Phenix user workshop, July 29 2022

Ligands

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What is a ligand?

- Small molecule (more than 1 atom, ligand, sugar, covalent modification, buffer molecules,…)
- Binds to a macromolecule
- Can serve a biological purpose or was designed to bind
- Can bind “by chance” (buffer molecule)
- Binding: non-covalent and/or covalent bonds

193k models in the PDB

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146k out of 193k models in the PDB have at least one ligand (”distinct non-polymer entity)

→ Chances are that you will have to deal with ligands!

“Dealing” with ligands

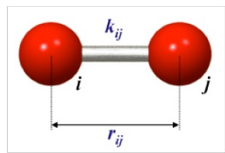
1) Find out what ligand(s) are in the crystal structure
2) Refine the ligand along with the macromolecule

We need restraints for the ligand

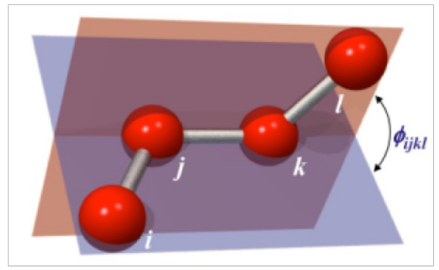
a priori knowledge that increases the number of observations

Stereochemical restraints stipulate ideal values for:

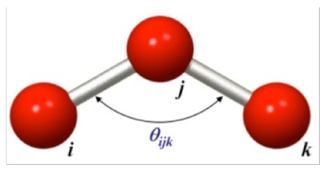
- Bond lengths
- Angles
- Torsions
- Planes
- Chiral volumes



$\Sigma_{bonds}\ \omega(d_{model} - d_{ideal})^2$

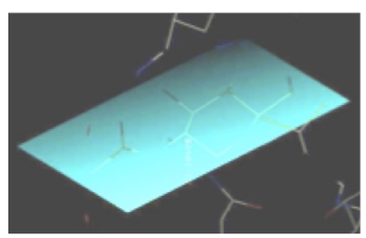


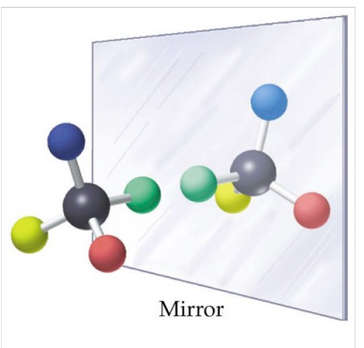
$\Sigma_{dihedrals}\ \omega(1 + \cos(n\chi_{model} + \chi_{shift}))$



$\Sigma_{angles}\ \omega(\theta_{model} - \theta_{ideal})^2$

Images from PumMa web site (http://www.pumma.nl)



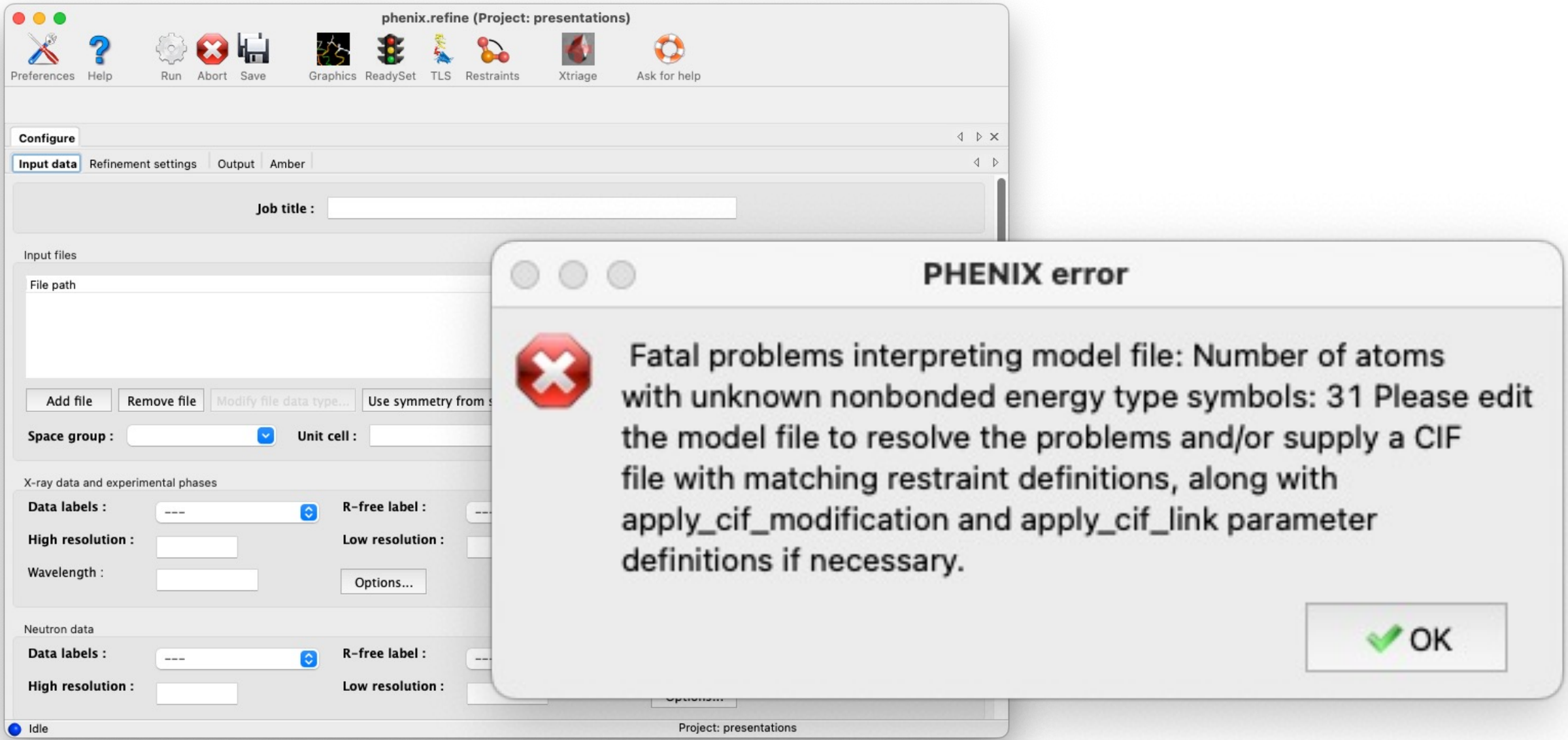


Volume (V) = $(r_N\text{-}r_{CA})$ •
$[(r_C\text{-}r_{CA}) \times (r_{CB}\text{-}r_{CA})]$

$E = \Sigma_{planes}\ \Sigma_{atoms}\ w\ (m \bullet r - d)^2$

Ligands need restraints

If you try to run phenix.refine and a ligand has no restraints…



(This message also occurs if atom names don’t match!)

How to get restraints

- Amino acids: Engh&Huber dictionary
- Nucleic Acids: CCP4 monomer library
- Common ligands: libraries, such as the CCP4 monomer library
- Novel ligands → Use a dictionary generator

Ligand libraries

- **Chemical Components library** (CCL):
  - Has an entry for every entity in PDB entries
  - Contains SMILES (absolute topology)
  - Two sets of xyz – One from the “best, first” model in PDB and one calculated by PDBx as the ideal
  - Atom names needed for restraints generation and lookup

- **CCP4 monomer library**:

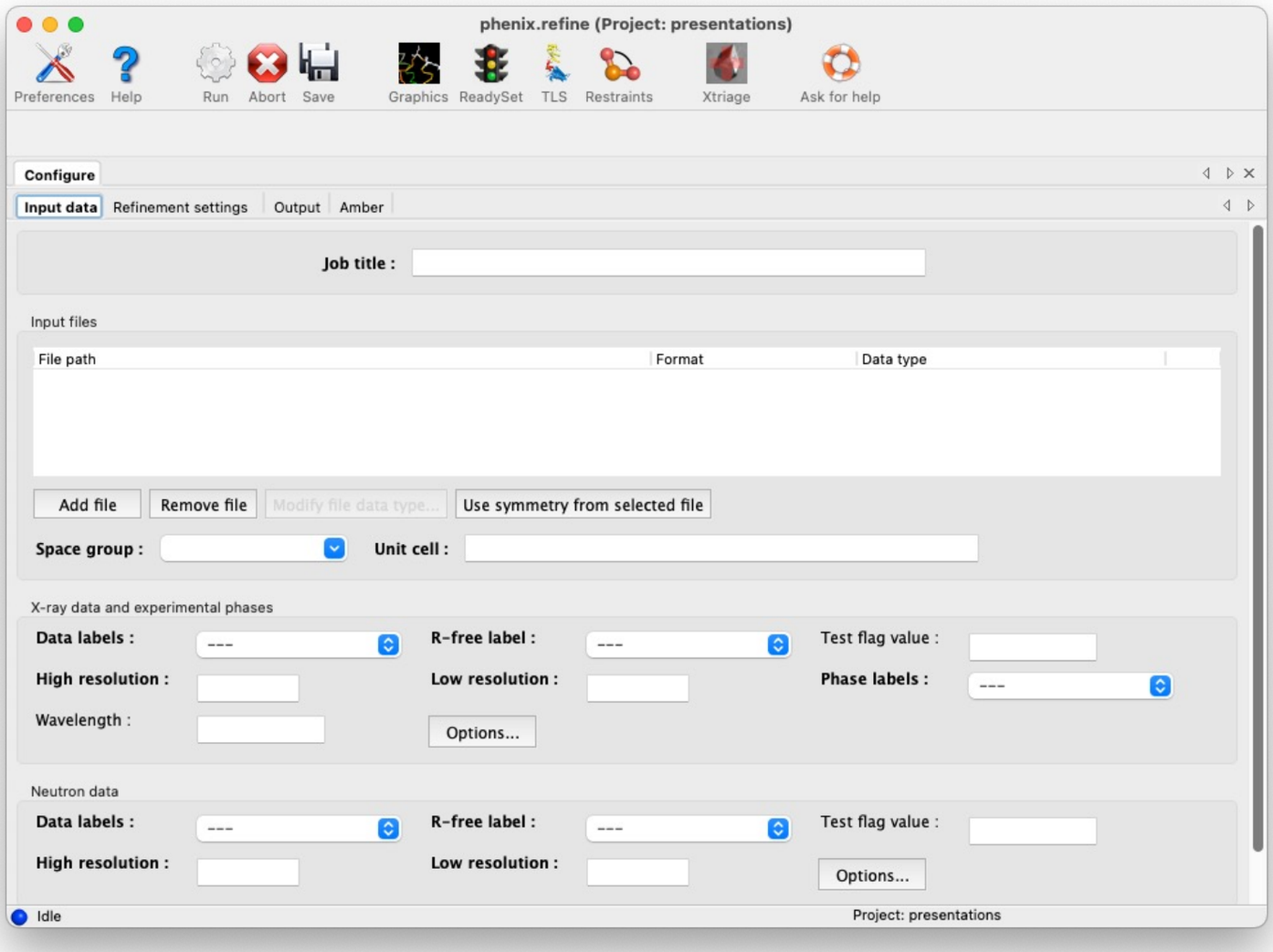
  All ligands in the CCL

- **GeoStandard** (GeoStd):
  - Geometry obtained by QM minimization (PBE-3c with CPCM solvent)
  - Validated by Mogul (library of molecular geometry derived from small molecules)
  - About 15k ligands

Standard library in Phenix: GeoStd

Phenix comes with the GeoStd library and a trimmed version of CCP4 monomer library

→ No need to generate novel restraints if your ligand is in the libraries.



...But it is always good to have a look at the restraints and check if there is something unexpected (i.e. atoms not in a plane)

Novel ligands

If the ligand is not in the libraries that ship with Phenix:

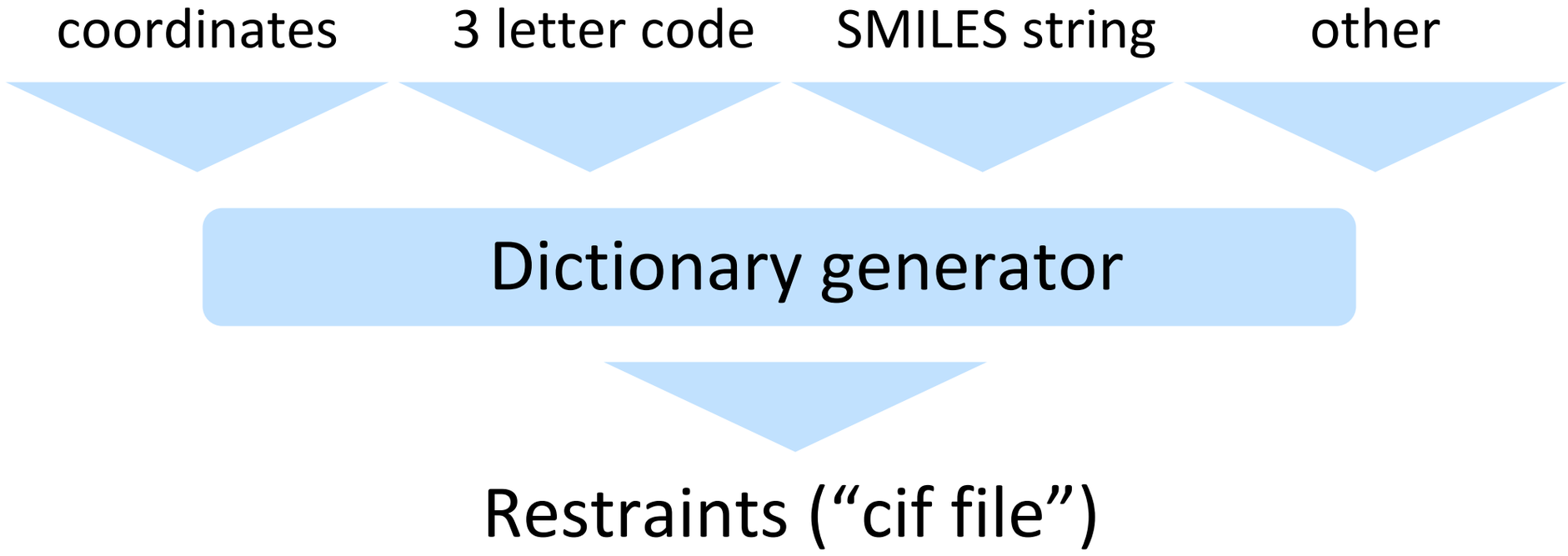
- Check other libraries
- If relying on 3 letter code: double check that it is the correct one

If still not found:

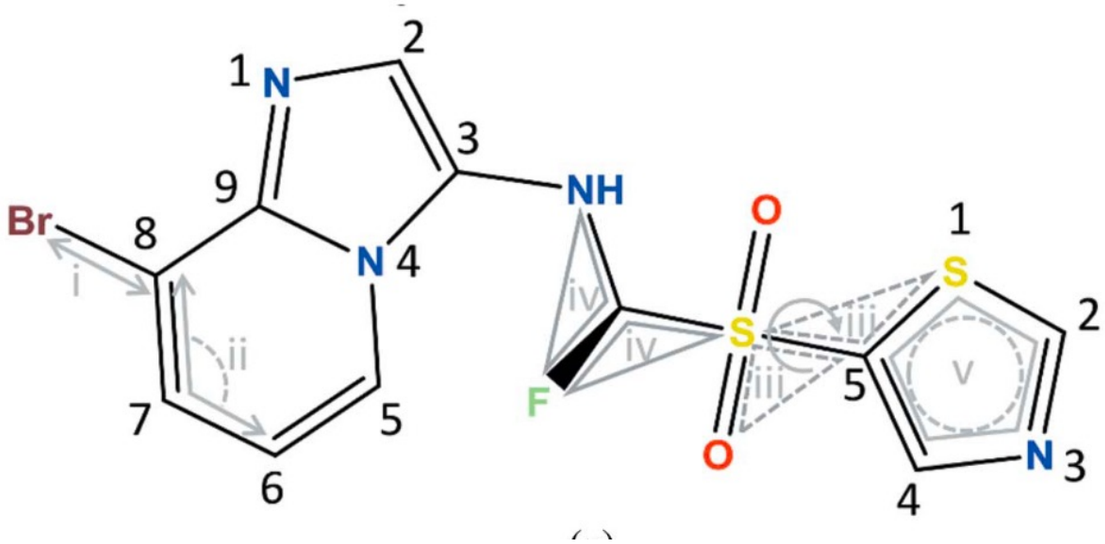
→ Get restraints from a dictionary generator

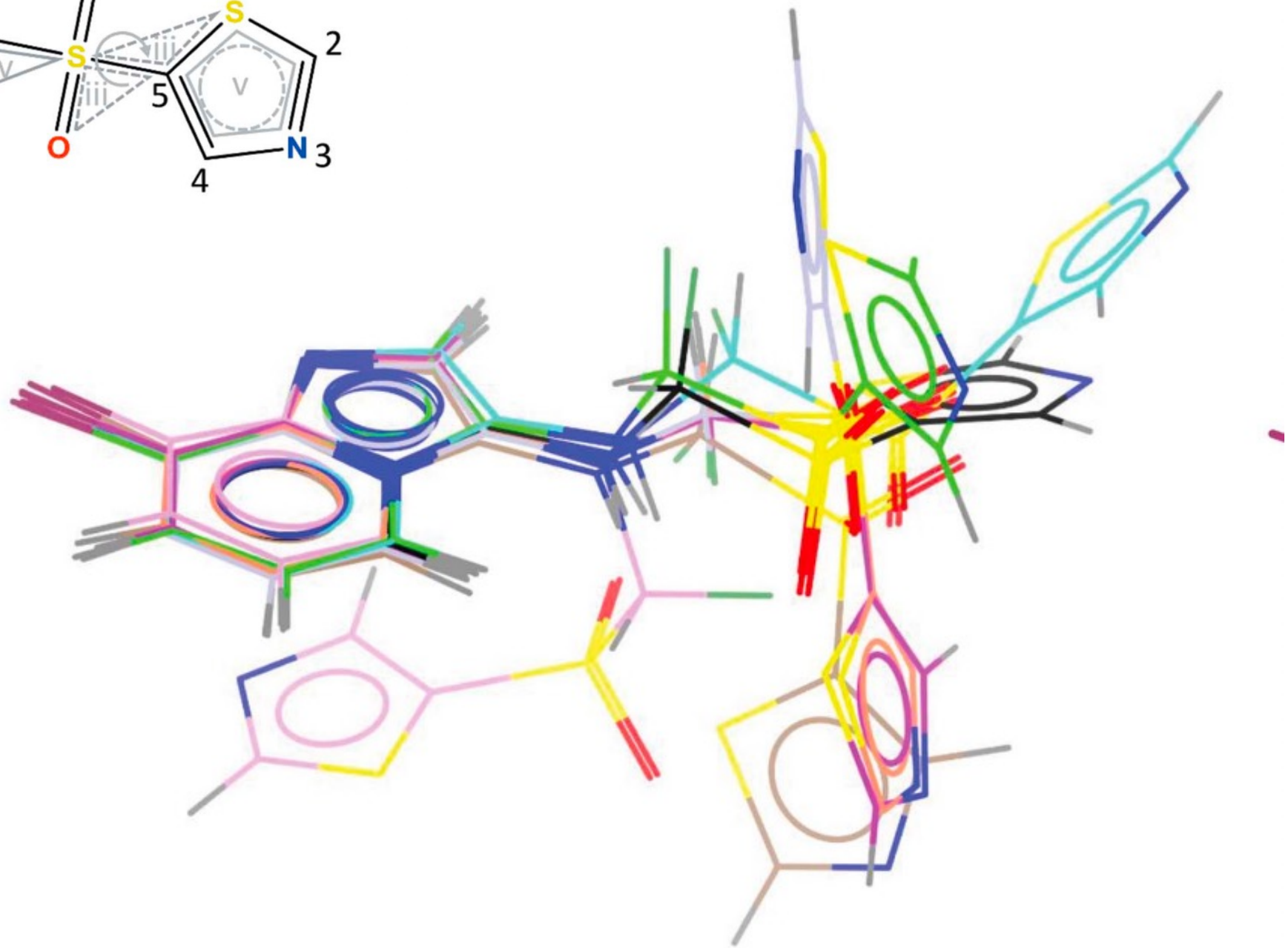
Dictionary generator

Create restraints programmatically by using experimental information (wwPDB CCD, CS, COD) or by molecular-simulation.



Restraints can vary





Steiner, R. A. & Tucker, J. A. (2017). *Acta Crystallogr. Sect. Struct. Biol.* **73**, 93–102.

Ligand restraints for Phenix

Restraints are needed for

- Refinement (any kind of geometry minimization)
- Validation

Tools for ligand restraints in Phenix

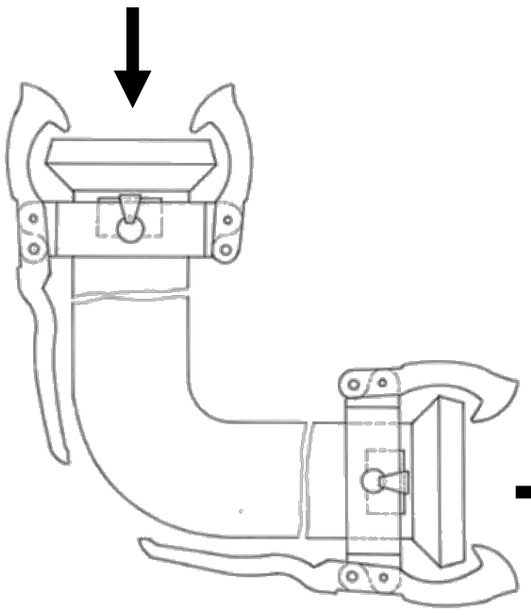
- eLBOW
- ReadySet!

eLBOW

eLBOW = electronic Ligand Builder and Optimisation Workbench

- Automated generation of restraints for ligands
- Fast, simple and flexible procedure
- Reduces the tedium of building 3D ligand geometries

Chemical input



Chemical restraints (CIF)
Cartesian coordinates (PDB)

eLBOW

Acta Crystallographica Section D
Biological Crystallography
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electronic Ligand Builder and Optimization Workbench (*eLBOW*): a tool for ligand coordinate and restraint generation

Acta D paper

Nigel W. Moriarty,[a]* Ralf W. Grosse-Kunstleve[a] and Paul D. Adams[a,b]

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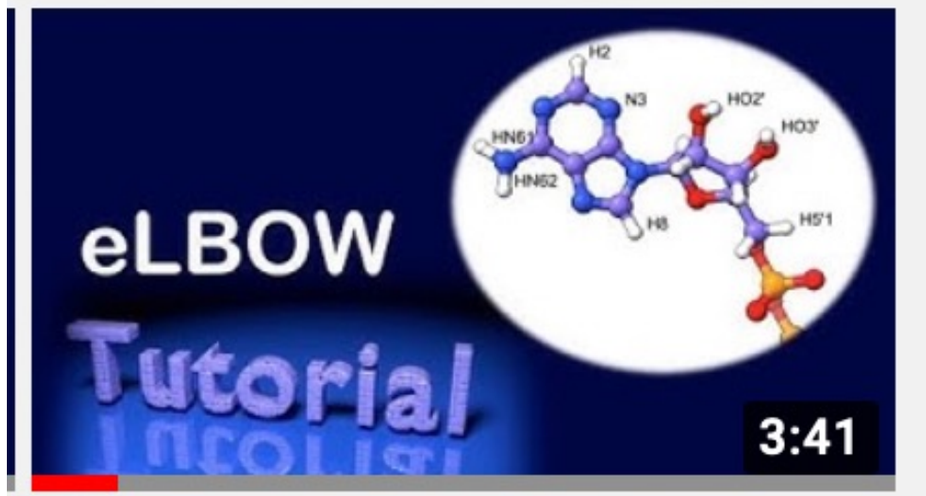
The *electronic Ligand Builder and Optimization Workbench* (*eLBOW*) is a program module of the *PHENIX* suite of computational crystallographic software. It is designed to be a flexible procedure that uses simple and fast quantum-chemical techniques to provide chemically accurate information for novel and known ligands alike. A variety of input formats and options allow the attainment of a number of diverse goals including geometry optimization and generation of restraints.

Received 27 April 2009
Accepted 23 July 2009

Video tutorial on the Phenix YouTube channel



Phenix *Python-based Hierarchical ENvironment for Integrated Xtallography*

Generating ligand structures and restraints in the eLBOW GUI

Overview

The electronic Ligand Builder and Optimization Workbench (eLBOW) is the primary tool for generating non-standard ligand restraints in Phenix. In addition to existing as a standalone program, it is also used internally by the LigandFit wizard and phenix.ready_set (integrated with the `phenix.refine` GUI). In addition to eLBOW, a separate standalone graphical restraint editor is available for advanced customization of restraints and structures.

Online documentation

https://phenix-online.org/documentation/reference/elbow_gui.html

N. W. Moriarty, R. W. Grosse-Kunstleve, P. D. Adams, (2009). Acta Cryst. D 65, 1074-1080.

ReadySet!

Prepare a model file for refinement (ReadySet! Refine!!!)

- Add H to protein with *phenix.reduce*.
- Add H to ligands with eLBOW.
- Optional: add H to water, add H/D to neutron model.
- Generate metal coordination files.
- Use the .cif file for the unknown molecule.

Model file

ReadySet!

Restraints, model file

ReadySet!

Contents

Author

Nigel W. Moriarty

Purpose

https://phenix-online.org/documentation/reference/ready_set.html

Other tools for ligands in Phenix

- **LigandFit**: Flexible ligand fitting into difference maps.

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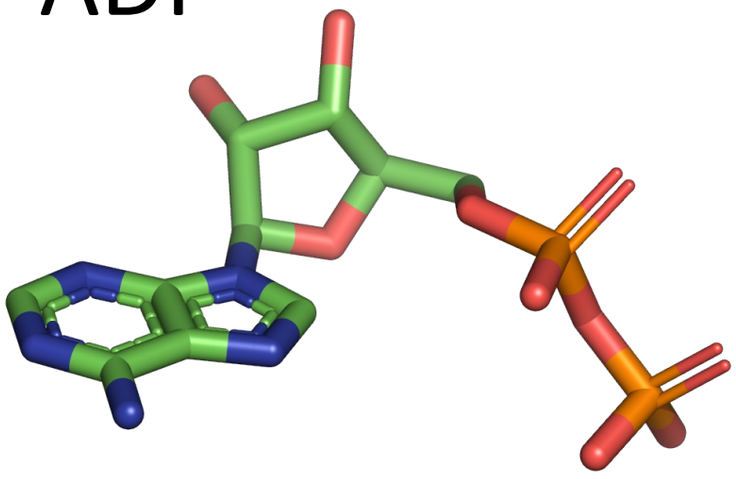
Automated ligand fitting by core-fragment fitting and extension into density

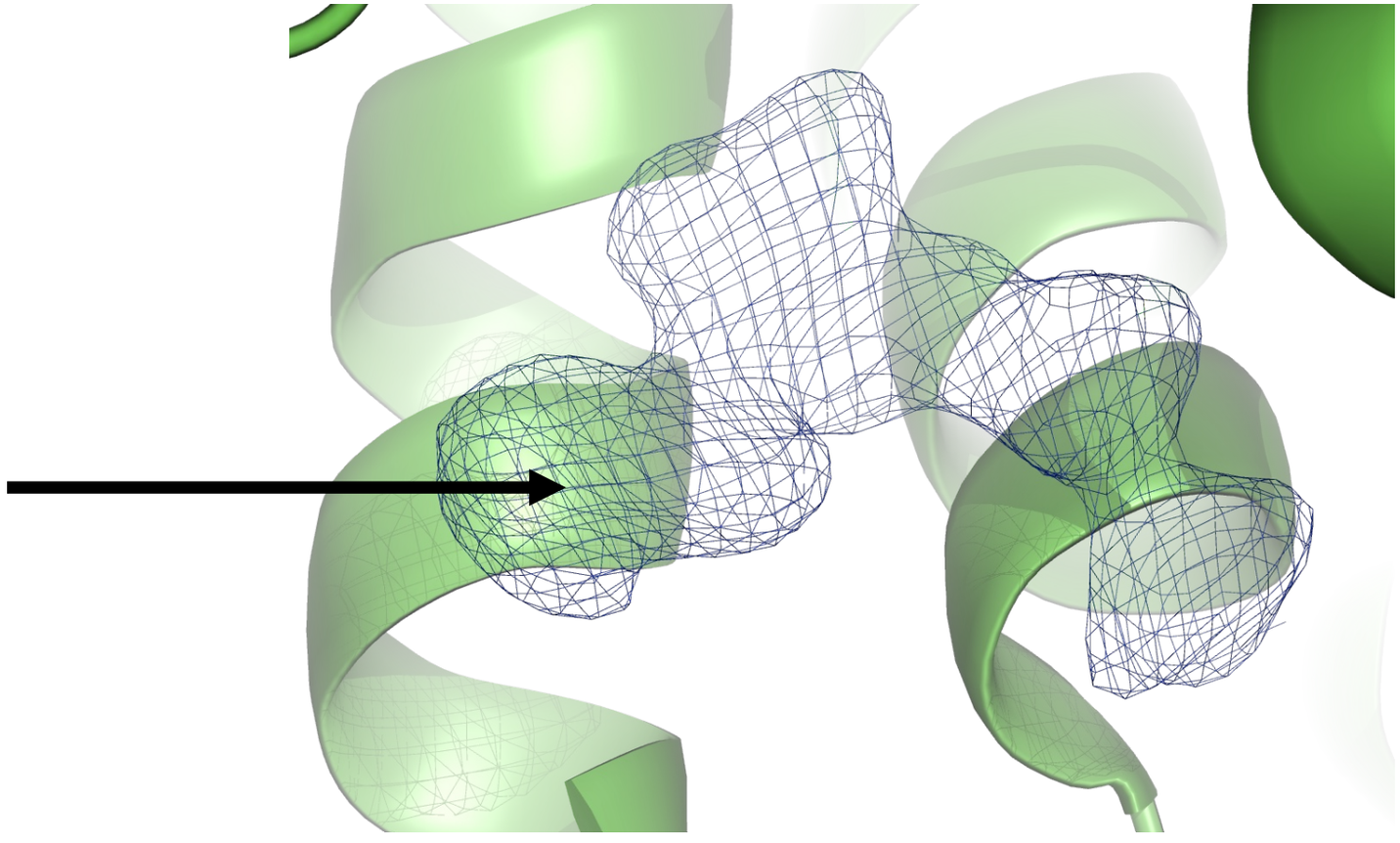
Thomas C. Terwilliger,[a]* Herbert Klei,[b] Paul D. Adams,[c] Nigel W. Moriarty[c] and Judith D. Cohn[a]

A procedure for fitting of ligands to electron-density maps by first fitting a core fragment of the ligand to density and then extending the remainder of the ligand into density is presented. The approach was tested by fitting 9327 ligands

Received 26 January 2006
Accepted 9 May 2006

ADP





Other tools for ligands in Phenix

- **LigandFit**: Flexible ligand fitting into difference maps.

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- **Guided ligand replacement**: facilitates ligand placement when a similar protein:ligand complex is known; often employed in structure-assisted drug design.

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Ligand placement based on prior structures: the guided ligand-replacement method

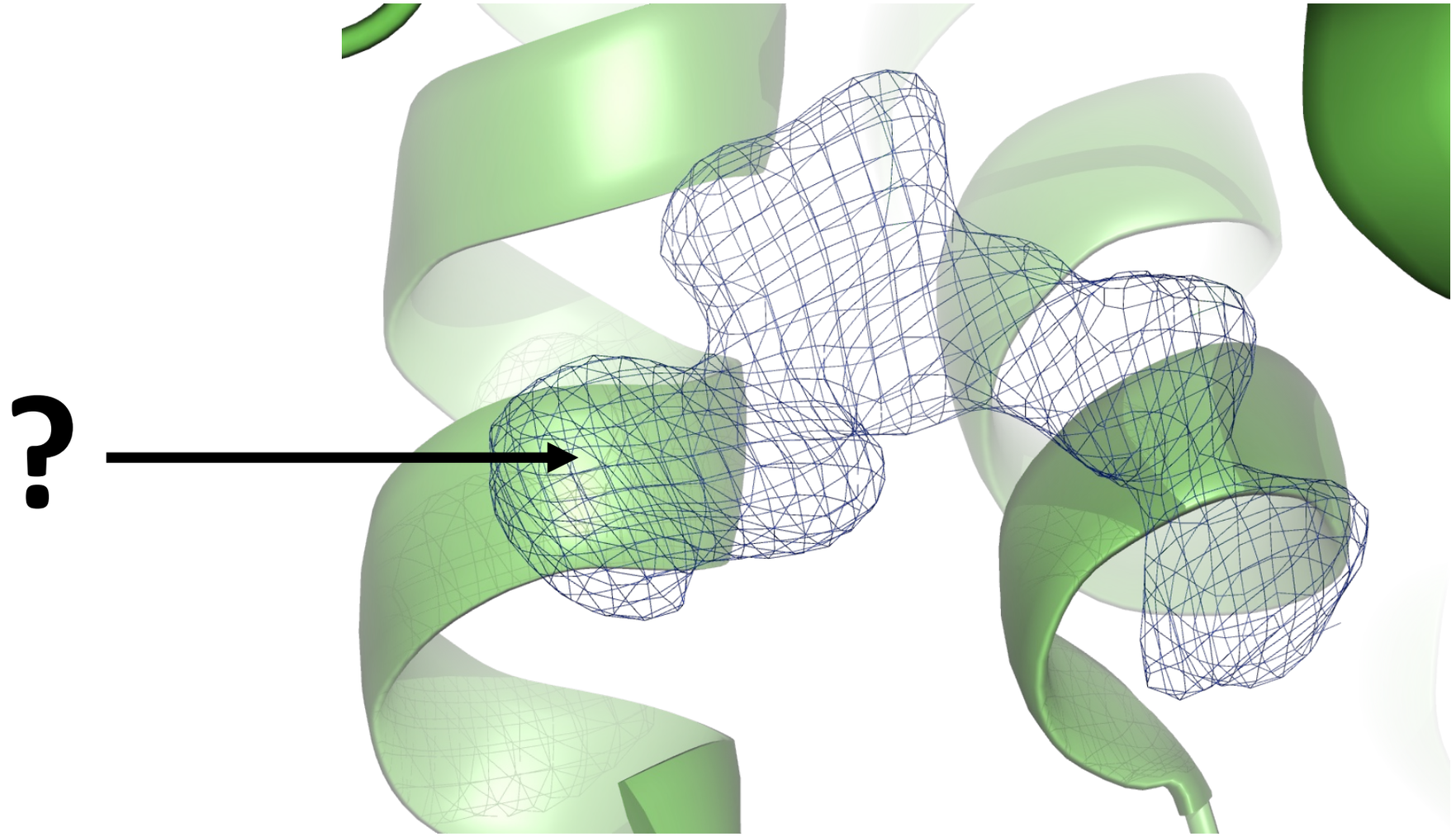
Herbert E. Klei,[a,b]‡ Nigel W. Moriarty,[a]*‡ Nathaniel Echols,[a] Thomas C. Terwilliger,[c] Eric T. Baldwin,[b,d] Matt Pokross,[b] Shana Posy[b] and Paul D. Adams[a,e]

The process of iterative structure-based drug design involves the X-ray crystal structure determination of upwards of 100 ligands with the same general scaffold (*i.e.* chemotype) complexed with very similar, if not identical, protein targets. In conjunction with insights from computational models and assays, this collection of crystal structures is analyzed to

Received 10 September 2013
Accepted 2 November 2013

Other tools for ligands in Phenix

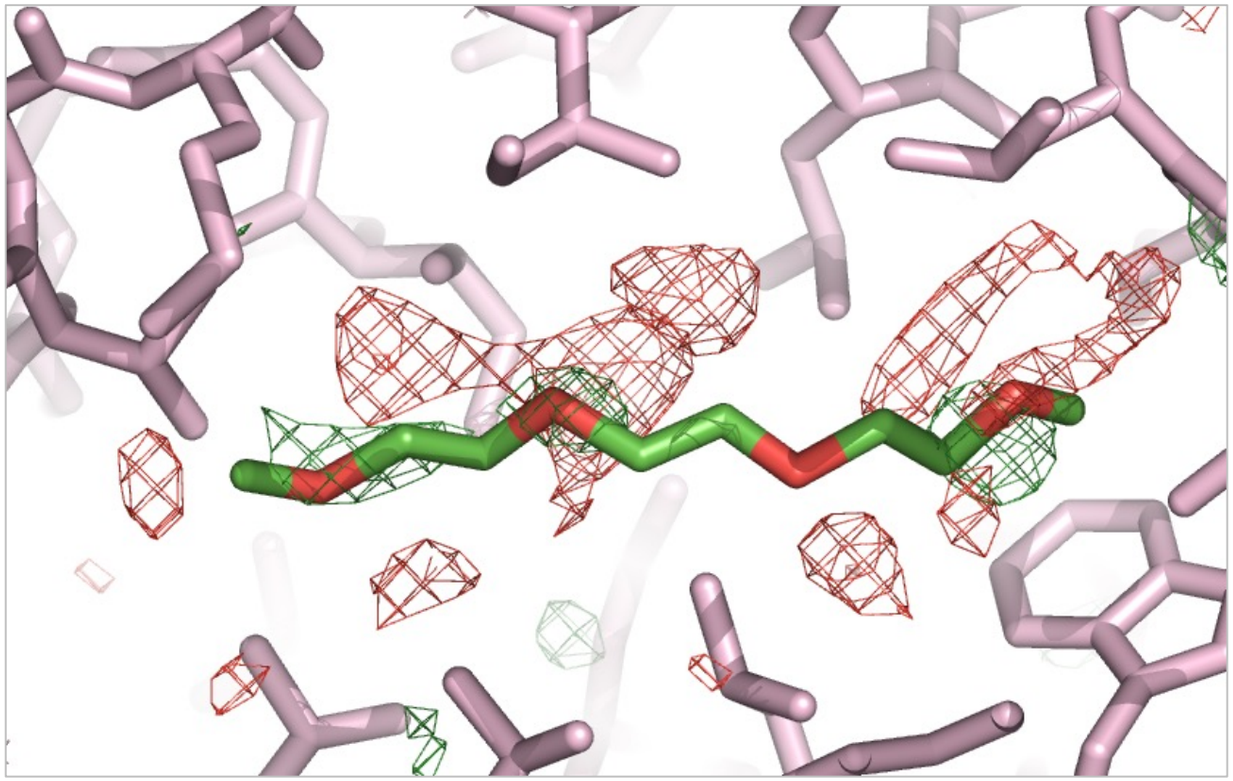
- **Ligand identification**: identifies an unknown ligand by analyzing difference density peaks. Uses a library of the 180 most frequently observed ligands in the PDB to rank each molecule by density fit and chemical interaction with the macromolecule.



Other tools for ligands in Phenix

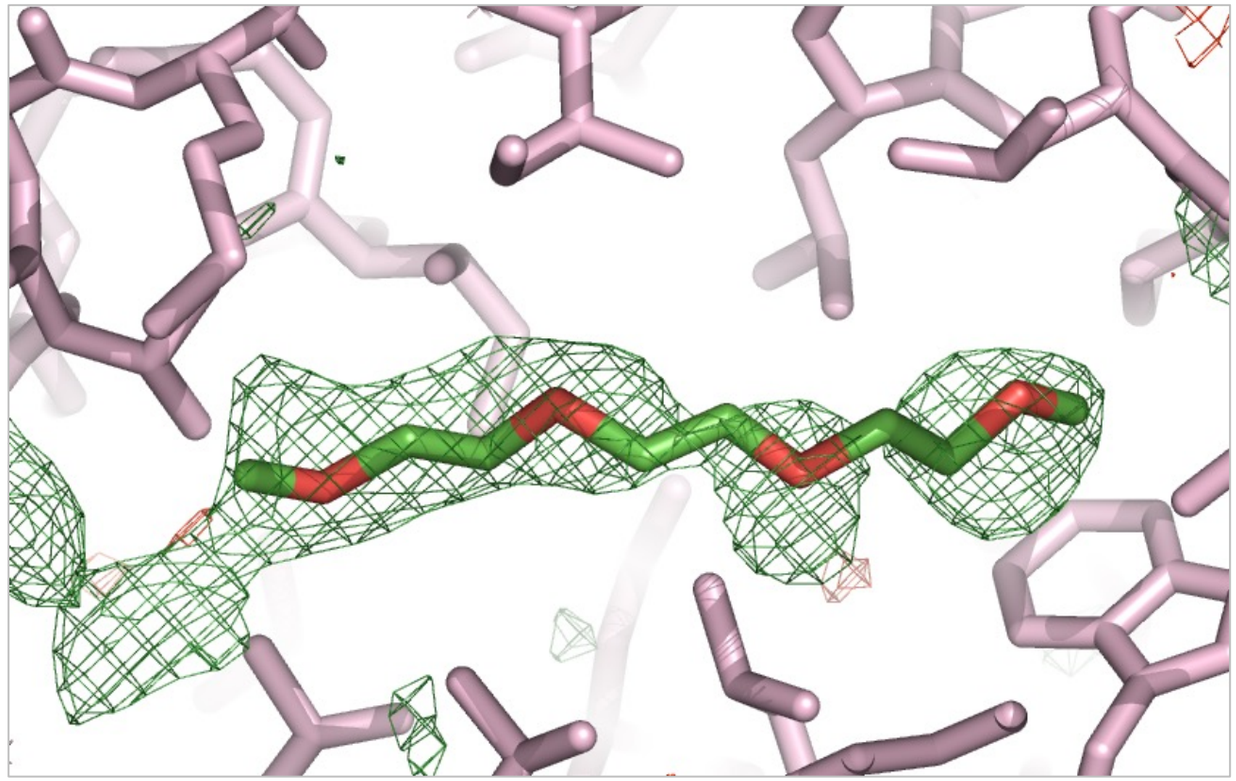
- **Polder maps**: OMIT map that excludes the bulk solvent around the ligand to visualize weak densities.

OMIT map



Contour +/- 3 rms

Polder map



Contour +/- 3 rms

Model: 1iWN
Resolution: 2.2 Å
Ligand: PG5 201

The Phenix Project

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