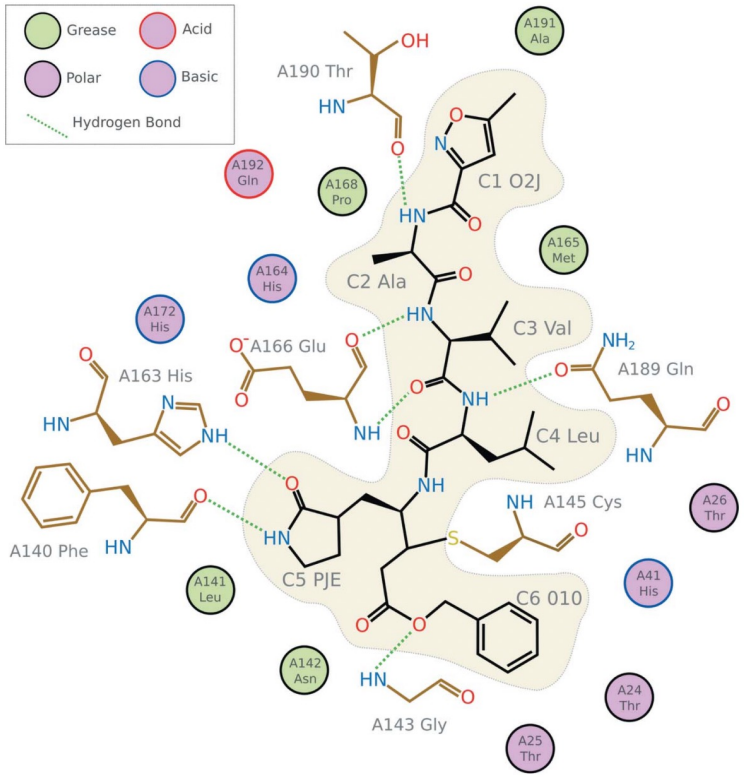
Acta Cryst D STRUCTURAL BIOLOGY
ISSN 2059-7983

The missing link: covalent linkages in structural models

Robert A. Nicholls,[a]* Marcin Wojdyr,[b] Robbie P. Joosten,[c,d] Lucrezia Catapano,[a,e] Fei Long,[a] Marcus Fischer,[f] Paul Emsley[a] and Garib N. Murshudov[a]*

[a]Structural Studies, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, United Kingdom, [b]Global Phasing Limited, Sheraton House, Castle Park, Cambridge CB3 0AX, United Kingdom, [c]Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands, [d]Oncode Institute, The Netherlands, [e]Randall Centre for Cell and Molecular Biophysics, Faculty of Life Sciences and Medicine, King's College London, London SE1 9RT, United Kingdom, and [f]Chemical Biology and Therapeutics and Structural Biology, St Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA. *Correspondence e-mail: nicholls@mrc-lmb.cam.ac.uk, garib@mrc-lmb.cam.ac.uk

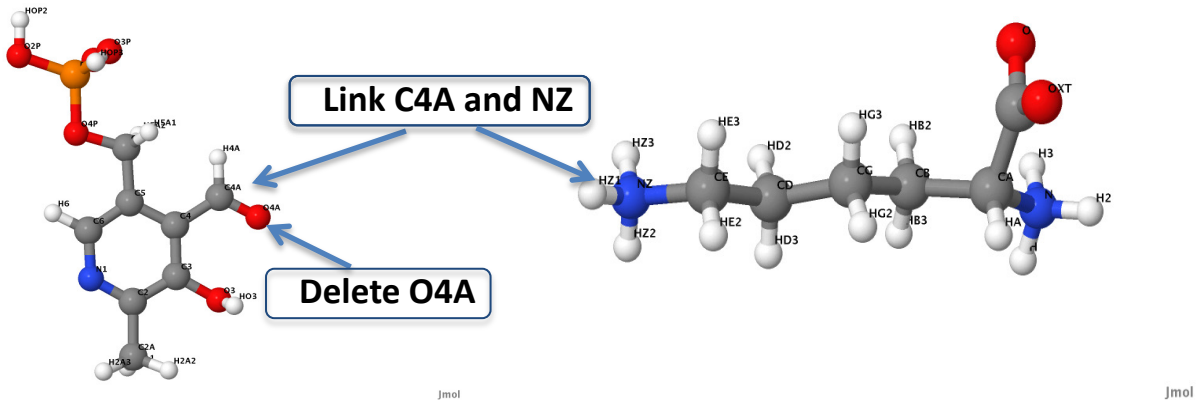
Received 14 December 2020
Accepted 13 April 2021



- Analysis of potential links in the PDB
  - Be careful when interpreting linkage info from PDB models…
- Includes summary of links in the CCP4-ML

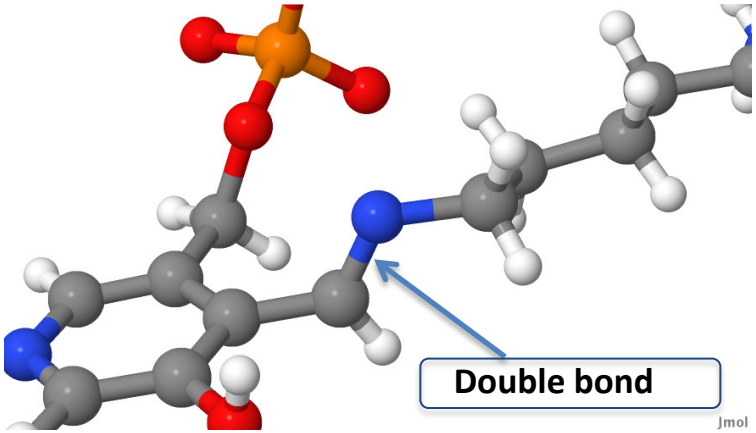
Covalent linkage example: LYS – PLP

1. Delete atom O4A from PLP
2. Create double bond between PLP-C4A and LYS-NZ

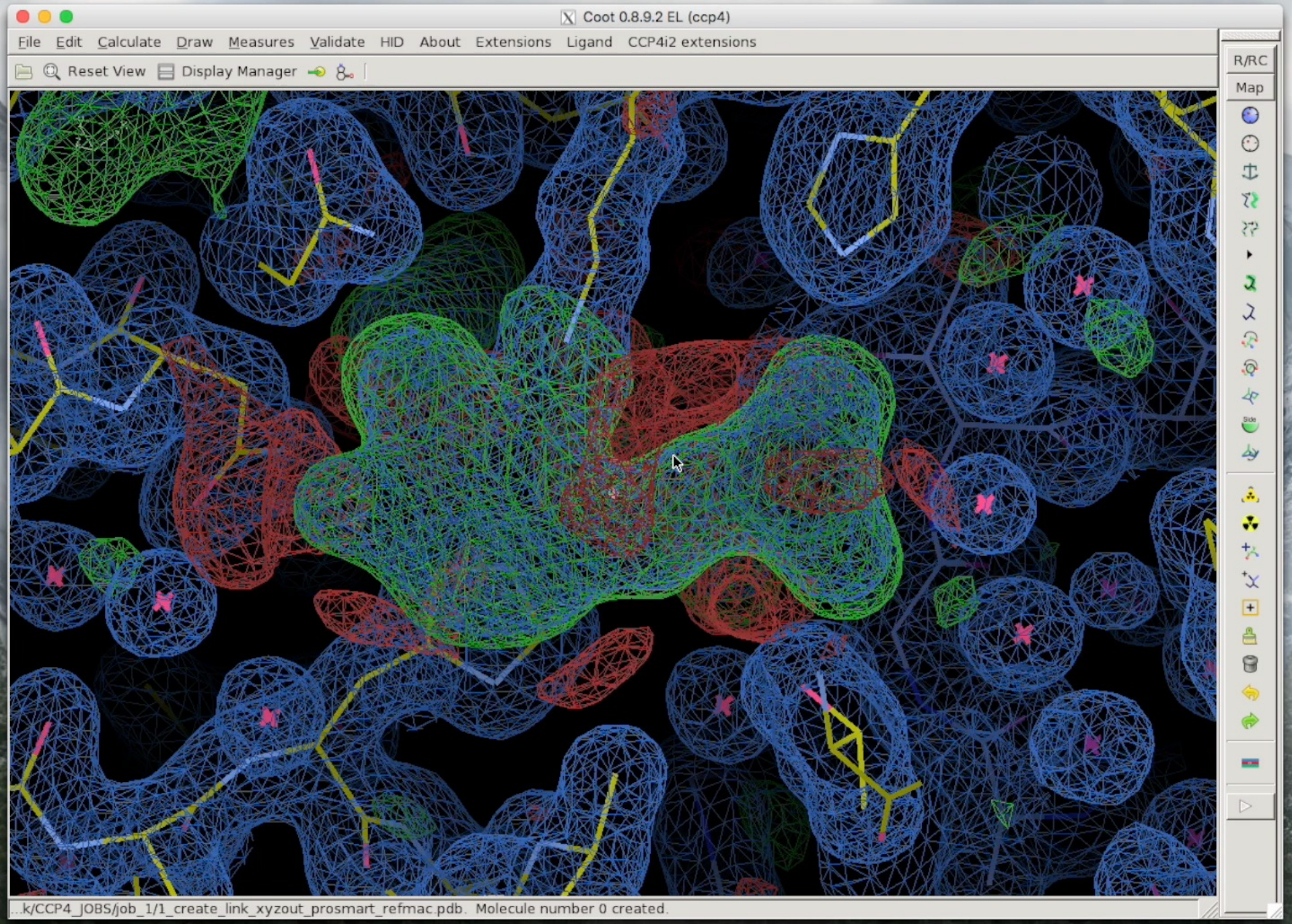


PLP

LYS



Linked molecule


Coot 0.8.9.2 EL (ccp4)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand CCP4i2 extensions
Reset View
Display Manager
R/RC
Map
...k/CCP4_JOBS/job_1/1_create_link_xyzout_prosmart_refmac.pdb. Molecule number 0 created.

Covalent Links in CCP4

STRUCTURAL BIOLOGY

ISSN 2059-7983

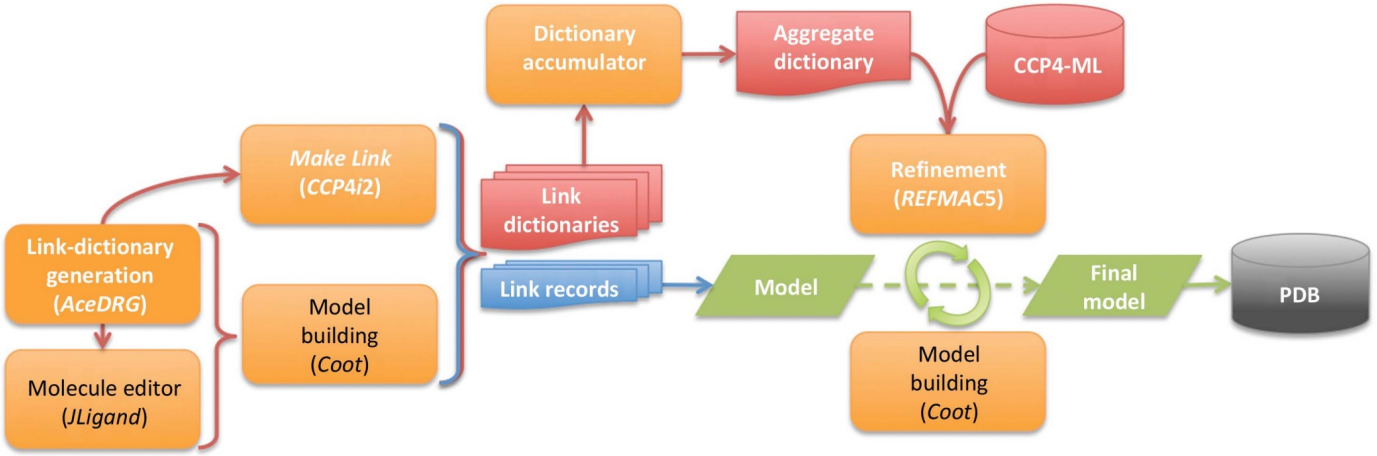
Modelling covalent linkages in *CCP*4

Robert A. Nicholls,[a]* Robbie P. Joosten,[b,c] Fei Long,[a] Marcin Wojdyr,[d] Andrey Lebedev,[e] Eugene Krissinel,[e] Lucrezia Catapano,[a,f] Marcus Fischer,[g] Paul Emsley[a] and Garib N. Murshudov[a]*

[a]Structural Studies, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, United Kingdom, [b]Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands, [c]Oncode Institute, The Netherlands, [d]Global Phasing Limited, Sheraton House, Castle Park, Cambridge CB3 0AX, United Kingdom, [e]CCP4, STFC Rutherford Appleton Laboratory, Chilton, Didcot OX11 0QX, United Kingdom, [f]Randall Centre for Cell and Molecular Biophysics, Faculty of Life Sciences and Medicine, King's College London, London SE1 9RT, United Kingdom, and [g]Chemical Biology and Therapeutics and Structural Biology, St Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA. *Correspondence e-mail: nicholls@mrc-lmb.cam.ac.uk, garib@mrc-lmb.cam.ac.uk

Received 4 November 2020
Accepted 12 February 2021

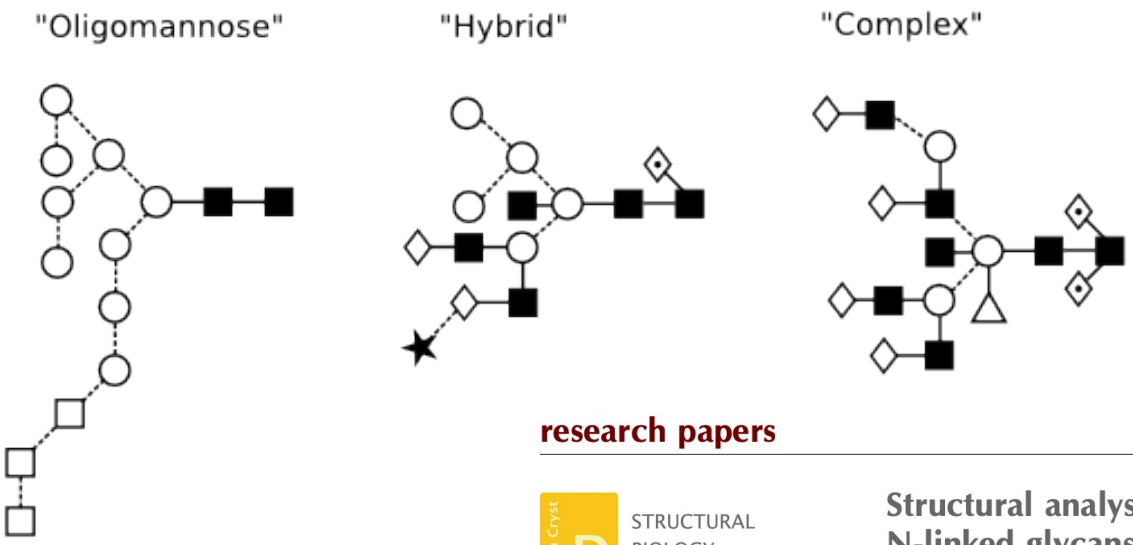
Edited by J. Agirre, University of York, United Kingdom



Carbohydrate building in Coot

LO/Carb – Linking Oligosaccharides/Carbohydrates

- Ease building complex N-linked carbohydrate structures
- Uses a dictionary of standard links
- Automatic and semi-automatic oligomannose builder



N-linked glycosylation trees specific to expression system

research papers

Acta Cryst D STRUCTURAL BIOLOGY

ISSN 2059-7983

Structural analysis of glycoproteins: building N-linked glycans with *Coot*

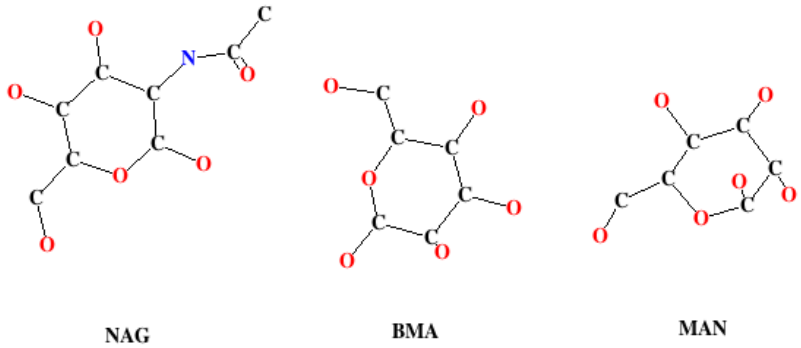
Paul Emsley[a]* and Max Crispin[b]

[a]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, England, and [b]Centre for Biological Sciences and the Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, England. *Correspondence e-mail: pemsley@mrc-lmb.cam.ac.uk

Received 4 August 2017
Accepted 29 March 2018

Carbohydrate building in Coot

Example: Semi-automatic polysaccharide building



NAG : N-acetyl-D-glucosamine
BMA : beta-D-mannose
MAN : alpha-D-mannose

We know:

- Refined model & data (PDB & MTZ)
- Protein has one glycosylation site – N-linked, residue ASN 112
- Polysaccharide comprises NAG, BMA & MAN

We want to:

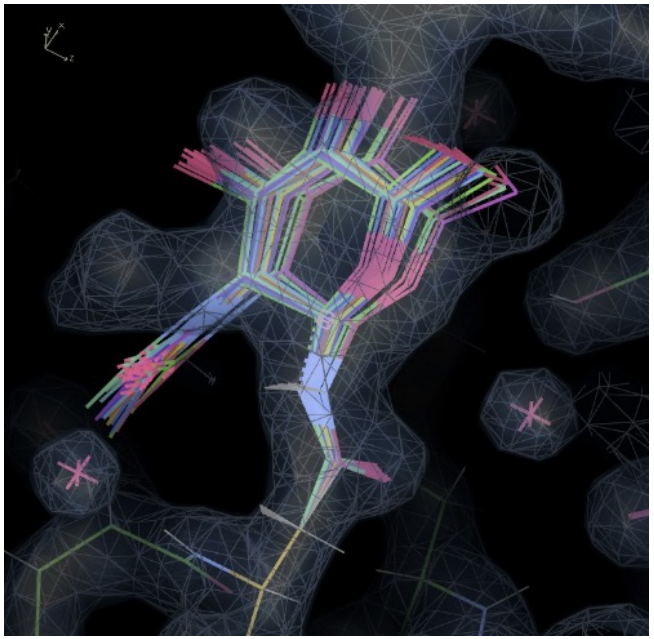
- Build the polysaccharide
- Create the link to ASN 112

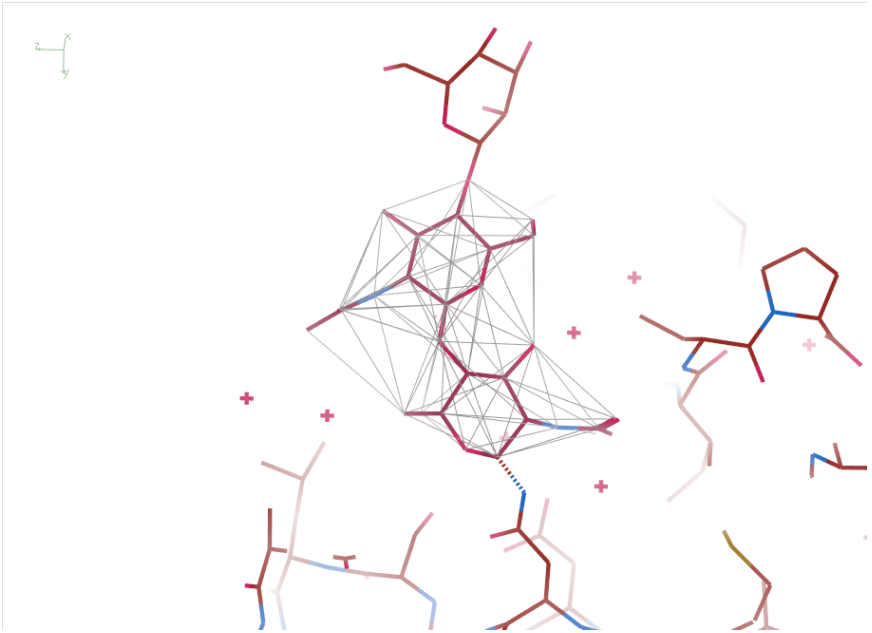
PDB code: 4gos (1.6Å)

Carbohydrate building in Coot

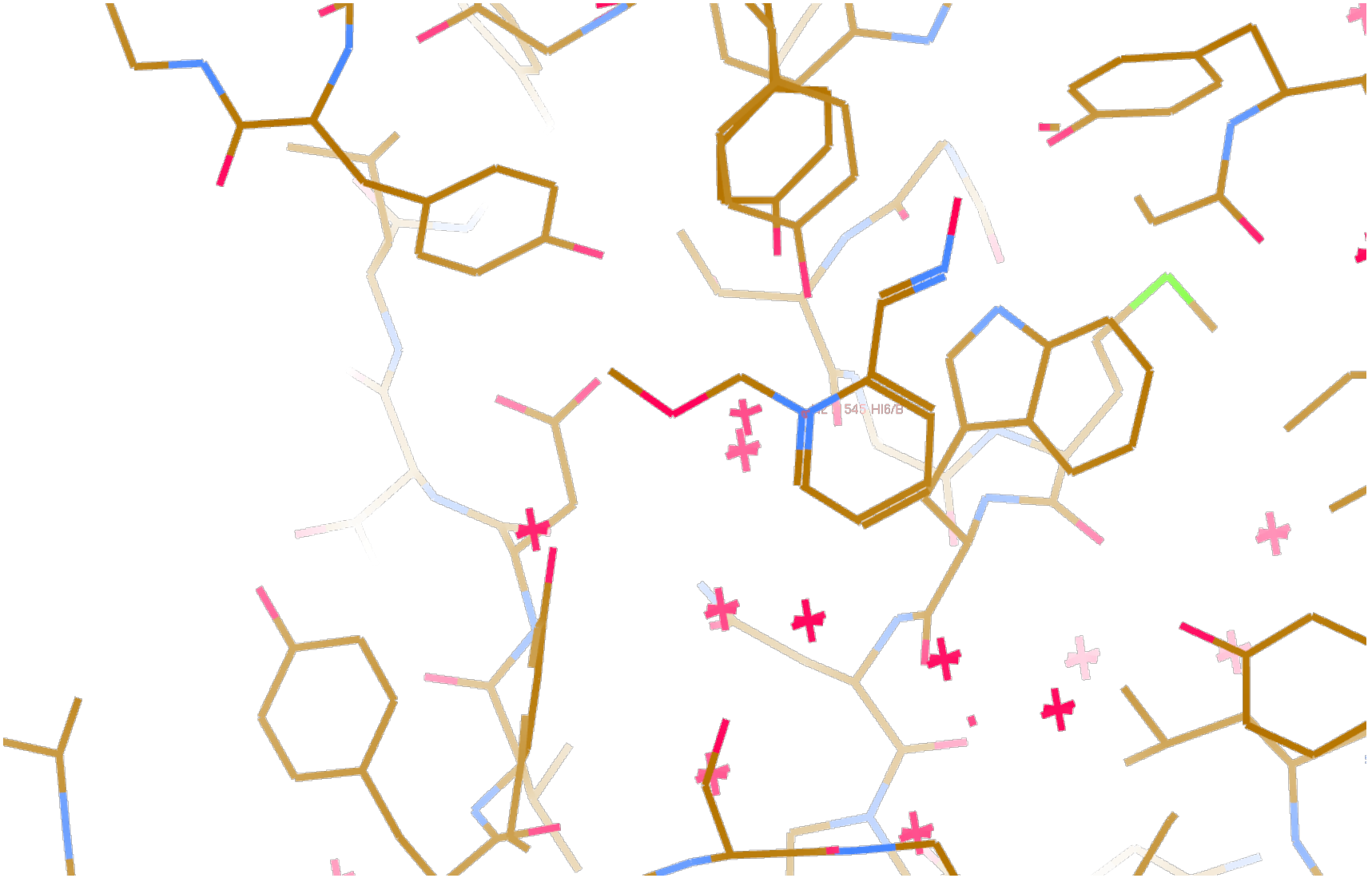
LO/Carb – Linking Oligosaccharides/Carbohydrates

- Performs torsion angle refinement
- Uses restraints generated from analysis of disaccharides in the PDB
  (robust ProSMART-like restraints as used in low-res refinement)



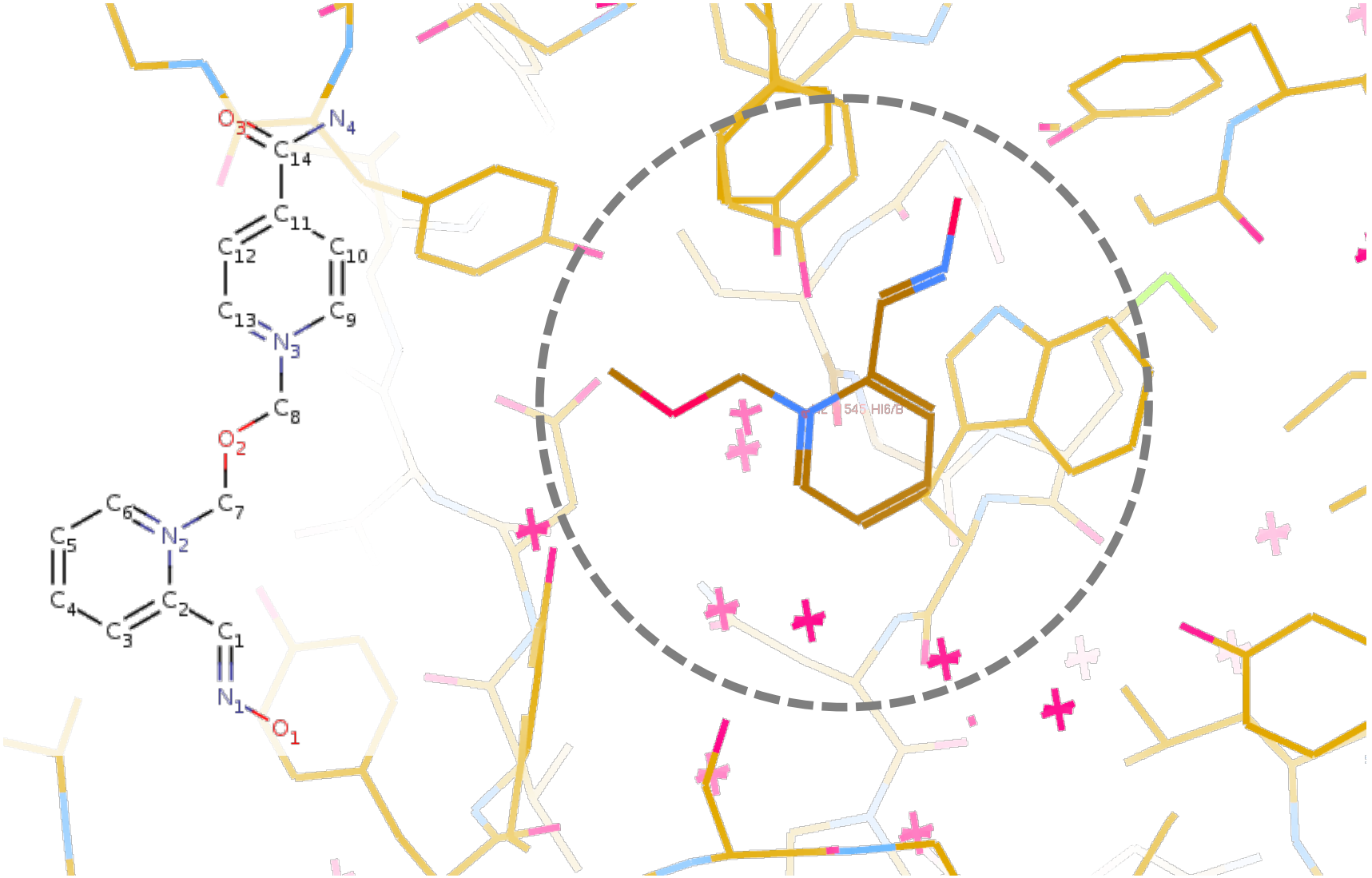


Map Blurring



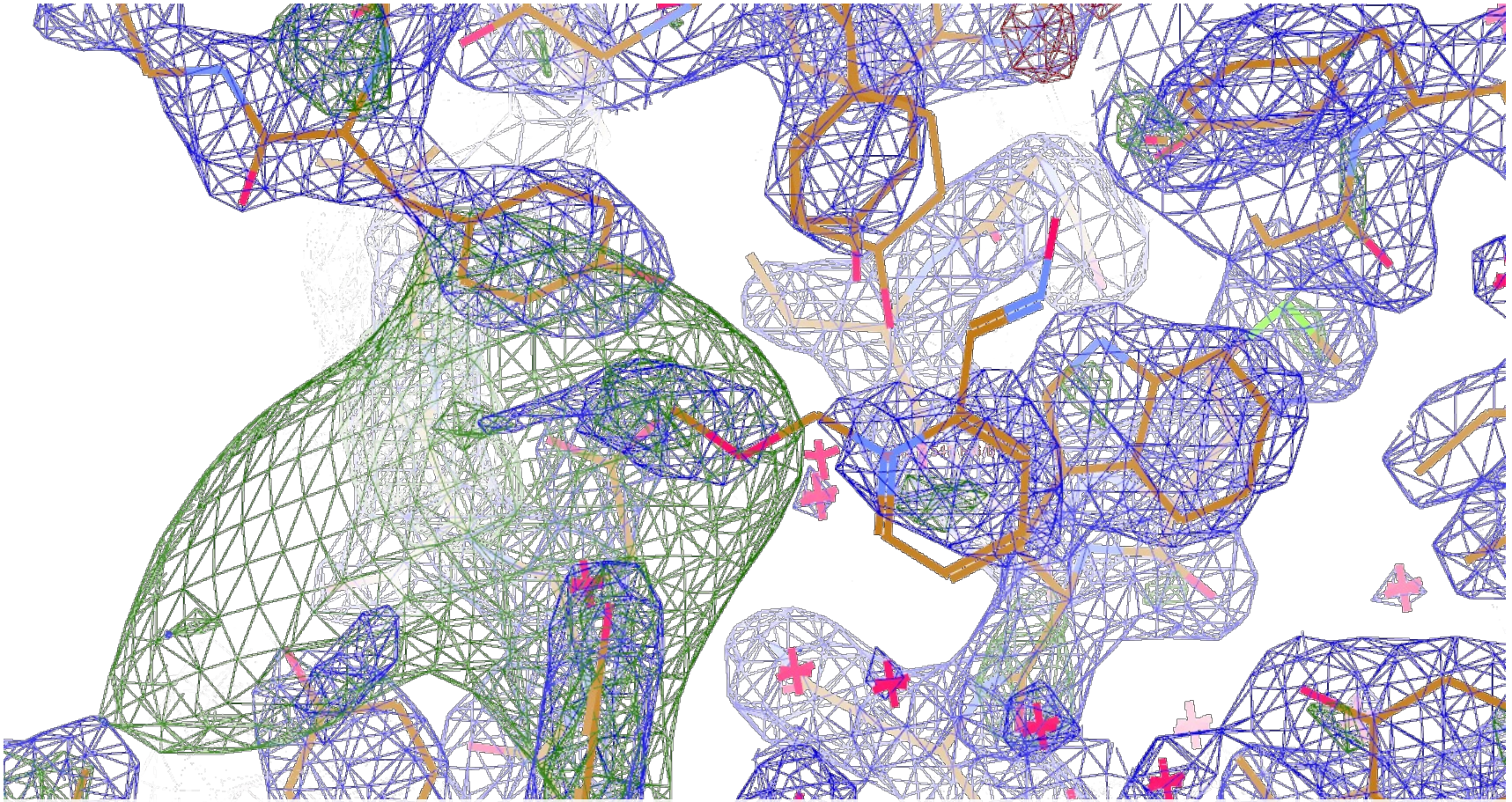
2whq – 2.15Å

Map Blurring



2whq – 2.15Å

Map Blurring



Electron density at 1.7σ; Residual density at 3.5σ
Blurred residual map, B = 200

2whq – 2.15Å

Summary

In CCP4, we have:

- **AceDRG** – dictionary, conformer and link generation
- **Jligand & Lidia** – manual sketching
- **Coot** – ligand fitting, model building and validation
- **REFMAC5** – refinement

Final points:

- Refine the rest of the model before looking at ligands
- Look at the crystallization conditions – is it your ligand or your buffer?
- In MX, incorrect building can cause model bias (not in cryoEM)

  -> misleading density

  -> poor model
- In future we need to deal better with metals…
- PDB-REDO's Platonyzer for Zn/Na/Mg ion sites

Acknowledgements

CCP4 Core

Eugene Krissinel
Andrey Lebedev
Charles Ballard
Ronan Keegan
Ville Uski
Maria Fando

MRC-LMB Computational Structural Biology Group

Garib Murshudov - *REFMAC5*
Keitaro Yamashita - REFMACAT
Fei Long - *AceDRG*
Paul Emsley - *Coot*
Lucrezia Catapano - *Coot*

CCP-EM

Tom Burnley
Colin Palmer
Rangana Warshamanage
Agnel Joseph
Martyn Wynn

Other Collaborators

Marcin Wojdyr (Global Phasing Ltd)
Oleg Kovalevskiy
Robbie Joosten - pyranose geometry analysis
Jon Agirre - pyranose geometry analysis
Marcus Fischer
Alan Brown
Ben Bax
Martin Noble
Stuart McNicholas