Restraints in Phenix

Generating & modifying for all scenarios

Nigel W. Moriarty

User's Meeting
University of Montana, June 2024

Ligands in Phenix

Generating & modifying for all scenarios

Nigel W. Moriarty

User's Meeting
University of Montana, June 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

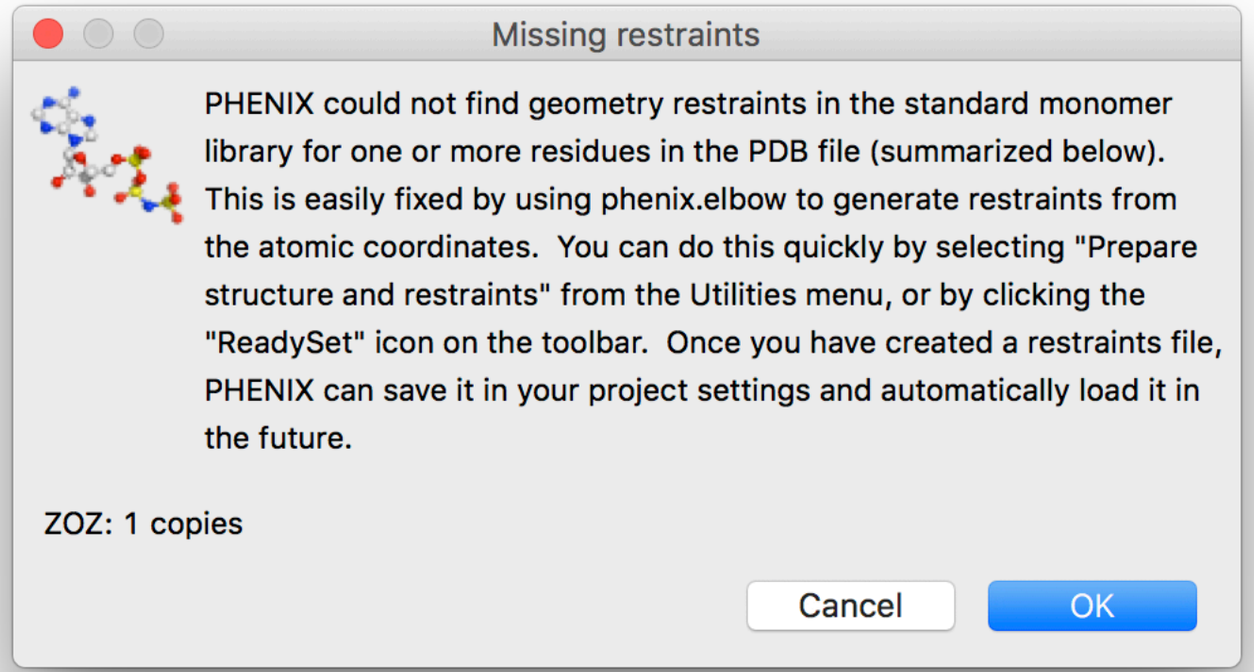
Restraints in Action

- 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 – 37k 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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Overview

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Ligands in crystallography

Generate ligand restraints

Fit ligand to density

Refine macromolecule and ligand

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

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.

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

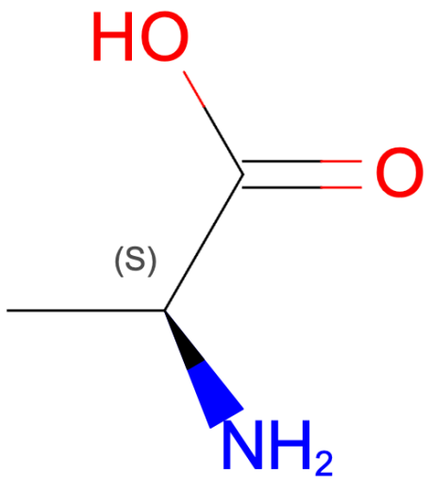
Ligand Expo / Chemical Component Library

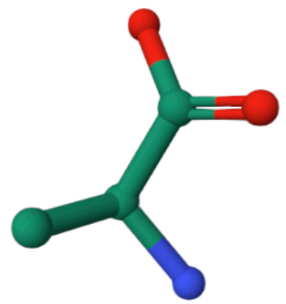
- List of all the entities in the Protein Data Bank
  - Amino acids, Nucleic acids
  - Ligands, Small molecule
  - Metal clusters
- In CIF format
  - Contents chemical information
    - SMILES, atom names, bonds
  - Not restraints

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Amino Acid





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)

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Water

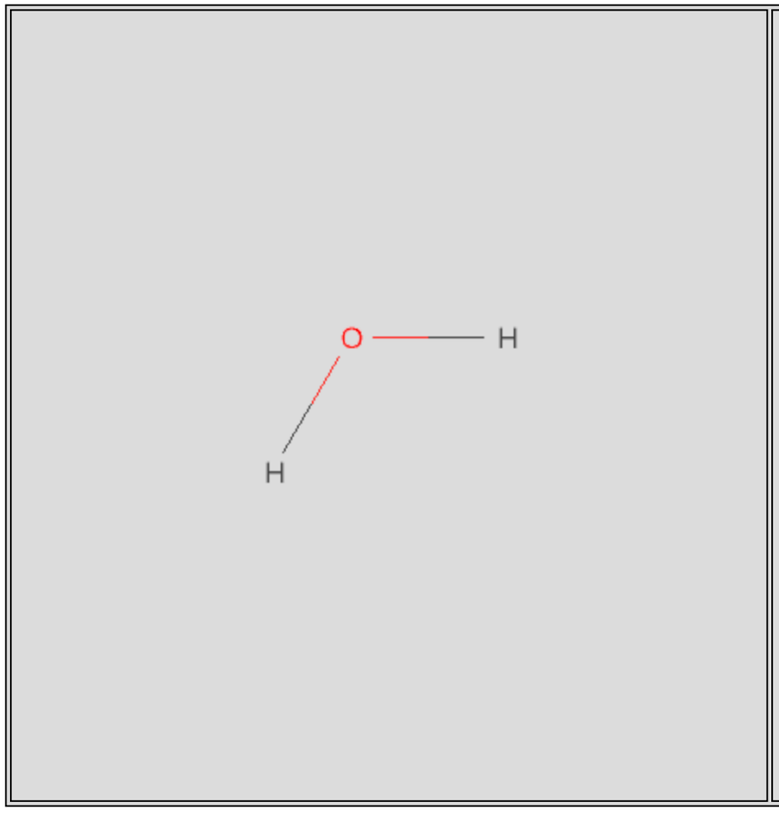
RCSB PDB
PROTEIN DATA BANK
RCSB PDB | Contact Us

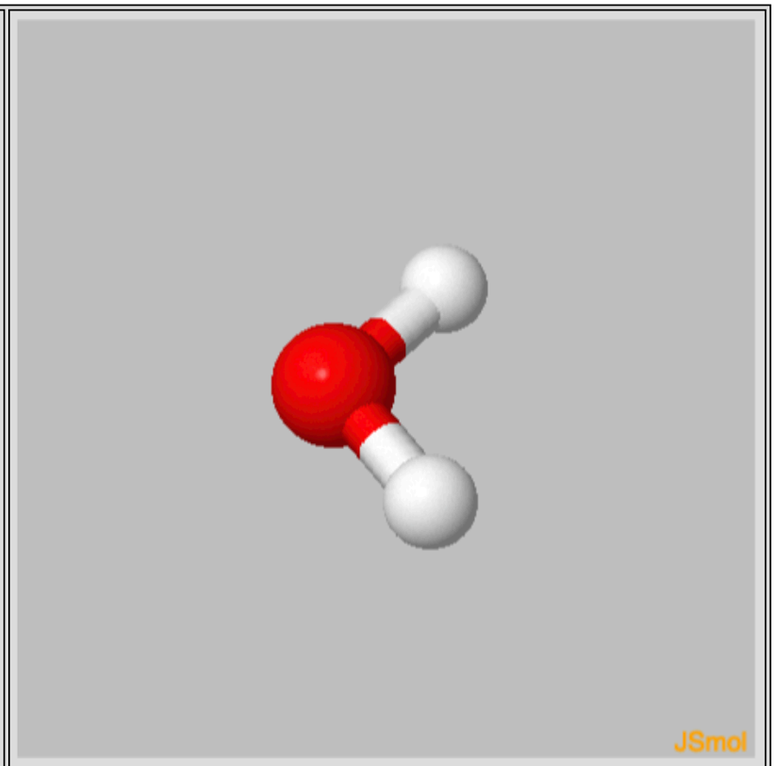
Ligand Expo

Home Search Browse Download Ligand Expo Help

Chemical Details | Geometry | Atom Nomenclature | Downloads | Related Resources

PDB Chemical Component HOH





Ideal Model

Chemical Description

Name	WATER
Formula	H2 O
Formal charge	0
Molecular weight	18.015 g/mol
Component type	NON-POLYMER

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Metal clusters

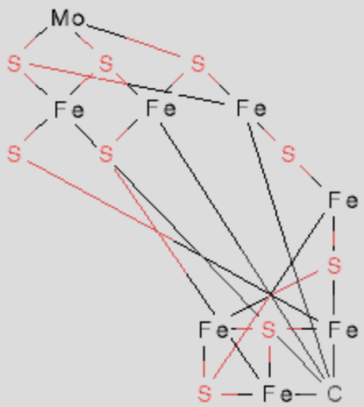
RCSB PDB
PROTEIN DATA BANK
RCSB PDB | Contact Us

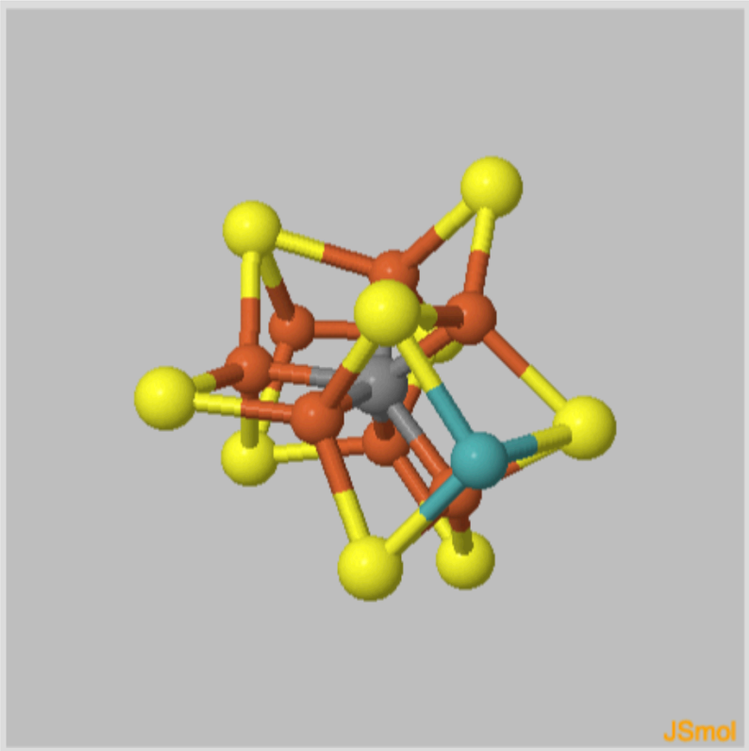
Ligand Expo

Home Search Browse Download Ligand Expo Help

Chemical Details | Geometry | Atom Nomenclature | Downloads | Related Resources

PDB Chemical Component ICS



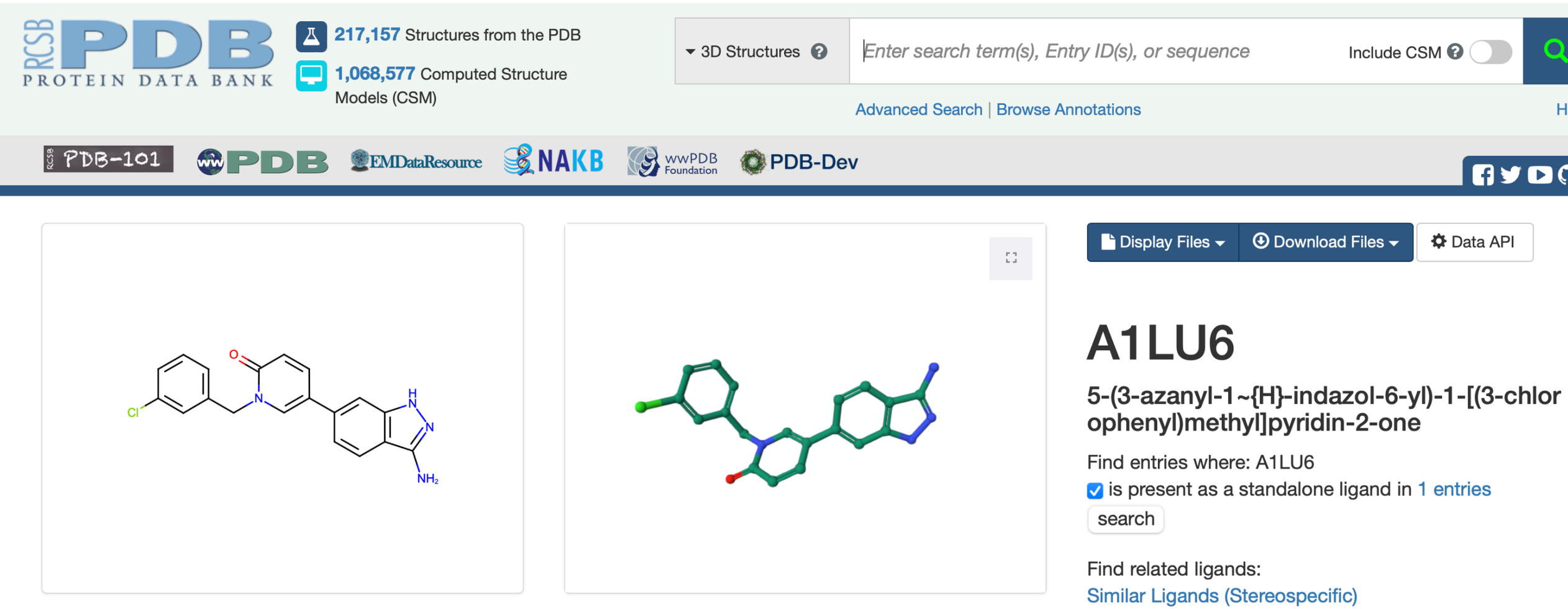


Ideal Model

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

5-letter codes



- 49k combinations for 3-letter codes

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Human readable

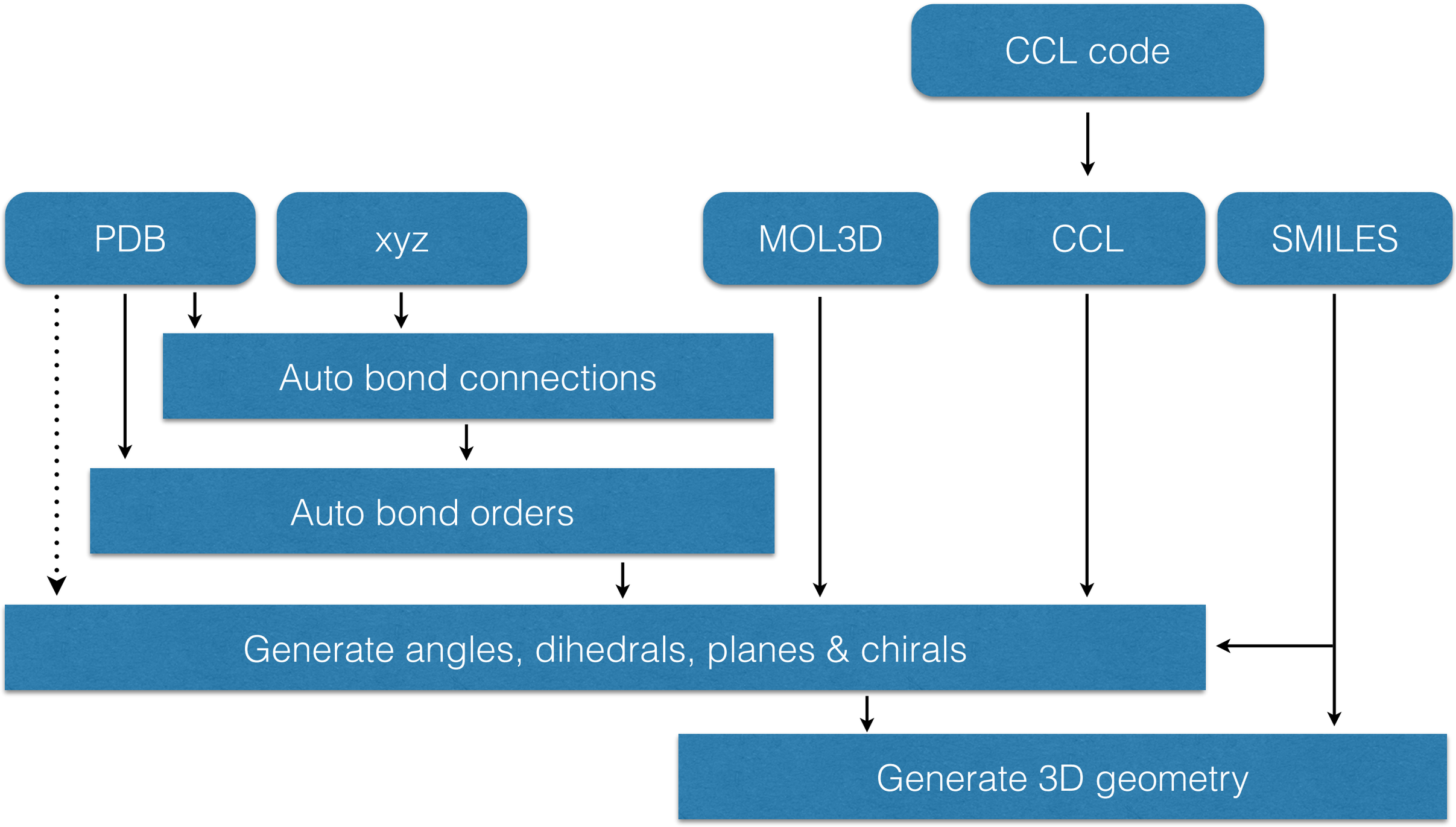
Standard		Human readable	
Uppercase	Lowercase	Uppercase	Lowercase
1OI0	1oi0	1oi0	1oi0
1IJJ	1ijj	1iJJ	1ijj
4OCL	4ocl	4oCL	4ocL
5SS2	5ss2	5ss2	5ss2

- Confusable letters are case-forced
  - L is always uppercase
  - i, o are always lowercase
- Somewhat confusable – “5” and “S”

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Topology



BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Optimisation

Topology information - Atoms, bonds, angles, ...

Simple force field geometry optimisation ⟷ Add hydrogens

Advanced geometry optimisation

Output geometry (PDB) and restraints (CIF)

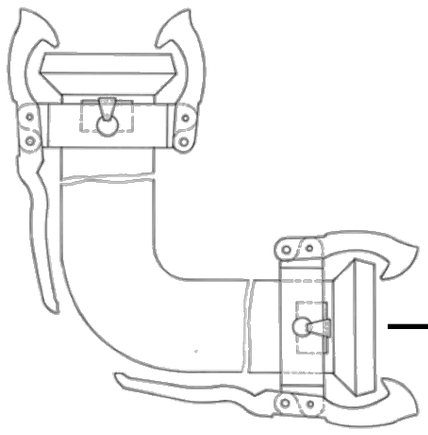
BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Getting ready to refine

- Many details needed to prepare for structure refinement

Chemical input



Chemical restraints (CIF)
Cartesian coordinates (PDB)

Protein Information

ReadySet!

Experimental data

refinement

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

ReadySet!

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

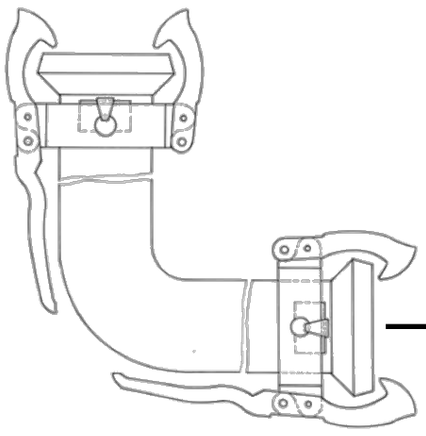
BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Restraints editing

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

Chemical input



Chemical restraints (CIF)
Cartesian coordinates (PDB)

REEL

Protein Information

ReadySet!

Reflection data

phenix.refine

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

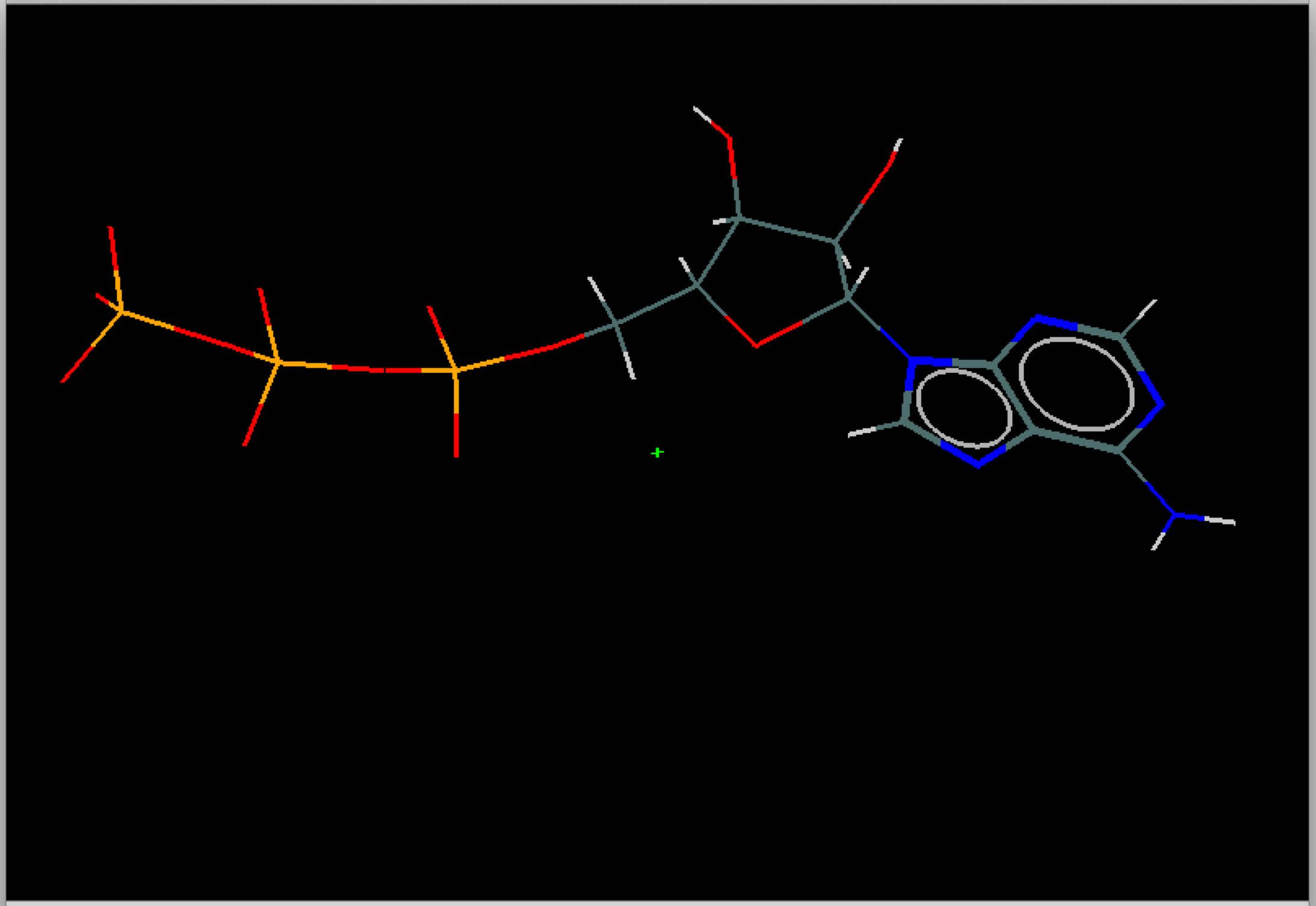
Restraints Editor, Essentially Ligands

- 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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

REEL



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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Restraints Editor Especially Ligands (REEL)

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

ATP

Angles(78) | Dihedrals(30) | Planes(17) | Chirals(4) | CisTrans | Chirals Implicit(7) | BoatChair | **Ribose(1)**

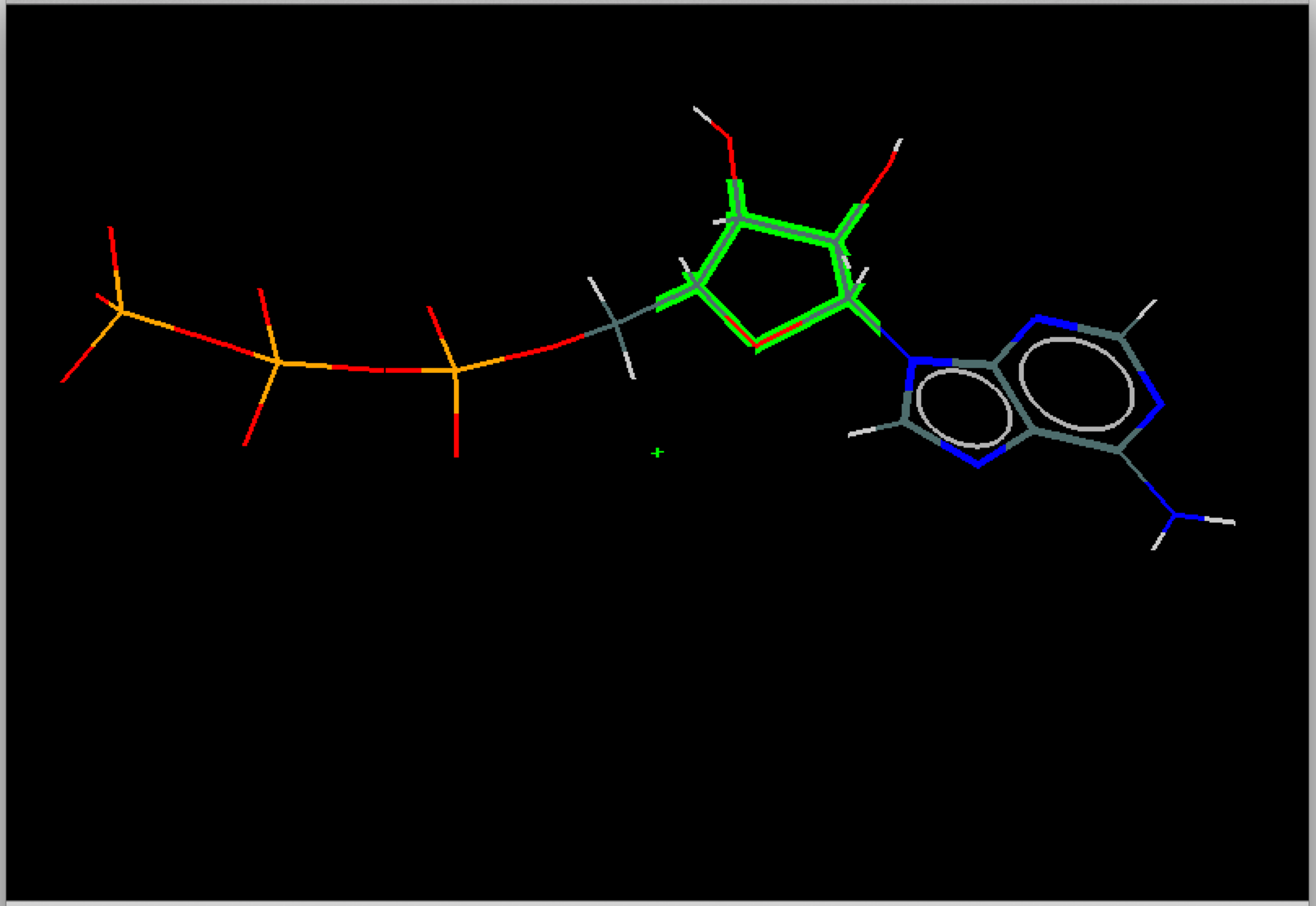
	?	atom_id_1	atom_id_2	atom_id_3	atom_id_4	atom_id_5	restrained_to	Pucker
1		C4'	C3'	C2'	C1'	O4'		-+-+-

View preferences loaded

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

REEL



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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Restraints Editor Especially Ligands (REEL)

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

LIG

Atoms(12) | Bonds(11) | Angles(18) | Dihedrals(3) | Planes(6) | Chirals | **CisTrans(1)** | Chirals Implicit | BoatChair | Ribose

	?	atom_id_1	atom_id_2	restrained_to	E/Z
1		C03	C02	E	E

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Chemical Components Search

View in REEL | View info | View similar | View help

	Code	Name
1	HOH	WATER
2	1CU	COPPER ION, 1 WATER COORDINATED
3	2OF	FERROUS ION, 2 WATERS COORDINATED
4	3OF	HYDRATED FE (III) ION, 2 WATERS COORDINATED
5	543	CALCIUM ION, 6 WATERS PLUS ETHANOL COORDINATED
6	CD1	CADMIUM ION, 1 WATER COORDINATED
7	CD3	CADMIUM ION, 3 WATERS COORDINATED
8	CD5	CADMIUM ION, 5 WATERS COORDINATED
9	CO5	COBALT ION,5 WATERS COORDINATED
10	DOD	DEUTERATED WATER
11	HC0	2 IRON/2 SULFUR/6 CARBONYL/1 WATER INORGANIC CLUST...
12	HC1	2 IRON/2 SULFUR/5 CARBONYL/2 WATER INORGANIC CLUST...
13	HCN	2 IRON/2 SULFUR/3 CARBONYL/2 CYANIDE/WATER/METHYLE...
14	KO4	POTASSIUM ION, 4 WATERS COORDINATED
15	MN5	MANGANESE ION, 5 WATERS COORDINATED
16	MN6	MANGANESE ION, 6 WATERS COORDINATED
17	MO1	MAGNESIUM ION, 1 WATER COORDINATED
18	MO2	MAGNESIUM ION, 2 WATERS COORDINATED
19	MO3	MAGNESIUM ION, 3 WATERS COORDINATED
20	MO4	MAGNESIUM ION, 4 WATERS COORDINATED
21	MO5	MAGNESIUM ION, 5 WATERS COORDINATED
22	MO6	MAGNESIUM ION, 6 WATERS COORDINATED
23	MTO	BOUND WATER
24	MW1	MANGANESE ION, 1 WATER COORDINATED

Input search string | Search

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Ligand Fitting

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

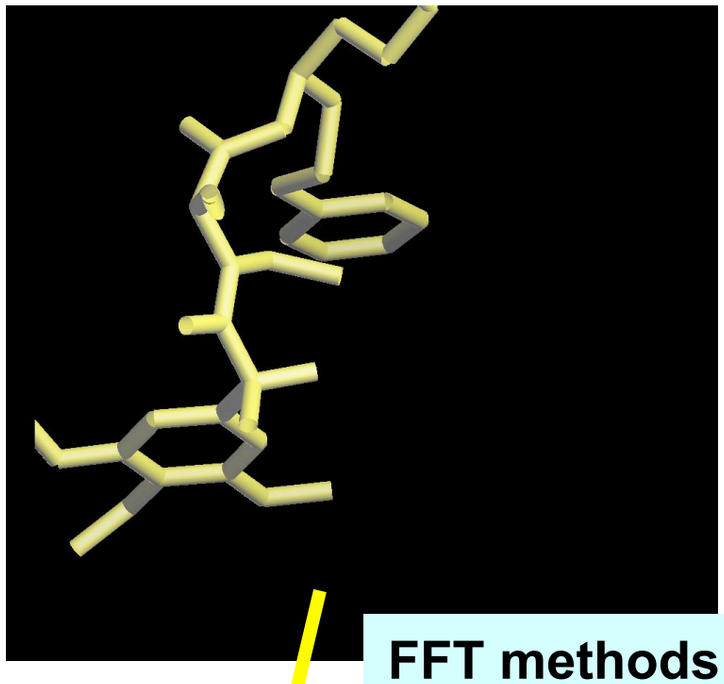
Approach

- 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

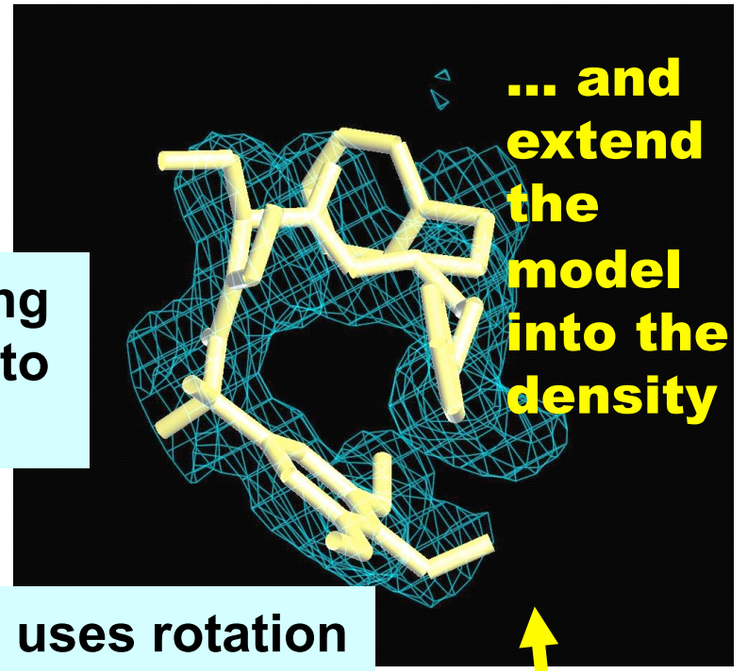
BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

Automated Ligand Fitting

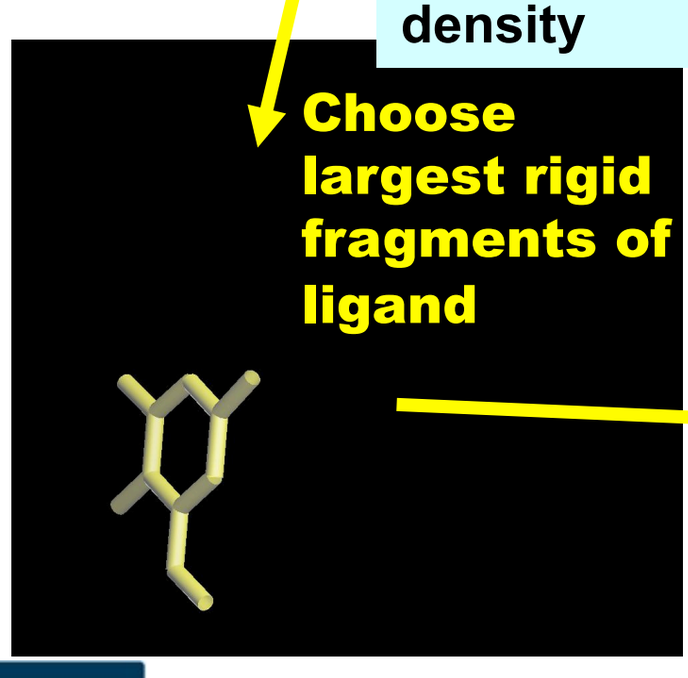


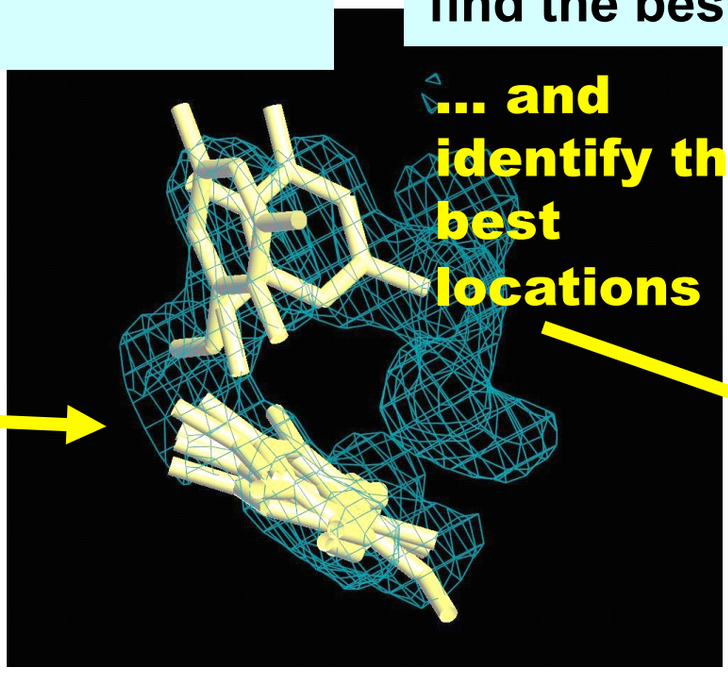
The best fit is measured using the correlation of the model to the electron density

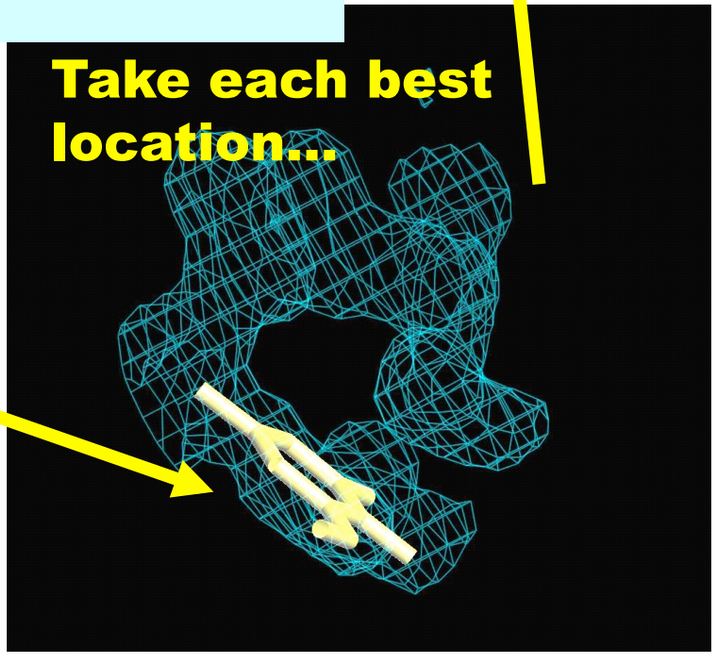


FFT methods are used to rapidly fit fragments to density

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







BERKELEY LAB
Lawrence Berkeley National Laboratory

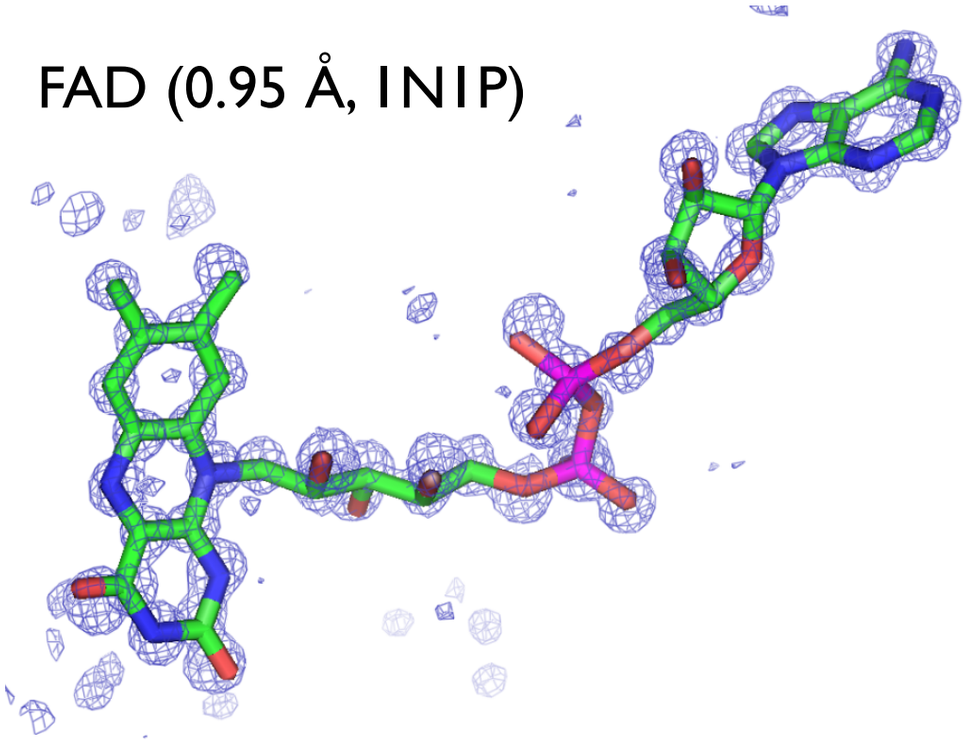
Tom Terwilliger, LANL

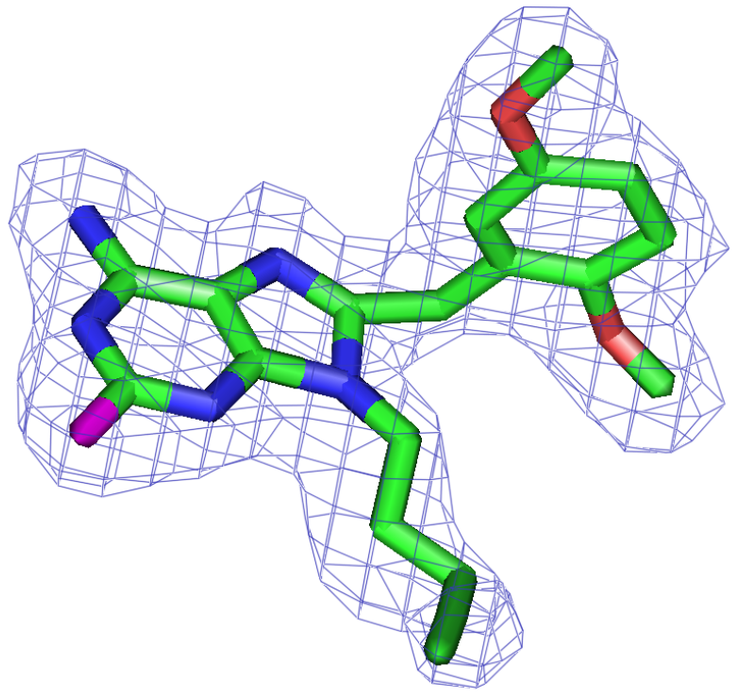
Phenix

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

Fitting Over a Range of Resolutions

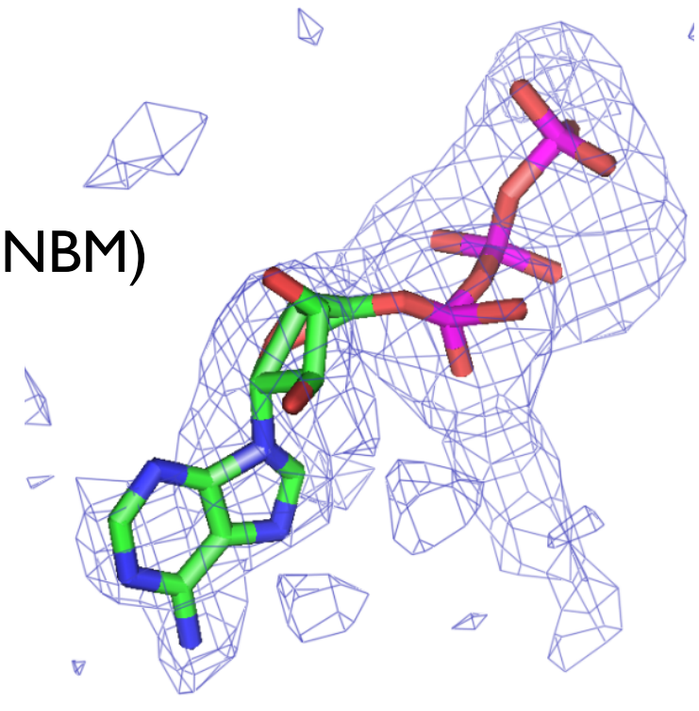
FAD (0.95 Å, 1NIP)

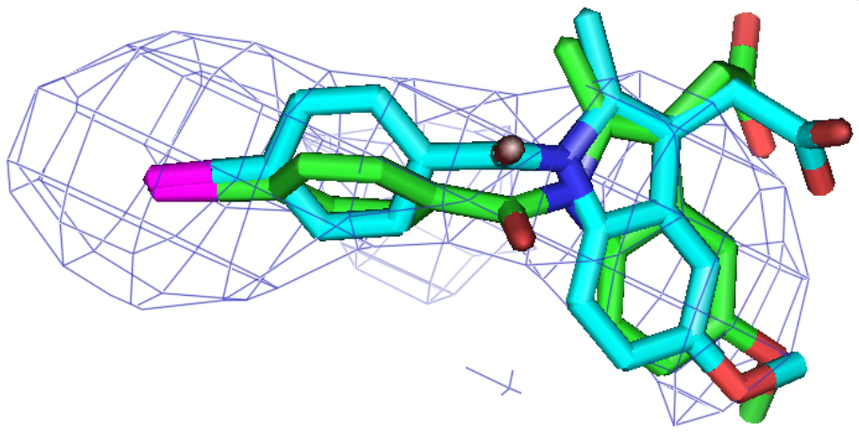




8-(2,5-DIMETHOXY-BENZYL)-2-FLUORO-9-PENT-9H-PURIN-6-YLAMINE (2.2 Å 1UYI)

ATP (3 Å, 1NBM)





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

BERKELEY LAB
Lawrence Berkeley National Laboratory

Phenix

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

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

BERKELEY LAB
Lawrence Berkeley National Laboratory

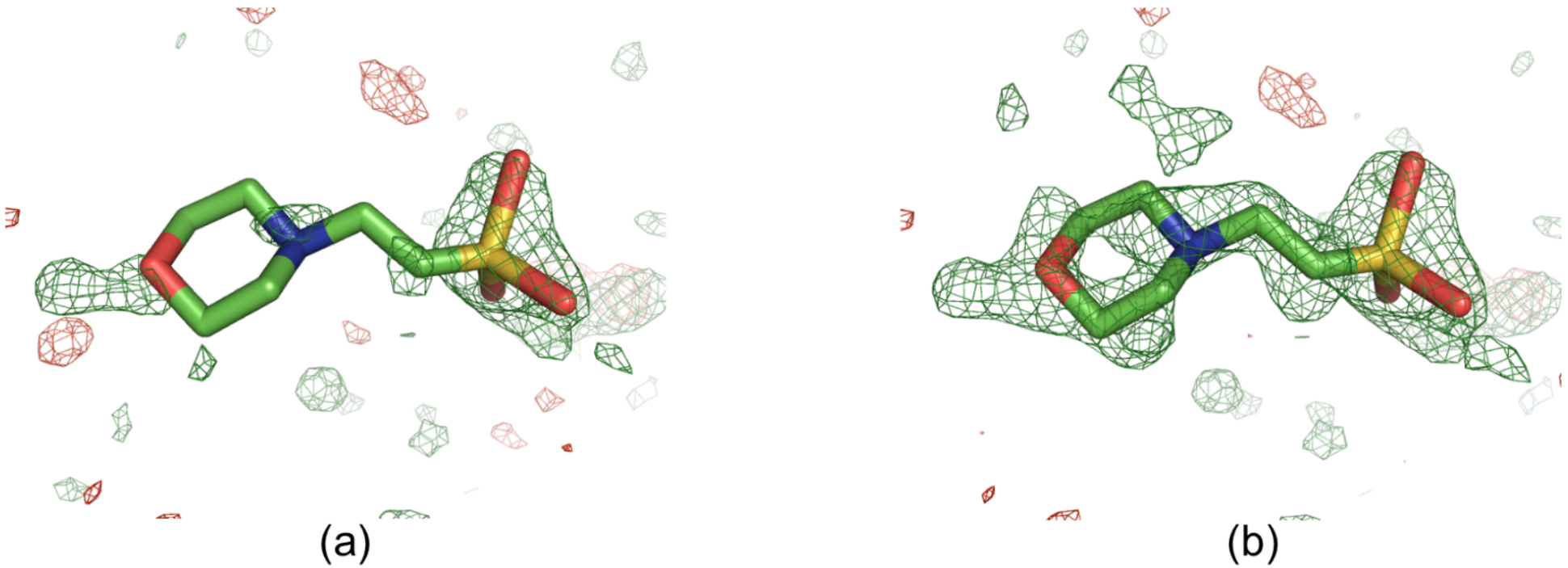
Phenix

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

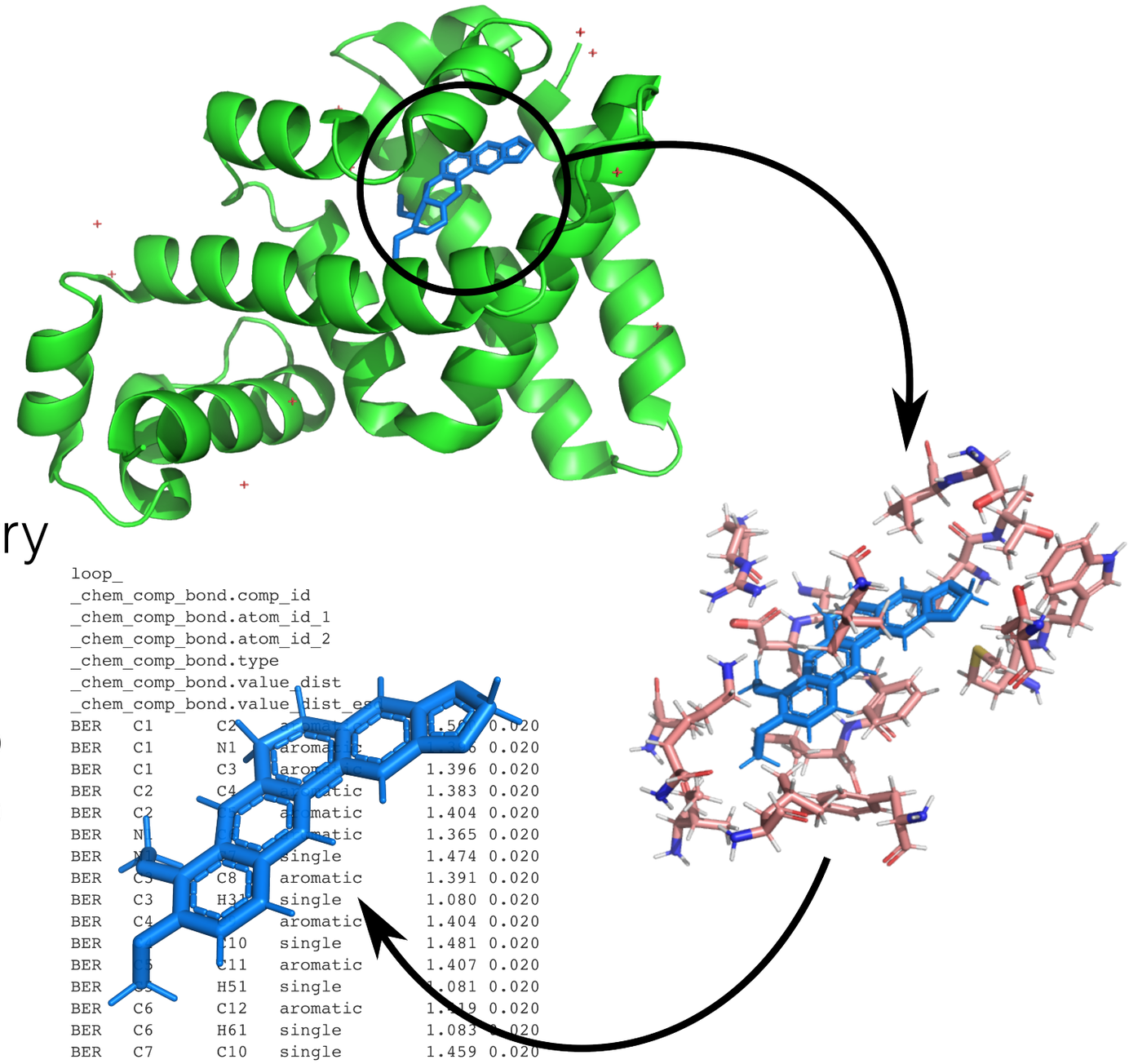
QM Restraints

- 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



QI

- phenix.fetch_pdb 4W53 --mtz
- phenix.ready_set 4W53.pdb
- mmtbx.quantum_interface 4W53.updated.pdb format=qi write_qmr_phil=True

The result is a PHIL scope for MBN QMR restraints:

- 4W53.updated_A_200_MBN.phil

QMR phil

```
qi.qm_restraints {
  selection = "chain A and resid  200  and resname MBN"
  run_in_macro_cycles = *first_only first_and_last all last_only test
  buffer = 3.5

  calculate = *in_situ_opt starting_energy final_energy starting_strain final_strain starting_bound
final_bound starting_higher_single_point final_higher_single_point

  write_files = *restraints pdb_core pdb_buffer pdb_final_core *pdb_final_buffer

package {
  program =  *mopac test
  charge = Auto
  multiplicity = Auto
  method = Auto
  basis_set = Auto
  solvent_model = None
  nproc = 1
  read_output_to_skip_opt_if_available = True
  ignore_input_differences = False
  view_output = None}}
```

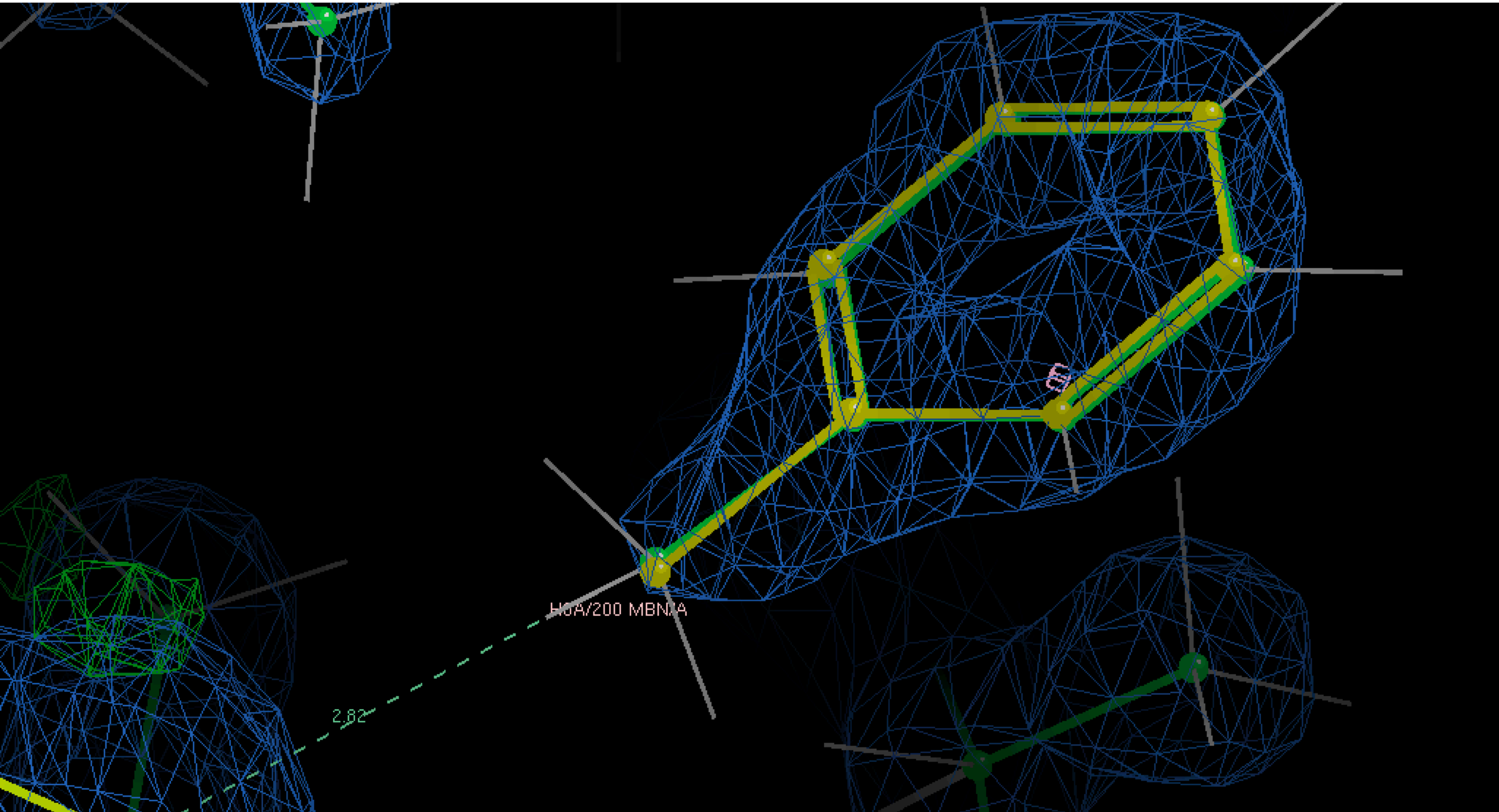
Run

- mmtbx.quantum_interface 4W53.updated.pdb 4W53.updated_A_200_MBN.phil run_qmr=True

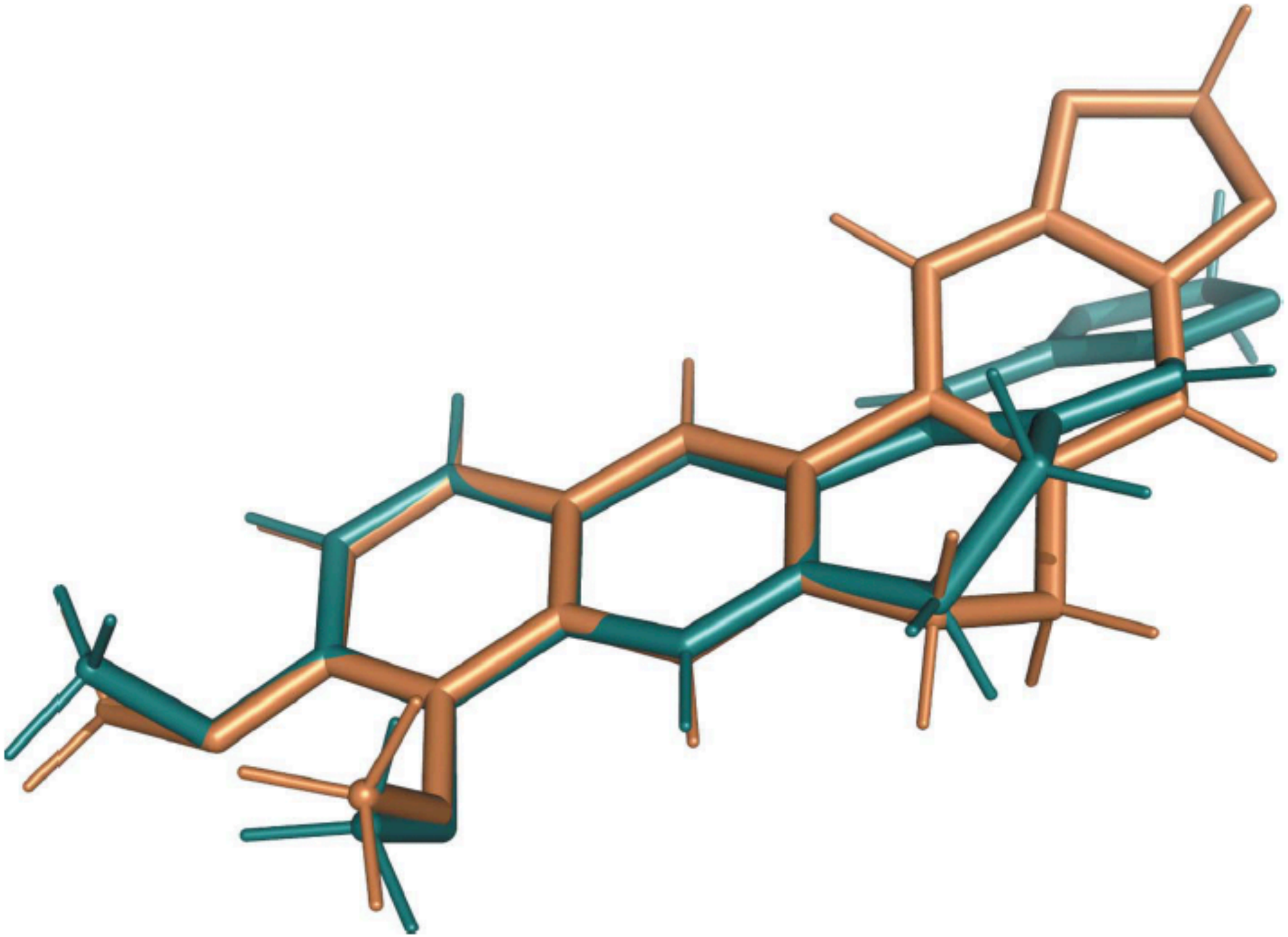
```
QM energies

  "chain A and resid  200  and resname MBN"
    Macro cycle 1
      strain                              2.073 kcal/mol (atoms    15)
      opt                           -405946.999 kcal/mol (atoms   246)
```

In situ



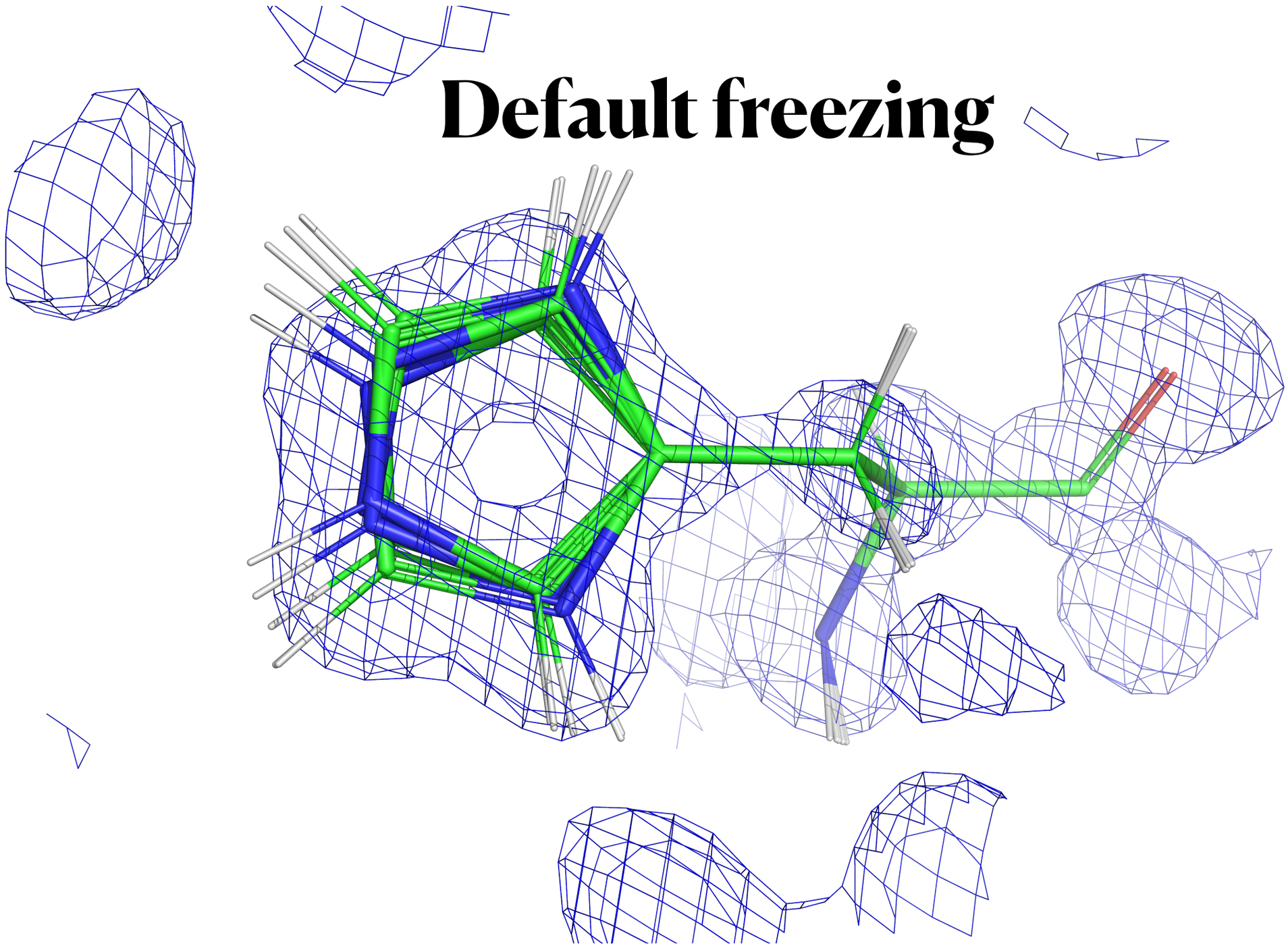
BER in 3vw2



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

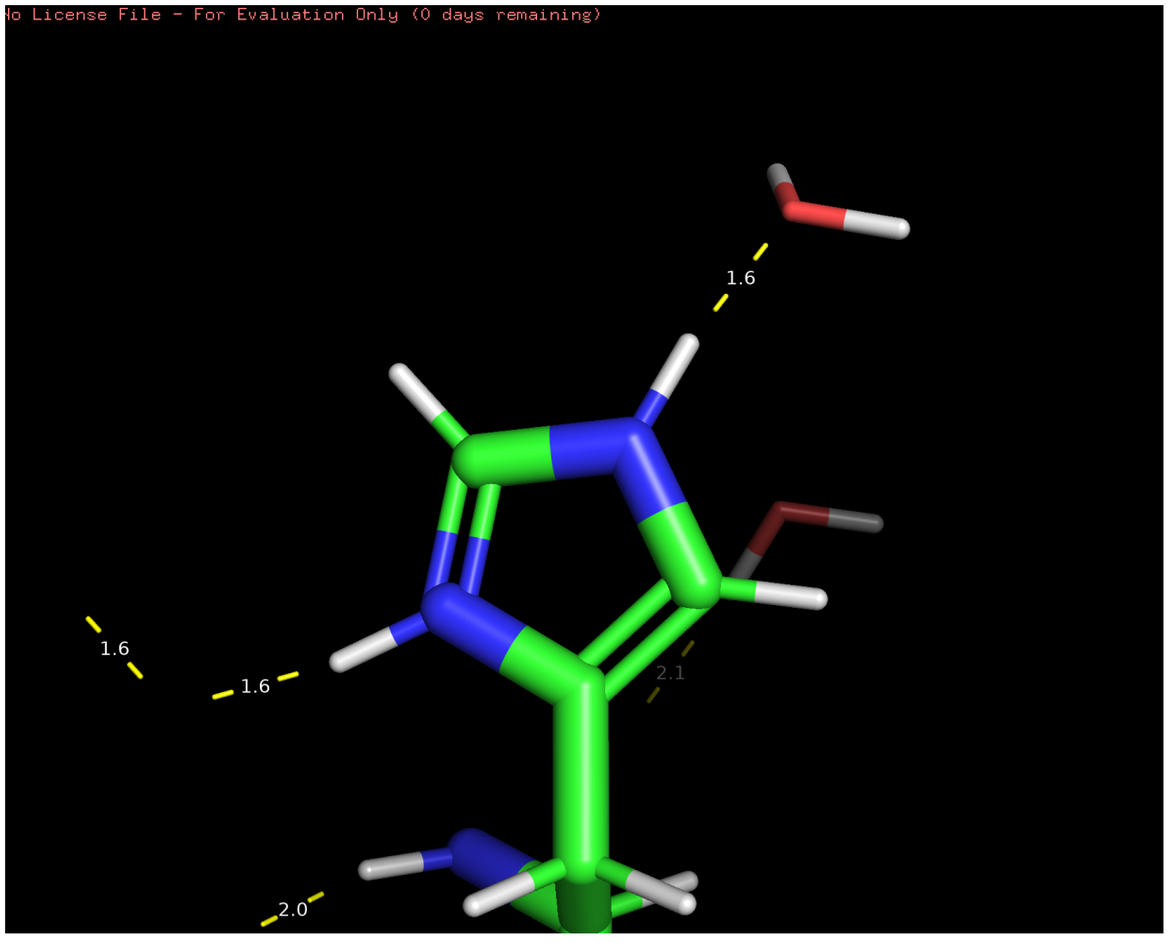


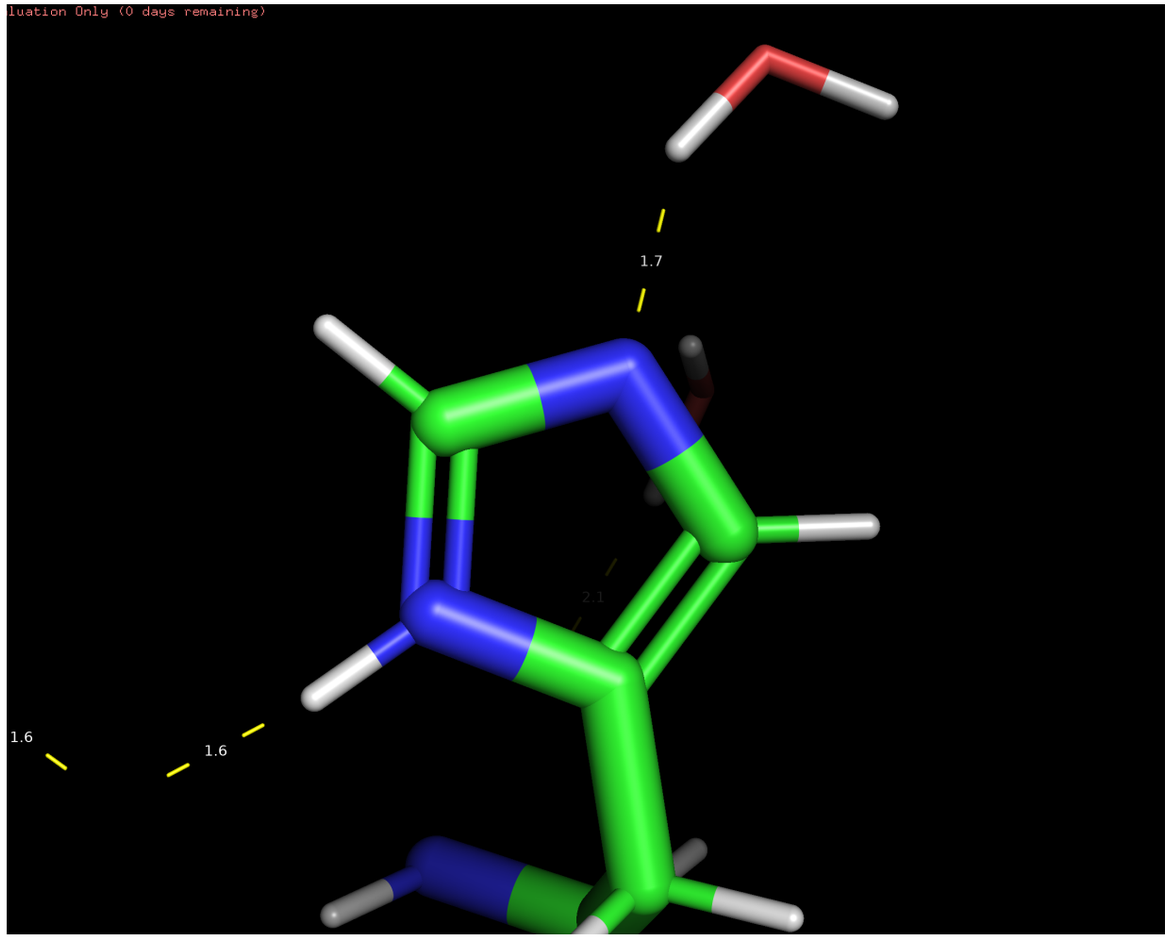
Metrics

	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

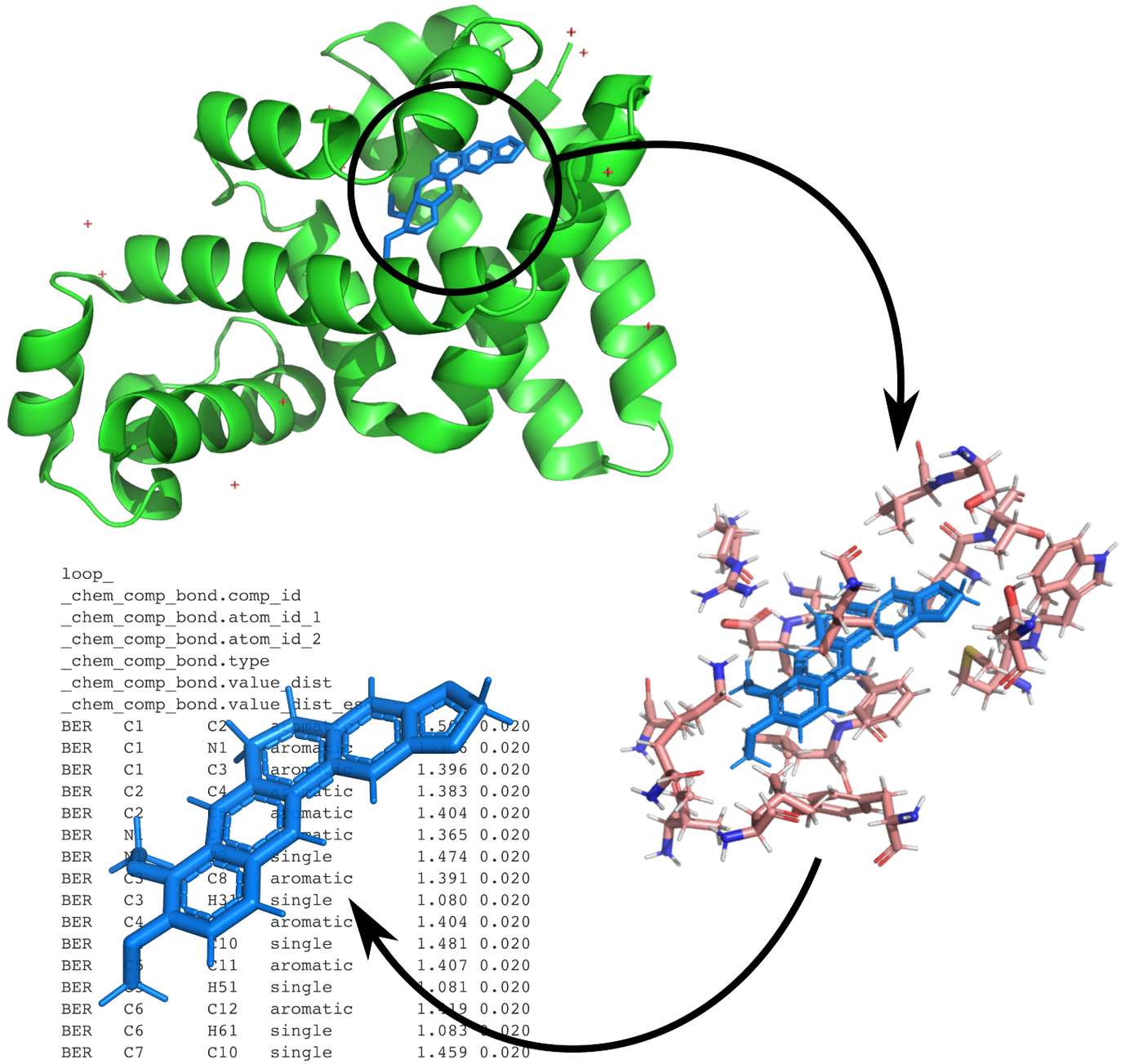
Minimised geometry of two histidine configurations of histidine in chain A and resid 4 of PDB 4rj2.





QM Energies

- Strain energy
  - Relaxation of ligand to local minimum
- Bound energy
  - Includes binding energy



```
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      1.50  0.020
BER   C1      N1      aromatic      1.[illegible]  0.020
BER   C1      C3      aromatic      1.396 0.020
BER   C2      C4      aromatic      1.383 0.020
BER   C2      [illegible]  aromatic      1.404 0.020
BER   N1      [illegible]  aromatic      1.365 0.020
BER   N1      [illegible]  single        1.474 0.020
BER   C3      C8      aromatic      1.391 0.020
BER   C3      H31     single        1.080 0.020
BER   C4      [illegible]  aromatic      1.404 0.020
BER   [illegible]  C10     single        1.481 0.020
BER   [illegible]  C11     aromatic      1.407 0.020
BER   [illegible]  H51     single        1.081 0.020
BER   C6      C12     aromatic      1.[illegible]9 0.020
BER   C6      H61     single        1.083 0.020
BER   C7      C10     single        1.459 0.020
```

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