AsCA meeting,
6 December 2025

Ligands

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Lawrence Berkeley Laboratory

Ligands in macromolecular models

- 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” (from solvent solutions).
- Binding: non-covalent and/or covalent bonds.

244k models in the PDB[1]

[1]October 2025

Ligands in macromolecular models

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185k out of 244k models in the PDB[1] have at least one ligand (”distinct non-polymer entity”)

75%

[1]October 2025

Ligands in macromolecular models

- Small molecule (more than 1 atom, ligand, sugar, covalent modification, buffer molecules,…).
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30k electron microscopy models in the PDB[1]

[1]October 2025

Ligands in macromolecular models

- 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” (from solvent solutions).
- Binding: non-covalent and/or covalent bonds

18.5k out of 30k electron microscopy models in the PDB[1] have at least one ligand.
62%

→ Many samples contain ligands!

[1]October 2025

Working with ligands

1) Find out what ligand(s) are in the structure.
2) Add the ligand to the model.
3) Refine the ligand along with the macromolecule

We need restraints for the ligand.

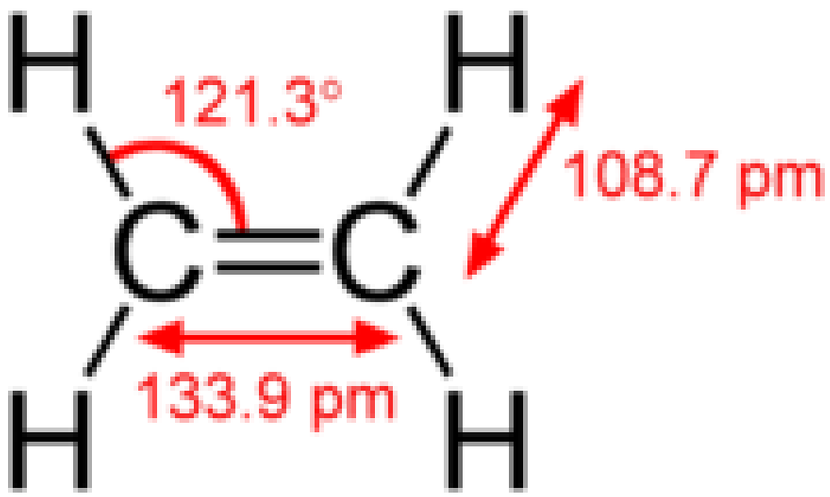
↓

a priori knowledge

Restraints modify the target function by creating relationships between independent parameters.

Restraints: *a priori* knowledge

Example: restrained bond lengths

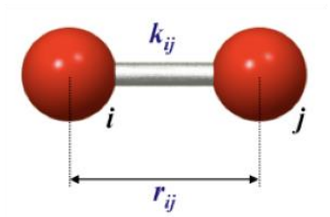


- the coordinates of the two atoms are independent
- restraint keeps their distance within a certain target value
- imposes a penalty if it deviates too much.

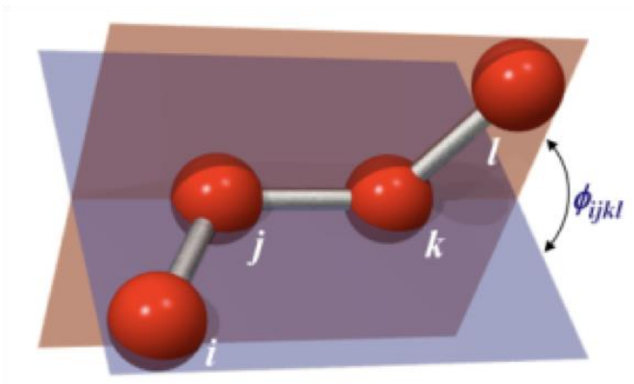
Ligands need restraints

Stereochemical restraints specify ideal values for:

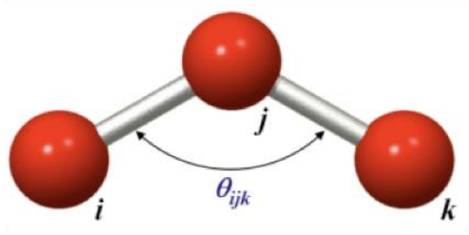
- Bond lengths
- Angles
- Torsions
- Planes
- Chiral volumes



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

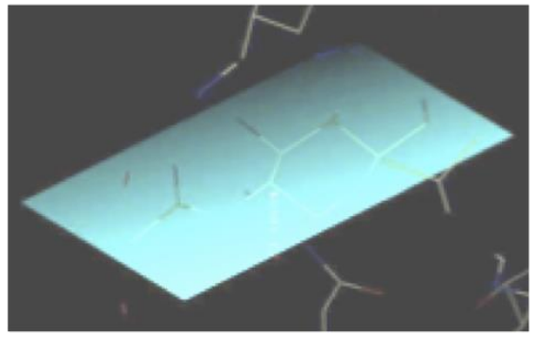


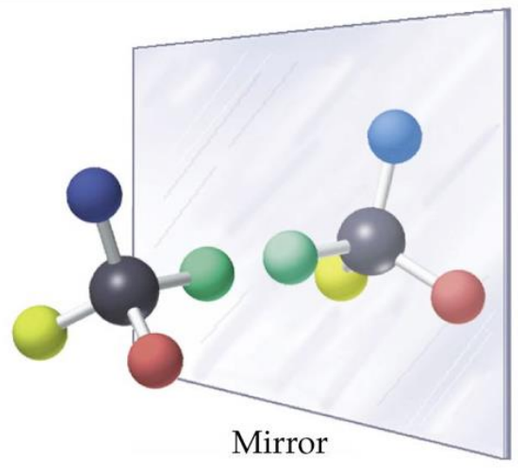
$\Sigma_{dihedrals}\ \omega(1 + \cos(n\chi_{model} + \chi_{shift}))$



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

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



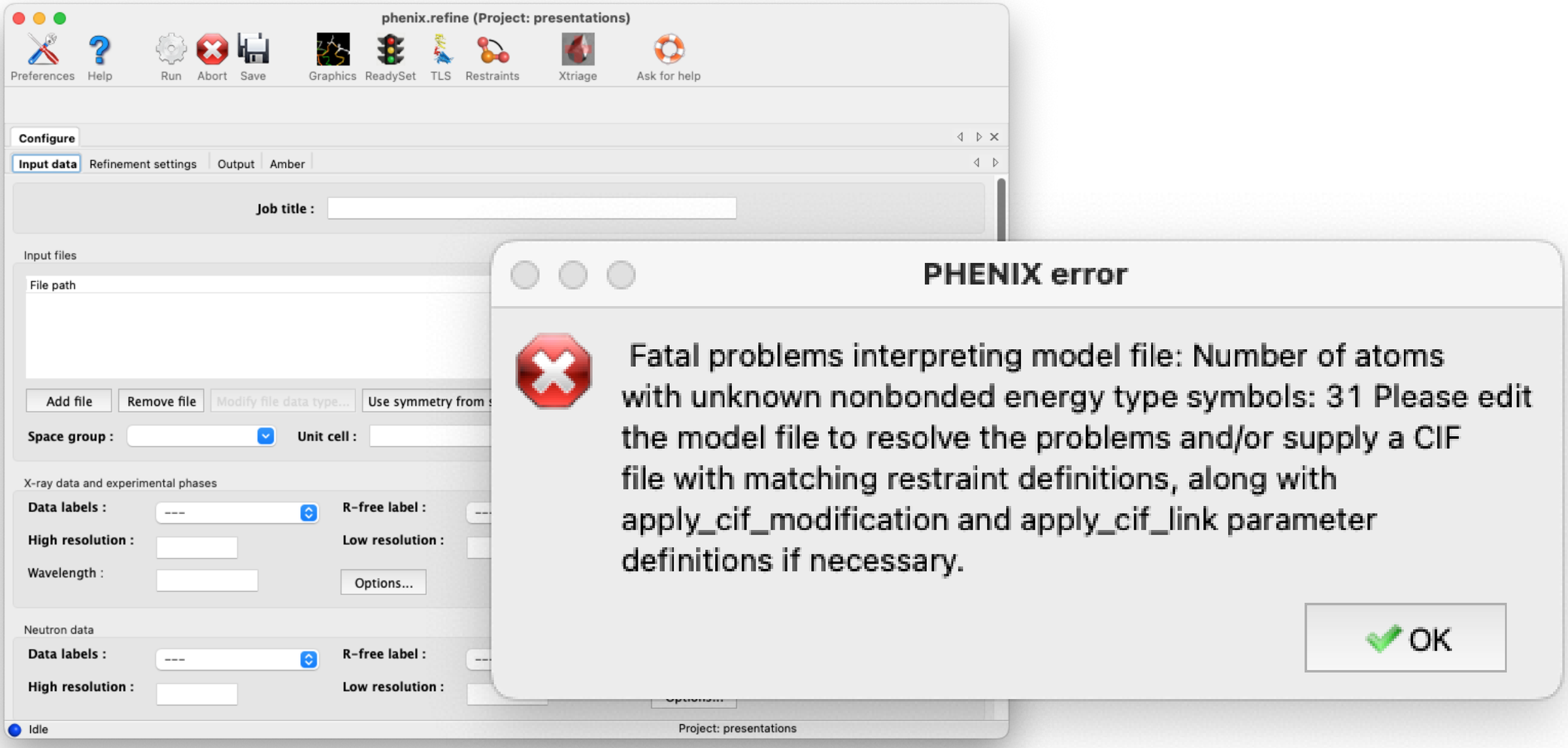


Volume (V) = $(r_N - r_{CA}) \bullet [(r_C - r_{CA}) \times (r_{CB} - r_{CA})]$

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

Ligands need restraints

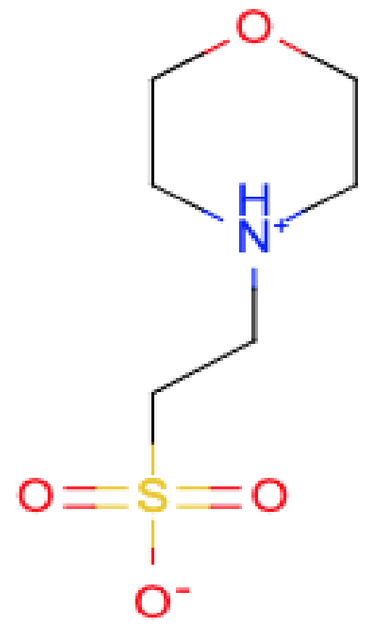
If you try to run phenix.refine and a ligand has no restraints…

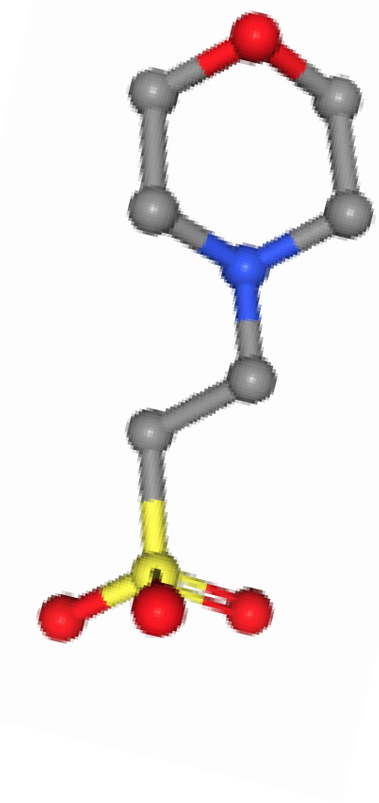


(This message also occurs if atom names don’t match!)

Restraints: Ligands

Restraints of common ligands are included in libraries.





If novel ligand:

- **eLBOW**
  ligand builder
- **ReadySet**!
  Wrapper for eLBOW with additional features (add H atoms)
- **REEL**
  GUI for editing restraints

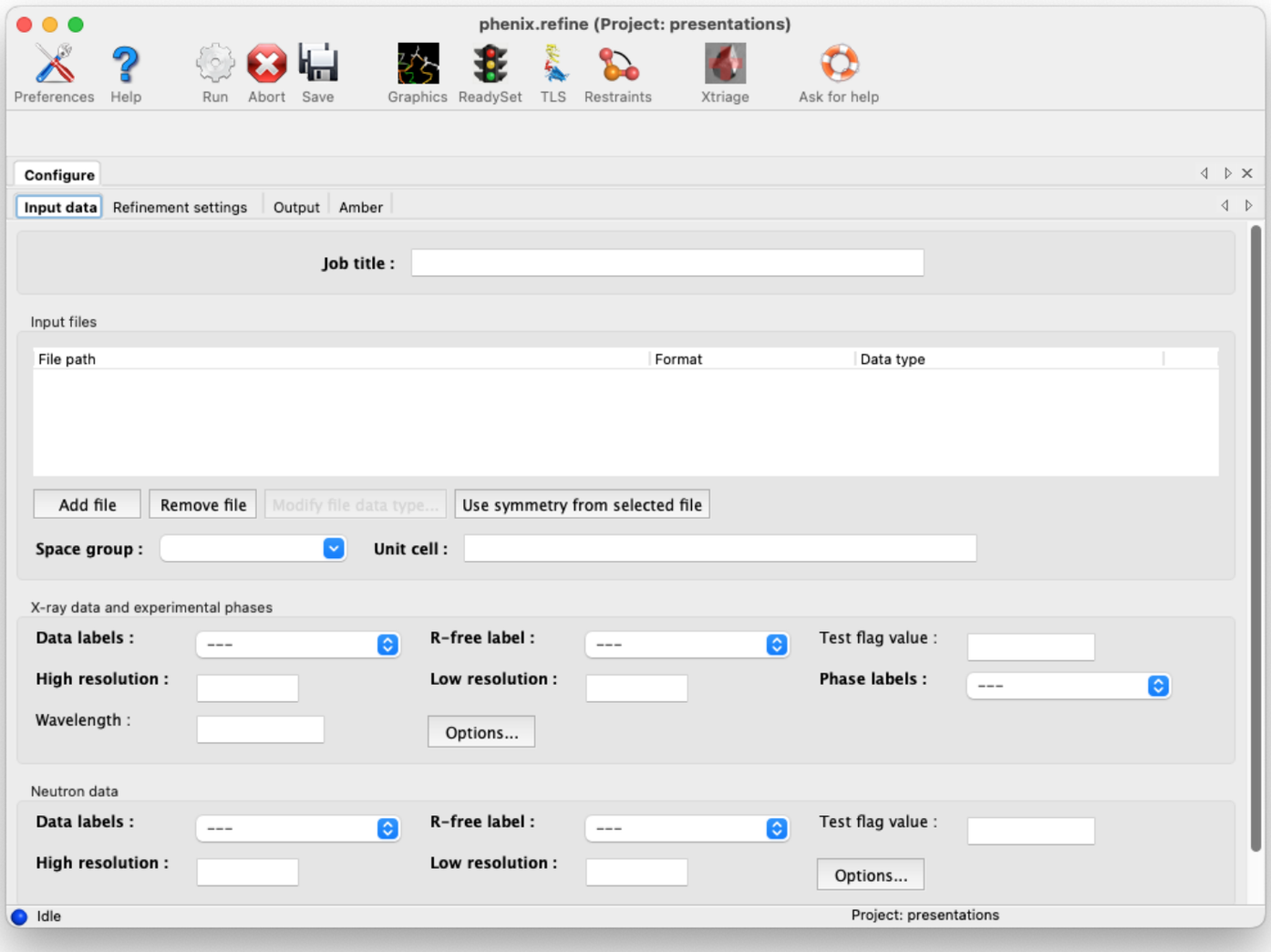
Restraints libraries

- Amino acids: Engh&Huber dictionary
- Nucleic Acids: E.g., CCP4 monomer library
- Common ligands: GeoStandard (Geostd)
- Novel ligands → Use a dictionary generator

Standard library in Phenix: GeoStd

Phenix comes with the GeoStd library (37k).

→ 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.

Novel ligands

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

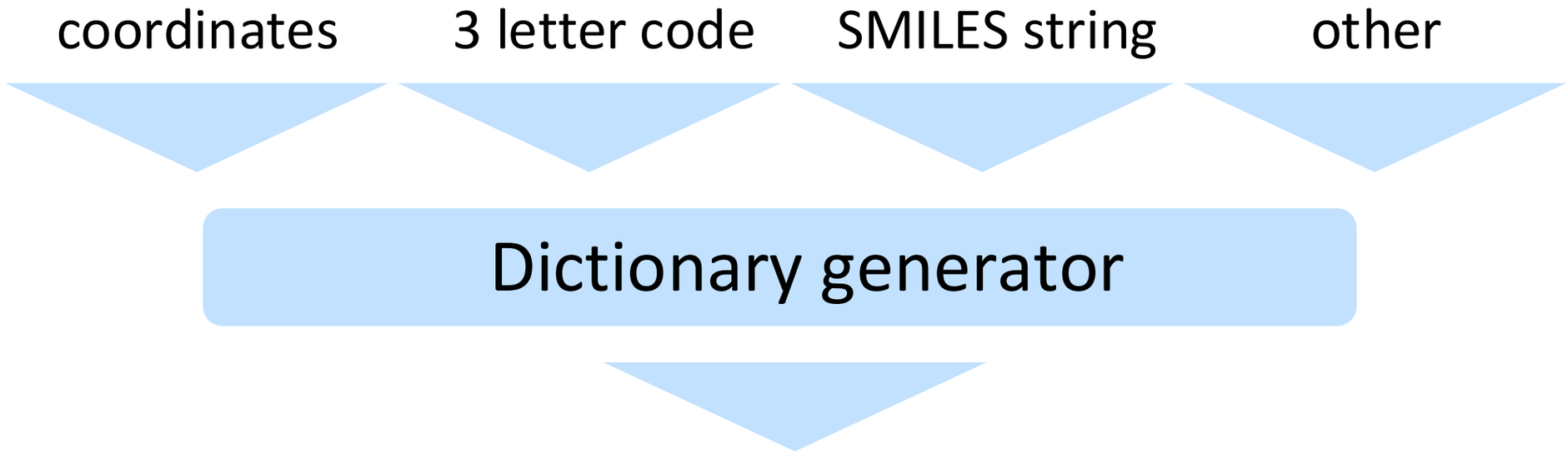
- Check other libraries
- If relying on 3 letter code: double check that it is the correct one

If still not found:

→ Get restraints from a dictionary generator

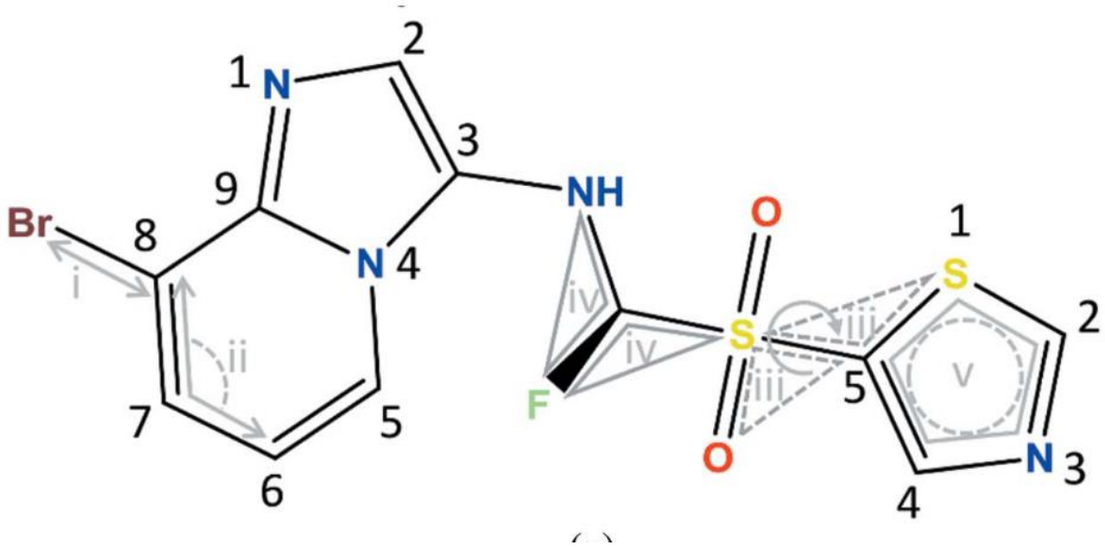
Dictionary generator

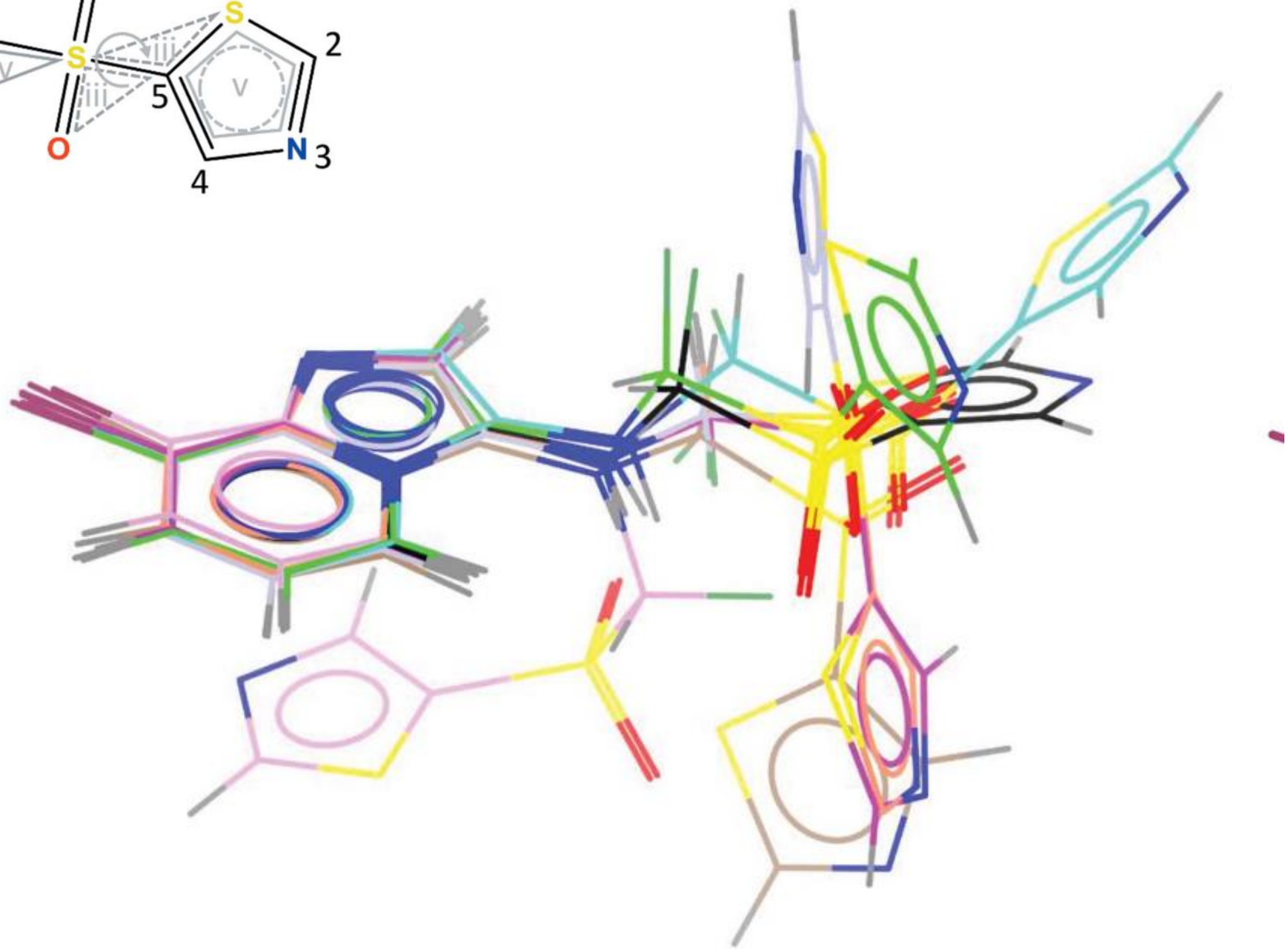
Create restraints programmatically by using experimental information (wwPDB, CCD, CS, COD) or by molecular-simulation.



Don’t confuse this with a model file (mmcif) or a reflection file (sf-cif)

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

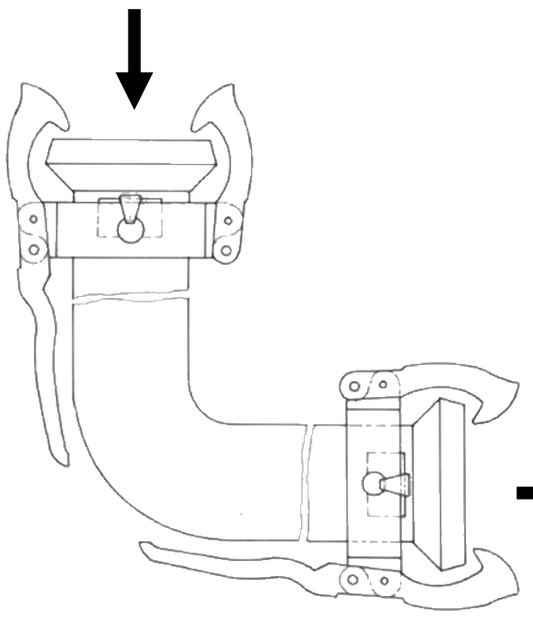
- eLBOW
- ReadySet!

eLBOW

eLBOW = electronic Ligand Builder and Optimisation Workbench

- Automated generation of restraints for ligands
- Fast, simple and flexible procedure
- geometry can be optimized, e.g., via semi-empirical quantum mechanical method (AM1)

Chemical input



Chemical restraints (CIF)
Cartesian coordinates (PDB)

eLBOW

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

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

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

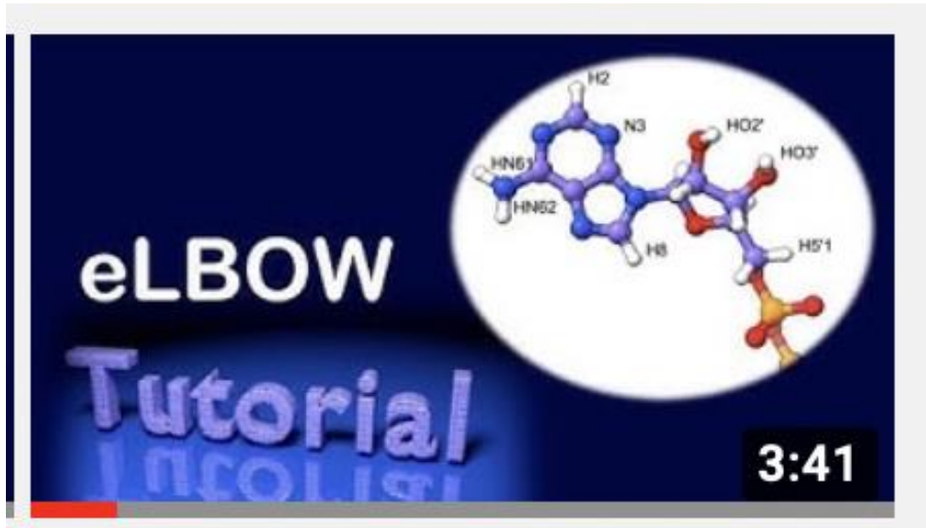
[a]Lawrence Berkeley National Laboratory, One Cyclotron Road, Mailstop 64R0246, Berkeley, CA 94720, USA, and [b]Department of Bioengineering, UC Berkeley, CA 94720, USA

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

Acta D paper

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.

Model file

ReadySet!

Restraints, model file

ReadySet!

Contents

Author

Nigel W. Moriarty

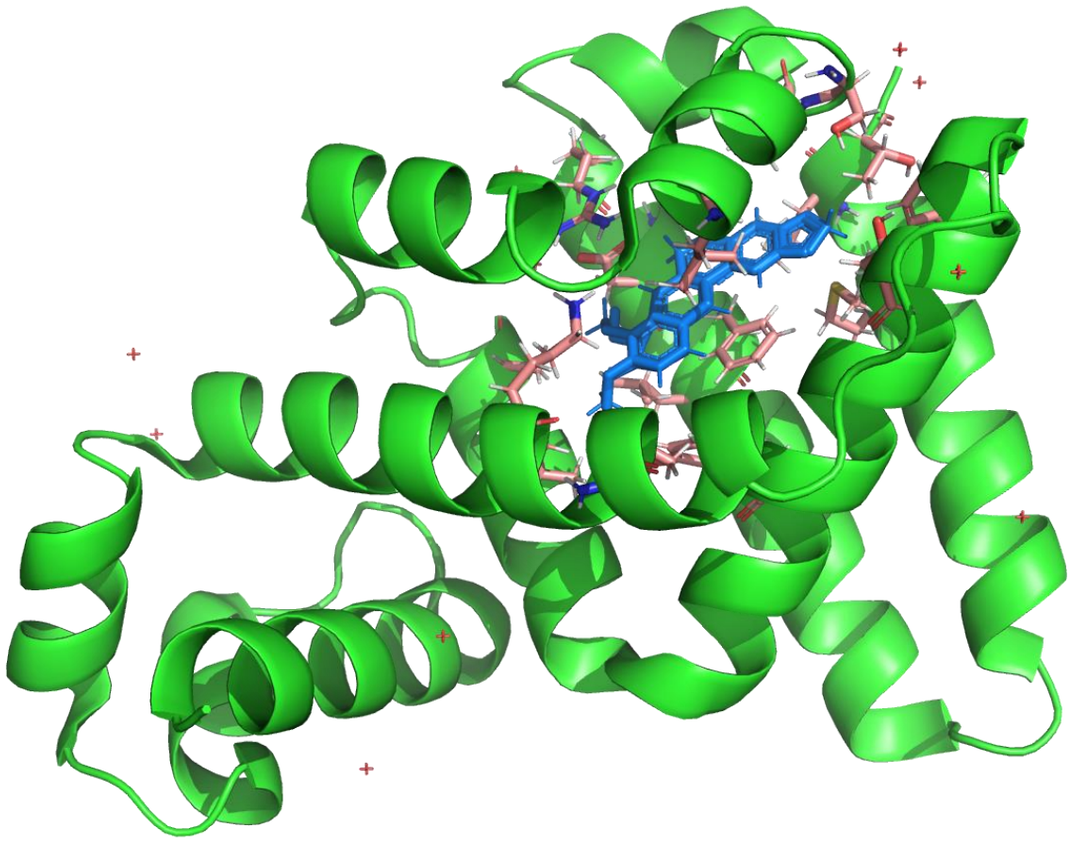
Purpose

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

QMR – quantum mechanical restraints

Restraint generators don’t predict

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



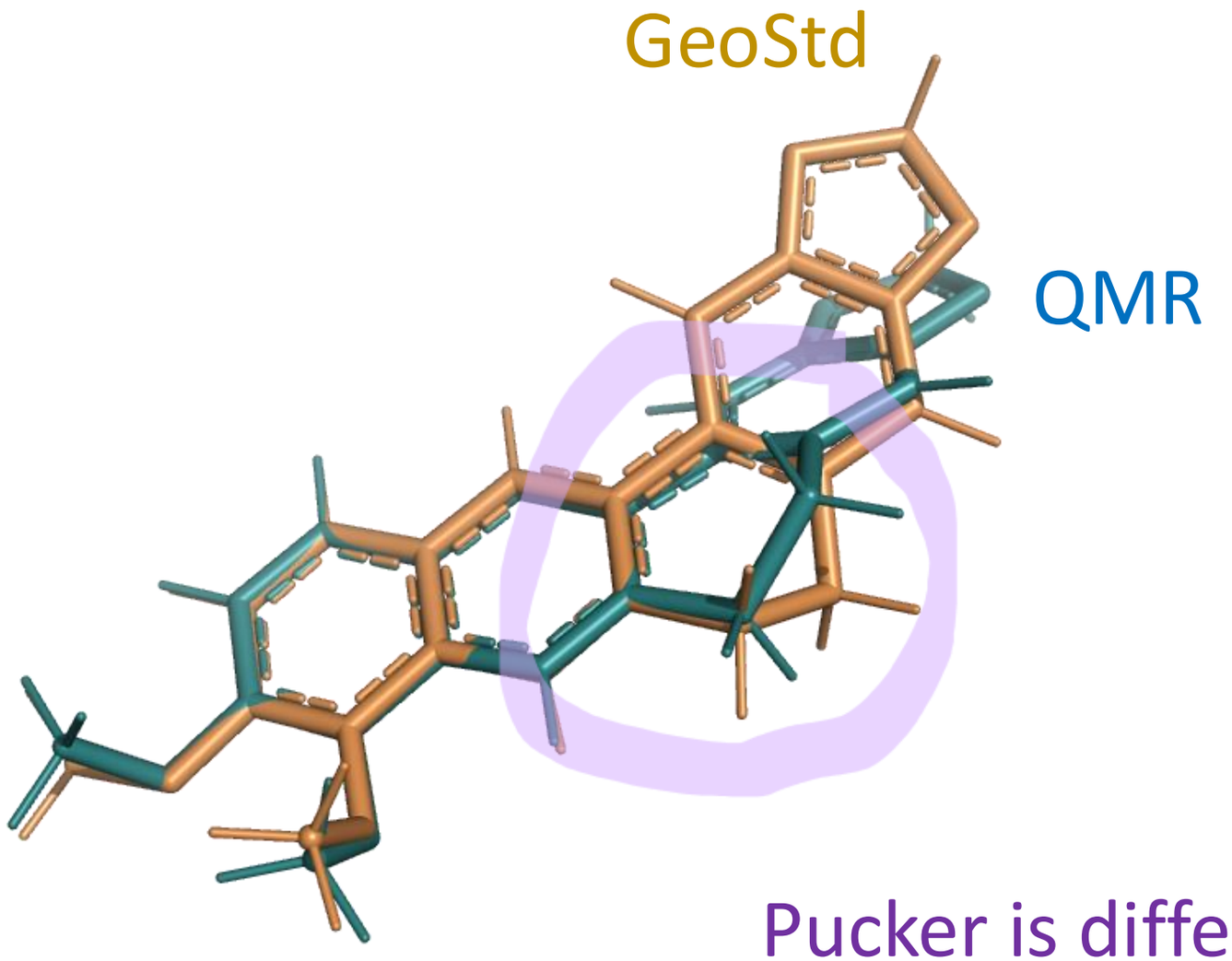
BER in 3vw2

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)

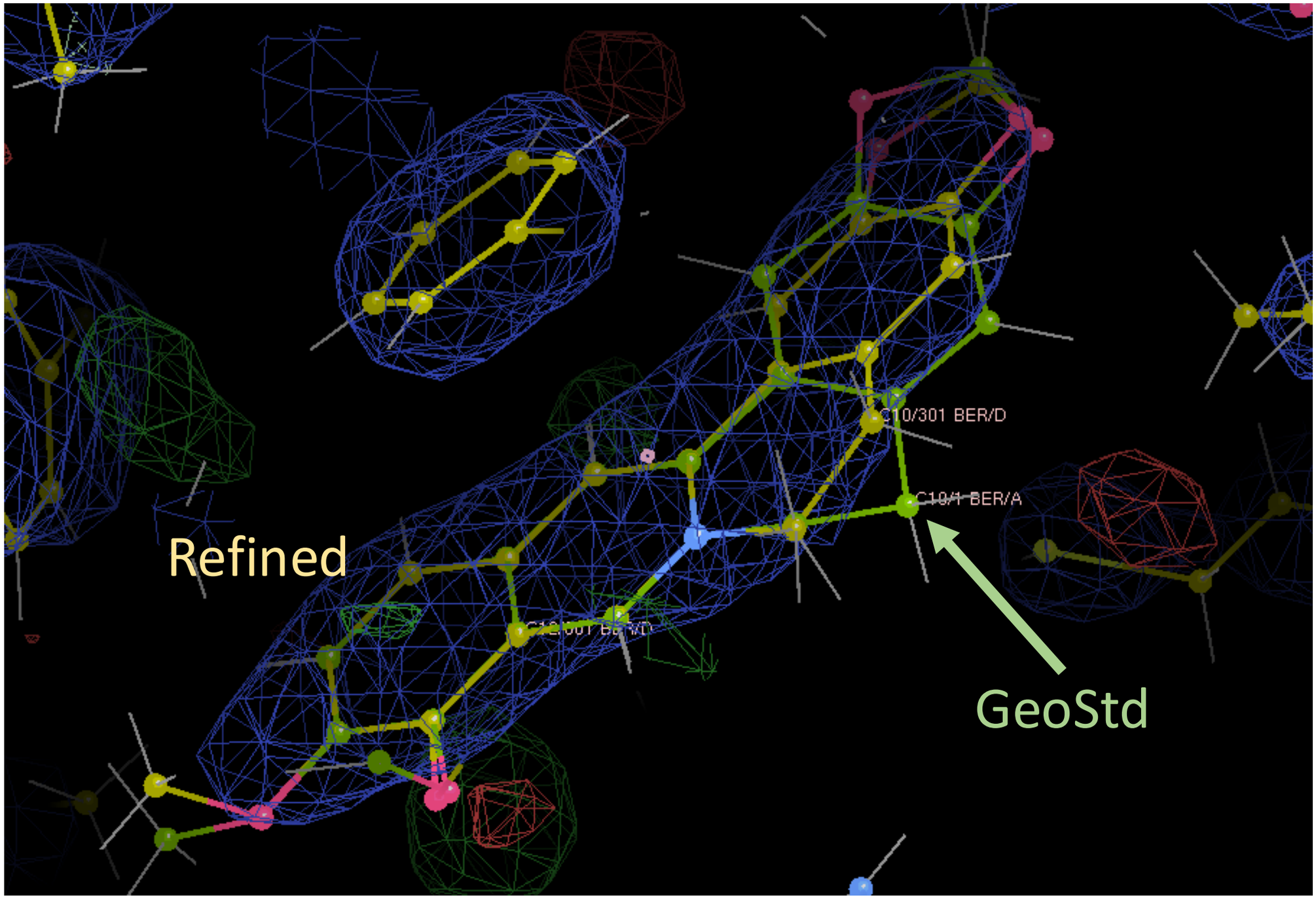
Liebschner D., Moriarty, N.W., Poon, B. K. & Adams, P. D., In situ ligand restraints from quantum-mechanical methods. *Acta Cryst. D*. **79**, 100–110, (2023)

GeoStd vs QMR



Pucker is different!

GeoStd structure in the density



QMR restraints are chemically plausible.

Other tools for ligands in Phenix

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

Acta Crystallographica Section D
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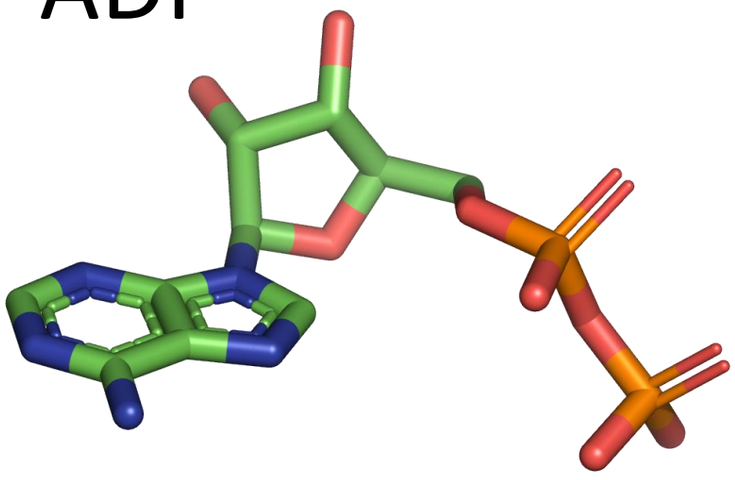
Automated ligand fitting by core-fragment fitting and extension into density

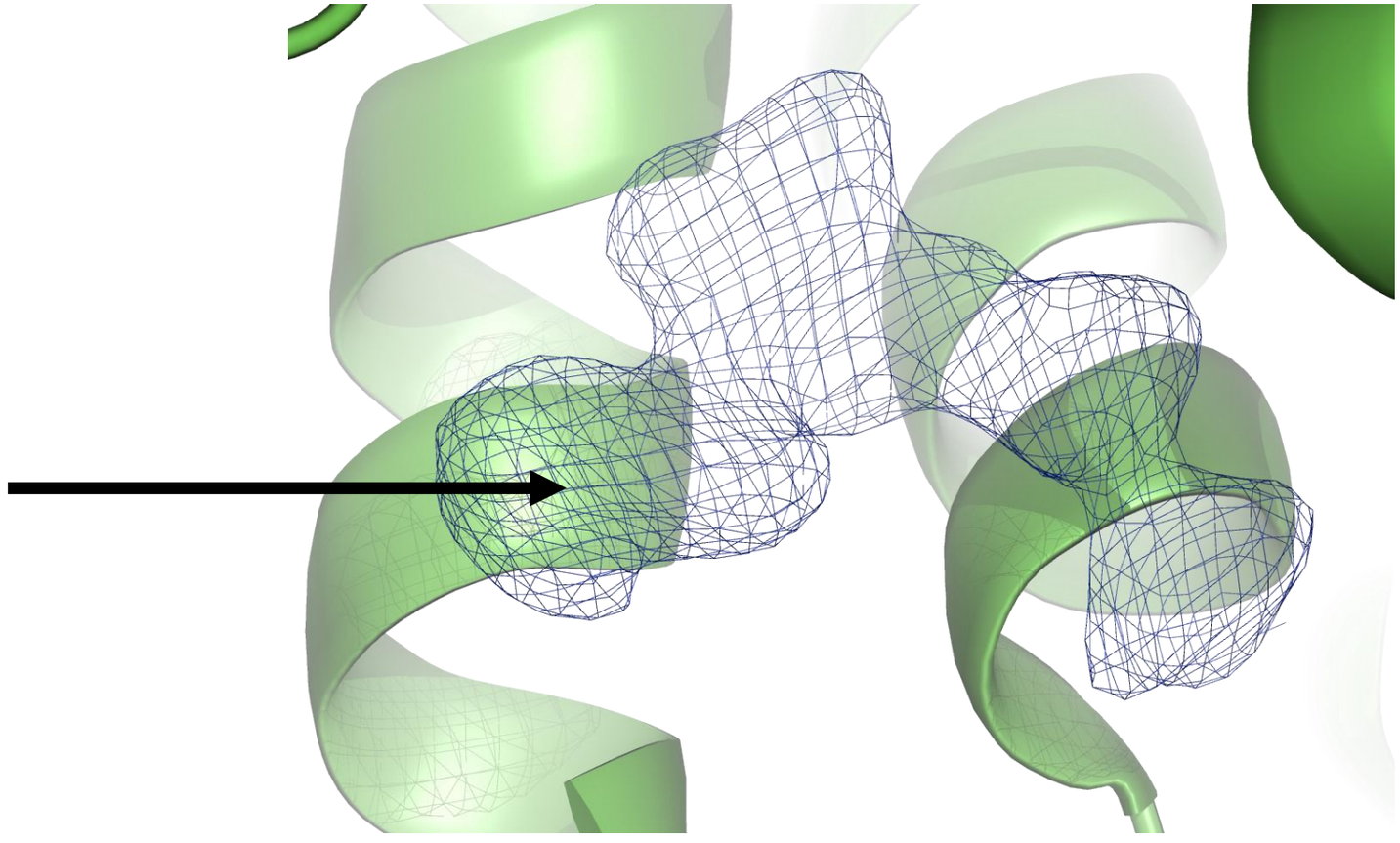
Thomas C. Terwilliger,[a]* Herbert Klei,[b] Paul D. Adams,[c] Nigel W. Moriarty[c] and Judith D. Cohn[a]

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





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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.

Acta Crystallographica Section D
Biological Crystallography
ISSN 1399-0047

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

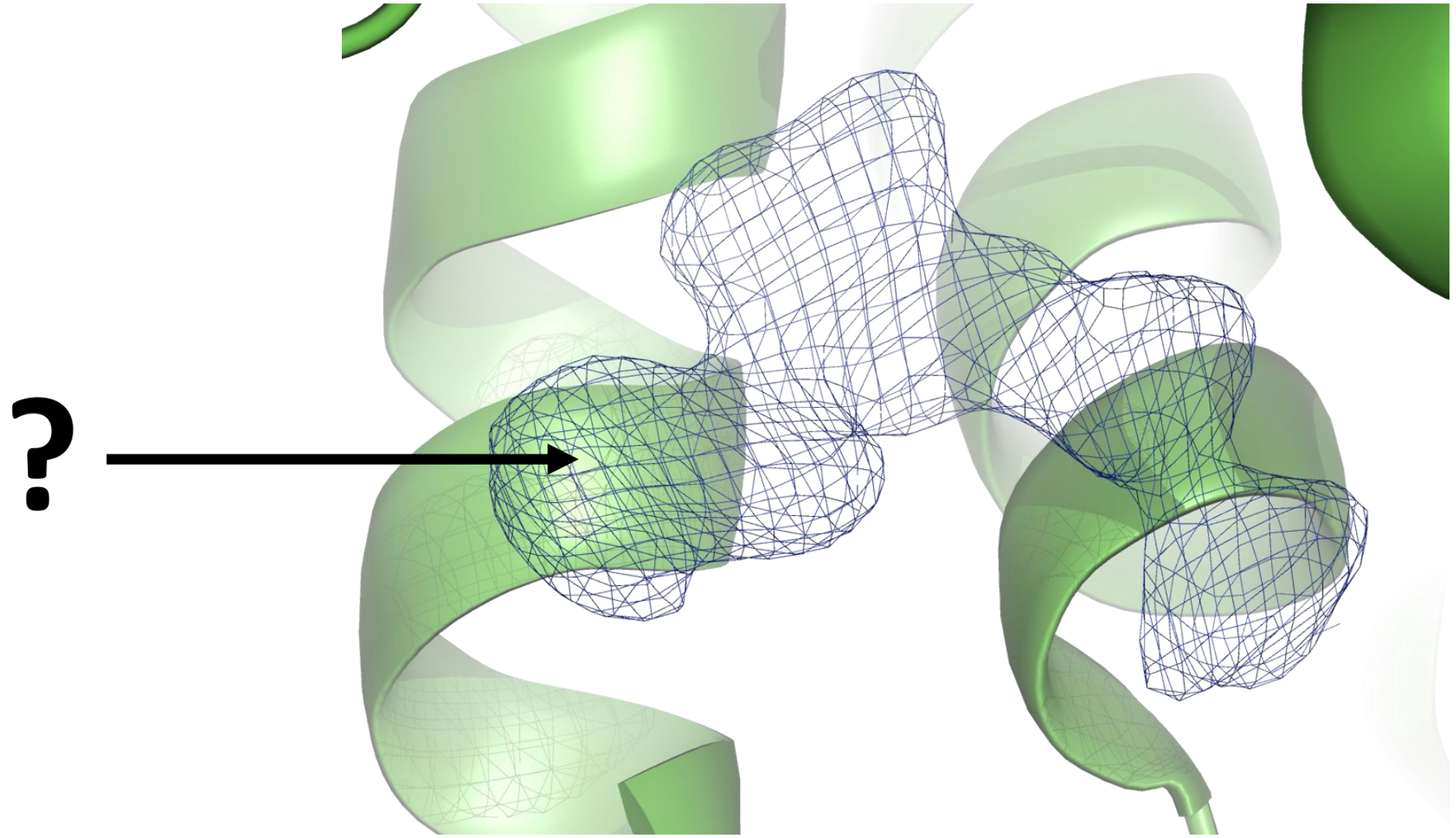
Herbert E. Klei,[a,b]‡ Nigel W. Moriarty,[a]*‡ Nathaniel Echols,[a] Thomas C. Terwilliger,[c] Eric T. Baldwin,[b,d] Matt Pokross,[b] Shana Posy[b] and Paul D. Adams[a,e]

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

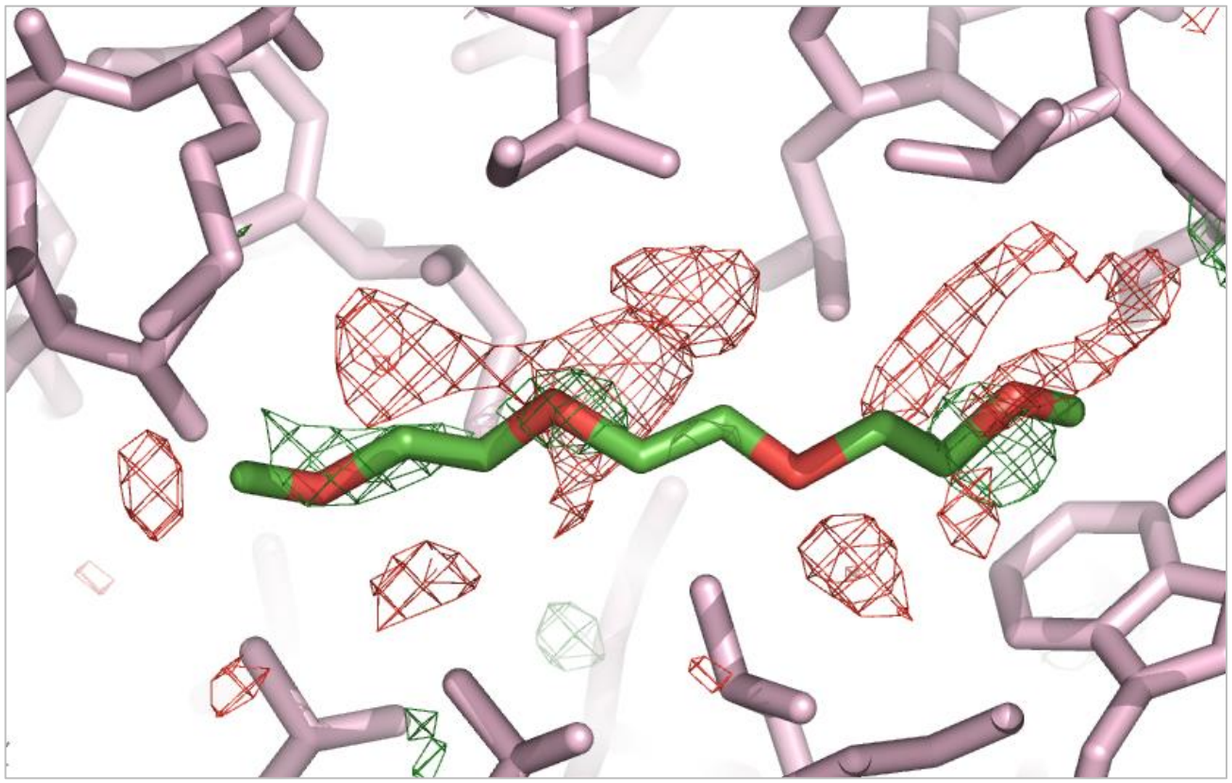
- **Ligand identification**: identifies an unknown ligand by analyzing difference density peaks. Uses a library of the 180 most frequently observed ligands in the PDB to rank each molecule by density fit and chemical interaction with the macromolecule.



Other tools for ligands in Phenix

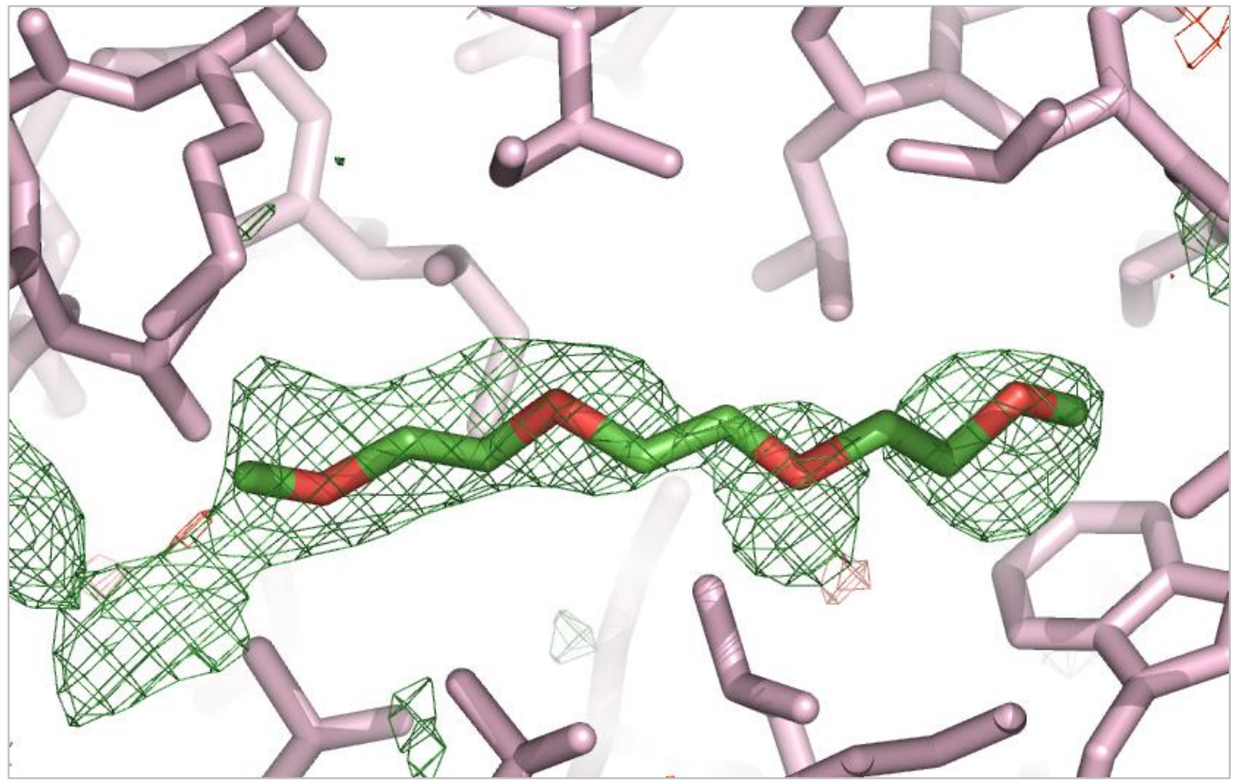
- **Polder maps**: OMIT map that excludes the bulk solvent around the ligand to visualize weak densities.

OMIT map



Contour +/- 3 rms

Polder map



Contour +/- 3 rms

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