

*Phenix User Workshop, Pittsburgh Diffraction Conference,  
October 14th 2023*



# Ligands

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# What is a ligand?

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- 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

# What is a ligand?

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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

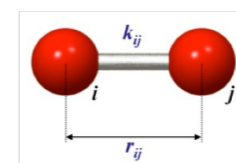
We need **restraints** for the ligand



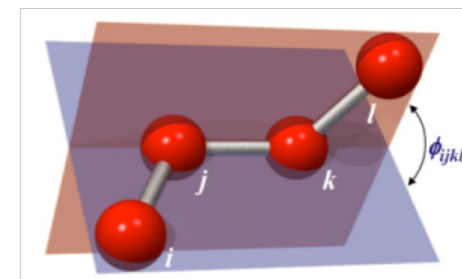
*a priori* knowledge

Stereochemical restraints stipulate ideal values for:

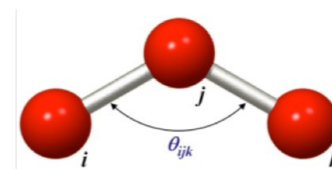
- Bond lengths
- Angles
- Torsions
- Planes
- Chiral volumes



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

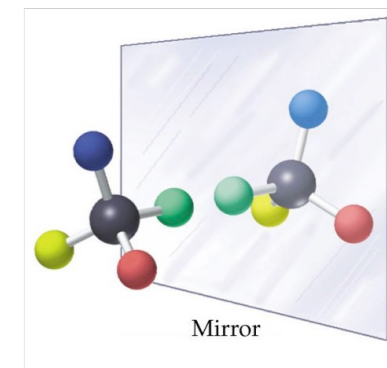


$$\sum_{\text{dihedrals}} \omega (1 + \cos(n\chi_{\text{model}} + \chi_{\text{shift}}))$$

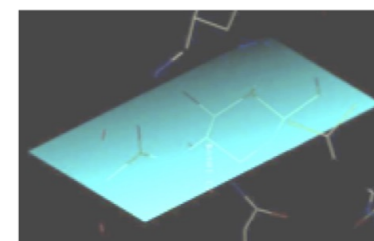


$$\sum_{\text{angles}} \omega (\theta_{\text{model}} - \theta_{\text{ideal}})^2$$

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



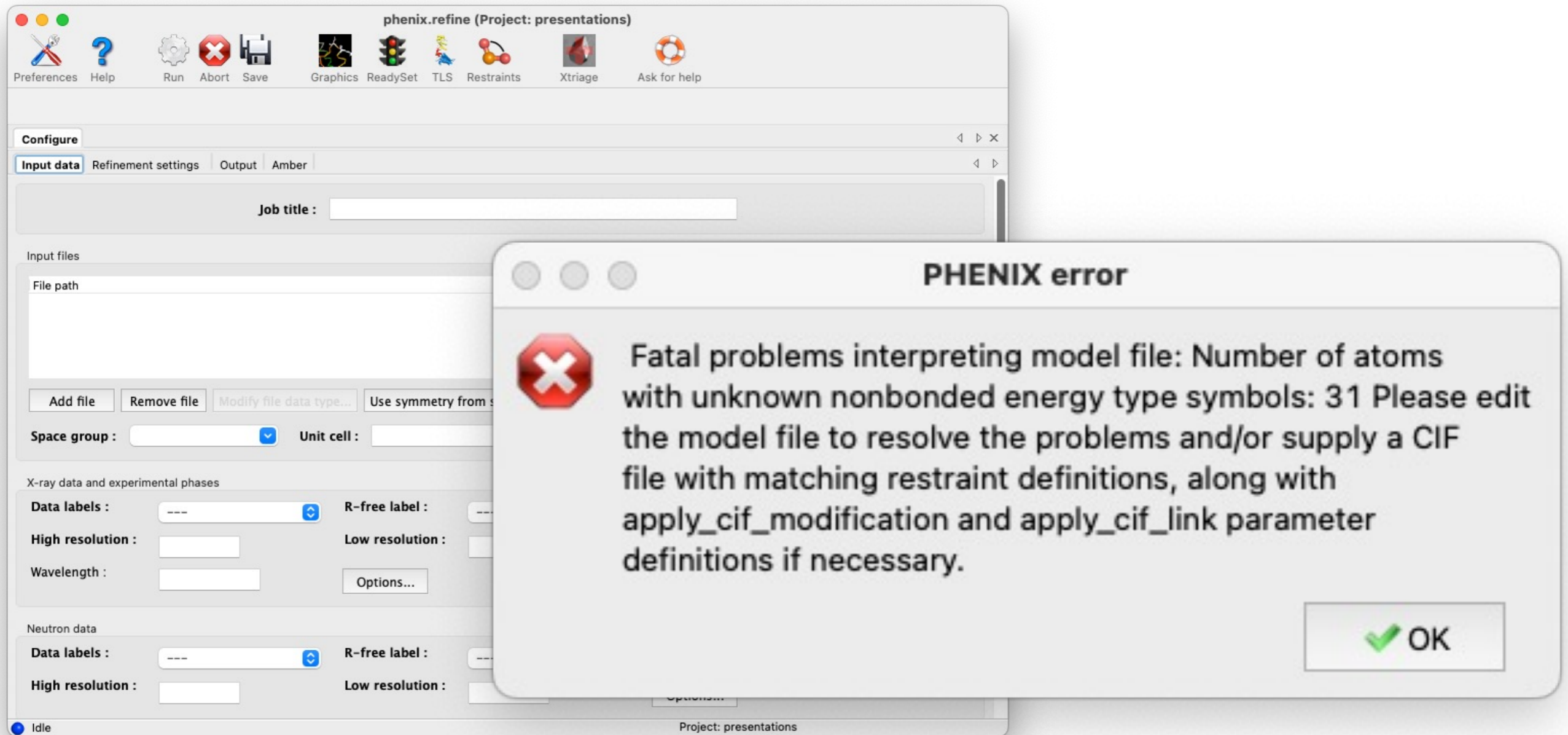
$$\text{Volume (V)} = (r_N - r_{CA}) \cdot [(r_C - r_{CA}) \times (r_{CB} - r_{CA})]$$



$$E = \sum_{\text{planes}} \sum_{\text{atoms}} W (m \cdot r - d)^2$$

# 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

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

# Ligand libraries

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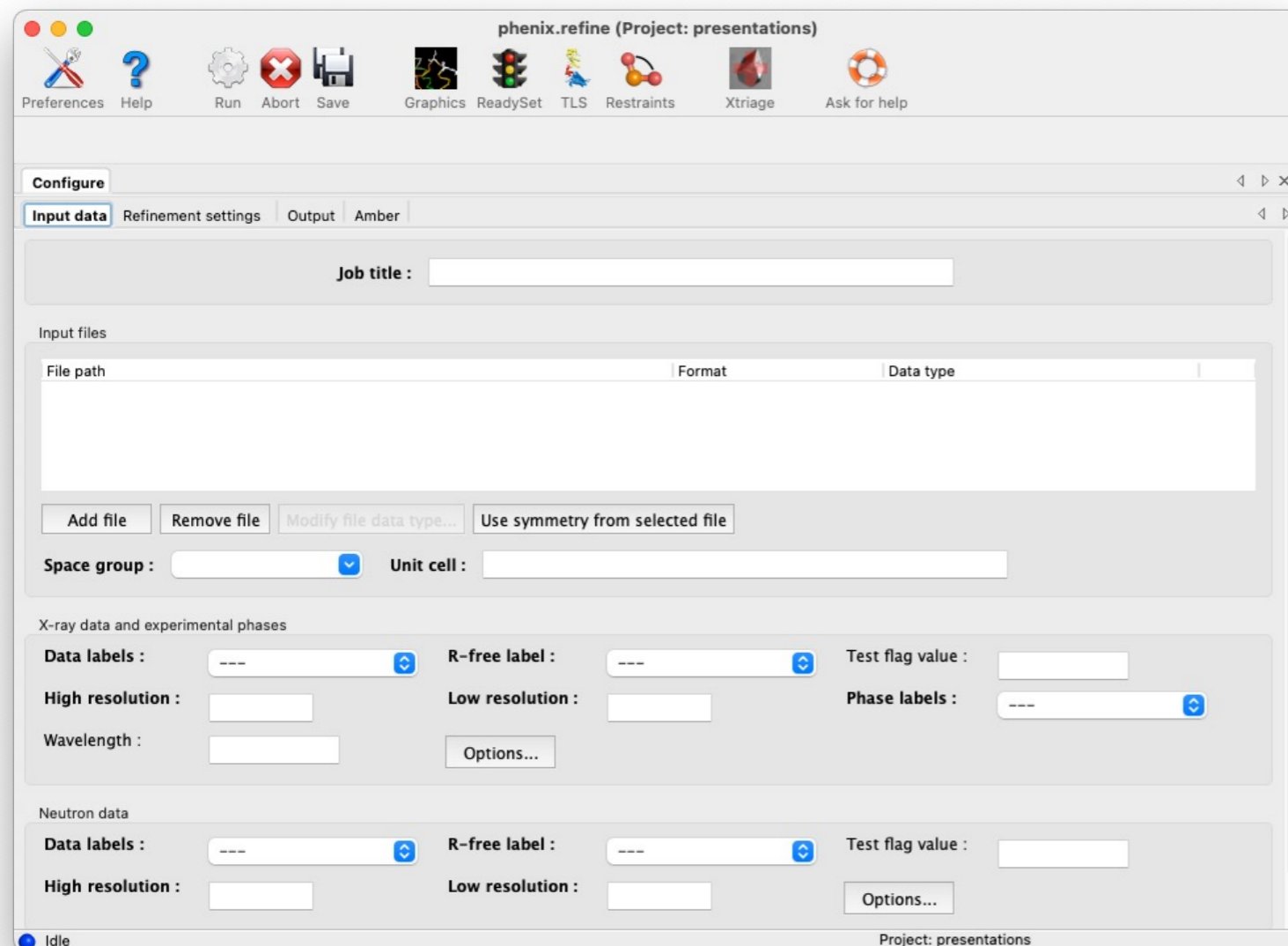
- **Chemical Components library (CCL):**
  - Has an entry for every entity in PDB entries
  - Contains SMILES (absolute topology)
  - Two sets of xyz – One from the “best, first” model in PDB and one calculated by PDBx as the ideal
  - Atom names needed for restraints generation and lookup
- **CCP4 monomer 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.



# Novel ligands

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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

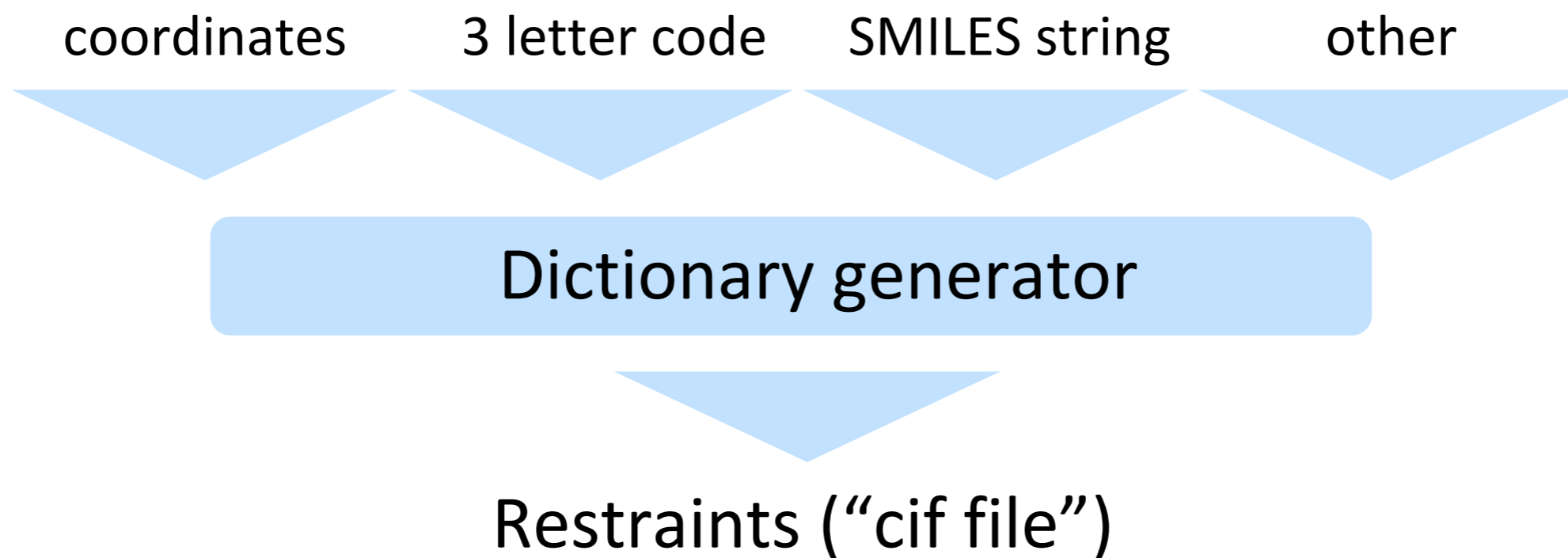
If still not found:

→ Get restraints from a dictionary generator

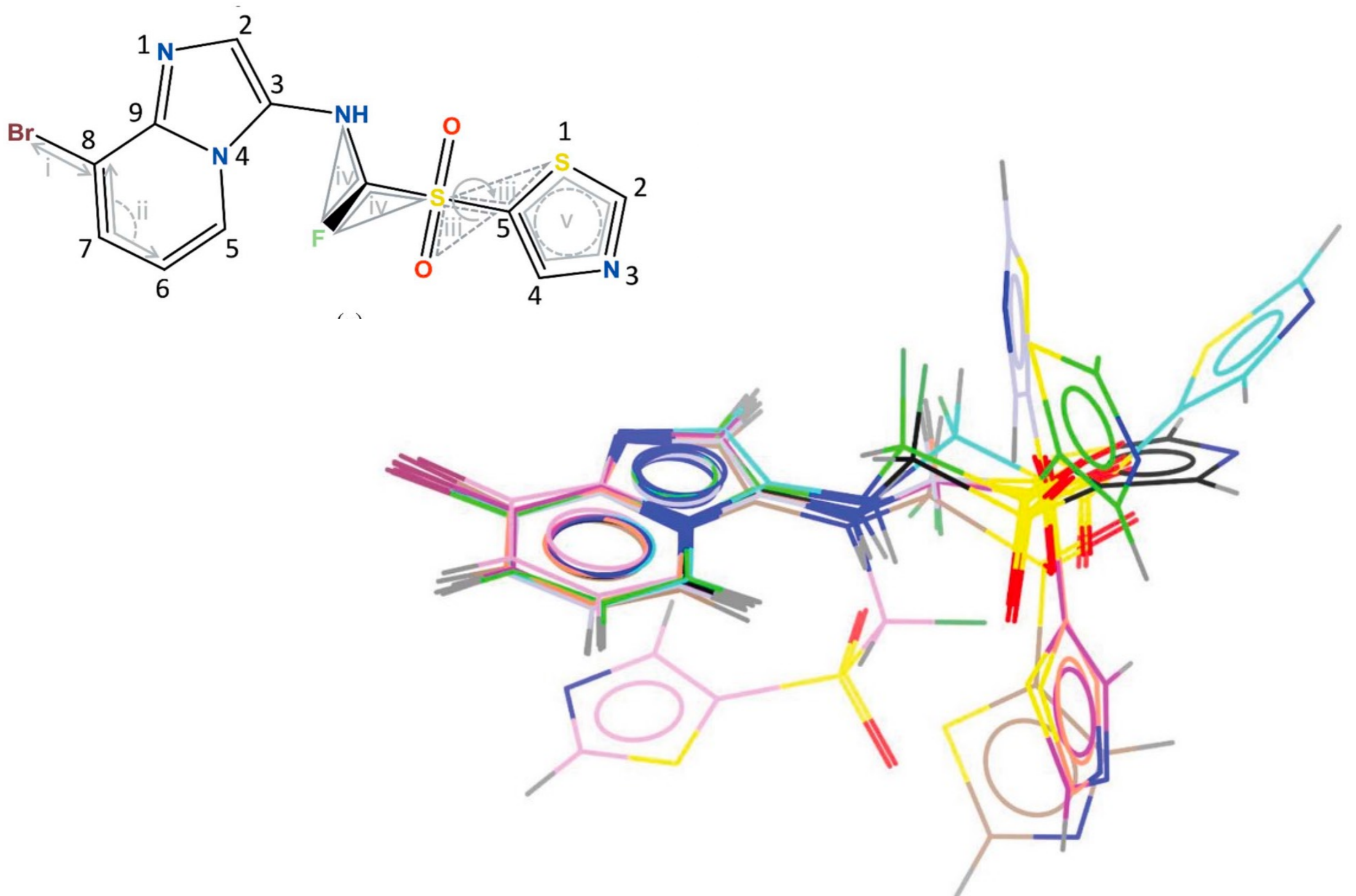
# Dictionary generator

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



# Restraints can vary



# Ligand restraints for Phenix

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Restraints are needed for

- Refinement (any kind of geometry minimization)
- Validation

Tools for ligand restraints in Phenix

- eLBOW
- ReadySet!

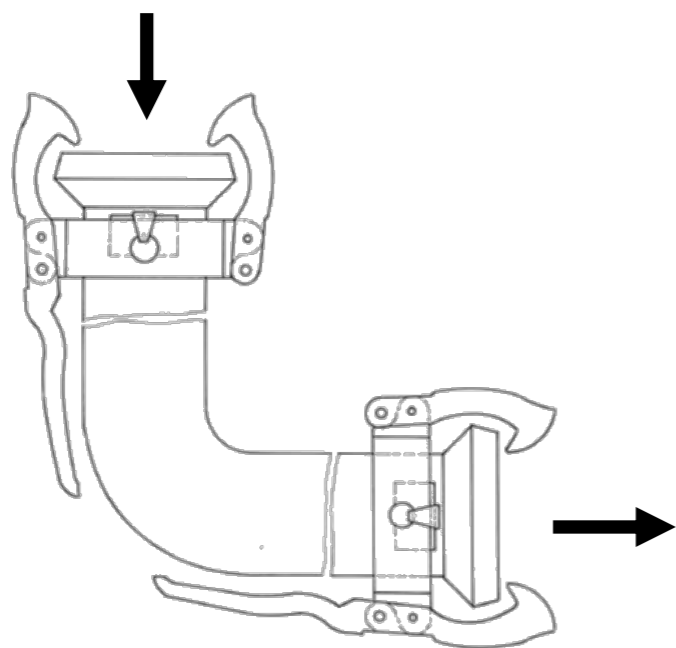
# eLBOW

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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*

Nigel W. Moriarty,<sup>a\*</sup> Ralf W. Grosse-Kunstleve<sup>a</sup> and Paul D. Adams<sup>a,b</sup>

<sup>a</sup>Lawrence Berkeley National Laboratory, One Cyclotron Road, Mailstop 64R0246, Berkeley, CA 94720, USA, and <sup>b</sup>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



Python-based **H**ierarchical **E**Nvironment for **I**ntegrated **X**tallography

### 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](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.

The thumbnail features a dark blue background. On the left, the text "eLBOW Tutorial" is displayed in a large, white, sans-serif font. To the right is a circular inset showing a ball-and-stick model of a ligand with various atoms labeled (H2, N3, HO2', HO3', H5', H8, HN61, HN62). In the bottom right corner of the thumbnail, a white box contains the text "3:41". Below the thumbnail, the video title "Basic Phenix eLBOW tutorial" is shown in a bold, black font, followed by "2.8K views • 5 years ago" in a smaller, grey font.

Basic Phenix eLBOW tutorial

2.8K views • 5 years ago

# ReadySet!

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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.

***ReadySet!***

Model file

ReadySet!

Restrains, model file

## Contents

- [Author](#)
- [Purpose](#)
- [General Procedure](#)
- [Ligand hydrogen addition](#)
- [Metal coordination](#)
- [Neutron exchange addition](#)
- [List of all available keywords](#)

## **Author**

Nigel W. Moriarty

## **Purpose**

# Quantum Mechanical Restraints (QMR)

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Challenges for ligand restraints:

- large chemical variability  
(can't predict configuration or puckers)
- possible novel nature
- specific interaction with the protein binding pocket

Full QM minimization or refinement is time-consuming.



# Quantum Mechanical Restraints (QMR)

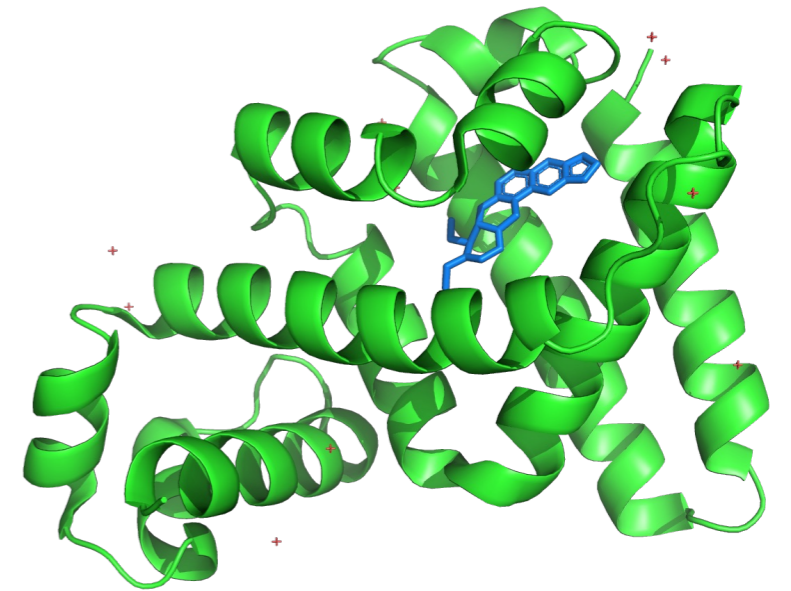
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## Idea:

minimize the ligand geometry in the binding pocket (MOPAC)

Account for the influence of

- the local energy minima of the ligand
- the in-situ environment

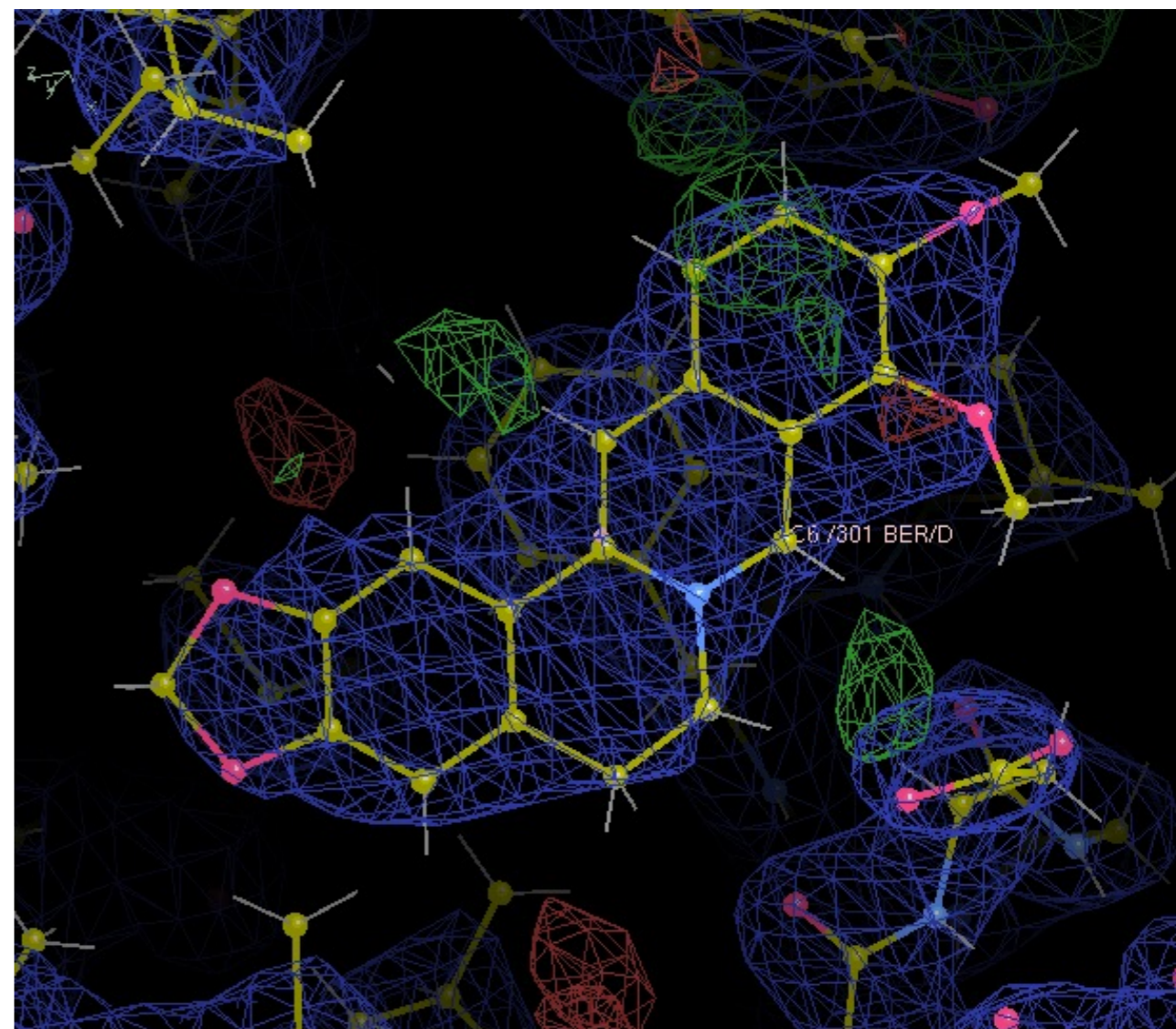
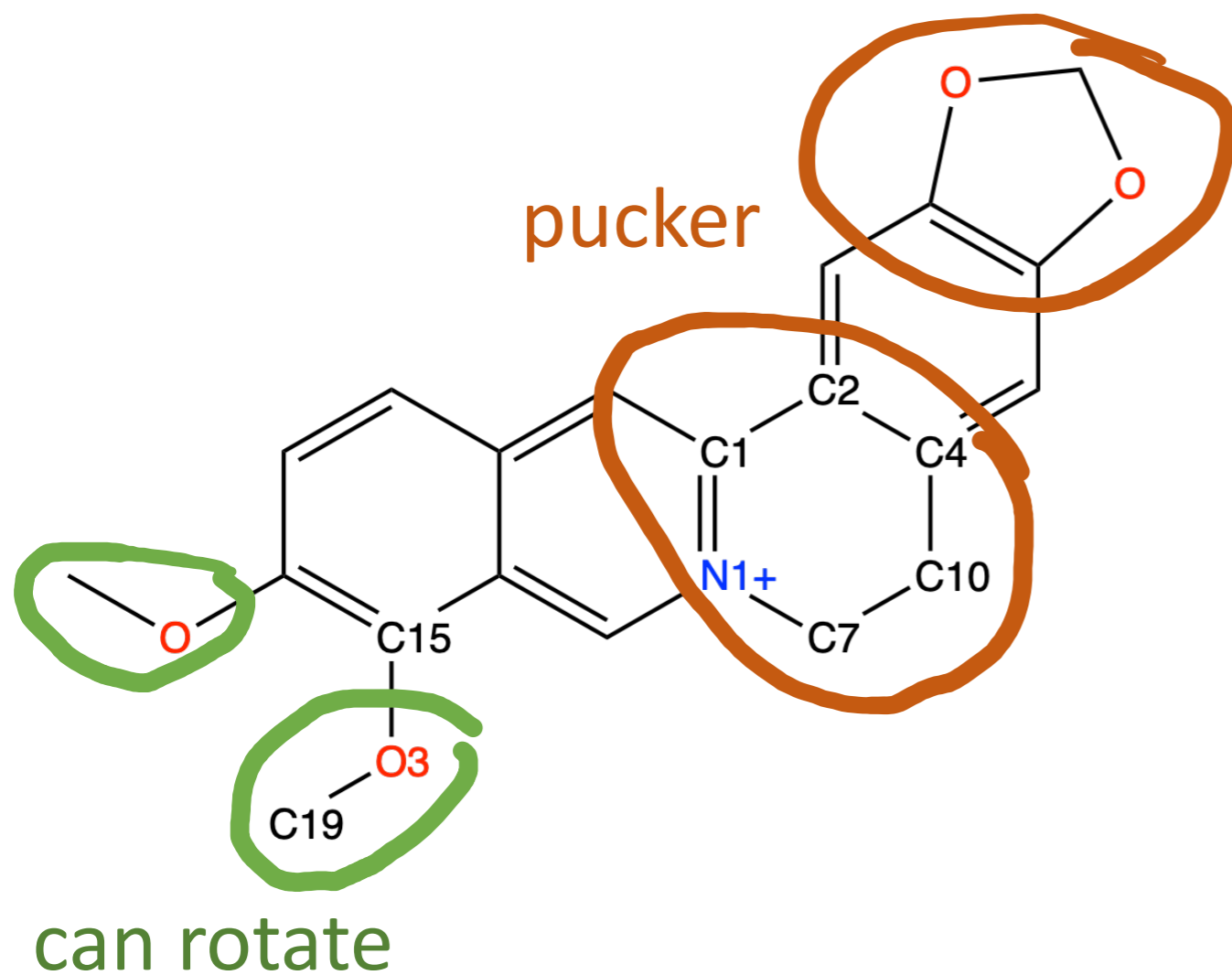


→ Use the minimized ligand geometry as target values for restraints.

# QMR example: BER in 3vw2

2.34 Å resolution

Ligand BER in every chain



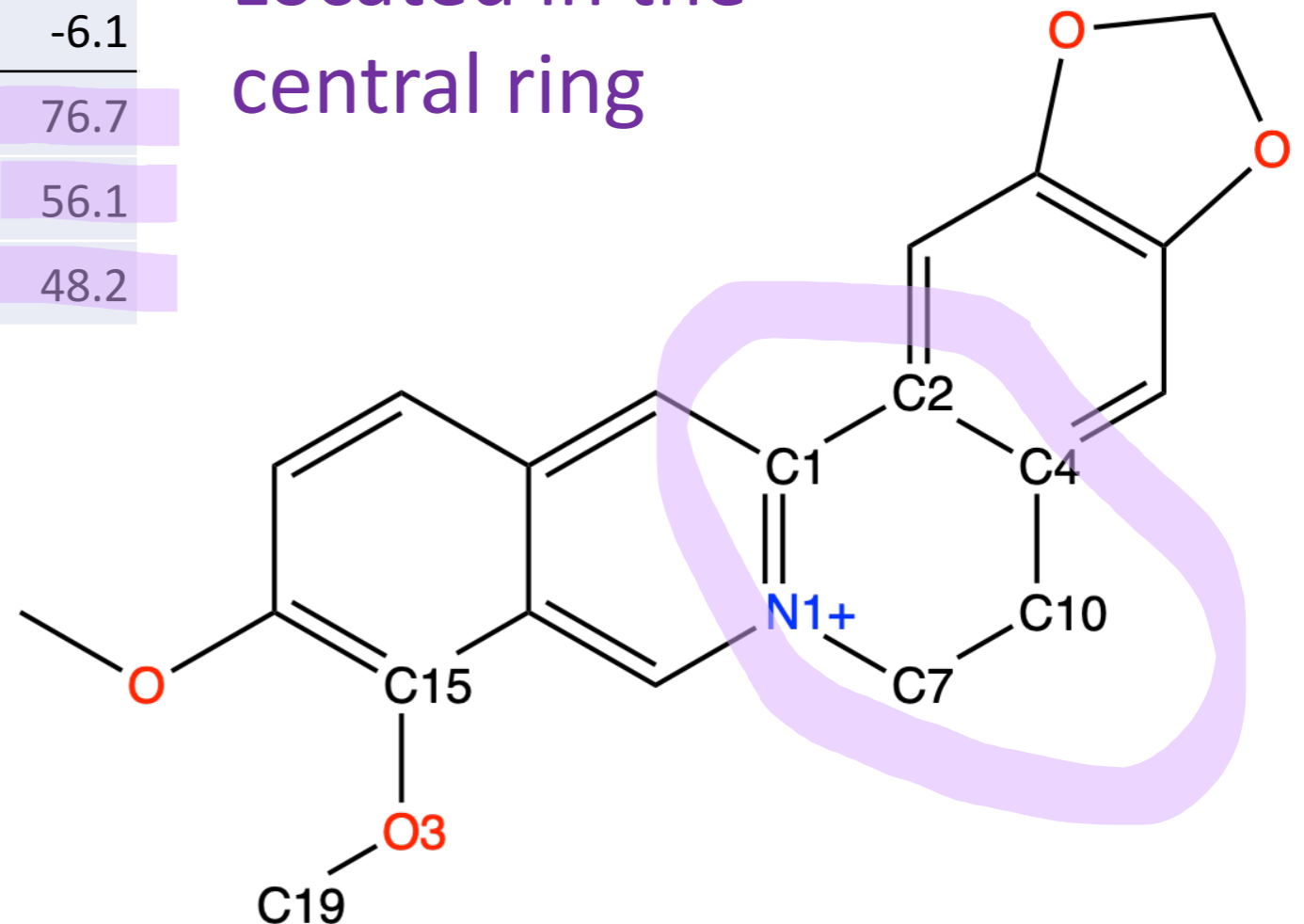
Density looks OK.

# QMR example: Geometry after refinement

Use GeoStd restraints and refine.

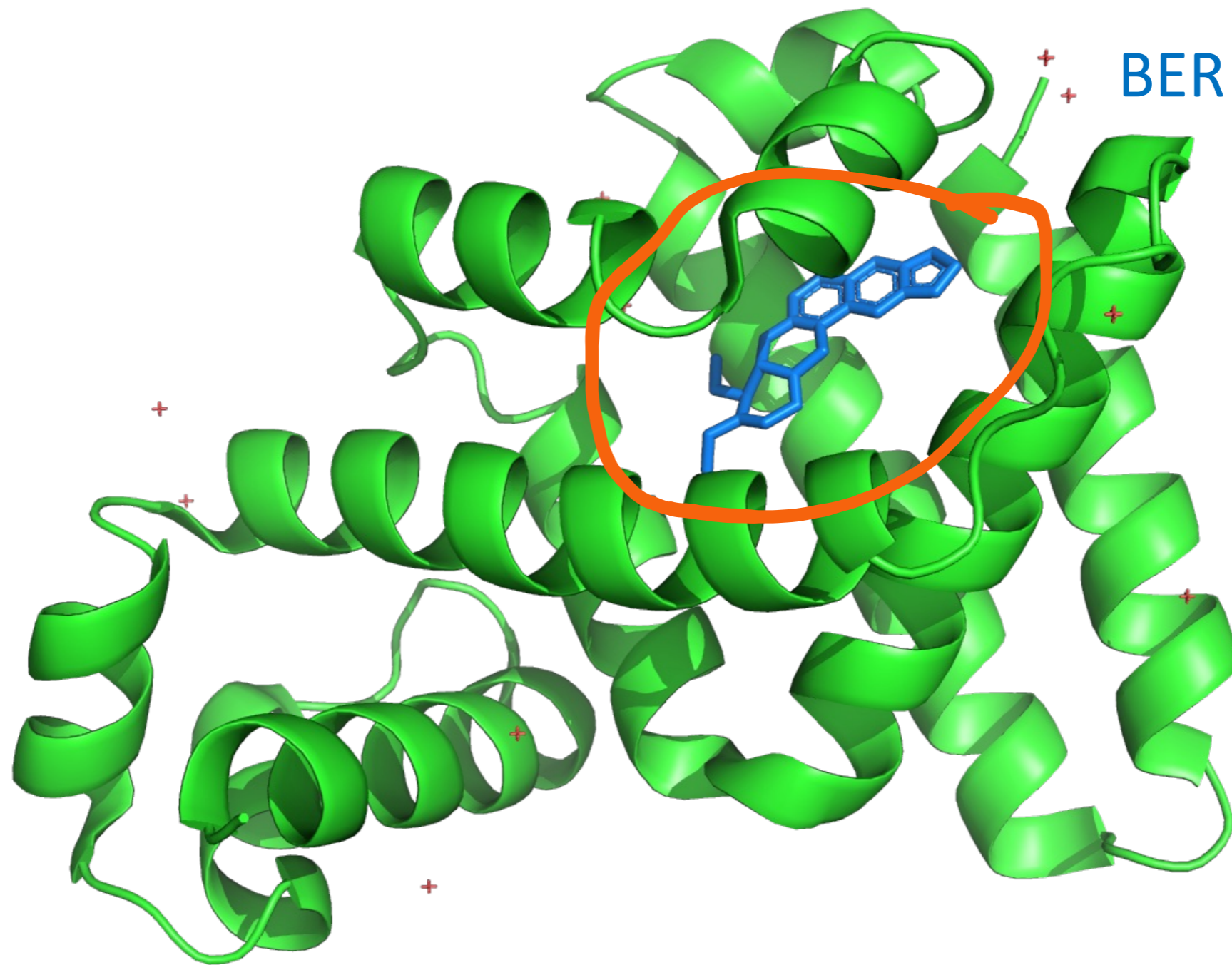
	mean		ideal	delta
angles	2.4	C10 - C7 - N1	109.7	-7.6
		C4 - C10 - C7	109.0	-6.7
		C15 - O3 - C19	115.2	-6.1
torsions	48.2	C4 - C10 - C7 - N1	58.0	76.7
		C1 - C7 - N1 - C10	43.0	56.1
		C2 - C10 - C4 - C7	35.8	48.2

Located in the central ring



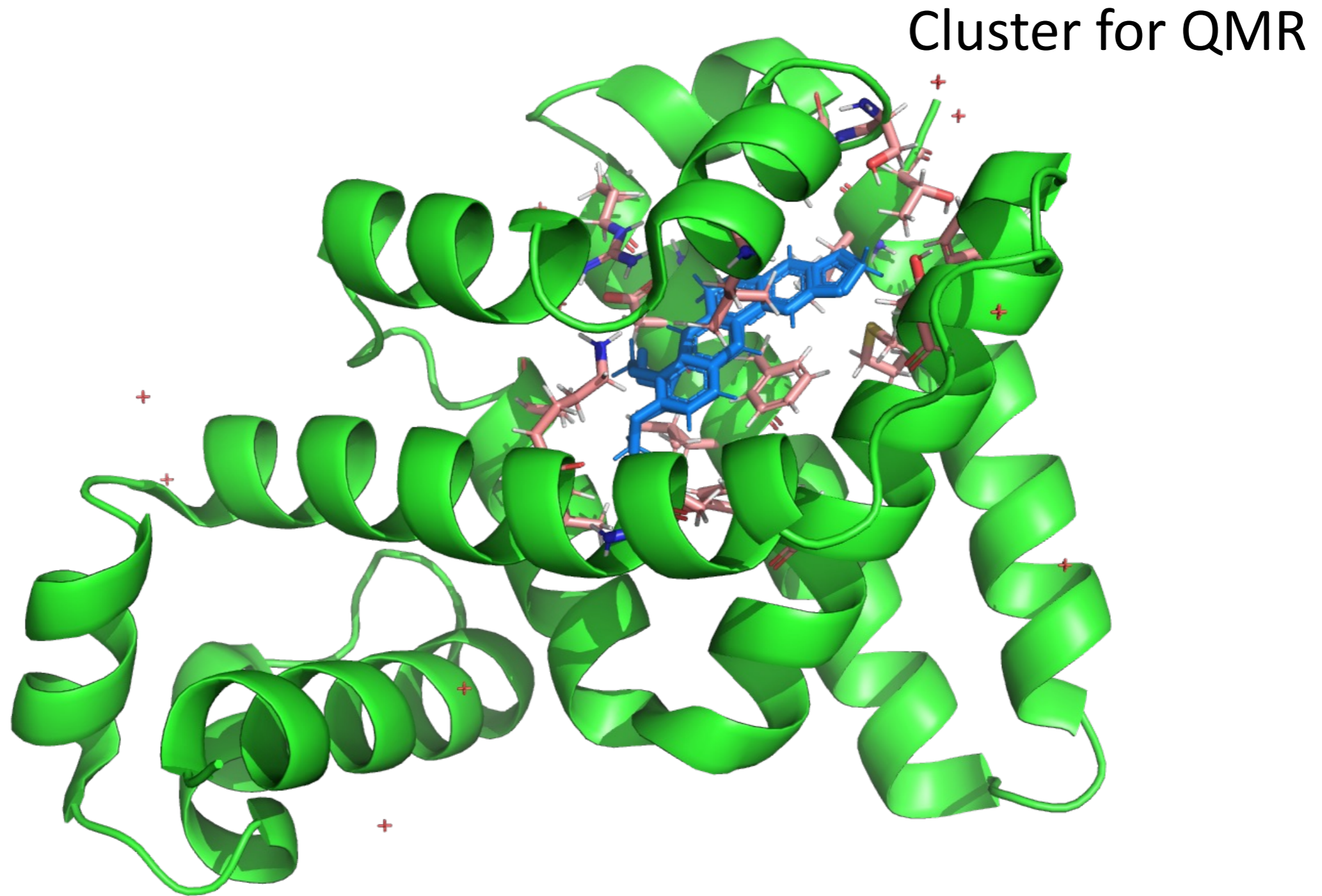
# QMR example: BER in 3vw2

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# QMR example: BER in 3vw2

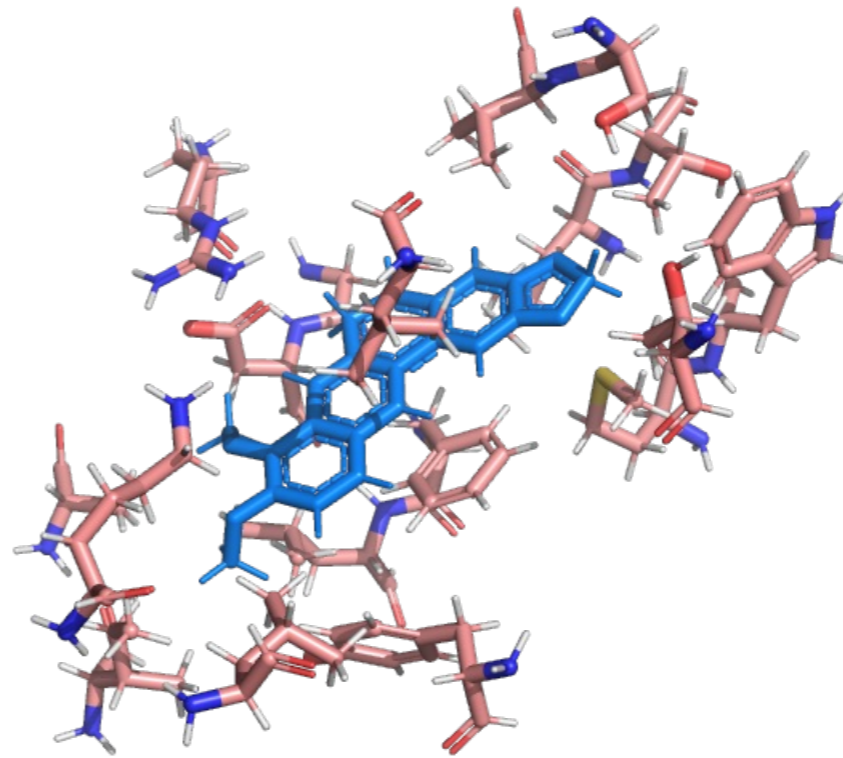
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# QMR example: BER in 3vw2

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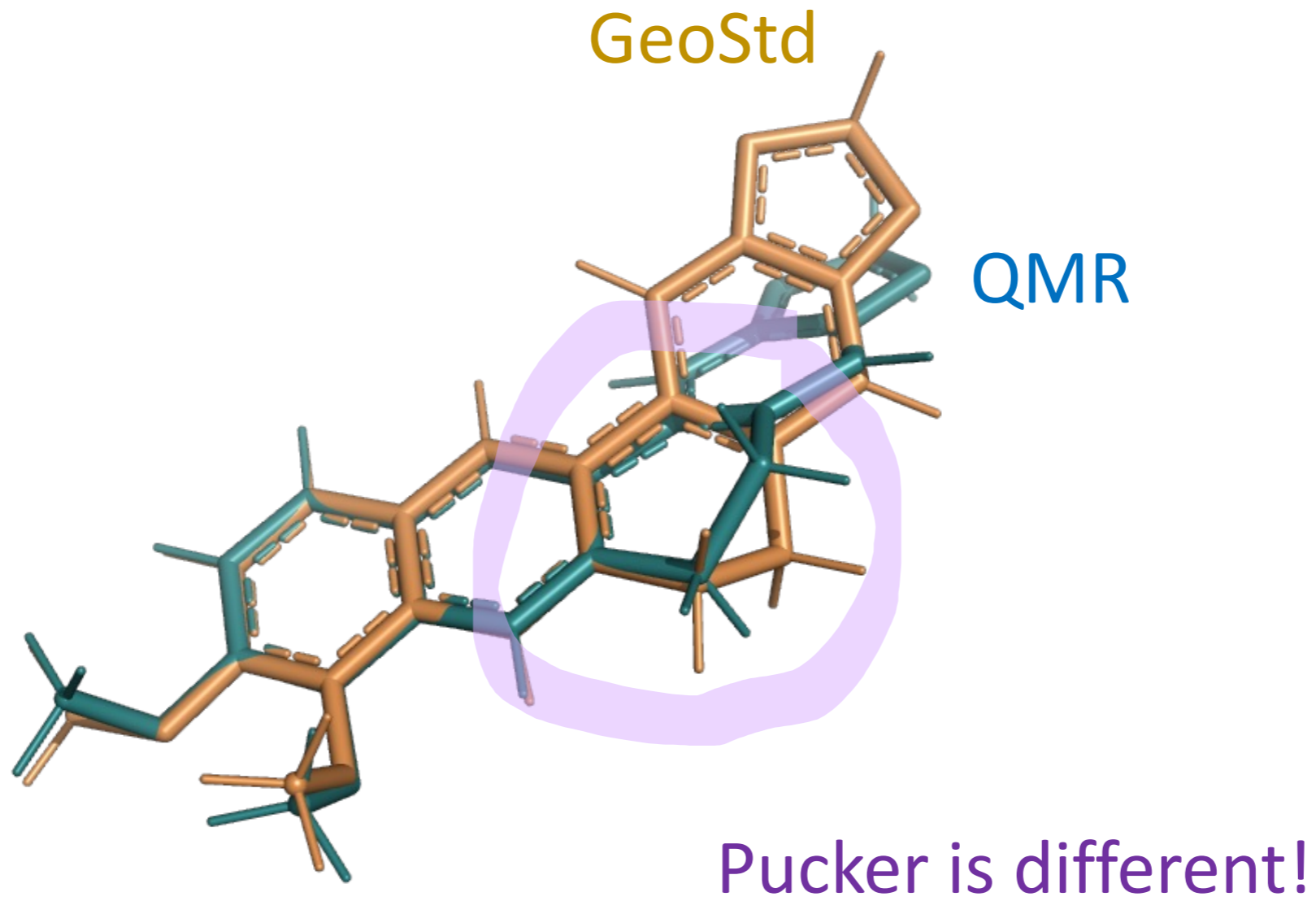
Cluster for QMR



→ Use minimized ligand geometry as targets for restraints.  
(forget about what happened with the residues)

# GeoStd vs QMR

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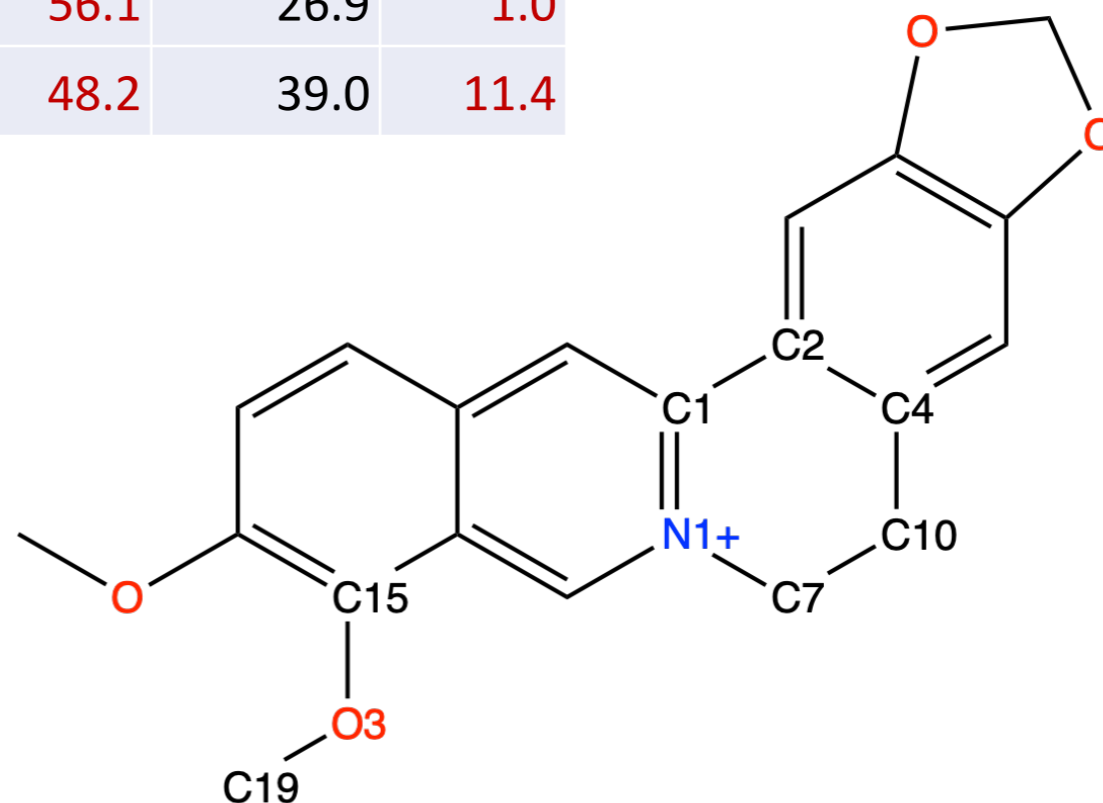


# GeoStd vs QMR

Refinement using GeoStd restraints vs QMR restraints:

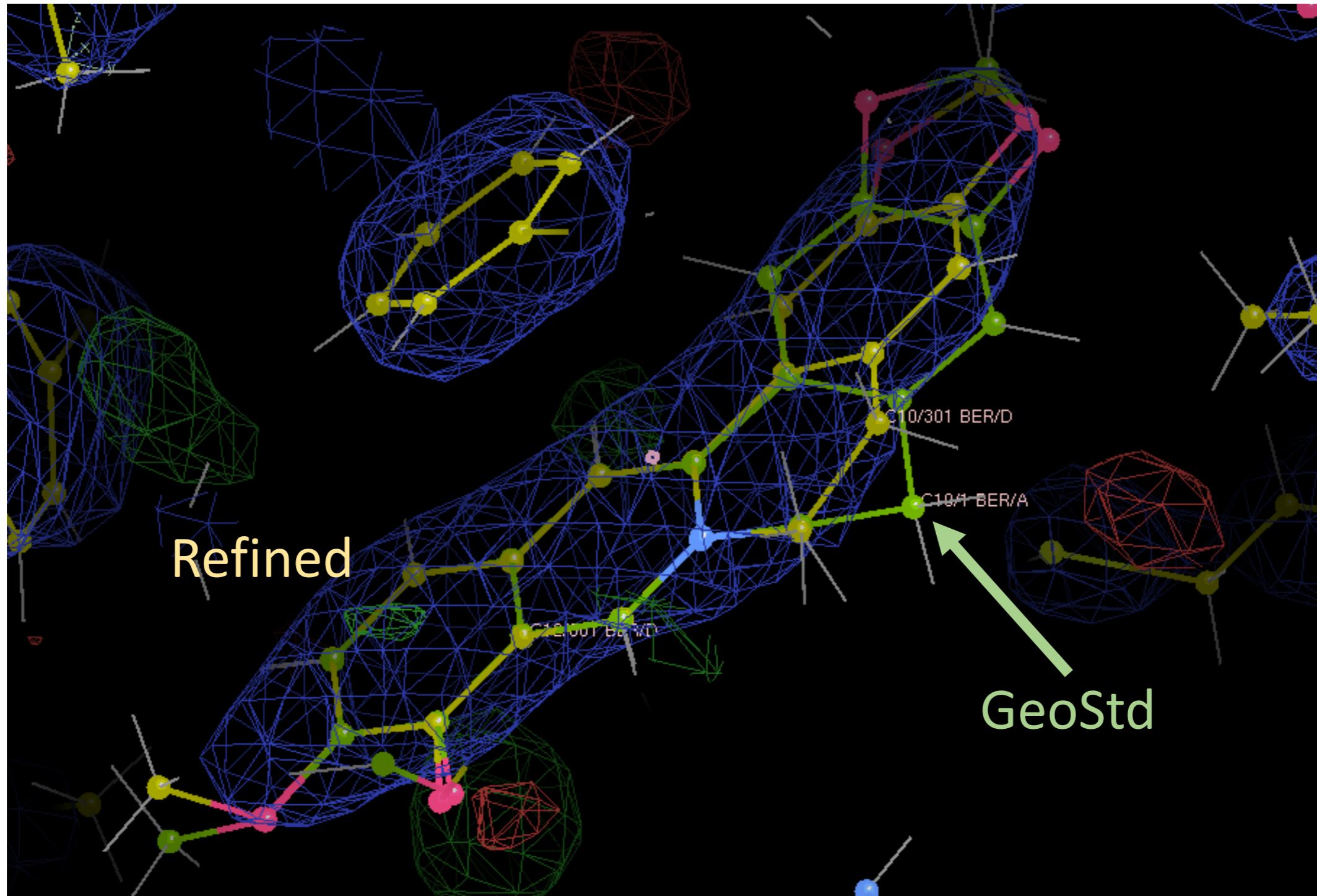
	GeoStd	QMR		GeoStd		QMR	
	mean	mean		ideal	delta	ideal	delta
angles	2.4	0.8	C10 - C7 - N1	109.7	-7.6	111.7	2.2
			C4 - C10 - C7	109.0	-6.7	110.9	2.4
			C15 - O3 - C19	115.2	-6.1	112.6	1.3
torsions	48.2	7.1	C4 - C10 - C7 - N1	58.0	76.7	50.7	10.4
			C1 - C7 - N1 - C10	43.0	56.1	26.9	1.0
			C2 - C10 - C4 - C7	35.8	48.2	39.0	11.4

Systematic improvement with QMR restraints.





# GeoStd structure in the density



QMR restraints are chemically plausible.

# Other tools for ligands in Phenix

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

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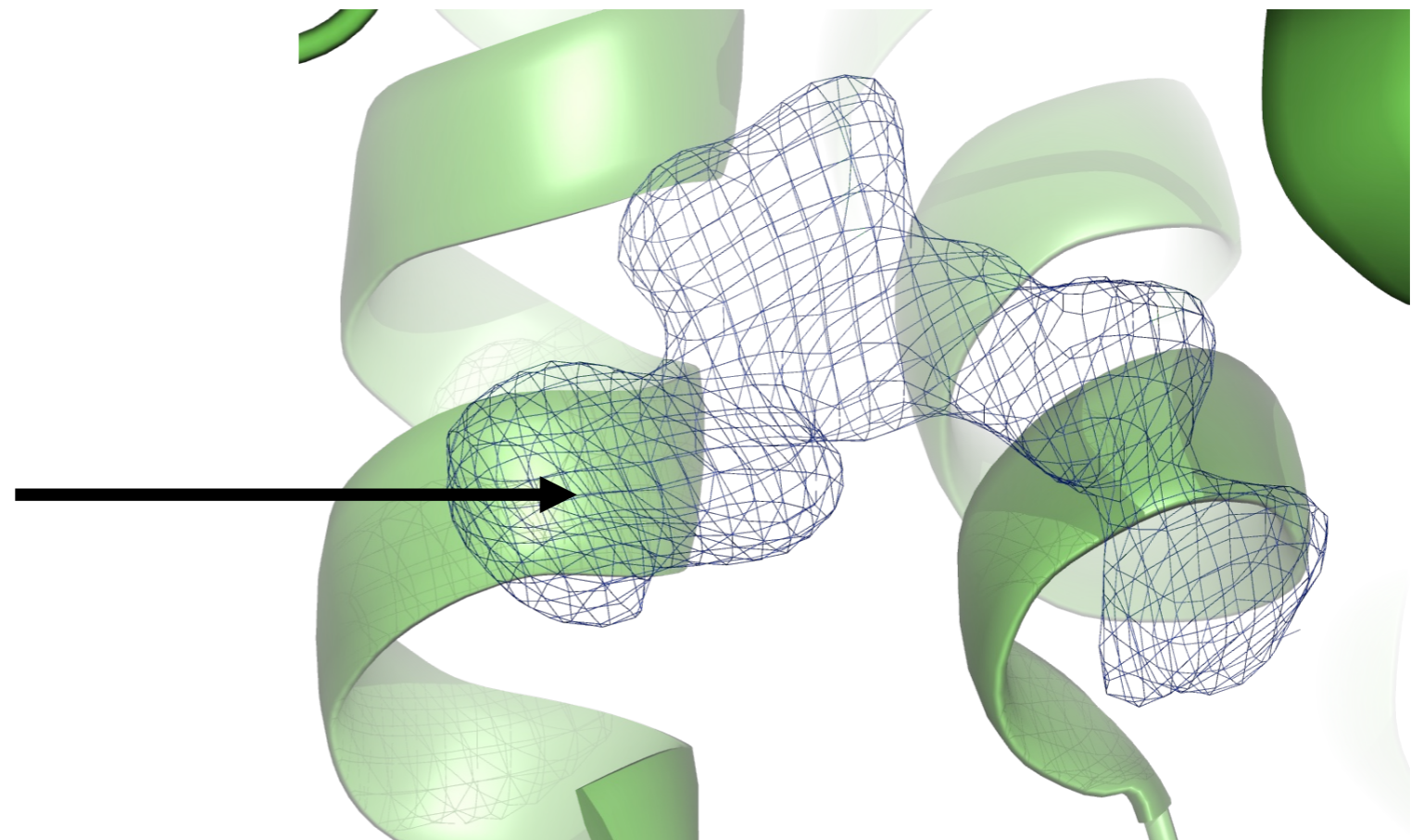
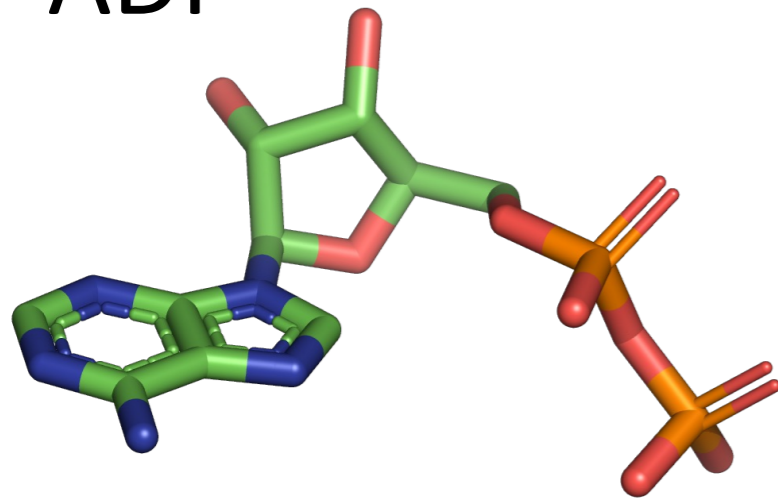
## Automated ligand fitting by core-fragment fitting and extension into density

Thomas C. Terwilliger,<sup>a\*</sup> Herbert  
Klei,<sup>b</sup> Paul D. Adams,<sup>c</sup> Nigel W.  
Moriarty<sup>c</sup> and Judith D. Cohn<sup>a</sup>

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

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- **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,<sup>a\*</sup> Herbert Klei,<sup>b</sup> Paul D. Adams,<sup>c</sup> Nigel W. Moriarty<sup>c</sup> and Judith D. Cohn<sup>a</sup>**

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

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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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Crystallography**  
ISSN 1399-0047

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

**Herbert E. Klei,<sup>a,b,†</sup> Nigel W. Moriarty,<sup>a,\*</sup> Nathaniel Echols,<sup>a</sup> Thomas C. Terwilliger,<sup>c</sup> Eric T. Baldwin,<sup>b,d</sup> Matt Pokross,<sup>b</sup> Shana Posy<sup>b</sup> and Paul D. Adams<sup>a,e</sup>**

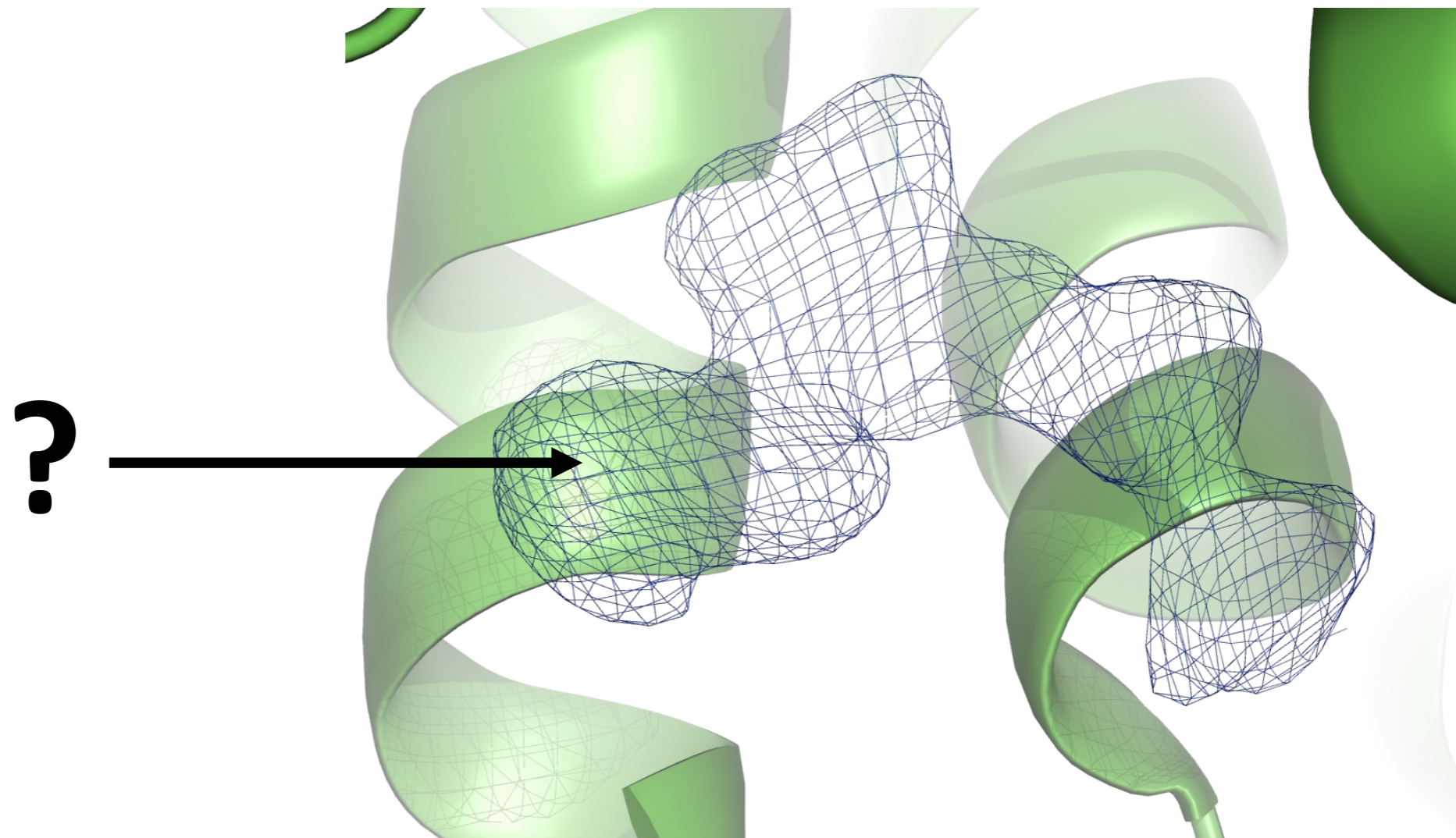
The process of iterative structure-based drug design involves the X-ray crystal structure determination of upwards of 100 ligands with the same general scaffold (*i.e.* chemotype) complexed with very similar, if not identical, protein targets. In conjunction with insights from computational models and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

Received 10 September 2013  
Accepted 2 November 2013

# Other tools for ligands in Phenix

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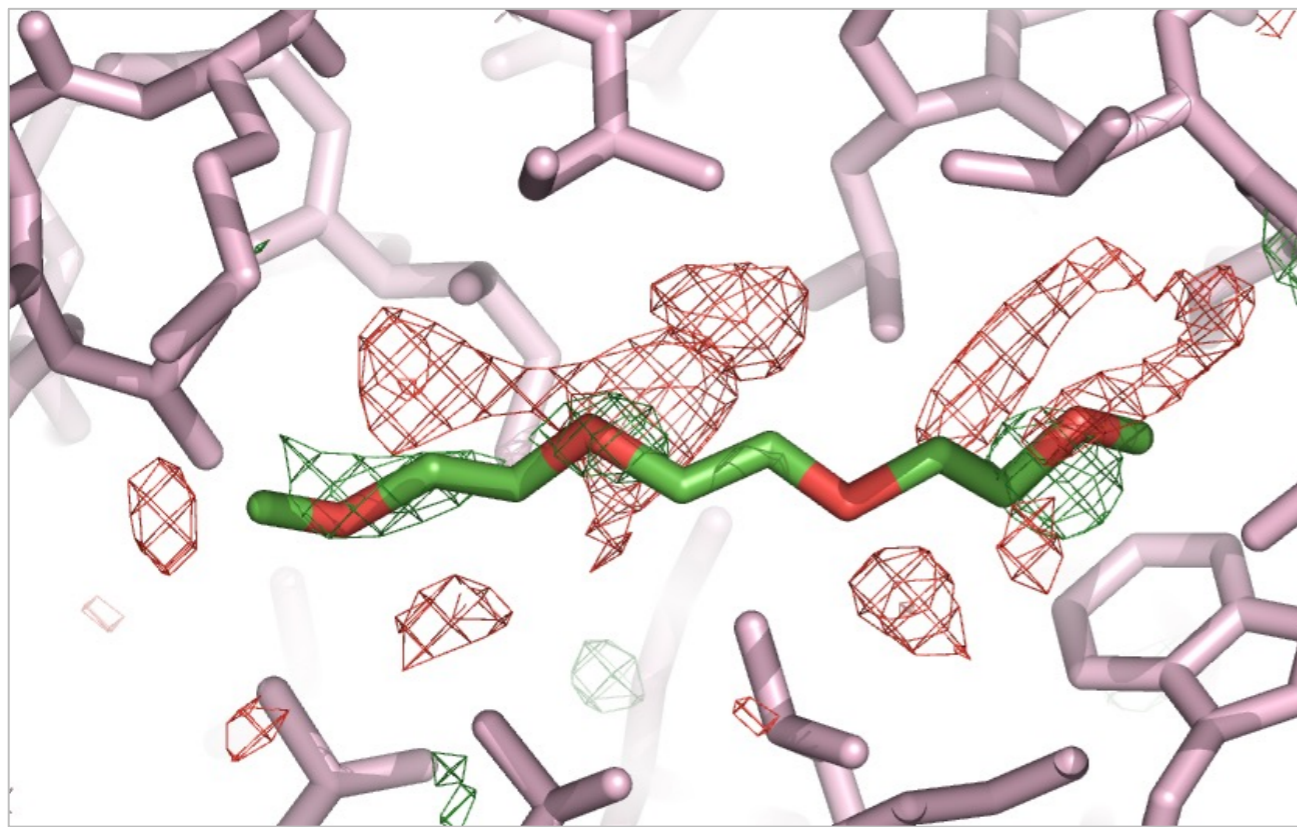
- **Ligand identification:** identifies an unknown ligand by analyzing difference density peaks. Uses a library of the 180 most frequently observed ligands in the PDB to rank each molecule by density fit and chemical interaction with the macromolecule.



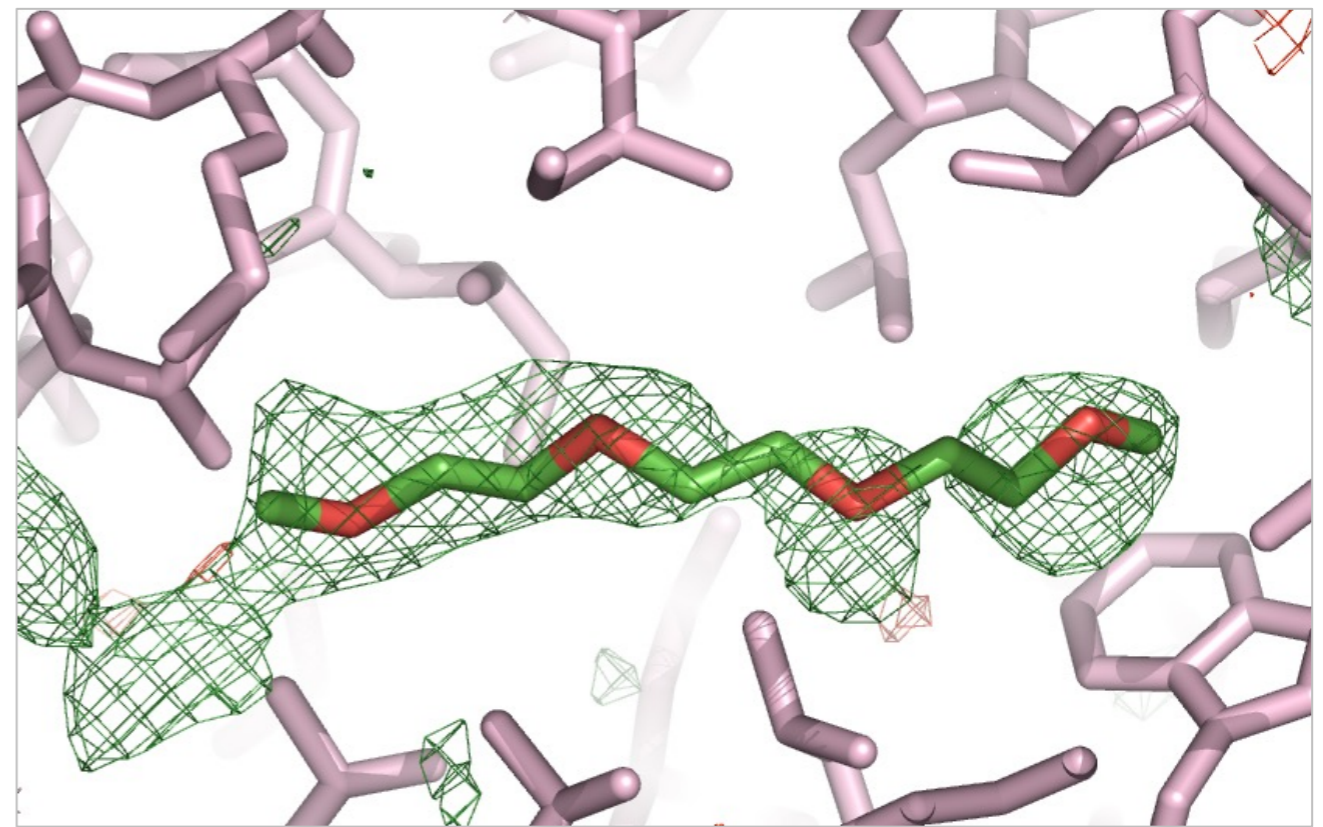
# Other tools for ligands in Phenix

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

OMIT map



Polder map



Contour +/- 3 rms

Contour +/- 3 rms

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

# The Project



## Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine,  
Dorothee Liebschner, Nigel  
Moriarty, Billy Poon,  
Christopher Schlicksup,  
Oleg Sobolev



## University of Cambridge

Randy Read, Airlie McCoy,  
Tristan Croll, Rob Oeffner



## Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



## UTHealth

Matt Baker, Corey Hyc



## 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