Dictionary of ligands

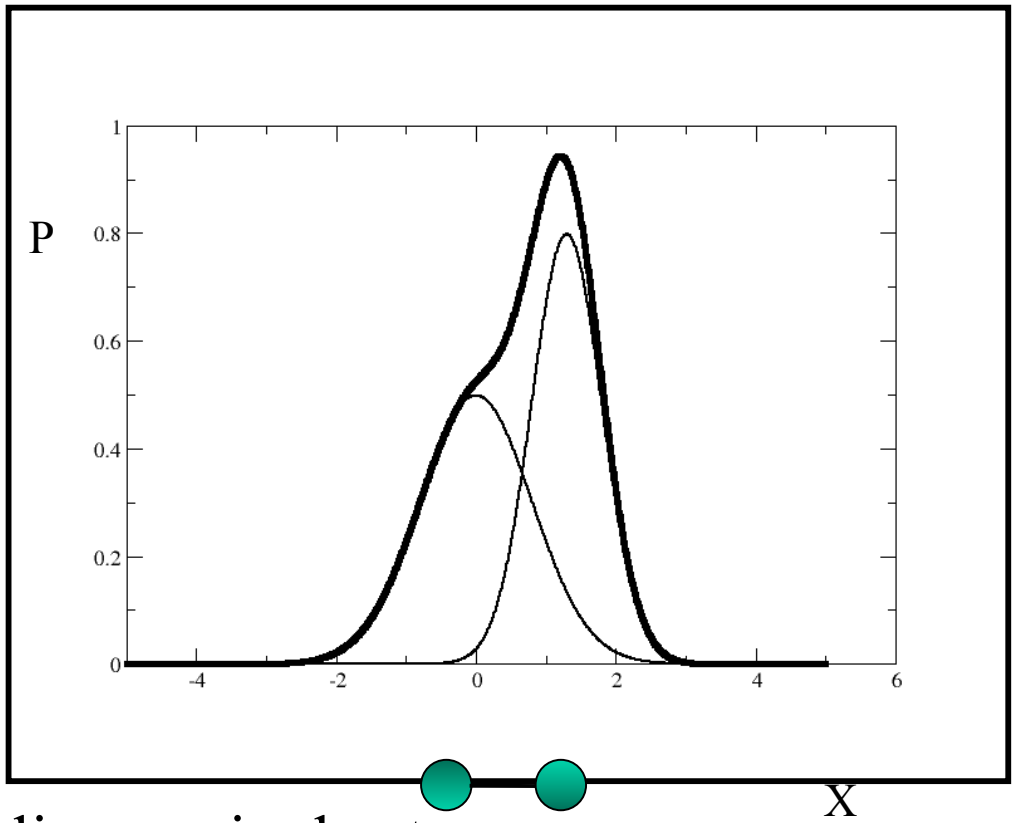
Some of the web and other resources

- Small molecules
- DrugBank: http://www.drugbank.ca/
- ZINC: http://zinc.docking.org/index.shtml
- PRODRUG: http://www.compbio.dundee.ac.uk/Web_Servers/prodrg_down.html
- CACTVS: http://www2.chemie.uni-erlangen.de/software/cactvs/
- Cambridge structural database - CSD: http://www.ccdc.cam.ac.uk/products/csd/

- Macromolecules
- PDB:
  - European EBI: http://www.ebi.ac.uk/msd/
  - USA RSCB: http://www.rcsb.org/pdb/download/download.do
- RASMOL (visualisation tool): http://rasmol.org/
- JMOL (Java based visualisation tool): http://jmol.sourceforge.net/

Why restraints:
Two atoms ideal case

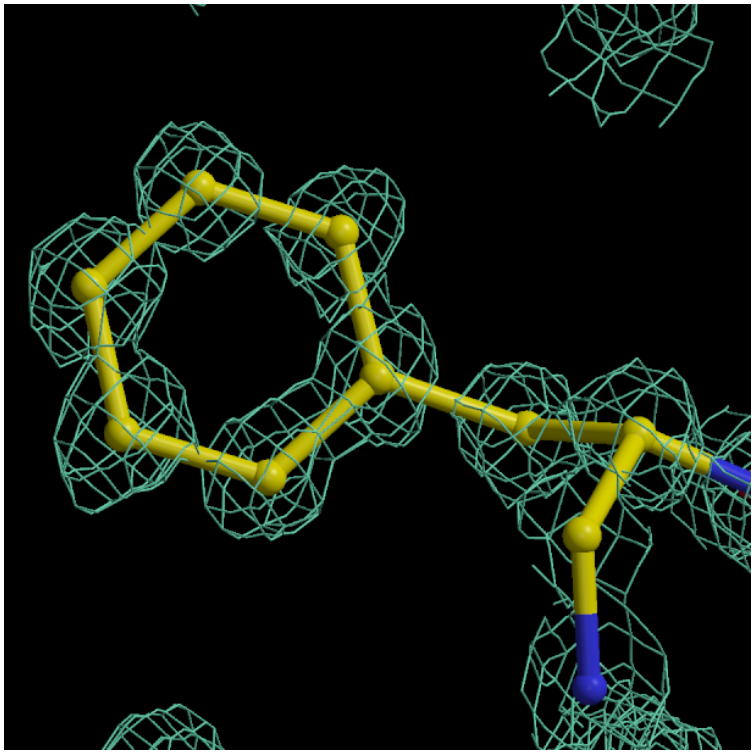
- Distance between atoms 1.3Å. B values 20 and 50



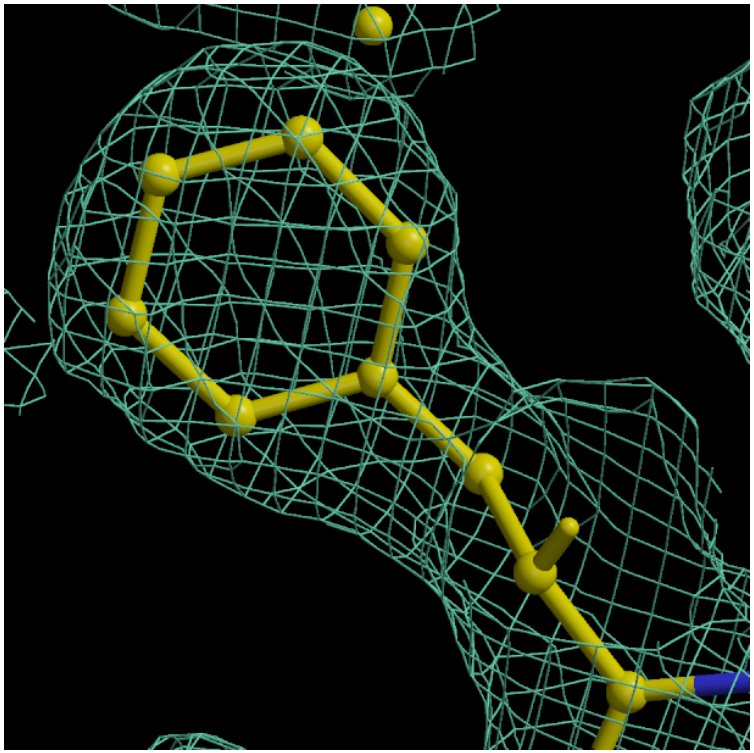
- Thin lines – single atoms
- Bold line - sum of the two atoms

Chemical information: Phe at two different resolutions

- 0.88 Å



2 Å and High mobility



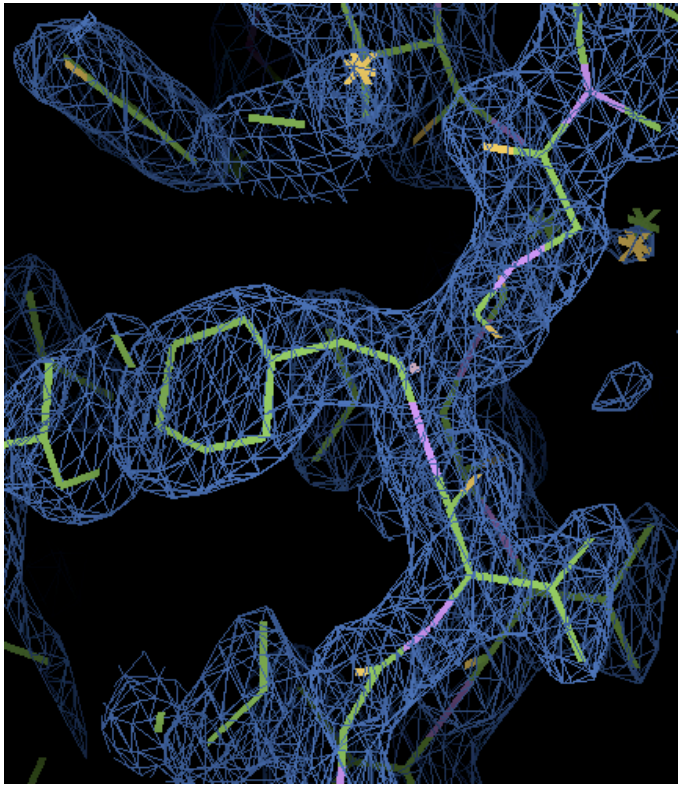
Role of restraints

- When atoms have high B values and/or data are at low resolution then electron density may not show separate peaks
- If restraints would not be used then chemistry of molecule would be unreasonable.
- Role of restraints is that to retain chemistry of atoms and at the same time describe electron density optimally.
- If atoms are close to each other it is unlikely that they will have hugely different B values

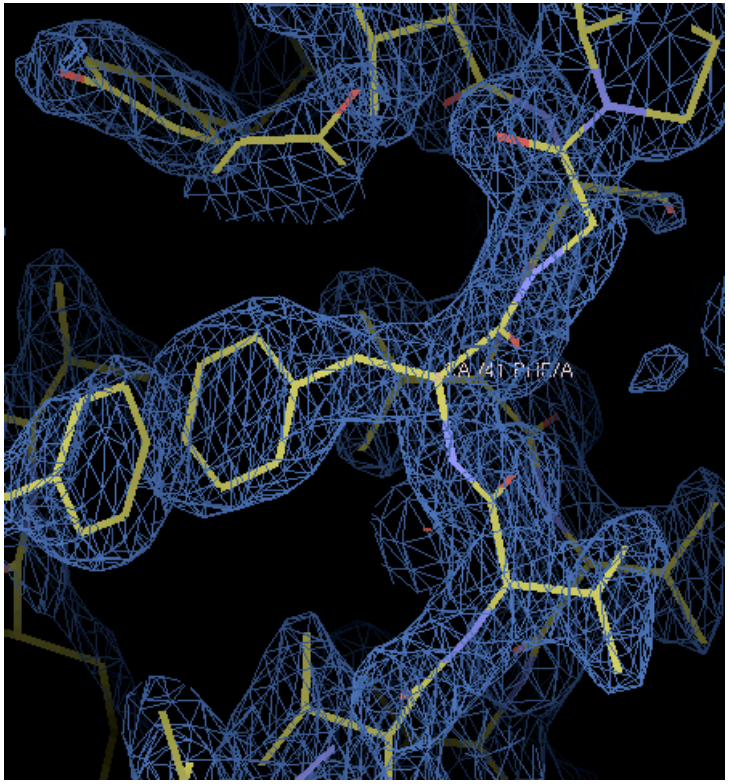
Example

- Data -
  1.9A

Unrestrained

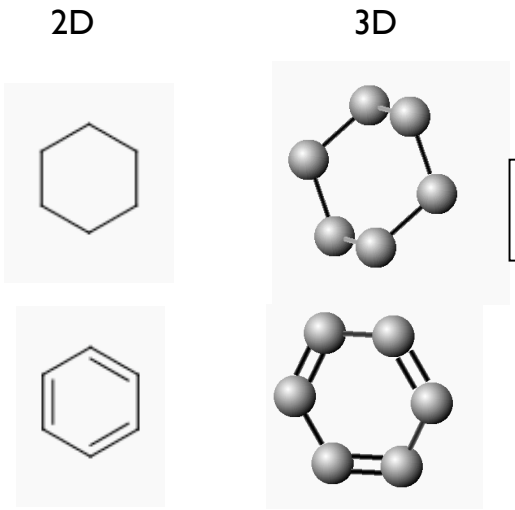


Restrained



Using restraints

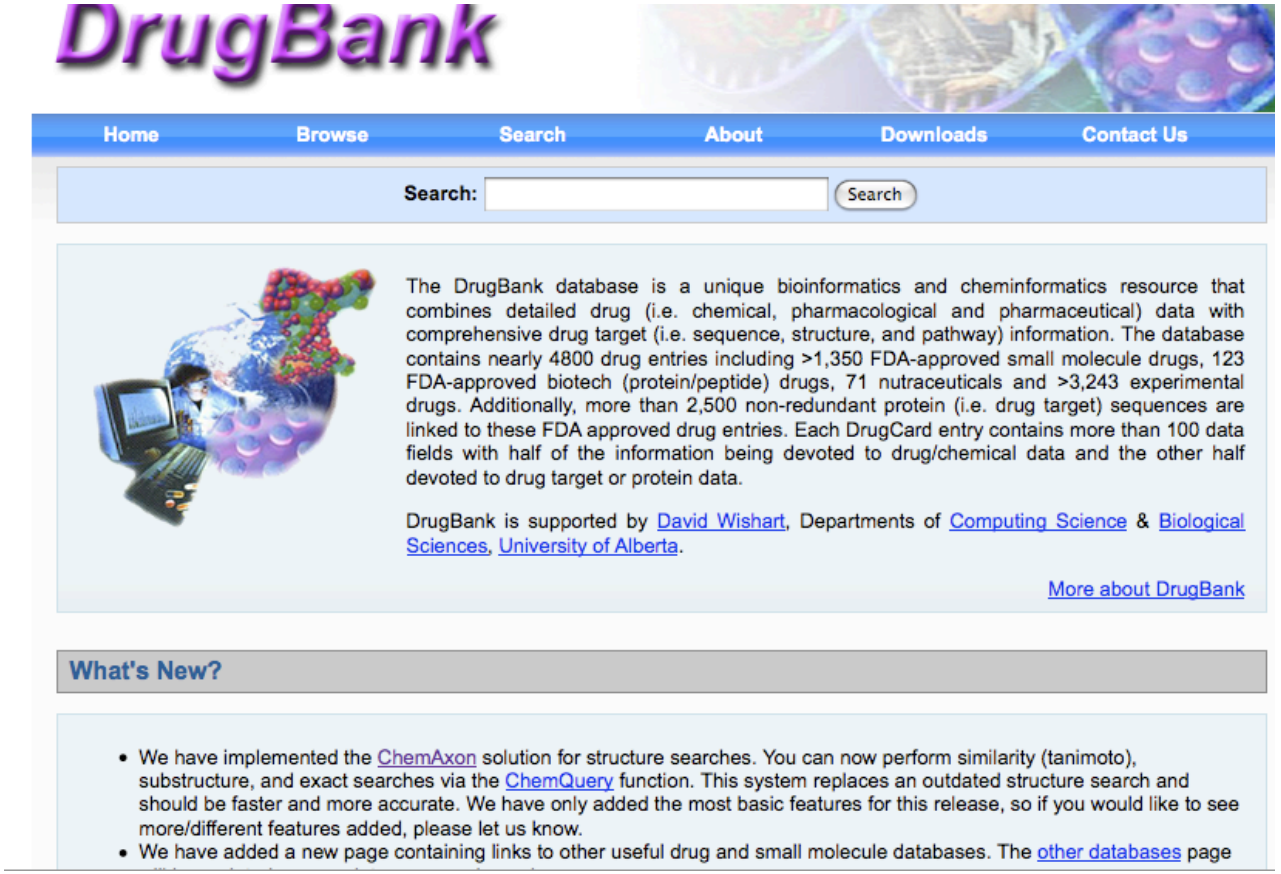
- Standard dictionary has description of around 1 500 small molecules. If one of them is in your crystal then the will be used automatically. In the new version there will be more than 8 000.
- What happens if you have a ligand that is not in the dictionary. Then it is your responsibility to create chemically sensible description.
- Before starting to create a description you need to study bonding structure of your ligand.



These two molecules will refine very differently (oviously)

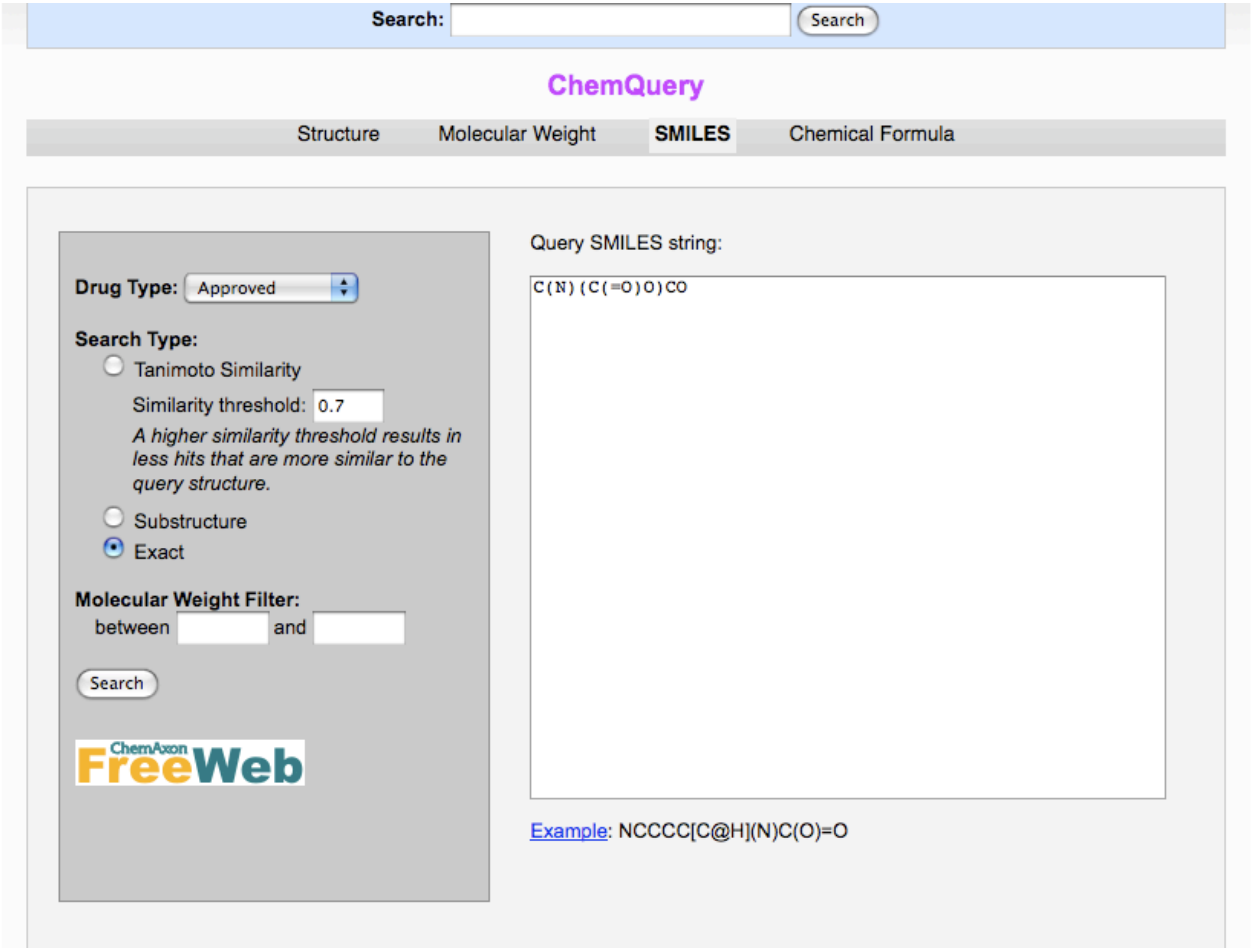
DrugBank

There are various options like “Search”, “Download”



DrugBank

Search can be performed using different tools. One of them is smile string Search can be exact or substructure



SMILES

SMILES notation is the most popular notation and almost all computational chemical websites, programs use this notation. They can read and write SMILES.

It is based on several simple rules. Full description of SMILES can be find from daylight websites.
http://www.daylight.com/dayhtml/doc/theory/theory.smiles.html

SMILES stands for Simplified Molecular Input Line Entry System.

It is concise and widely spread. It is very easy to learn. It was originally designed for manual input using text only editors. SMILES has become as a standard and it is a useful thing to know about.

SMILES

SMILES uses several very simple rules (these rules are sufficient to generate SMILES from structure and structure from SMILES).

Rules:

- Atomic symbols used for atoms
- Hydrogen atoms as a rule are implicit. They are deduced using valence information about atoms
- Neighbouring atoms stand one after another
- Single, double, triple and aromatic bonds are denoted using “-”, “=”, “#” and “:” respectively. Single and aromatic bonds are usually not shown.

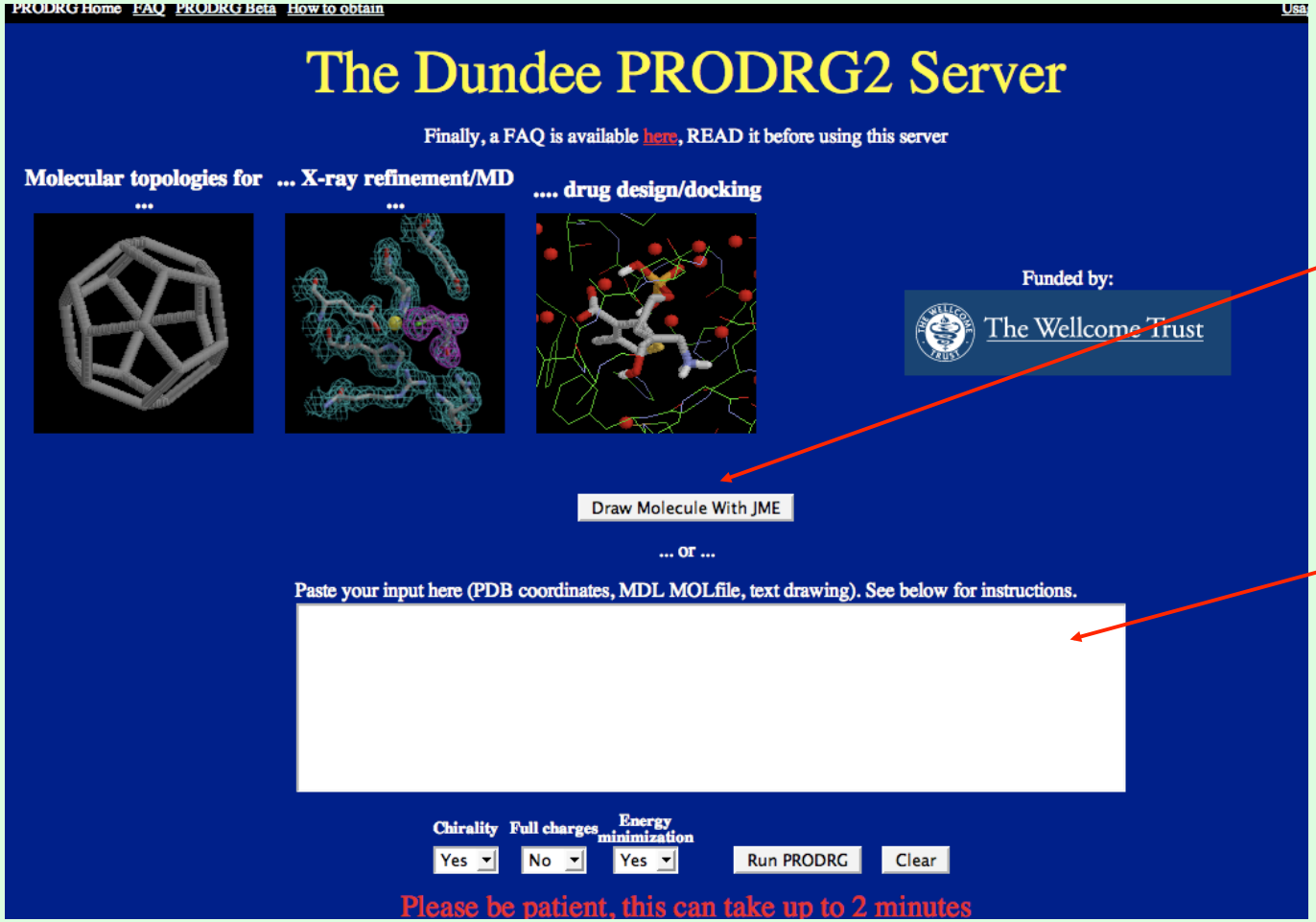
Branches represented by parentheses

Cycles are added by using matching digits on connecting atoms

- Aromatic atoms are denoted using lower cases.

These rules are sufficient to describe most of the cases. Let us consider some examples

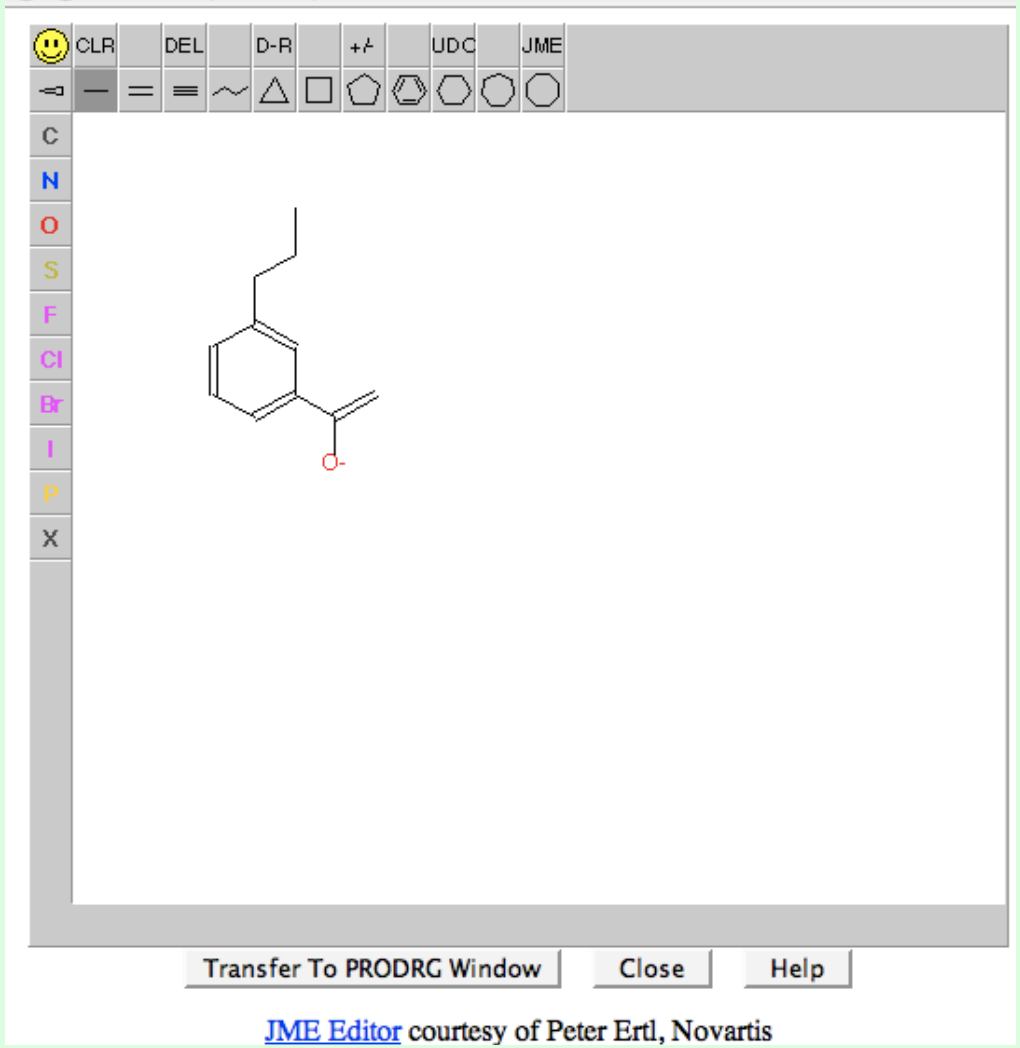
PRODRG server



JME

Load your file

PRODRG: JME



JME is java based program for 2D drawing of small compounds. It is used in PRODRG2, MSDchem etc

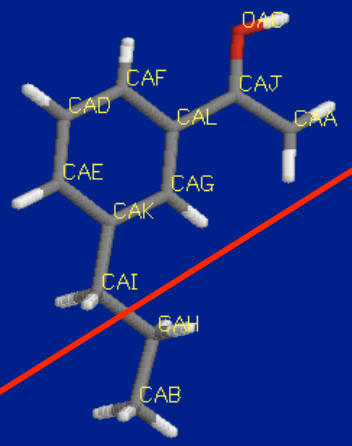
Draw your ligand, transfer to PRODRG window and run

PRODRG output

PRODRG Home FAQ PRODRG Beta How to obtain

```
PRODRG> Starting up PRODRG version 061128.0522
PRODRG> PRODRG written/copyrighted by Daan van Aalten
PRODRG> and Alexander Schuettelkopf
PRODRG>
PRODRG> Questions/comments to dava@davapc1.bioch.dundee.ac.uk
PRODRG>
PRODRG> When using this software in a publication, cite:
PRODRG> A. W. Schuettelkopf and D. M. F. van Aalten (2004).
PRODRG> PRODRG - a tool for high-throughput crystallography
PRODRG> of protein-ligand complexes.
PRODRG> Acta Crystallogr. D60, 1355--1363.
PRODRG>
PRODRG>
PRODRG> MOL mode detected.
PRODRG> No stereo information found in input file.
PRODRG> Molecule complexity index: 2.00.
PRODRG>    1 hydrogen(s) added.
PRODRG>   13 bonds                1 ambiguous
PRODRG>   16 bond angles           3 ambiguous
PRODRG>    9 improper dihedrals    1 ambiguous
PRODRG>    4 dihedrals             0 ambiguous
PRODRG>    2 partial charges       0 ambiguous
PRODRG> Net charge on molecule:    0.000
PRODRG> Using charge groups.
PRODRG> Writing GROMACS topology.
PRODRG> GROMACS topology quality on 0-10 scale:  7.7
GENDRG> Best structure was iteration  841 with   0.70210928
PRODRG> Spawning GROMACS version 3.2.1...
PRODRG> RMSD from GROMOS bond ideality (Angstrom) :    0.017
PRODRG> RMSD from GROMOS angle ideality (degrees) :    2.257
PRODRG> RMSD from GROMOS plane ideality (degrees) :    0.432
PRODRG> Number of improper improper dihedrals     :        0
PRODRG> Writing: SCRHWMMPG
PRODRG> Normal program end
```

Your molecule + added hydrogens



Click to go to the following output:

Coordinates

- PDB (all H's, polar H's only or no H's)
- MDL Molfile (all H's, polar H's only or no H's)
- GROMOS87/GROMACS (polar H's only)

X-ray refinement

- CNS (parameters and topology)
- REFMAC5
- SHELX
- O (pre-9.x torsion entry, pre-9.x refi dictionary and 9.x dictionary)

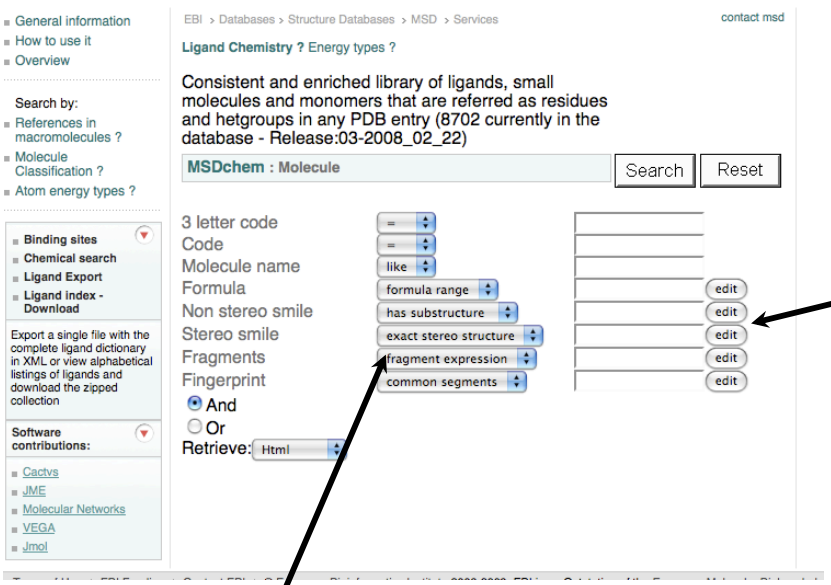
Done

It can write out representation in various formats suitable for various popular software

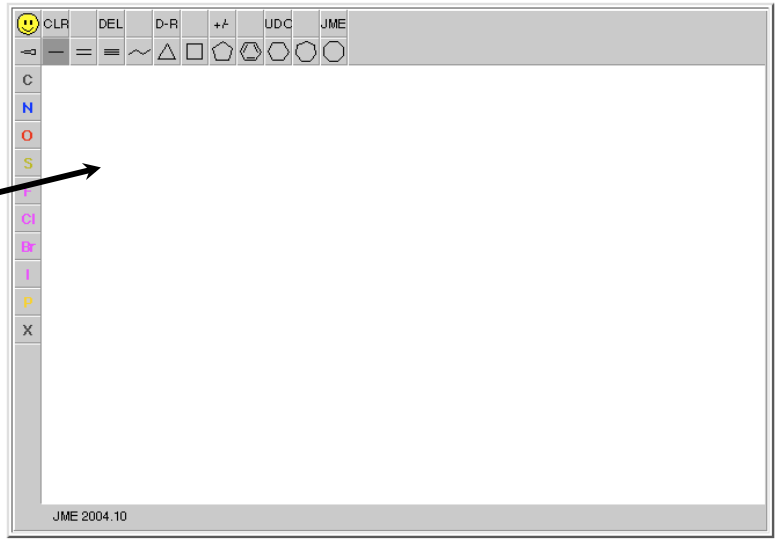
PDB

- PDB is Protein Data Bank. It has all macromolecular structures determined experimentally as well as theoretically. There are more than 56000 macromolecular structures available in the PDB.
- In many cases protein structures are determined with some ligands (small molecular compounds). These small molecular structures are available from PDB. There are 8000-9000 such small molecules in the PDB.
- There are websites that allows people to view macromolecular structures as well as small molecular compounds. These sites are located in USA, Europe and Japan.

PDB in Europe: PDBe at EBI, Cambridge



Substructure, common segment, exact stereo



Fragment expression

Load File Browse... (SDF,MOL,mmCif,PDB e.t.c.)

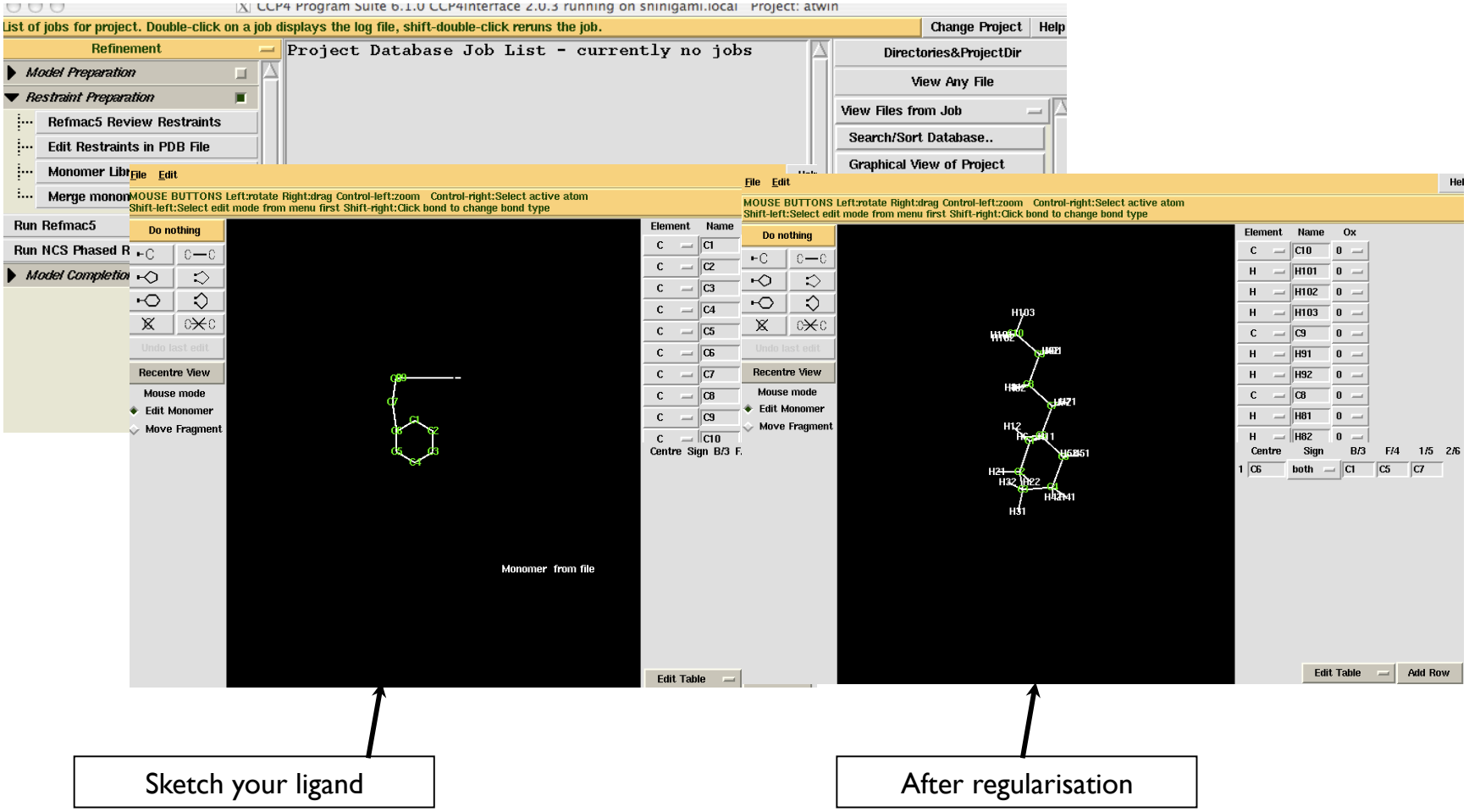
or give Smile String (i.e. c1ccccc1)

or give Code of Existing Molecule (i.e. ATP)

(Press the Load Button to Load the Molecule with that smile or 3 letter code, or file into the editor)

Using resources from ccp4

Sketcher is under Refinement/Restraint Preparation/Monomer library sketcher.

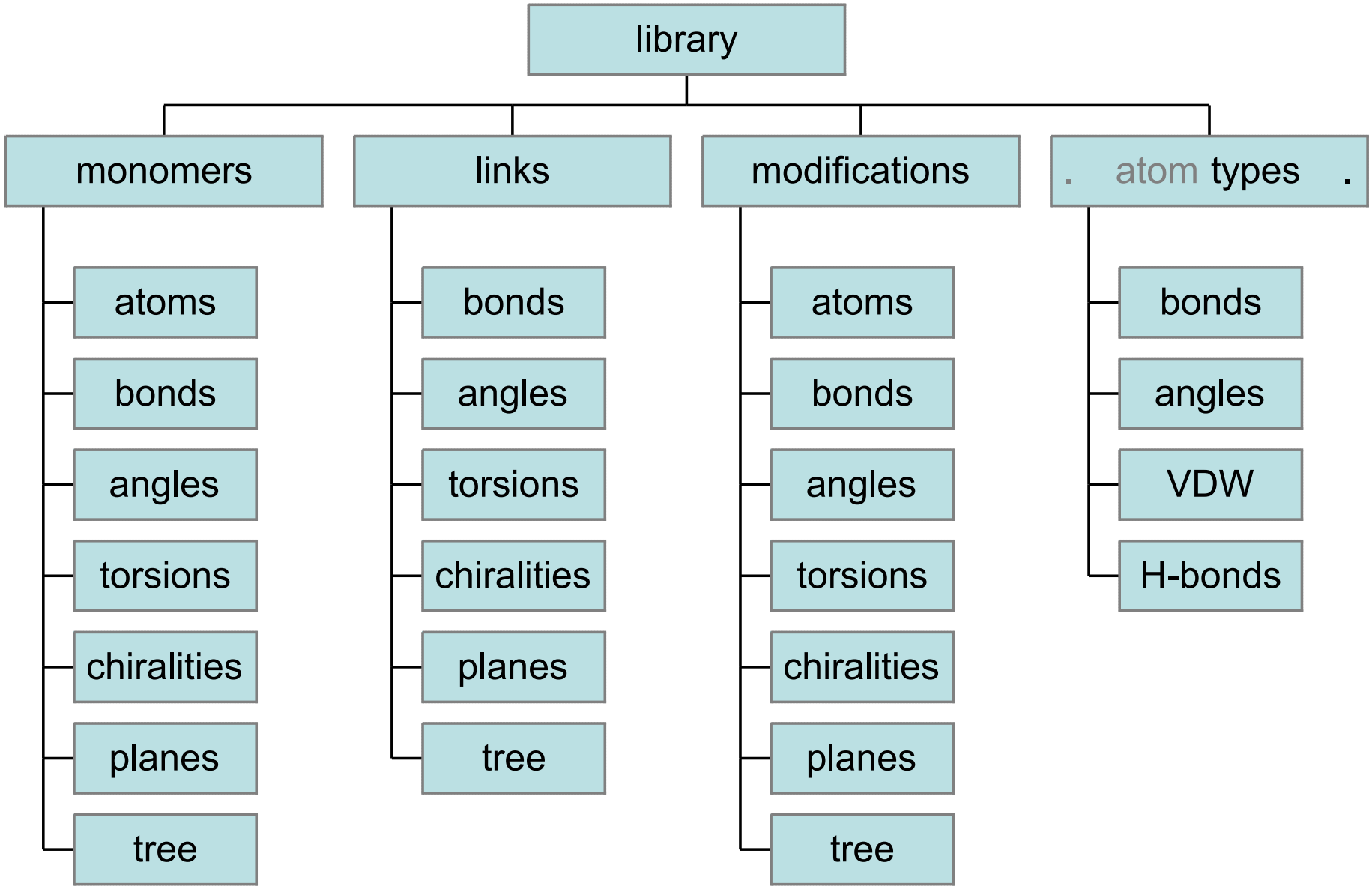


Jligand and Links

CCP4 monomer library: modfications and links

New link description

CCP4 monomer library (library of restraints)



Modifications and links

The idea of this mechanism is that
- while *monomer* records describe individual compounds
- *modifications* and *links* describe changes resulted from chemical reactions

Modification formalism allows to change a monomer

Link formalism allows to join modified monomers together

Generic links for peptides

Generic peptide modification "DEL-OXT":

```
NH3-(C-CH3)-COO   -> NH3-(C-CH3)-CO
```

Generic peptide modification "NH1":

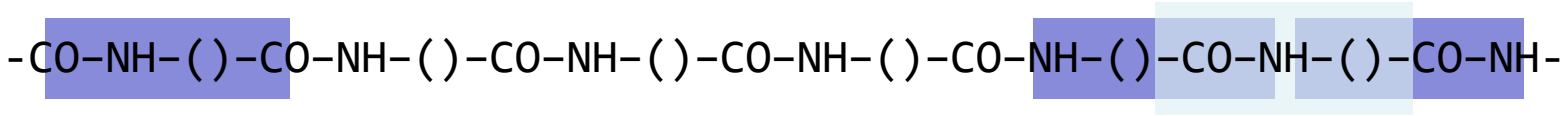
```
NH3-(C-CH3)-COO   -> NH-(C-CH3)-COO
```

Generic peptide link "TRANS":

```
NH3-(C-CH3)-CO   +   NH-(C-CH3)-COO -> NH3-(C-CH3)-CO–NH-(C-CH3)-CO
```

Thiese define: bond length, angles and a plane associated with the bond C-N

Specialised monomers vs. generic links



Specialised monomers:

NH3-()-CO2
-(trans)-NH-()-CO2
-(cis)-NH-()-CO2
NH3-()-CO-(trans)-
NH3-()-CO-(cis)-
-(trans)-NH-()-CO-(trans)-
-(trans)-NH-()-CO-(cis)-
-(cis)-NH-()-CO-(trans)-
-(cis)-NH-()-CO-(cis)-

---

9 versions
×
20 aminoacids
=
180 library entries

Generic links:

NH3-()-CO2

)-CO-(trans)-NH-(
)-CO-(cis)-NH-(
)-CO2
NH2-(

---

20 aminoacids
+
2 links
+
7 modifications
=
29 library entries

Links for peptides

generic

– peptide-peptide:	`TRANS, CIS`

generic from one side

– peptide-PRO:	`PTRANS, NMTRANS, PCIS, NMCIS`
– C-terminal modification:	`FOR_C-C, DFO_N-C, STA_N-C, ...`
– N-terminal modification:	`FOR_C-N, ACE_C-N, DFO_C-N, ...`
– pyranose-(ASP, THR, SER):	`NAG-SER, NAG-THR, NAG-ASN`

specialised

– S-S bridge:	`CYS-CYS`
– pyranose-peptide:	`XYS-SER, XYS-THR, XYS-ASN, ...`
– metal-peptide:	`ZN-CYS, FE-CYS`

Standard modifications and links (generic and specialised)

CCP4 library contains modifications for:

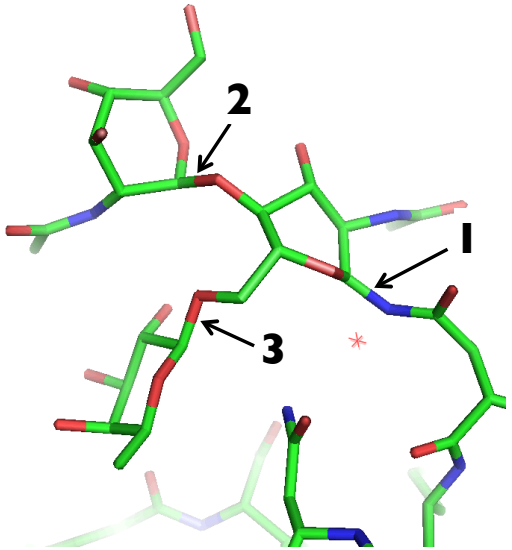
- terminal peptides and nucleotides
- methylated nucleotides
- deprotonated states

CCP4 library contains links and corresponding modifications for:

- polypeptide chains (CIS, TRANS), S-S bridges
- polynucleotide chains
- glycosylated proteins

Generic links for sugars

2xmb



For typical glycosylation cases

- necessary modifications and links are there in the standard ccp4 library

- by default REFMAC uses these library descriptions and does not need any additional instructions

NAG – NAG – ASN
|
FUL

FUL = Beta-L-Fucose
NAG = N-Acetyl-D-Glucosamine

Standard links used here:

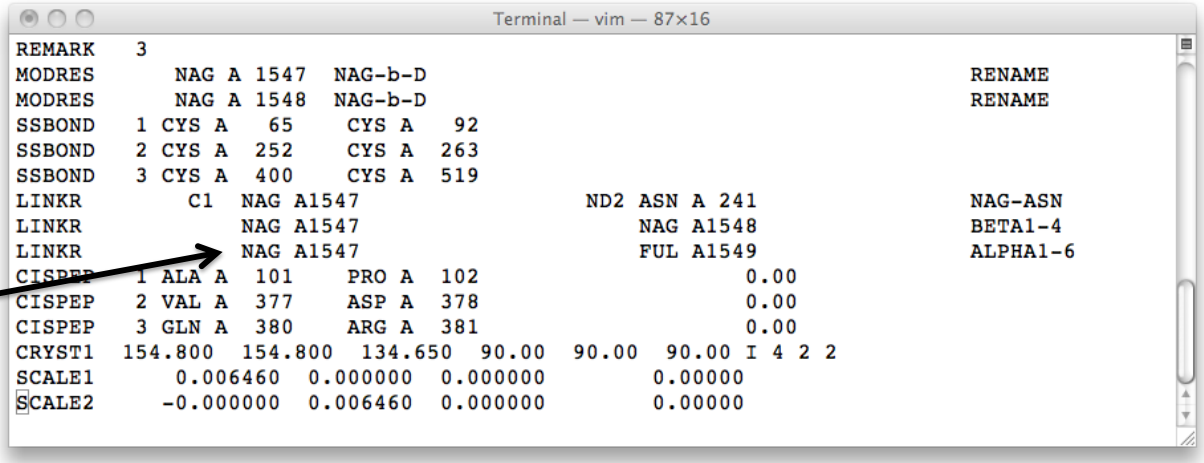
(1) "NAG-ASN"
(2) "BETA1-4"
(3) "ALPHA1-6"

Sugar links: refmac checkpoints

✓ refmac terminated normally

✓ output pdb-fail contains expected LINKR records, e.g.

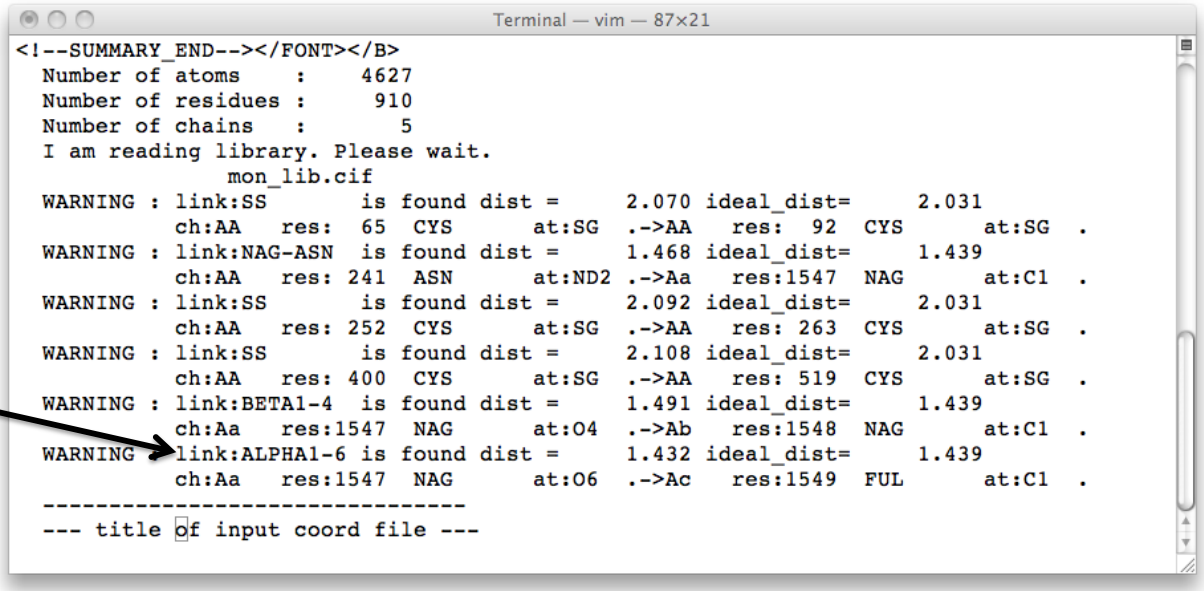
```
LINKR...
...NAG A1547  FUL
   A1549...
...ALPHA1-6
```



✓ log-file contains warnings saying e.g. that

```
... link:ALPHA1-6 is
        found
... res:1547  NAG  ...
... res:1549  FUL  ...
```

(WARNING = OK)



User-defined links

When new link descriptions are needed:

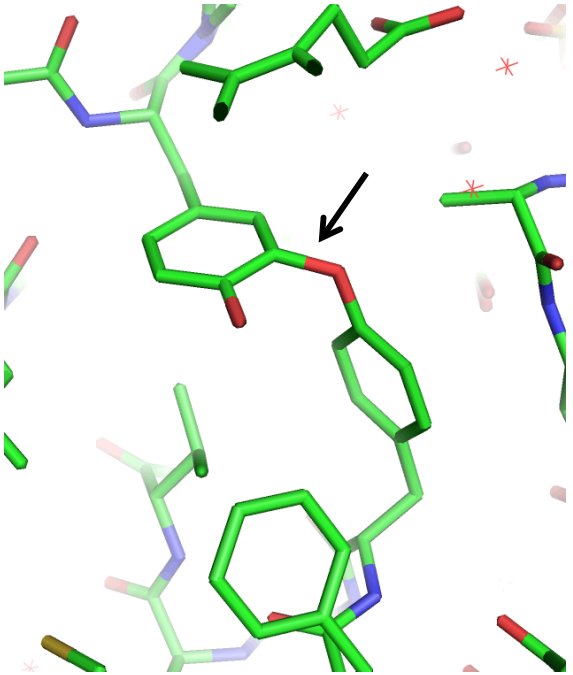
side chain – side chain (e.g. TYR – TYR on the figure)

side chain – main chain (e.g. LYS – Ubiquitin)

side chain – ligand (e.g. LYS – PLP)

JLigand:
– new GUI for LIBCHECK
– descriptions of monomers (functionality of SKETCHER)
– descriptions of links and corresponding modifications

TYR–TYR covalent link in
M. tuberculosis Hemoglobin O
PDB id 1ngk



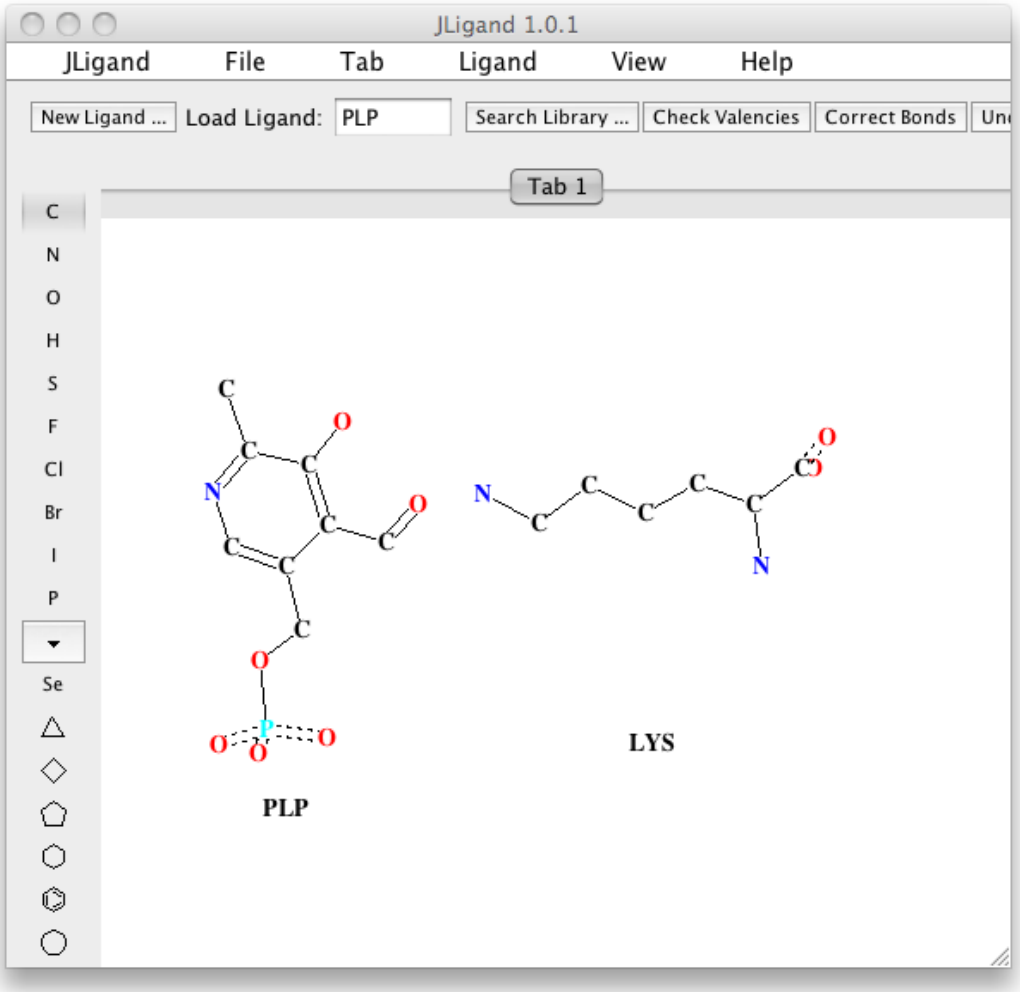
New link description

New link

Example:
– covalent linkage between LYS and Pyridoxal phosphate (PLP).
– describes PLP forming internal aldimine in aminotransferases.

Given:
- descriptions of LYS and PLP
  from the standard library

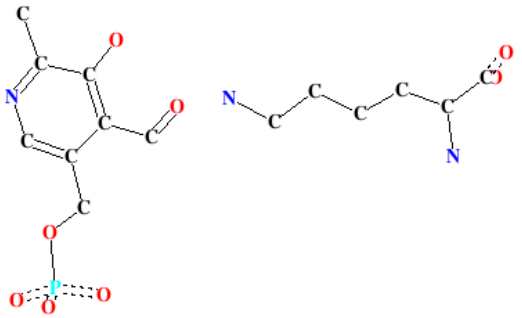
Needed:
- additional library file with
  the description of link LYS–PLP

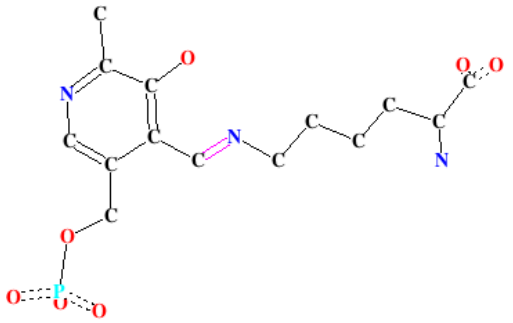


Creating a new link, as seen in JLigand GUI

The two monomers are in effect reacted in silico
Hydrogen atoms are dealt with automatically*)

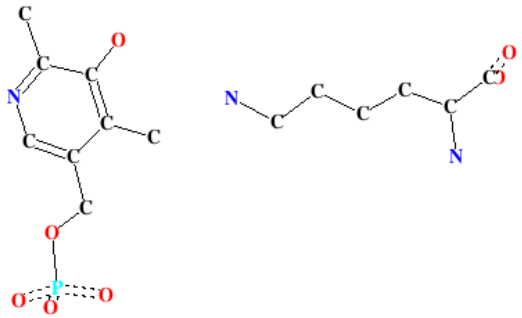
*) it is also possible to visualise H-atoms and deal with them explicitly



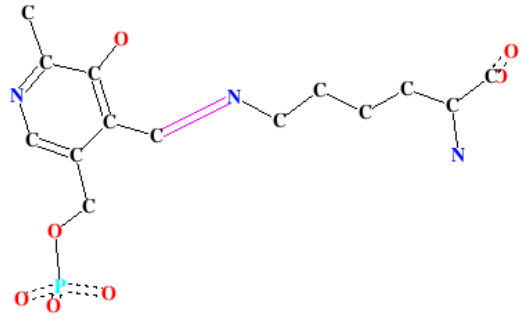


leaving O is removed

the new compound
is regularised

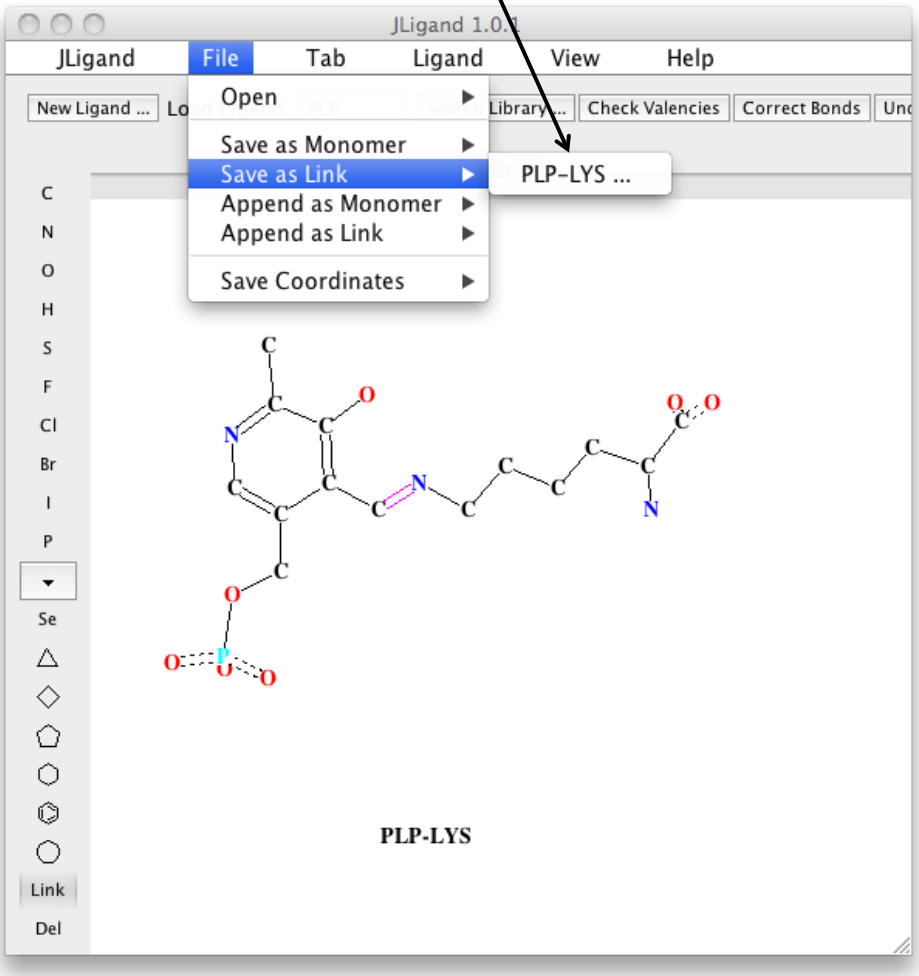


double bond C=N is added



The new link, "file view"

To save into CIF-file (additional library)



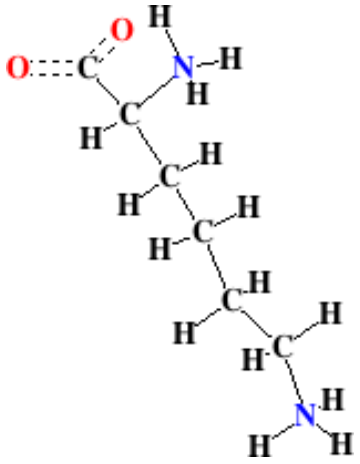
Contents:
(1) modification "PLPmod1"
(2) modification "LYSmod1"
(3) link "PLP-LYS"

No monomers

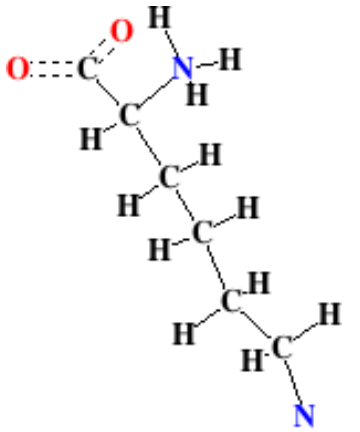
1
2
3

The new link, "file view"

LYS



LYSmod1



Modification "LYSmod1": changes to LYS

```
data_mod_LYSmod1

loop_
_chem_mod_atom.mod_id
_chem_mod_atom.function
_chem_mod_atom.atom_id
_chem_mod_atom.new_atom_id
_chem_mod_atom.new_type_symbol
_chem_mod_atom.new_type_energy
_chem_mod_atom.new_partial_charge
 LYSmod1  change NZ    .     .   N       0.000
 LYSmod1  delete HZ1   .     .   .         .
 LYSmod1  delete HZ2   .     .   .         .
 LYSmod1  delete HZ3   .     .   .         .
```

Atoms

```
loop_
_chem_mod_bond.mod_id
_chem_mod_bond.function
_chem_mod_bond.atom_id_1
_chem_mod_bond.atom_id_2
_chem_mod_bond.new_type
_chem_mod_bond.new_value_dist
_chem_mod_bond.new_value_dist_esd
 LYSmod1  change CE    NZ      .       1.455   0.020
 LYSmod1  delete NZ    HZ3     .         .     .
 LYSmod1  delete NZ    HZ2     .         .     .
 LYSmod1  delete NZ    HZ1     .         .     .
```

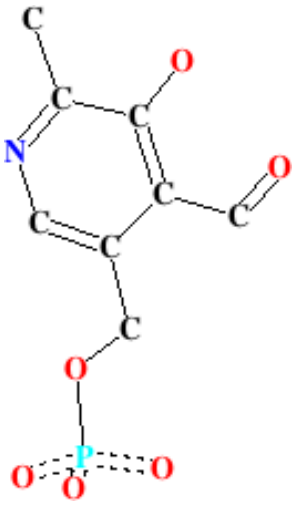
Bonds

Angles

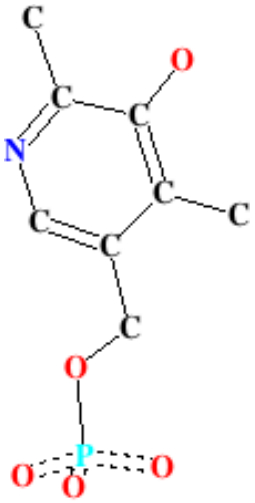
.....

The new link, "file view"

PLP



PLPmod1



Modification "PLPmod1": changes to PLP

```
data_mod_PLPmod1

loop_
_chem_mod_atom.mod_id
_chem_mod_atom.function
_chem_mod_atom.atom_id
_chem_mod_atom.new_atom_id
_chem_mod_atom.new_type_symbol
_chem_mod_atom.new_type_energy
_chem_mod_atom.new_partial_charge
 PLPmod1  delete O4A    .     .     .          .
```

Atom

```
loop_
_chem_mod_bond.mod_id
_chem_mod_bond.function
_chem_mod_bond.atom_id_1
_chem_mod_bond.atom_id_2
_chem_mod_bond.new_type
_chem_mod_bond.new_value_dist
_chem_mod_bond.new_value_dist_esd
 PLPmod1  delete C4A    O4A      .          .      .
```

Bond

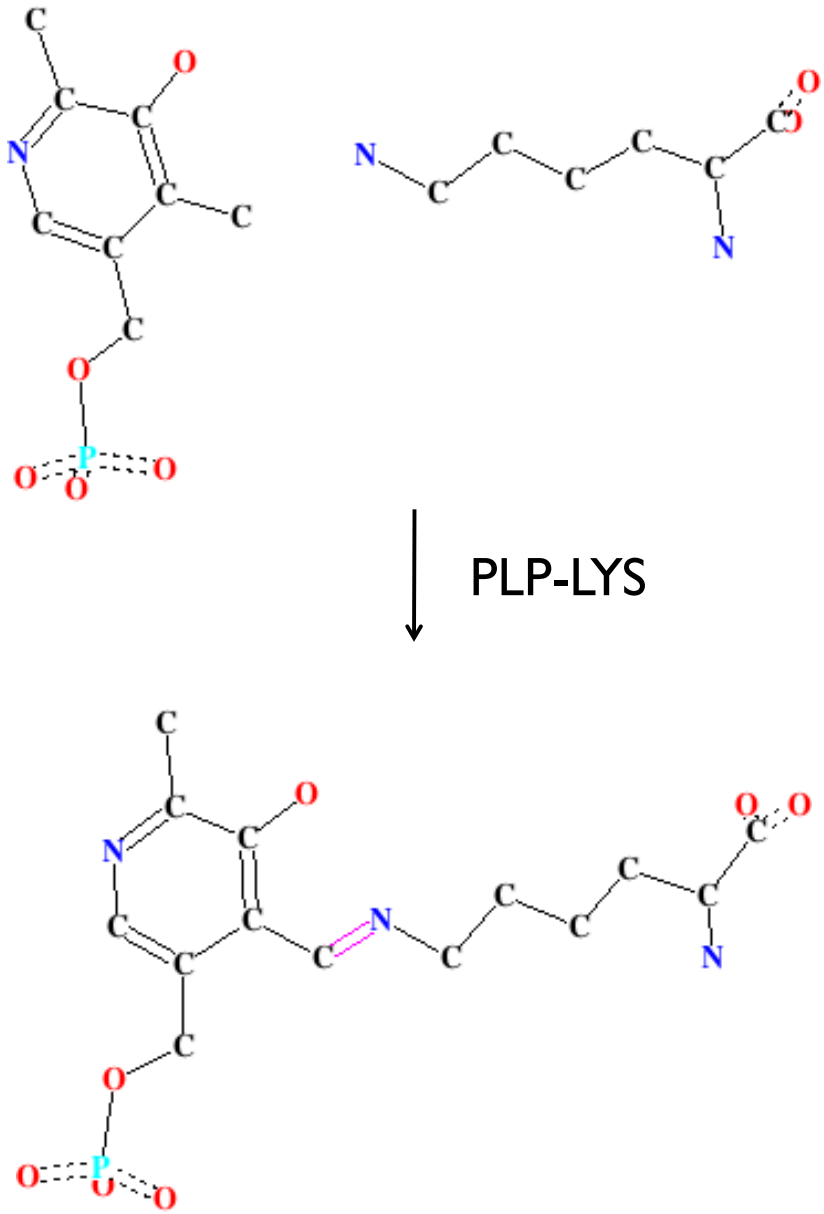
```
loop_
_chem_mod_angle.mod_id
_chem_mod_angle.function
_chem_mod_angle.atom_id_1
_chem_mod_angle.atom_id_2
_chem_mod_angle.atom_id_3
_chem_mod_angle.new_value_angle
_chem_mod_angle.new_value_angle_esd
 PLPmod1  delete H4A    C4A    O4A         .      .
 PLPmod1  delete C4     C4A    O4A         .      .
```

Angles

```
loop_
_chem_mod_plane_atom.mod_id
_chem_mod_plane_atom.function
_chem_mod_plane_atom.plane_id
_chem_mod_plane_atom.atom_id
_chem_mod_plane_atom.new_dist_esd
 PLPmod1  delete plan-2    C4          .
 PLPmod1  delete plan-2    C4A         .
 PLPmod1  delete plan-2    H4A         .
 PLPmod1  delete plan-2    O4A         .
```

Plane

The new link, "file view"



Link "PLP-LYS": changes associated with covalent linkage between modified PLP and LYS

```
data_link_PLP-LYS

loop_
_chem_link_bond.link_id
_chem_link_bond.atom_1_comp_id
_chem_link_bond.atom_id_1
_chem_link_bond.atom_2_comp_id
_chem_link_bond.atom_id_2
_chem_link_bond.type
_chem_link_bond.value_dist
_chem_link_bond.value_dist_esd
 PLP-LYS  1 C4A    2 NZ       double      1.260    0.020
```

Bond

```
loop_
_chem_link_angle.link_id
_chem_link_angle.atom_1_comp_id
_chem_link_angle.atom_id_1
_chem_link_angle.atom_2_comp_id
_chem_link_angle.atom_id_2
_chem_link_angle.atom_3_comp_id
_chem_link_angle.atom_id_3
_chem_link_angle.value_angle
_chem_link_angle.value_angle_esd
 PLP-LYS  1 C4A    2 NZ    2 CE     120.000    3.000
 PLP-LYS  1 H4A    1 C4A   2 NZ      120.000    3.000
 PLP-LYS  1 C4     1 C4A   2 NZ     120.000    3.000
```

Angles

```
loop_
_chem_link_plane.link_id
_chem_link_plane.plane_id
_chem_link_plane.atom_comp_id
_chem_link_plane.atom_id
_chem_link_plane.dist_esd
 PLP-LYS  plan-2    1 C4       0.020
 PLP-LYS  plan-2    1 C4A      0.020
 PLP-LYS  plan-2    1 H4A      0.020
 PLP-LYS  plan-2    2 CE       0.020
 PLP-LYS  plan-2    2 NZ       0.020
```

Plane

Utilising new link description

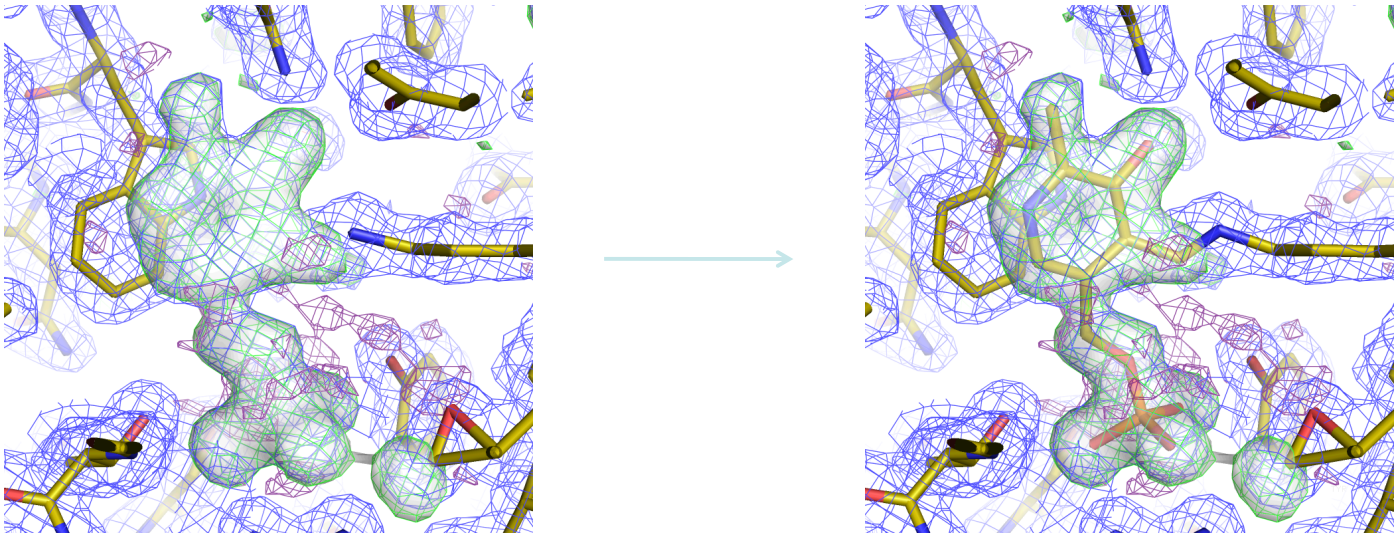
Three remaining steps:

- docking monomer(s) into electron density
- defining link in the pdb-file
- refinement of the structure with linked ligand using additional library

(1) Docking into the electron density

In our example, this is completely independent step: the additional library is not used.

– non-modified monomer is taken from the standard library
– docking is performed, e.g. using coot:

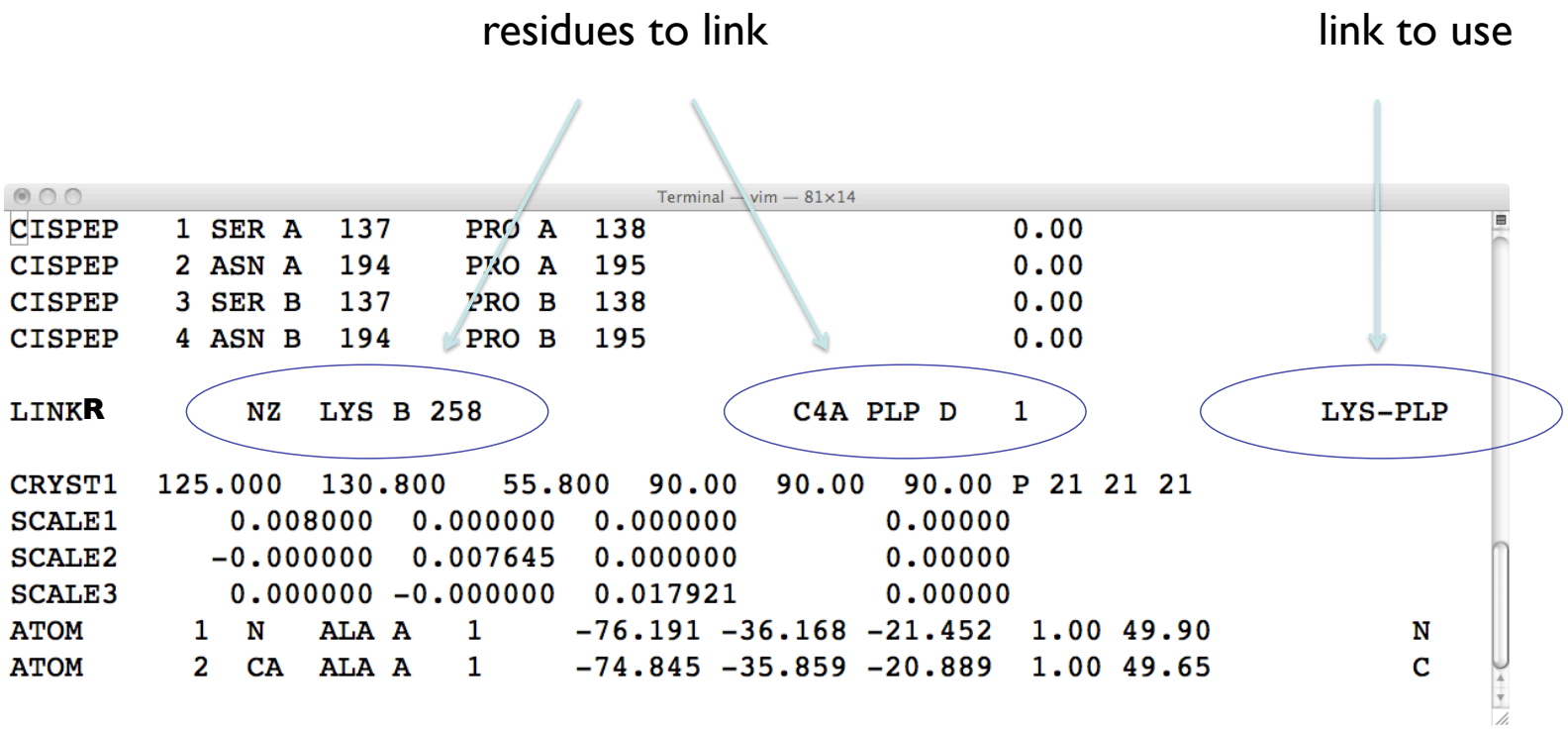


– leaving atoms (O4A of PLP in this example) are removed
– in our example, one of the monomers (LYS) is already in the model

(2) Defining link in the pdb-file

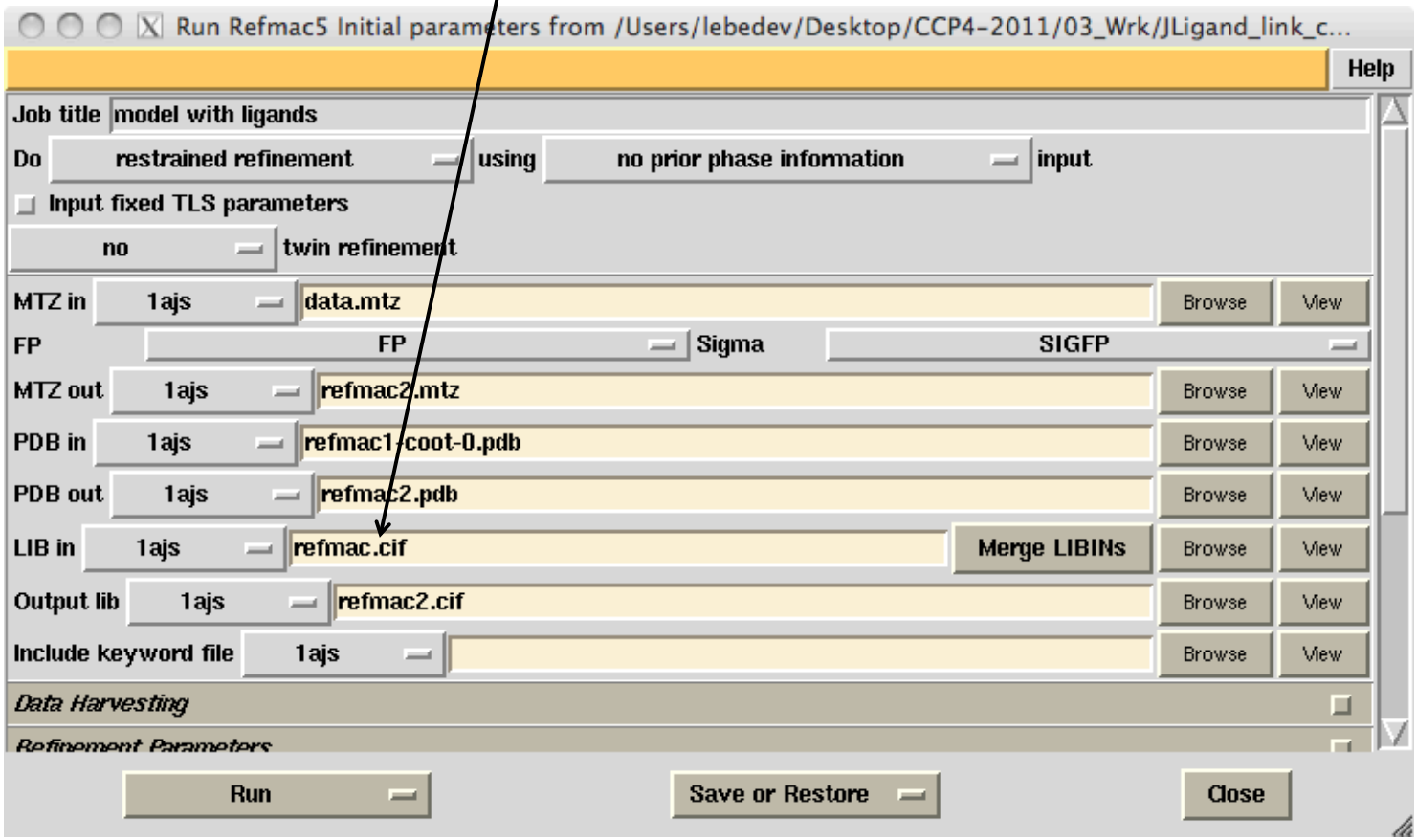
In general case, link cannot be applied automatically.
For example:
– e.g. the same two atoms of the same two compounds can form single or double bond
– H-atom are not defined in the PDB-file

Therefore REFMAC needs additional instructions:



(3) Refinement using additional library

Additional library is defined here



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Jligand is available from CCP4 or from York's ftp site:

www.ysbl.york.ac.uk/mxstat/JLigand

or google jligand

This and other presentations can be found on:

www.ysbl.york.ac.uk/refmac/Presentations/