Ligands in Phenix

Generating & modifying for all scenarios

Nigel W. Moriarty

User's Meeting
Duke, September 2024

What are restraints?

- Restraints are harmonic functions that provide (mostly) chemical information via residuals & gradients
- Needed because experimental data is rarely sufficient for structure determination
- Weighted with the experimental information to find the optimal result

Resolution dependence

- Ultra-hi res – Not needed
- Hi res – Can have large deviations because the experimental data dominates
- Lo res – Generally approaches the ideal values
    - If not, large scale problems

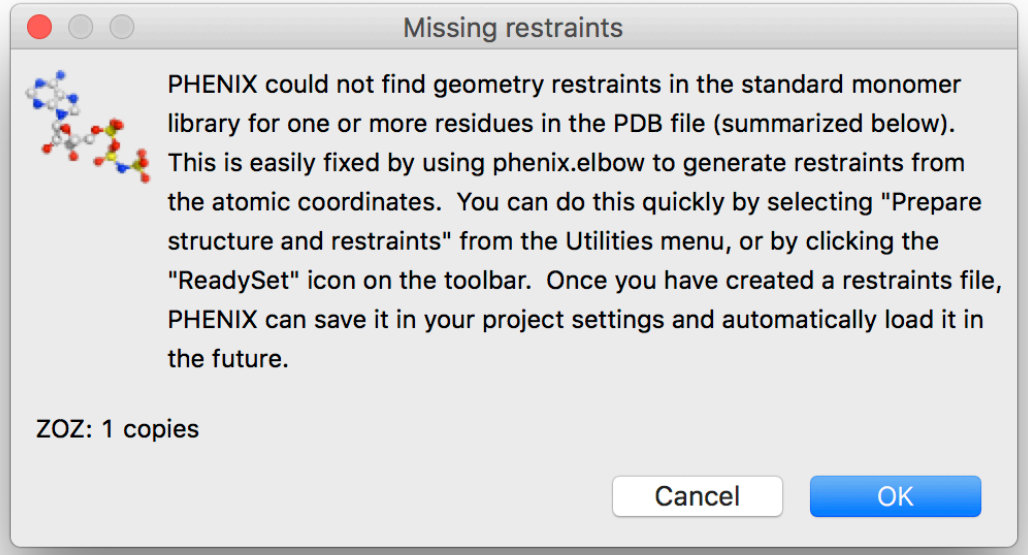
Restraints in Action

- 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

What you will see



Or

```
Sorry: Fatal problems interpreting model file:
  Number of atoms with unknown nonbonded energy type symbols: 21
    Please edit the model file to resolve the problems and/or supply a
    CIF file with matching restraint definitions, along with
    apply_cif_modification and apply_cif_link parameter definitions
    if necessary.
```

CIF

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

Confusion

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

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

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eLBOW goals

- Fast, simple and flexible procedure to include ligands
- Reduce the tedium of building 3D ligand models
- Automate generation of restraints for ligands
- Comparison of ligand structures

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

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Ligand Expo / Chemical Component Library

- List of all the entities in the Protein Data Bank
  - Amino acids, Nucleic acids
  - Ligands, Small molecule
  - Metal clusters
- In CIF format
  - Contents chemical information
    - SMILES, atom names, bonds
  - Not restraints

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Amino Acid

RCSB PDB PROTEIN DATA BANK

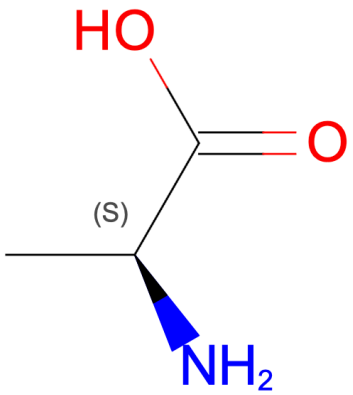
217,157 Structures from the PDB

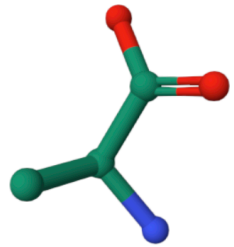
1,068,577 Computed Structure Models (CSM)

3D Structures | Enter search term(s), Entry ID(s), or sequence | Include CSM

Advanced Search | Browse Annotations | Help

PDB-101 | wwPDB | EMDataResource | NAKB | wwPDB Foundation | PDB-Dev





Toggle Hydrogen | Toggle Labels

Display Files | Download Files | Data API

ALA

ALANINE

Find entries where: ALA

- is present as a standalone ligand in 172 entries
- as a non-polymer is covalently linked to polymer or other heterogen groups 58 entries
- is present in a polymer sequence 210,908 entries

search

Find related ligands:

Similar Ligands (Stereospecific)
Similar Ligands (including Stereoisomers)
Similar Ligands (Quick Screen)
Similar Ligands (Substructure Stereospecific)
Similar Ligands (Substructure including Stereoisomers)

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5-letter codes

RCSB PDB PROTEIN DATA BANK

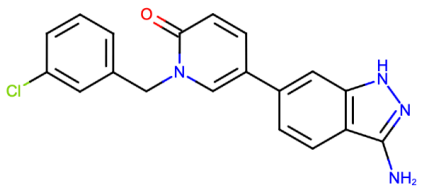
217,157 Structures from the PDB

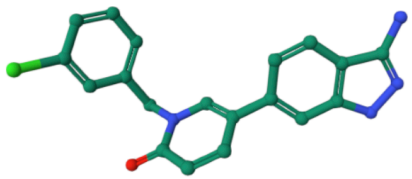
1,068,577 Computed Structure Models (CSM)

3D Structures | Enter search term(s), Entry ID(s), or sequence | Include CSM

Advanced Search | Browse Annotations | Help

PDB-101 | wwPDB | EMDataResource | NAKB | wwPDB Foundation | PDB-Dev





Toggle Hydrogen | Toggle Labels

Display Files | Download Files | Data API

A1LU6

5-(3-azanyl-1~{H}-indazol-6-yl)-1-[(3-chlorophenyl)methyl]pyridin-2-one

Find entries where: A1LU6

is present as a standalone ligand in 1 entries

search

Find related ligands:

Similar Ligands (Stereospecific)

Similar Ligands (including Stereoisomers)

Similar Ligands (Quick Screen)

Similar Ligands (Substructure Stereospecific)

Similar Ligands (Substructure including Stereoisomers)

- 49k combinations for 3-letter codes

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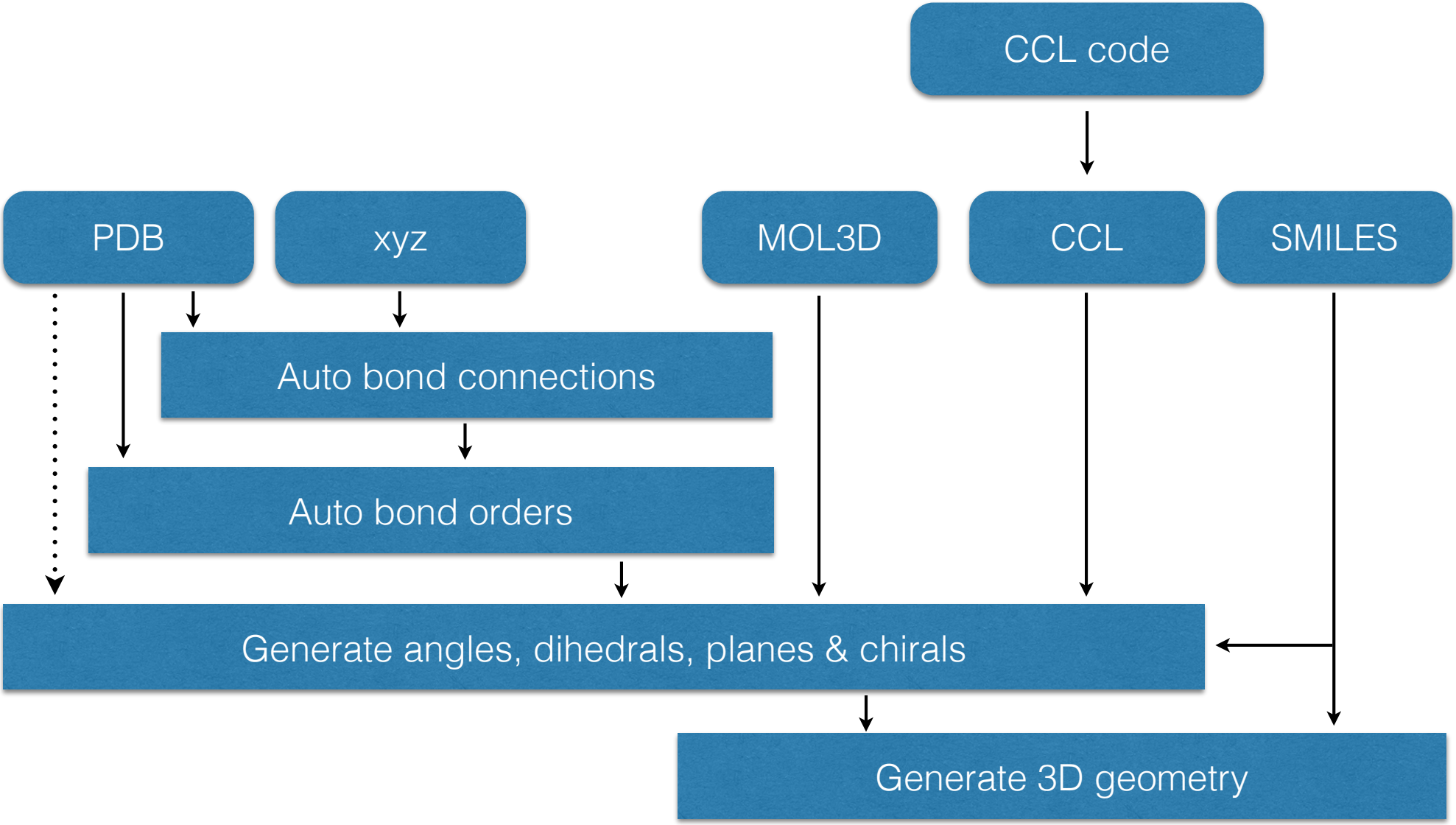
Topology



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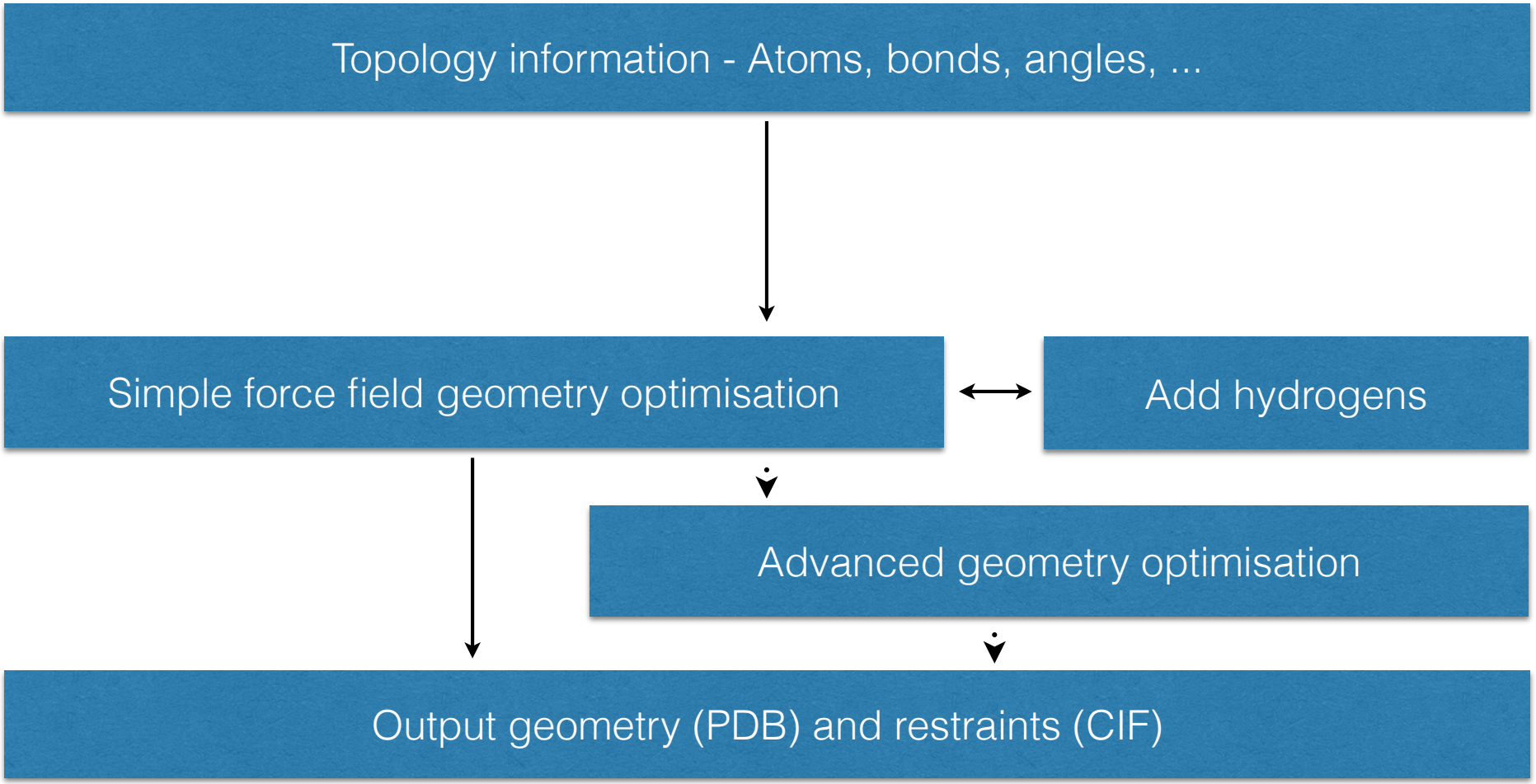
Optimisation



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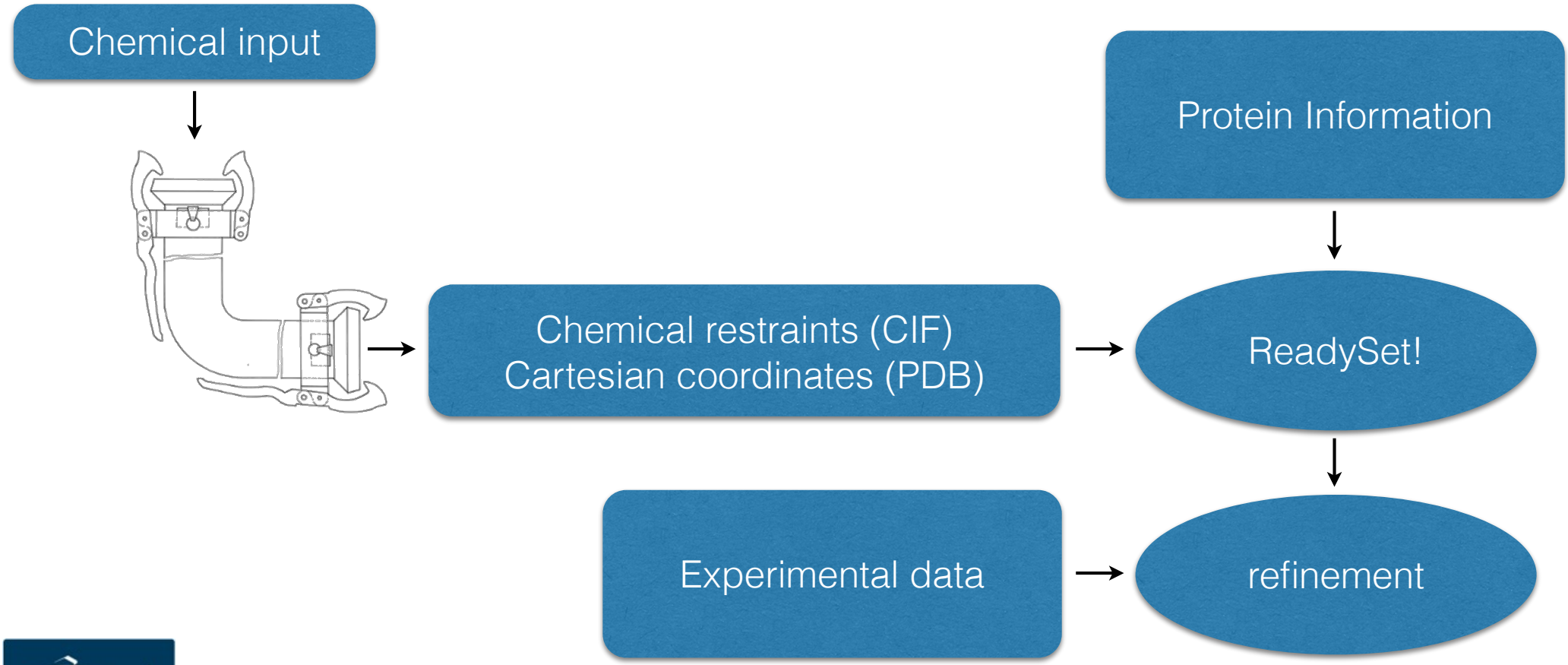
Getting ready to refine

- Many details needed to prepare for structure refinement



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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

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ReadySet!

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

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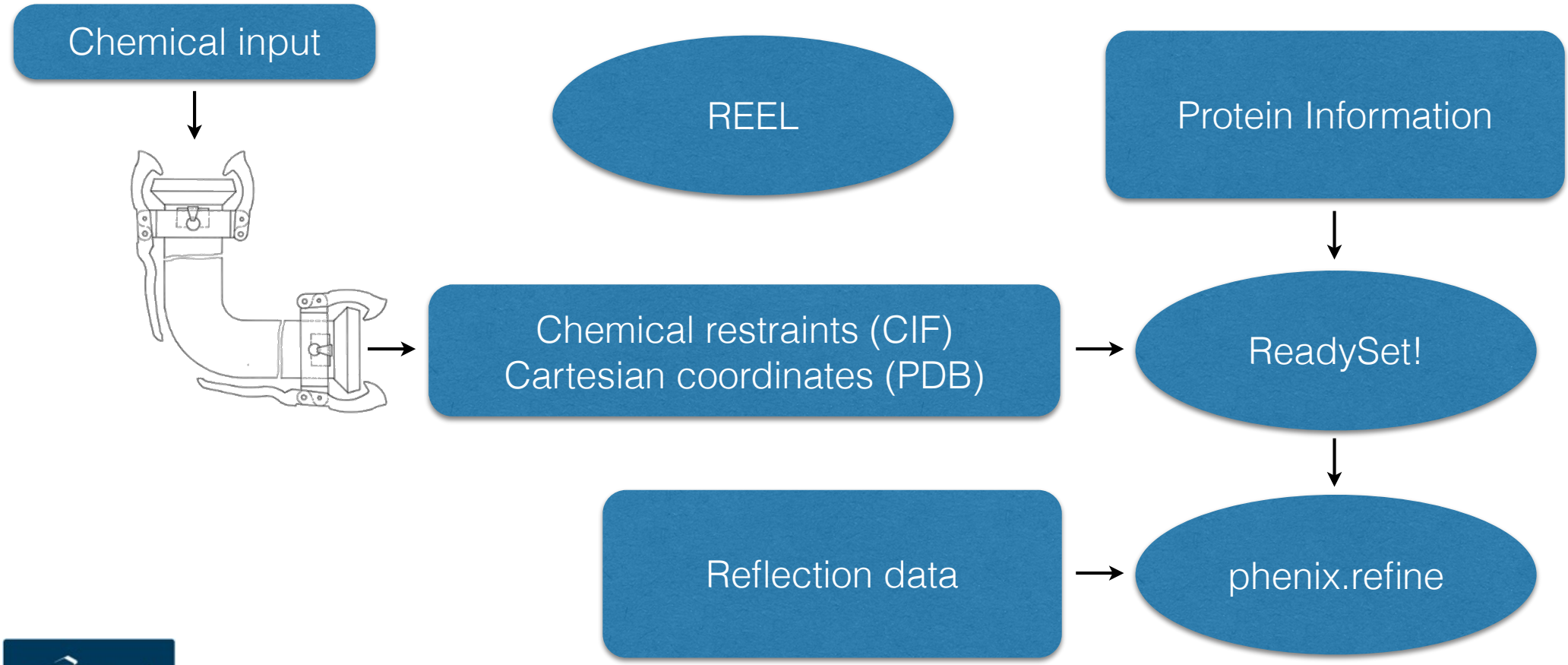
Restraints editing

- Visualisation of primary and secondary restraints
- Adjusting restraints to user preference



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Restraints Editor, Essentially Ligands

- 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

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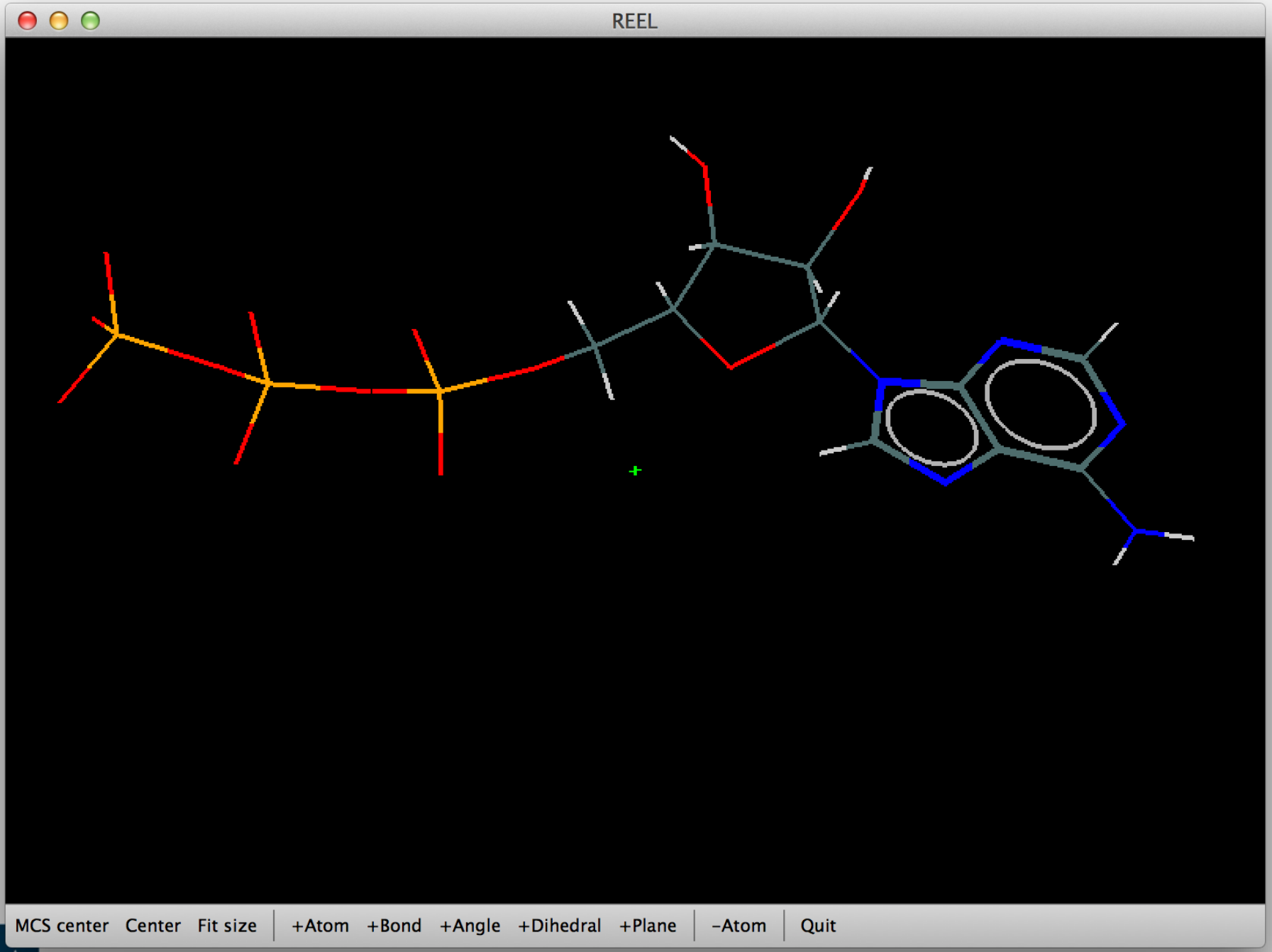

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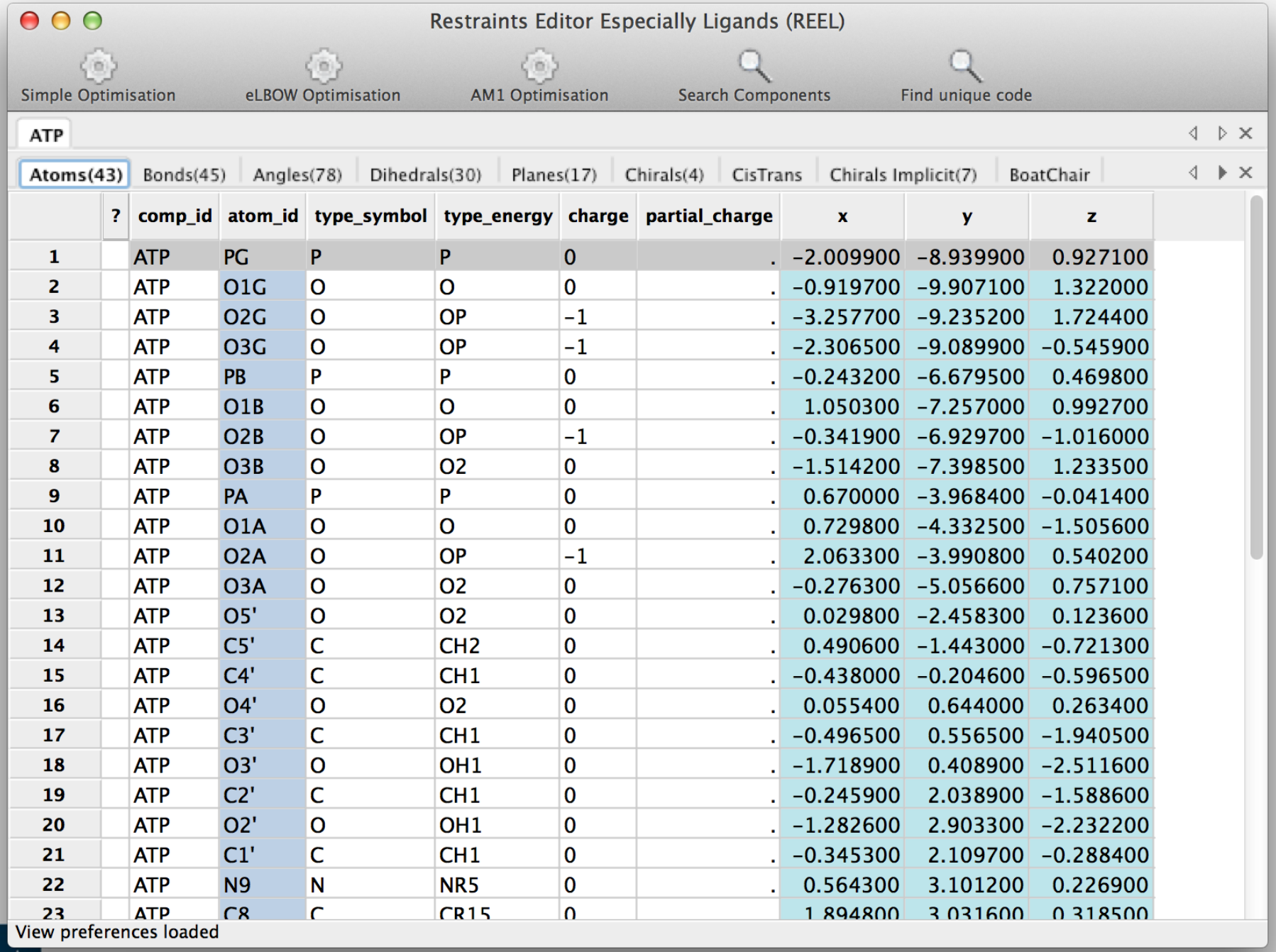

	?	comp_id	atom_id	type_symbol	type_energy	charge	partial_charge	x	y	z
1		ATP	PG	P	P	0	.	-2.009900	-8.939900	0.927100
2		ATP	O1G	O	O	0	.	-0.919700	-9.907100	1.322000
3		ATP	O2G	O	OP	-1	.	-3.257700	-9.235200	1.724400
4		ATP	O3G	O	OP	-1	.	-2.306500	-9.089900	-0.545900
5		ATP	PB	P	P	0	.	-0.243200	-6.679500	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

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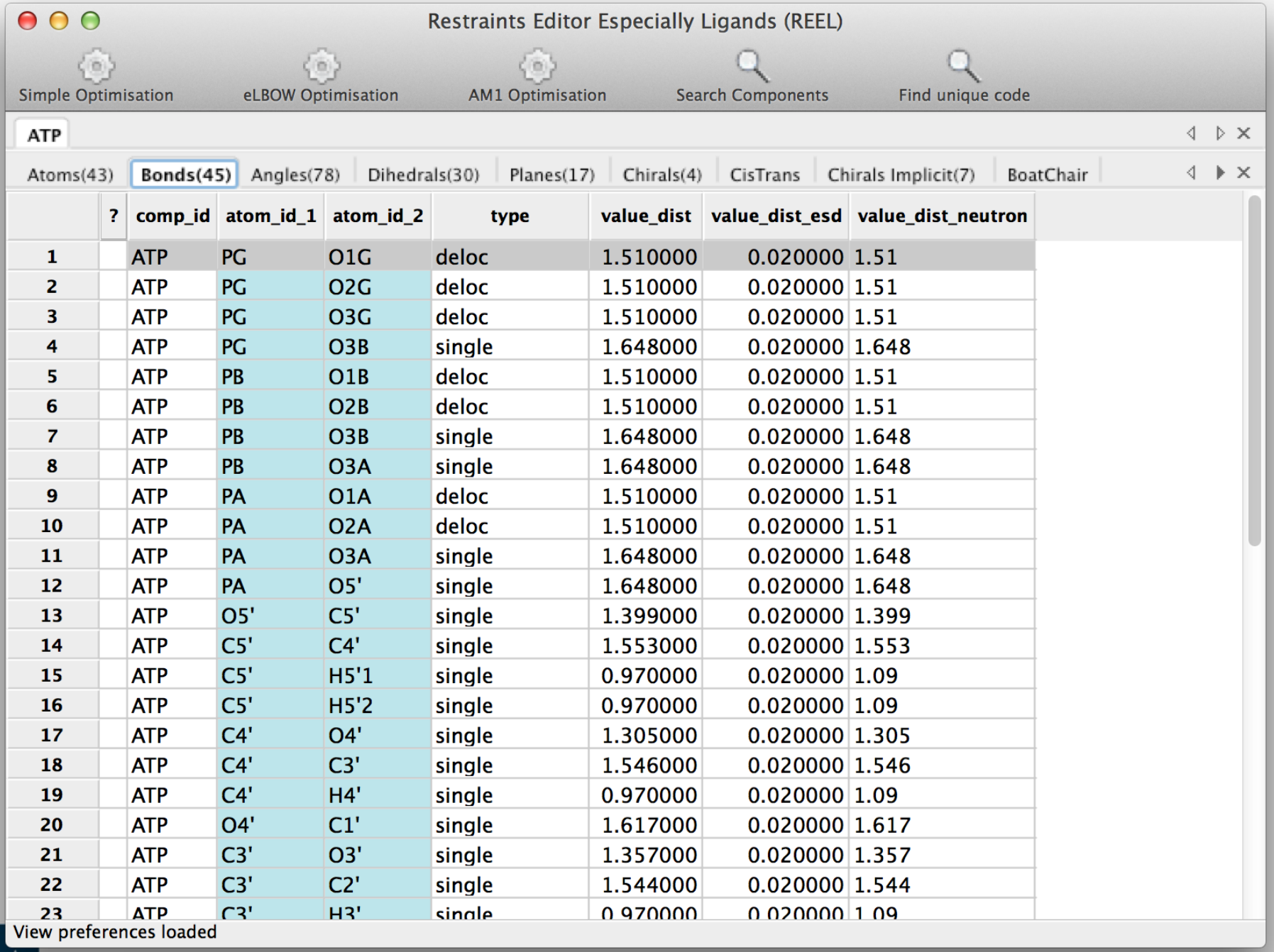

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Ligand Fitting

Phenix

Approach

- Where is the ligand?
  - Choose the largest region of contiguous density
- What are rotatable bonds?
  - Analyze ligand for allowed rotations
- What is the orientation of the ligand?
  - Fit core of ligand
- What is the conformation of the ligand?
  - Trace the ligand out from the core

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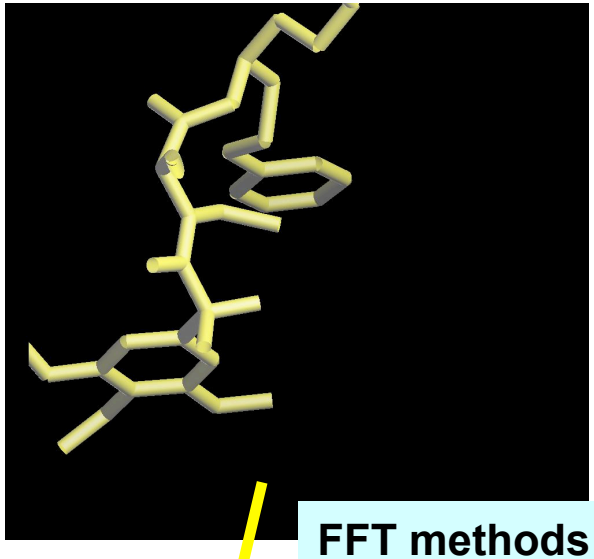
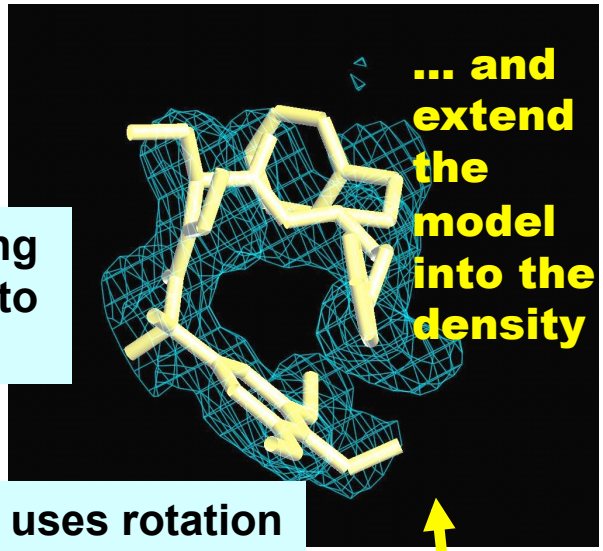
Automated Ligand Fitting

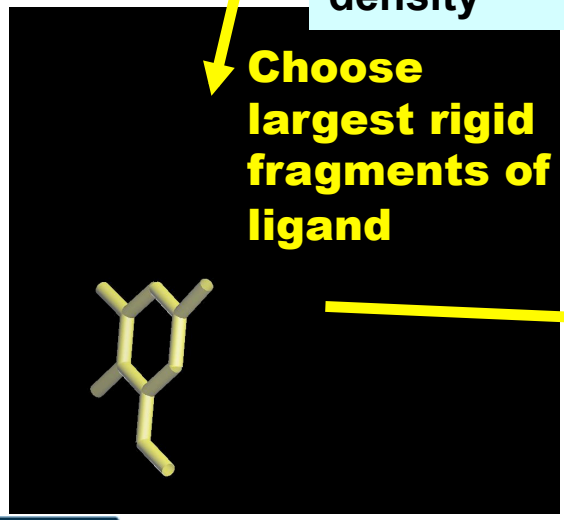


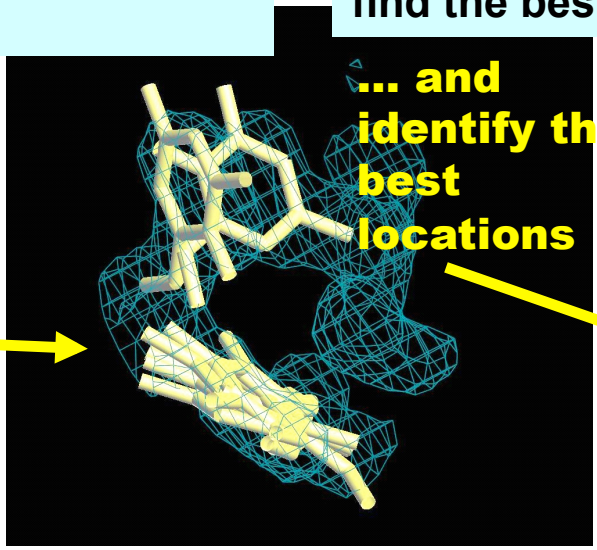
The best fit is measured using the correlation of the model to the electron density

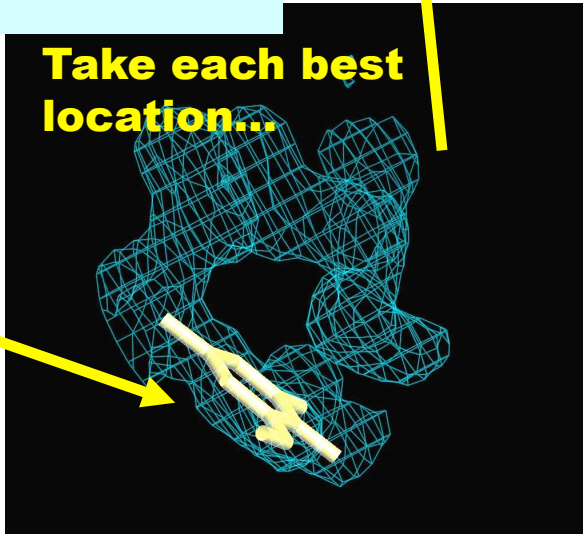


FFT methods are used to rapidly fit fragments to density

The extension uses rotation around torsion angles to find the best fit







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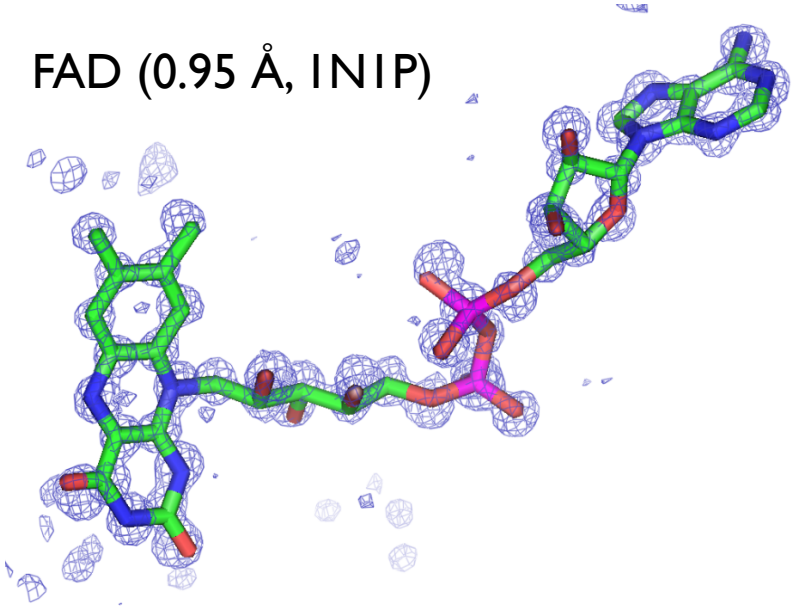
Tom Terwilliger, LANL

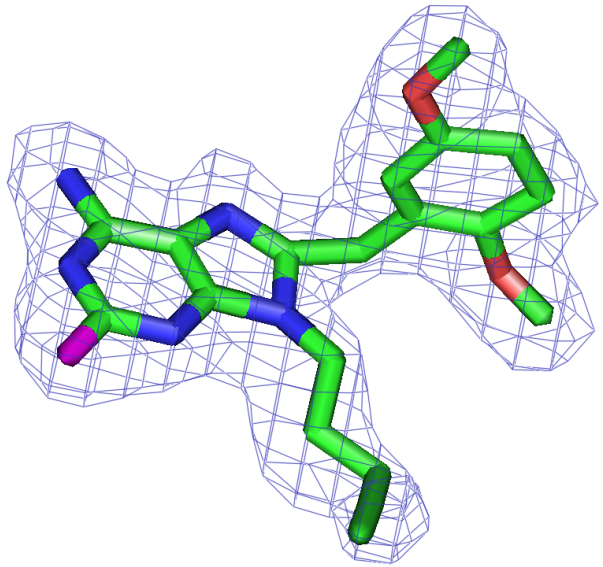
Phenix

Acta Cryst. 2006, **D62**:915-922.

Fitting Over a Range of Resolutions

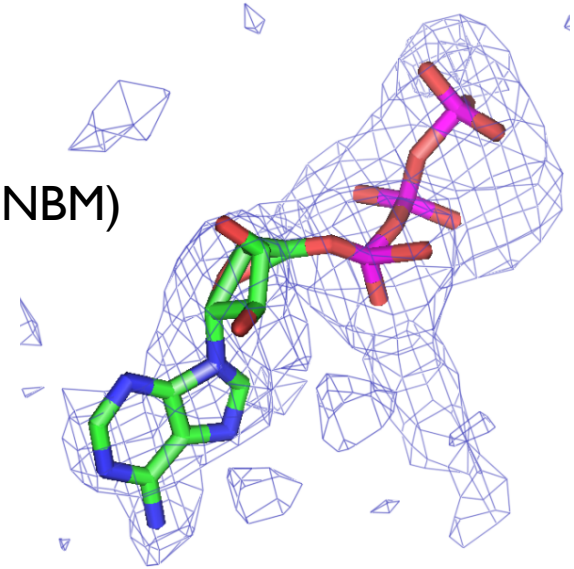
FAD (0.95 Å, 1N1P)

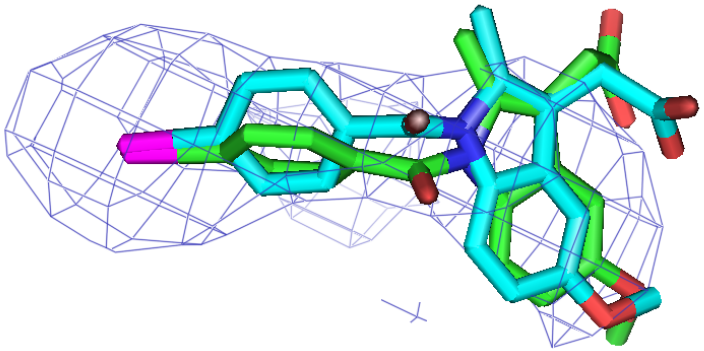




8-(2,5-DIMETHOXY-BENZYL)-2-FLUORO-9-PENT-9H-PURIN-6-YLAMINE (2.2 Å 1UYI)

ATP (3 Å, 1NBM)





(1-(4-IODOBENZOYL)-5-METHOXY-2-METHYL-INDOLE-3-ACETIC ACID (4.5 Å, 1PGF)

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Restraints in phenix.(real_space_)refine

- LINK records have no impact
- Automatically accesses the “standard” residues restraints
- Automatically links the “standard” residues
- Parameter “link_all=True” links
  - Covalent ligands
  - Carbohydrates
  - Metal ions

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Phenix

phenix.(real_space_)refine (continued)

- RNA/DNA restraints
  - Base pair hydrogen bonding
  - Base pair planarity
  - Base stacking (parallelity)
- Secondary Structure restraints
- NCS restraints
- Custom bonds & angles using edits
- Restraints are written to .geo file including non bonded interactions

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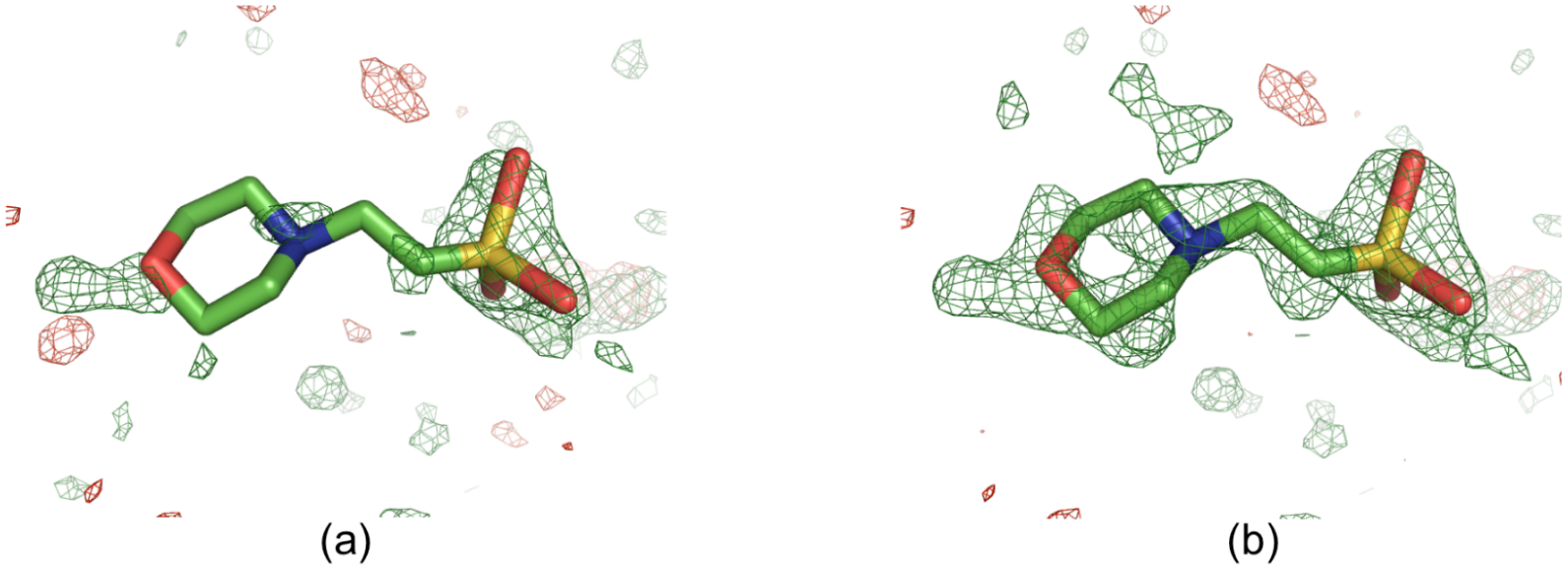
Ligands? Water?

- Be careful when inheriting a model
- Can contain ligands and water molecules
- Must be able to “see” them in the map

Ligands

Polder OMIT Maps



Solvent molecule MES88 in structure 1ABA. The positive and negative mFobs-DFmodel OMIT difference density is displayed in green and red, respectively. (a) OMIT map contoured at +/-3σ. (b) Polder map contoured at +/-3σ. In the OMIT map, there is only some density for the O, N and S atoms. The polder map shows difference electron density for the entire molecule

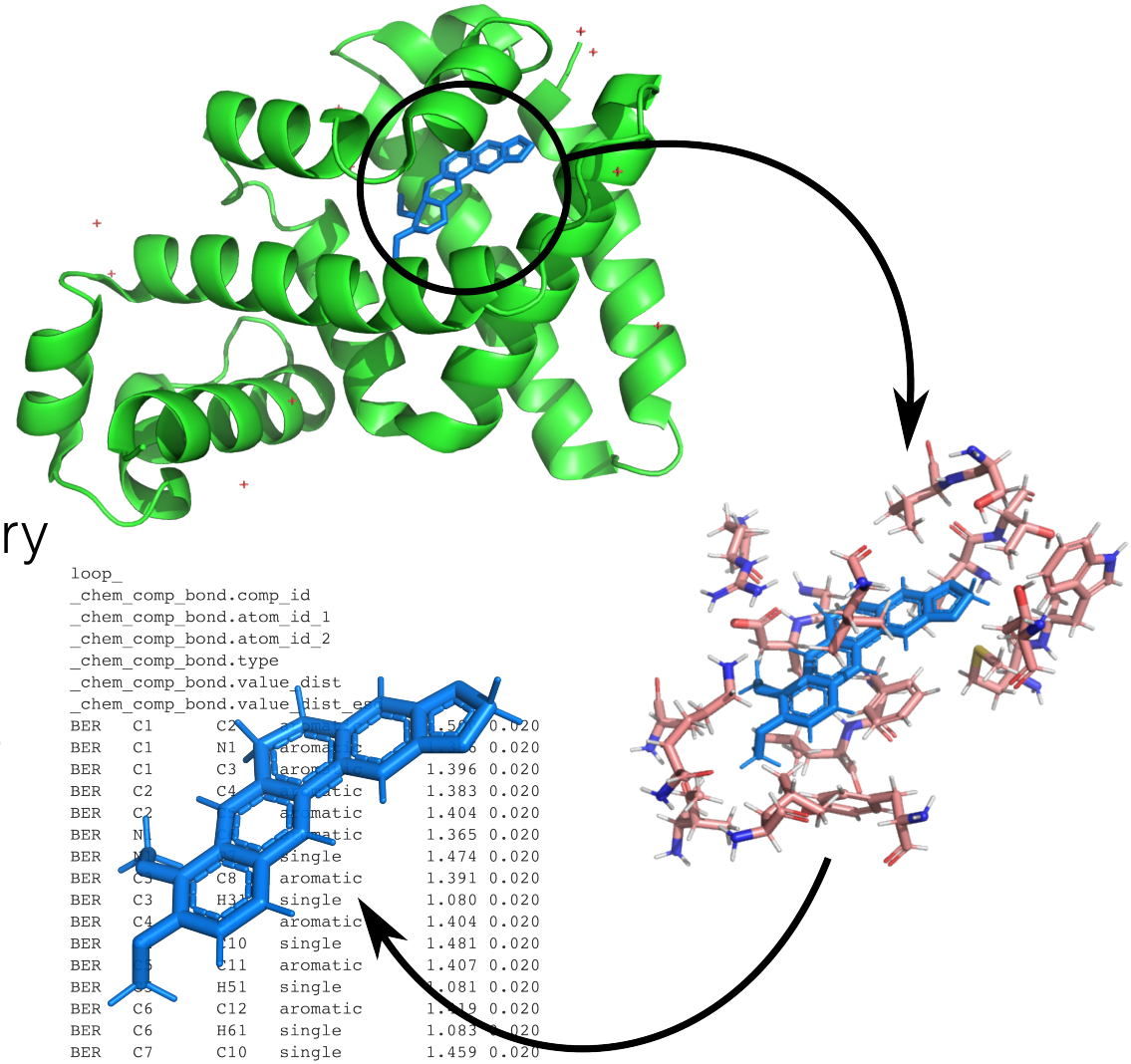
QM Restraints

- Generates restraints of ligands using Quantum Mechanics *in situ*
- There are two ways of using QMR
  - In *phenix.refine*
  - In a standalone program *mmtbx.quantum_interface*
- Python3 installers from the bottom of the download page
- For ORCA, set $PHENIX_ORCA

QMR

In situ restraints generation

- Carve out the ligand environment
- Minimise the ligand geometry *in situ*
- Transfer geometry values to restraints (and write to disk)
- Refinement with modified restraints



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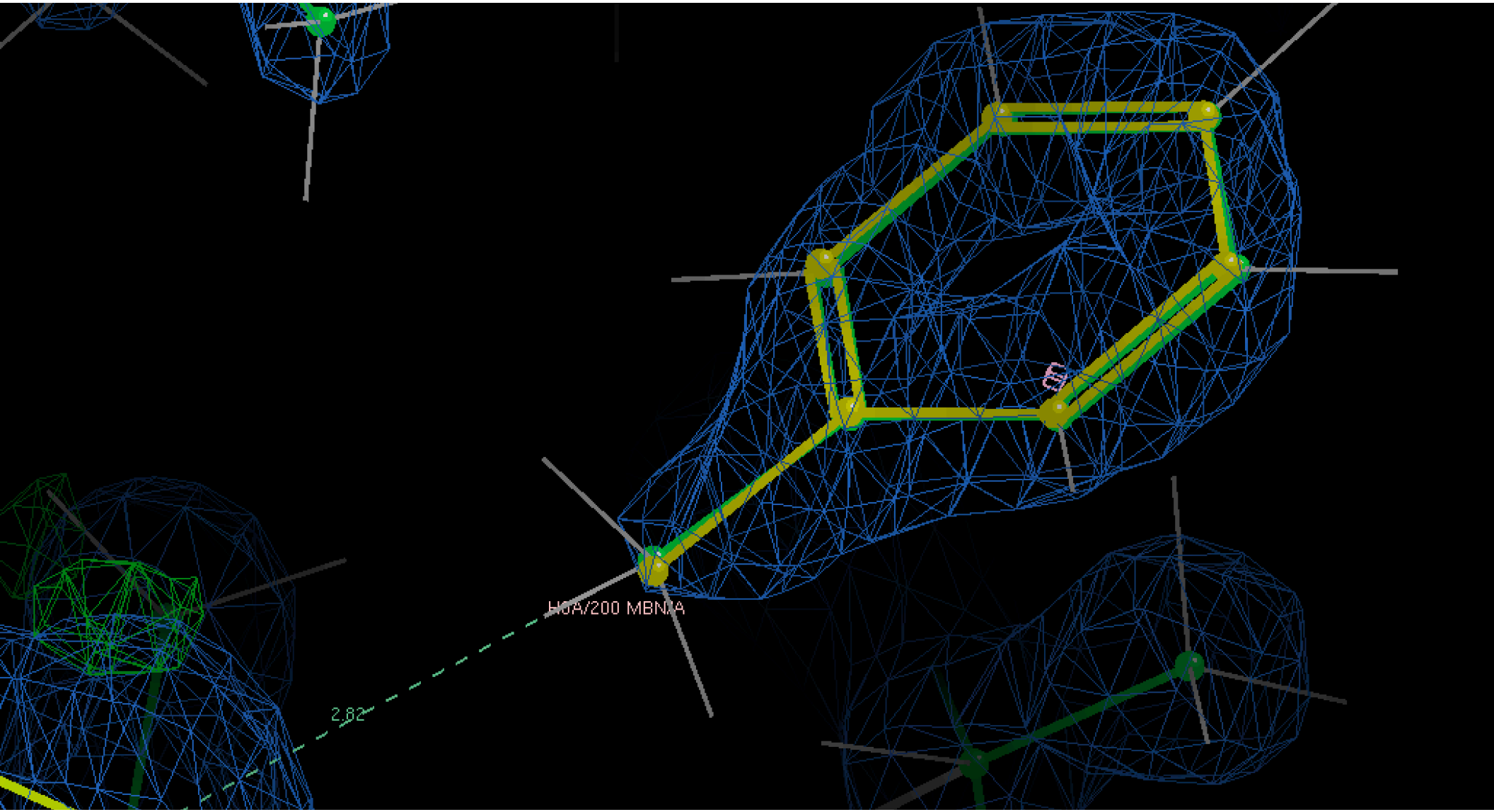
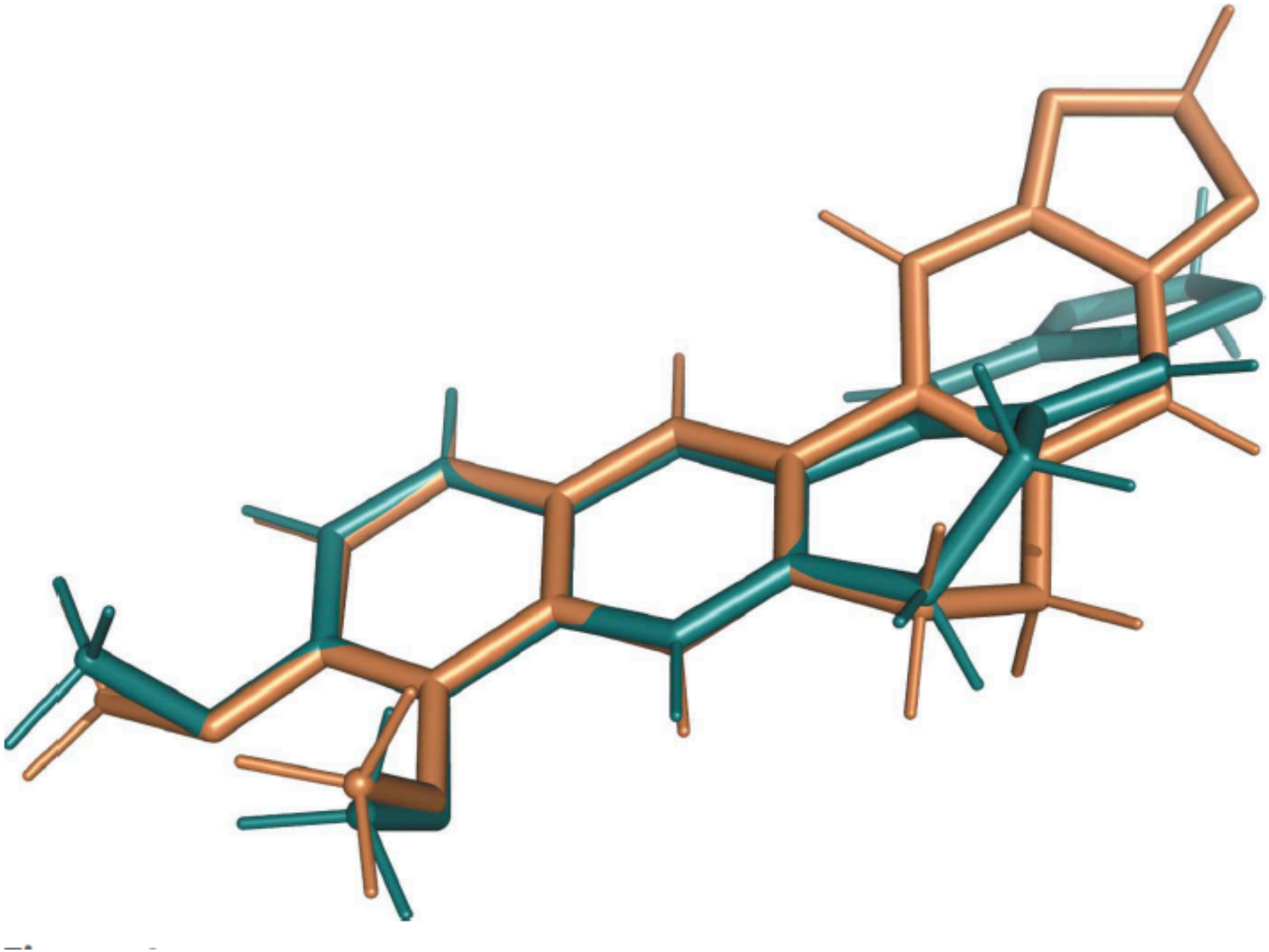
loop_
_chem_comp_bond.comp_id
_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
_chem_comp_bond.type
_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_es
BER   C1      C2    aromatic      1.396 0.020
BER   C1      N1    aromatic      1.396 0.020
BER   C1      C3    aromatic      1.396 0.020
BER   C2      C4    aromatic      1.383 0.020
BER   C2      C5    aromatic      1.404 0.020
BER   N1      C4    aromatic      1.365 0.020
BER   N1      C7    single        1.474 0.020
BER   C3      C8    aromatic      1.391 0.020
BER   C3      H31   single        1.080 0.020
BER   C4      C9    aromatic      1.404 0.020
BER   C5      C10   single        1.481 0.020
BER   C5      C11   aromatic      1.407 0.020
BER   C5      H51   single        1.081 0.020
BER   C6      C12   aromatic      1.419 0.020
BER   C6      H61   single        1.083 0.020
BER   C7      C10   single        1.459 0.020
```

In situ



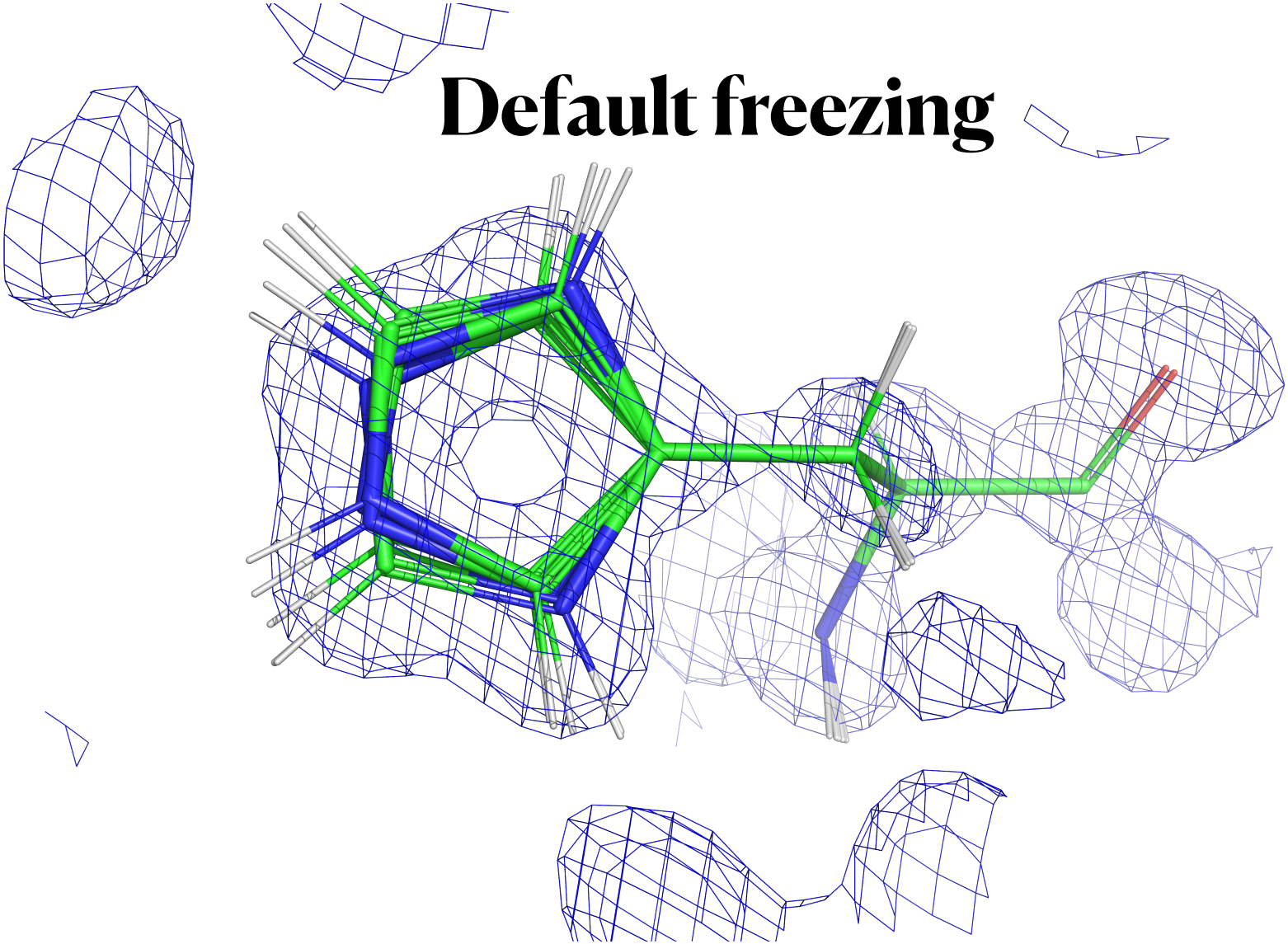
BER in 3vw2



QM Flipping

- Generate the three pronation states of HIS
- Flip chi-2 180 for total of six configurations
- Perform a QM geometry minimisation of the side-chain while freezing the heavy atoms (non-H) for rest of model
- Compare metrics
  - Energy
  - H-bonds
  - RMSD

Default freezing

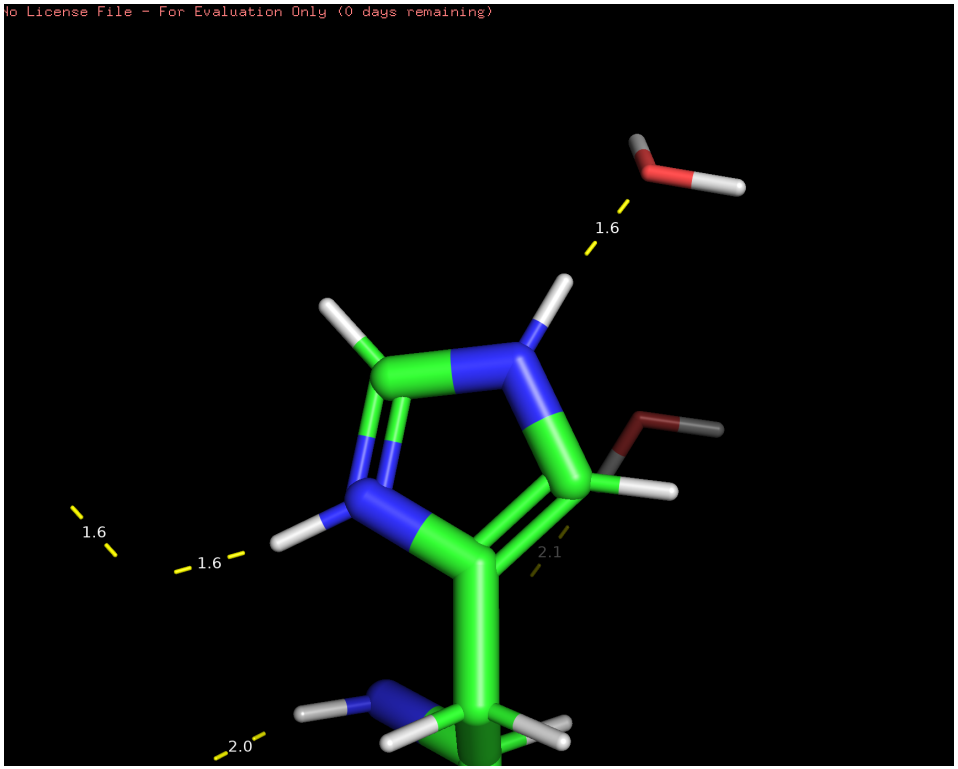


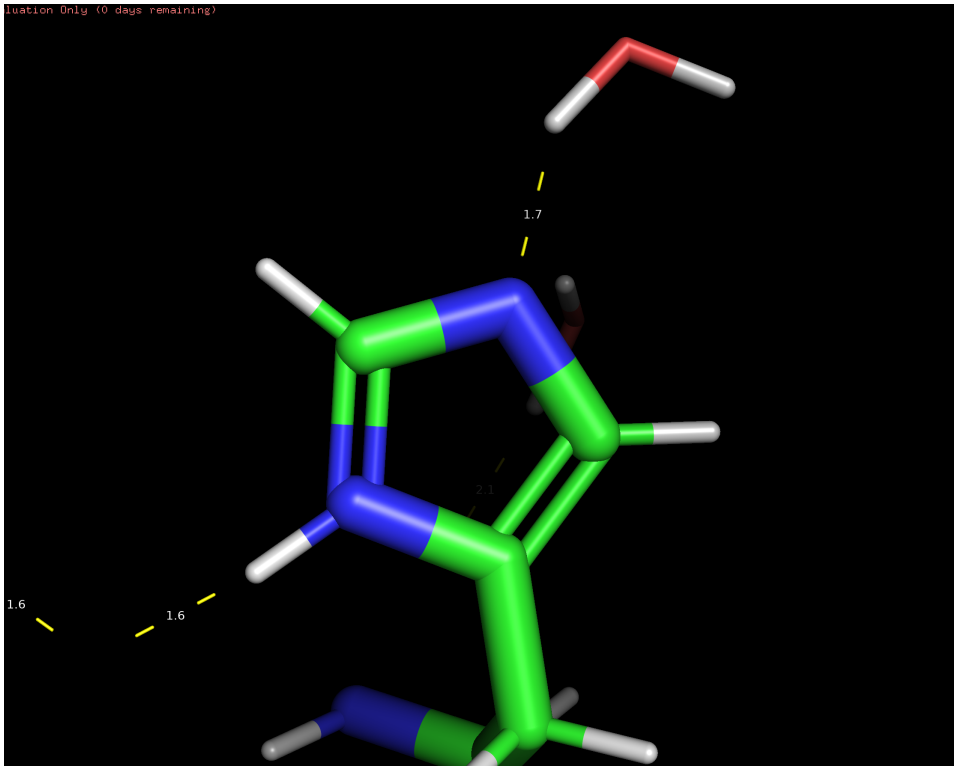
Metrics

	Configuratio	Energy (kcal/	ΔE	H bonds	r.m.s.d.	Rotamer
0	HD1, HE2					m90
1	HD1, HE2	-1019.6	2.6	14	0.04	m90
2	HD1 only	-1022.2	0.0	14	0.04	m90
3	HE2 only	-1003.0	19.3	13	0.05	m90
4	HD1, HE2	-1004.0	18.3	12	0.29	m-70
5	HD1 only	-1004.4	17.9	12	0.38	m-70
6	HE2 only	-1009.4	12.9	11	0.32	m-70

The QMF results for the histidine resseq 4 in chain A of PDB 4rj2

Minimised geometry of two histidine configurations of histidine in chain A and resid 4 of PDB 4rj2.





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

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