Ligands in Phenix

Generating & modifying for all scenarios

Oleg Sobolev

CBMS Structural Biology Workbenches
September 2024

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

215k models in the PDB

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163k out of 215k 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

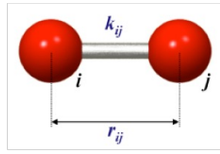
We need restraints for the ligand.

↓

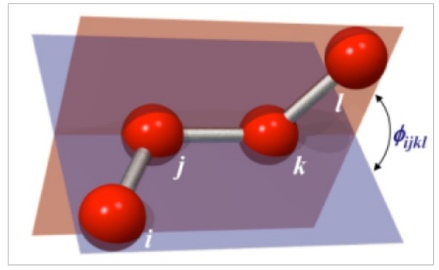
a priori knowledge

Stereochemical restraints specify ideal values for:

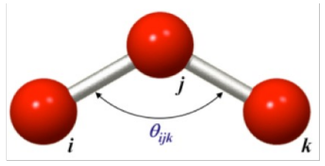
- Bond lengths
- Angles
- Torsions
- Planes
- Chiral volumes



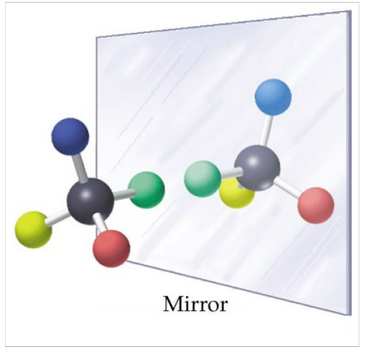
$\Sigma_{bonds}\ \omega(d_{model} - d_{ideal})^2$



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

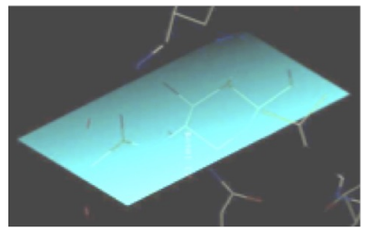


$\Sigma_{angles}\ \omega(\theta_{model} - \theta_{ideal})^2$



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

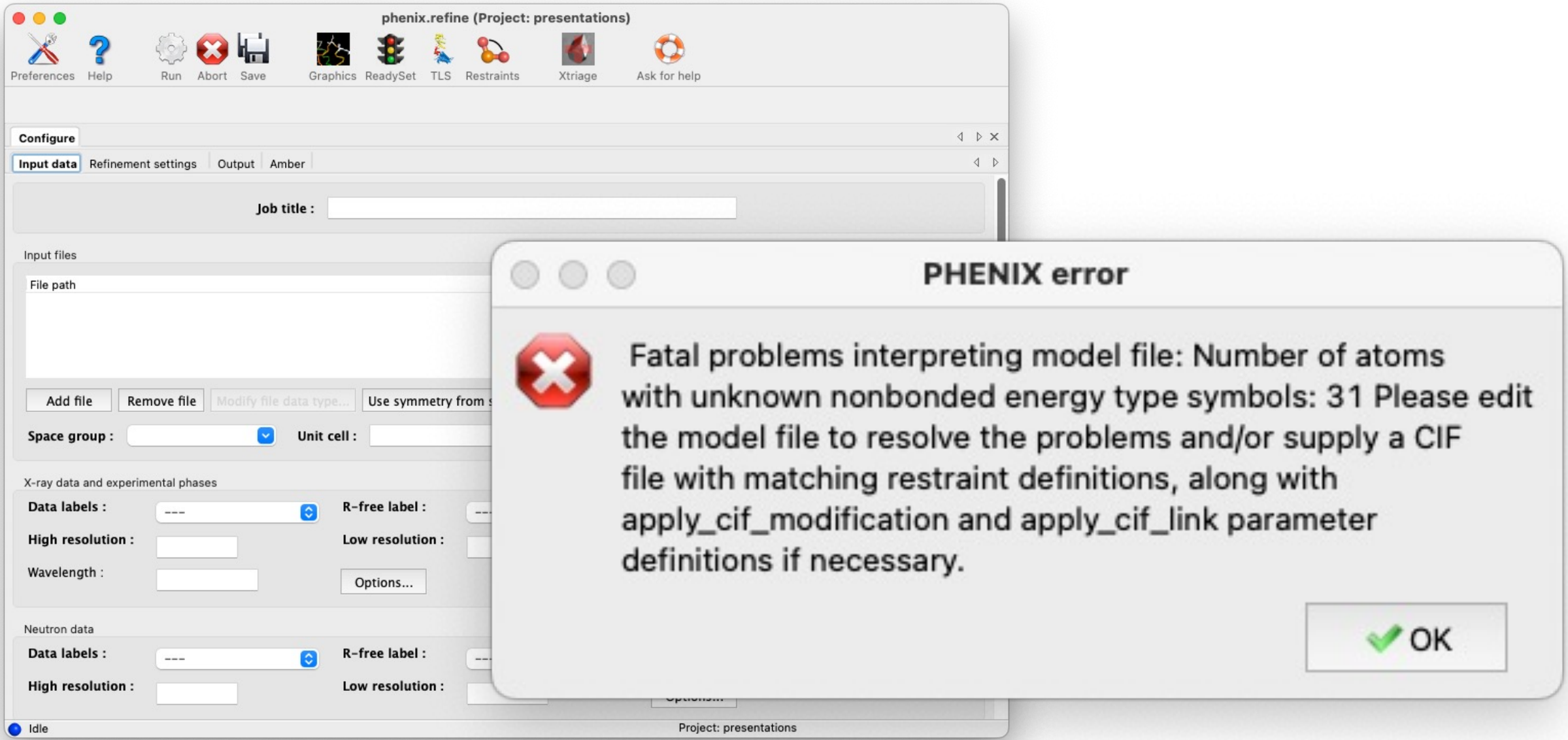
Images from PumMa web site (http://www.pumma.nl)



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

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!)

Sources of restraints

Libraries

- Monomer Library
- GeoStd

Algorithms

- Polymer
- Links

GeoStd

- All standard amino acids
- Current list of non-standard amino acids
- All standard RNA/DNA
- Current list of non-standard RNA/DNA
- Others – 34k Mogul validated restraints using PBEh-3c/CPCM and higher QM

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

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

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

Confusion

- All depositions of X-ray model use mmCIF from 1 July 2019
- “I need a CIF file.”
  - But what do you really need?

CIF

- Crystallographic Information File
- mmCIF – macro-molecular CIF
- Used for
  - Model
  - Data
  - Maps
  - Ligands
    - Information
    - Restraints

Restraints?

- Provide a reasonable geometry during refinement particularly at low resolution
  - Bonds, angles, dihedrals, chirals, planes, …
- Must be weighted against the experimental information

Overview

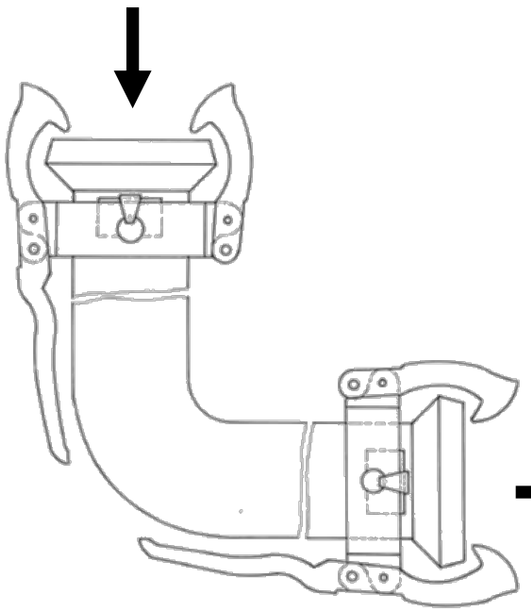
- eLBOW - electronic Ligand Builder & Optimisation Workbench
- ReadySet! - One-stop preparation for your refinement needs
- REEL - Restraints Editor Essentially Ligands

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

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electronic Ligand Builder and Optimization Workbench (*eLBOW*): a tool for ligand coordinate and restraint generation

Acta D paper

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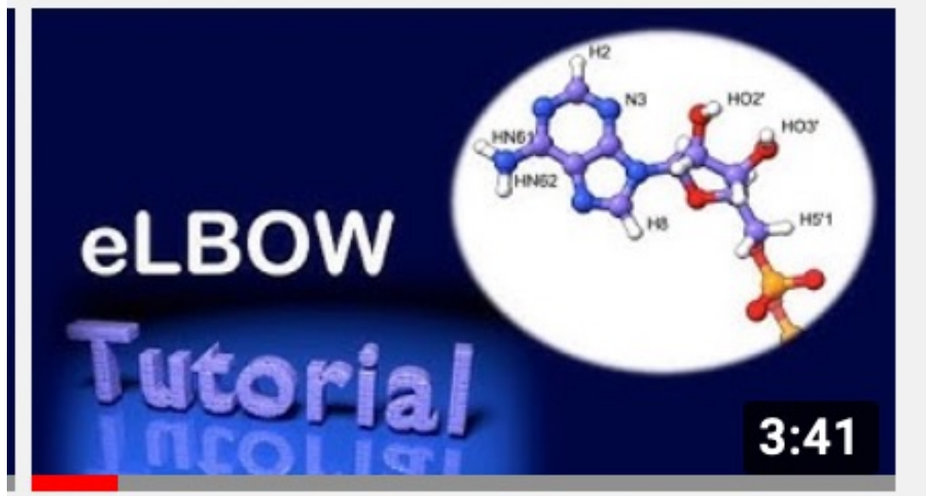
The *electronic Ligand Builder and Optimization Workbench* (*eLBOW*) is a program module of the *PHENIX* suite of computational crystallographic software. It is designed to be a flexible procedure that uses simple and fast quantum-chemical techniques to provide chemically accurate information for novel 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



Basic Phenix eLBOW tutorial

2.8K views • 5 years ago

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

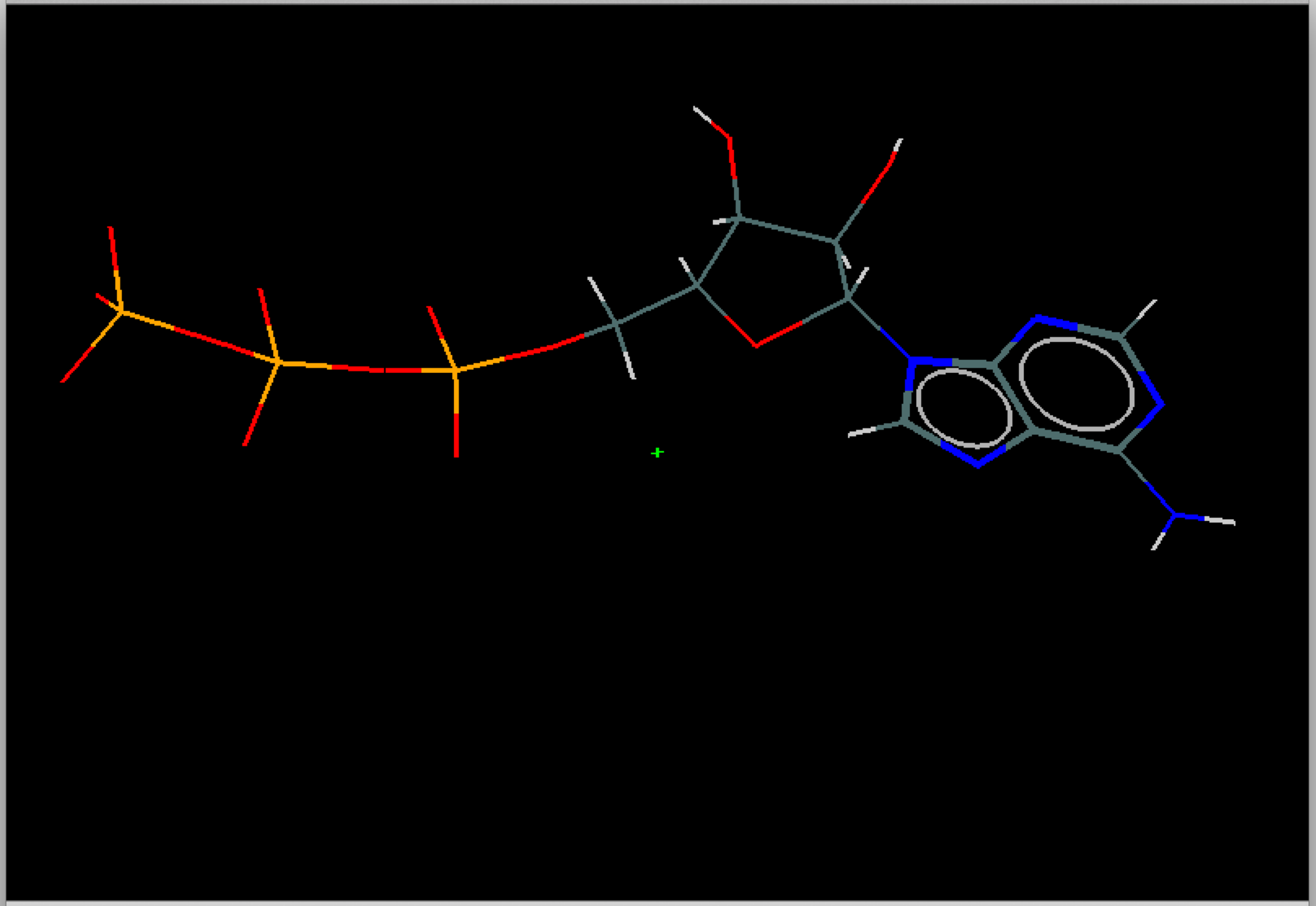
Purpose

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

Restraints Editor, Essentially Ligands (REEL)

- Generate a geometry for a set of restraints
- Modify restraints and generate new geometry
- Fast editing in menu items
- Highlight atom and restraints are highlighted
- Multiple ligands simultaneously
- Highlight restraint and atoms are highlighted
- Save restraints (CIF)
- Save geometry (PDB)
- Run eLBOW

REEL



MCS center | Center | Fit size | +Atom | +Bond | +Angle | +Dihedral | +Plane | -Atom | Quit

Restraints Editor Especially Ligands (REEL)

Simple Optimisation | eLBOW Optimisation | AM1 Optimisation | Search Components | Find unique code

ATP

Atoms(43) | Bonds(45) | Angles(78) | Dihedrals(30) | Planes(17) | Chirals(4) | CisTrans | Chirals Implicit(7) | BoatChair

	?	comp_id	atom_id	type_symbol	type_energy	charge	partial_charge	x	y	z
1		ATP	PG	P	P	0	.	-2.009900	-8.939900	0.927100
2		ATP	O1G	O	O	0	.	-0.919700	-9.907100	1.322000
3		ATP	O2G	O	OP	-1	.	-3.257700	-9.235200	1.724400
4		ATP	O3G	O	OP	-1	.	-2.306500	-9.089900	-0.545900
5		ATP	PB	P	P	0	.	-0.243200	-6.679500	0.469800
6		ATP	O1B	O	O	0	.	1.050300	-7.257000	0.992700
7		ATP	O2B	O	OP	-1	.	-0.341900	-6.929700	-1.016000
8		ATP	O3B	O	O2	0	.	-1.514200	-7.398500	1.233500
9		ATP	PA	P	P	0	.	0.670000	-3.968400	-0.041400
10		ATP	O1A	O	O	0	.	0.729800	-4.332500	-1.505600
11		ATP	O2A	O	OP	-1	.	2.063300	-3.990800	0.540200
12		ATP	O3A	O	O2	0	.	-0.276300	-5.056600	0.757100
13		ATP	O5'	O	O2	0	.	0.029800	-2.458300	0.123600
14		ATP	C5'	C	CH2	0	.	0.490600	-1.443000	-0.721300
15		ATP	C4'	C	CH1	0	.	-0.438000	-0.204600	-0.596500
16		ATP	O4'	O	O2	0	.	0.055400	0.644000	0.263400
17		ATP	C3'	C	CH1	0	.	-0.496500	0.556500	-1.940500
18		ATP	O3'	O	OH1	0	.	-1.718900	0.408900	-2.511600
19		ATP	C2'	C	CH1	0	.	-0.245900	2.038900	-1.588600
20		ATP	O2'	O	OH1	0	.	-1.282600	2.903300	-2.232200
21		ATP	C1'	C	CH1	0	.	-0.345300	2.109700	-0.288400
22		ATP	N9	N	NR5	0	.	0.564300	3.101200	0.226900
23		ATP	C8	C	CR15	0		1.894800	3.031600	0.318500

View preferences loaded

Restraints Editor Especially Ligands (REEL)

Simple Optimisation | eLBOW Optimisation | AM1 Optimisation | Search Components | Find unique code

ATP

Atoms(43) | **Bonds(45)** | Angles(78) | Dihedrals(30) | Planes(17) | Chirals(4) | CisTrans | Chirals Implicit(7) | BoatChair

	?	comp_id	atom_id_1	atom_id_2	type	value_dist	value_dist_esd	value_dist_neutron
1		ATP	PG	O1G	deloc	1.510000	0.020000	1.51
2		ATP	PG	O2G	deloc	1.510000	0.020000	1.51
3		ATP	PG	O3G	deloc	1.510000	0.020000	1.51
4		ATP	PG	O3B	single	1.648000	0.020000	1.648
5		ATP	PB	O1B	deloc	1.510000	0.020000	1.51
6		ATP	PB	O2B	deloc	1.510000	0.020000	1.51
7		ATP	PB	O3B	single	1.648000	0.020000	1.648
8		ATP	PB	O3A	single	1.648000	0.020000	1.648
9		ATP	PA	O1A	deloc	1.510000	0.020000	1.51
10		ATP	PA	O2A	deloc	1.510000	0.020000	1.51
11		ATP	PA	O3A	single	1.648000	0.020000	1.648
12		ATP	PA	O5'	single	1.648000	0.020000	1.648
13		ATP	O5'	C5'	single	1.399000	0.020000	1.399
14		ATP	C5'	C4'	single	1.553000	0.020000	1.553
15		ATP	C5'	H5'1	single	0.970000	0.020000	1.09
16		ATP	C5'	H5'2	single	0.970000	0.020000	1.09
17		ATP	C4'	O4'	single	1.305000	0.020000	1.305
18		ATP	C4'	C3'	single	1.546000	0.020000	1.546
19		ATP	C4'	H4'	single	0.970000	0.020000	1.09
20		ATP	O4'	C1'	single	1.617000	0.020000	1.617
21		ATP	C3'	O3'	single	1.357000	0.020000	1.357
22		ATP	C3'	C2'	single	1.544000	0.020000	1.544
23		ATP	C3'	H3'	single	0.970000	0.020000	1.09

View preferences loaded

Summary

- eLBOW & ReadySet! perform better when provided with better input. (GIGO)
- Need to know something about the ligand

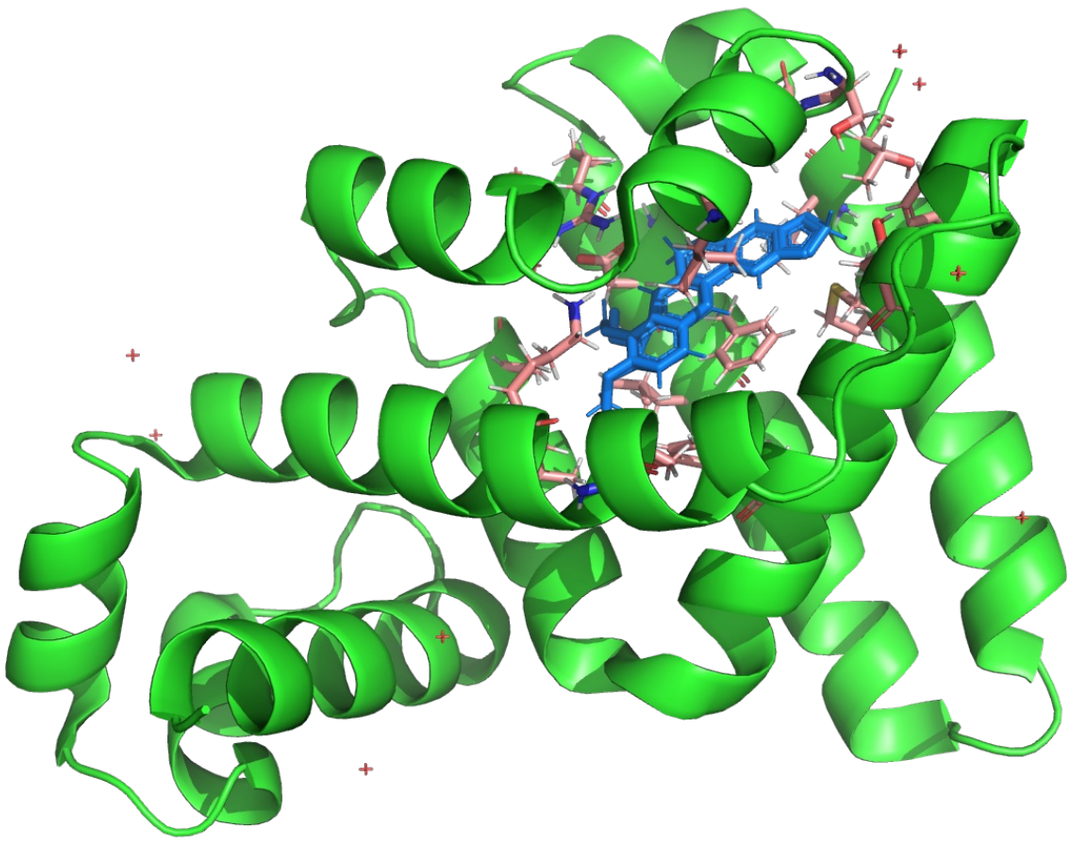
  Hierarchy of input file value

  - Check your .geo file for confirmation of restraints

QMR – quantum mechanical restraints

Restraint generators don’t predict

- Chemical variability (configuration or pucker)
- Specific binding interactions



BER in 3vw2

QMR approach:

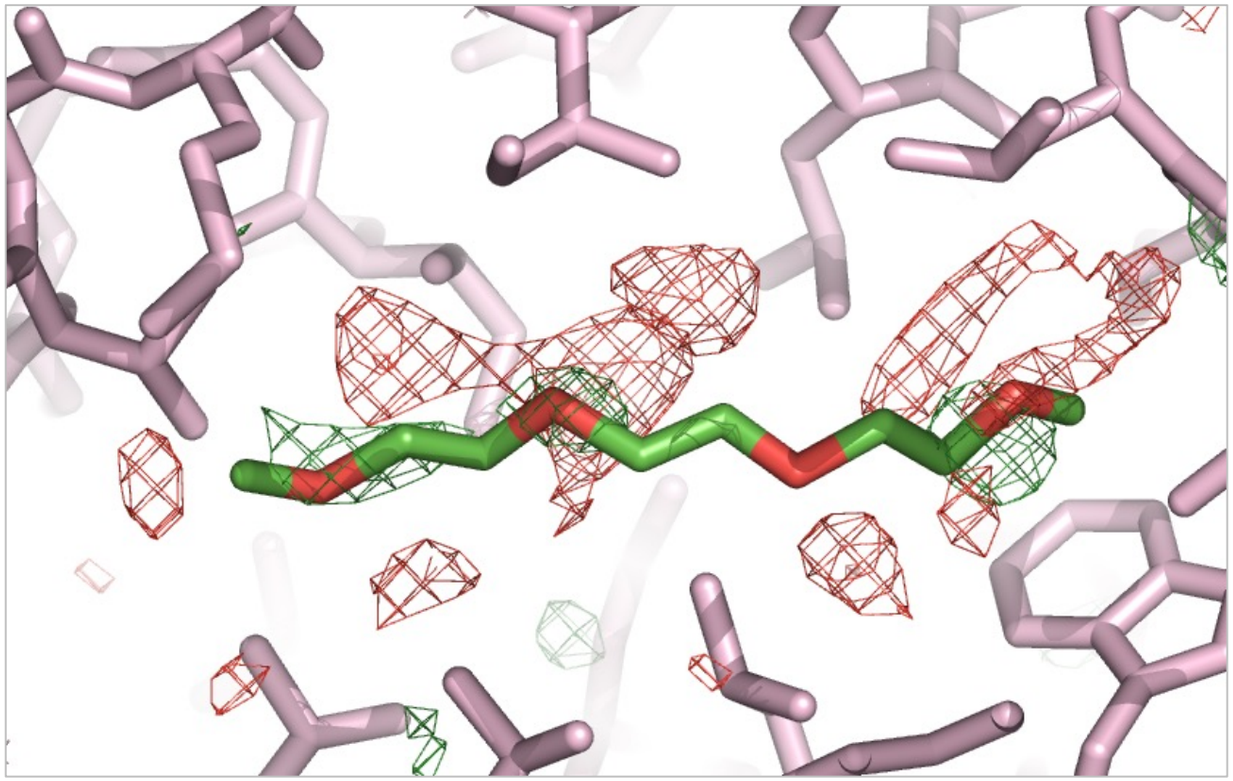
- Minimize the ligand geometry in the binding pocket.
- Use minimized ligand geometry as targets for restraints.
  (forget about what happened with the residues)

Liebschner D., Moriarty, N.W., Poon, B. K. & Adams, P. D., In situ ligand restraints from quantum-mechanical methods. *Acta Cryst. D*. **79**, 100–110, (2023)

Other tools for ligands in Phenix

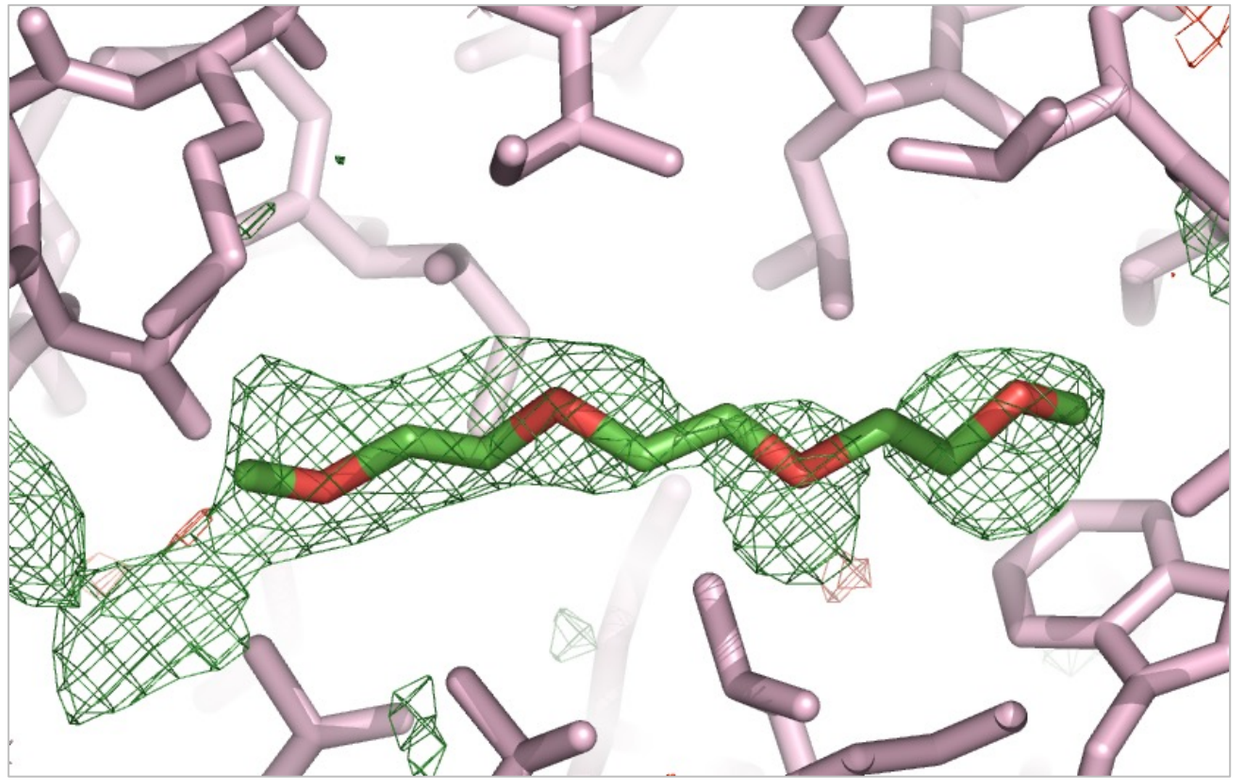
- **Polder maps**: OMIT map that excludes the bulk solvent around the ligand to visualize weak densities.

OMIT map



Contour +/- 3 rms

Polder map



Contour +/- 3 rms

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

Other tools for ligands in Phenix

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

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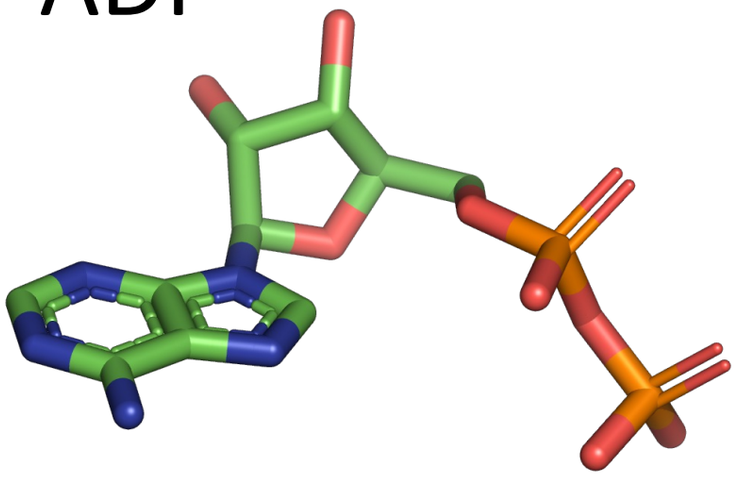
Automated ligand fitting by core-fragment fitting and extension into density

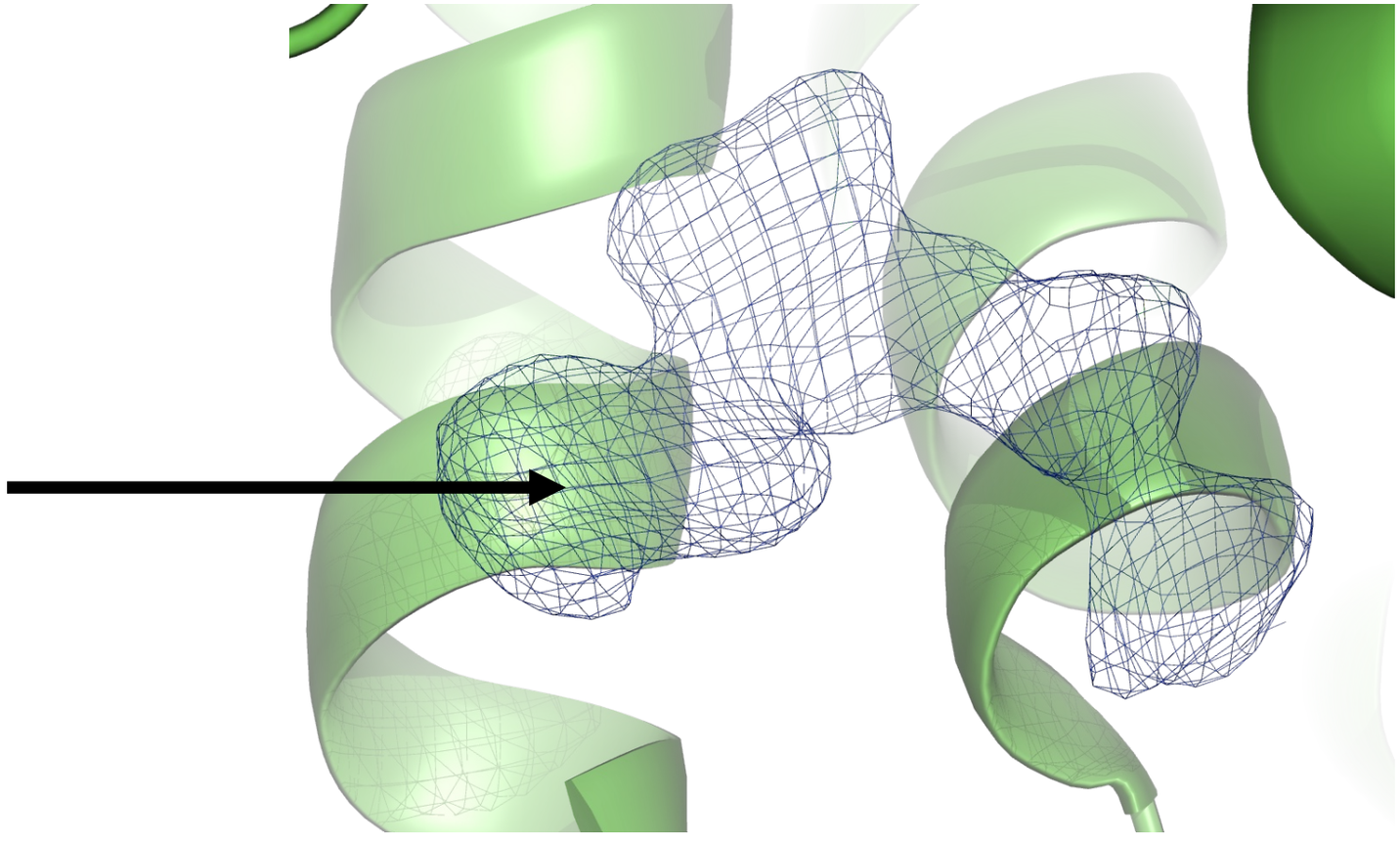
Thomas C. Terwilliger,[a]* Herbert Klei,[b] Paul D. Adams,[c] Nigel W. Moriarty[c] and Judith D. Cohn[a]

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





The Phenix Project

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Duke University

Jane & David Richardson, Christopher Williams, Vincent Chen

An NIH/NIGMS funded Program Project

Liebschner D, *et al*., Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. Acta Cryst. 2019 **D75**:861–877

ReadySet!

- Add hydrogens
    - Default: adds hydrogens to protein, ligands
        - Protein - Reduce
        - Ligands - eLBOW
        - Add hydrogens to water
        - Add deuteriums instead of hydrogens
        - Add hydrogen & deuteriums appropriately
- Generate restraints

ReadySet!

- Restraints CIF filename
- Restraints CIF directory
- LINKS to “edits”
- --dry-run to show ligand process pathway
- Metal coordination