AFITT

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CONTENTS

CHAPTER

ONE

OVERVIEW

AFITT is a crystallographic tool for correctly placing small molecules in real-space density. **AFITT** combines our flagship Shape technology with generic forcefields into a new forcefield that fits small molecules into crystallographic density while maintaining excellent chemistry.

AFITT also provides an interface to external refinement programs and tools to validate the refinement including interactive Ramachandran plots and real-space correlation coefficient calculation (RSCC).

CHAPTER TWO

APPLICATIONS

The **AFITT** distribution contains a single application:

- *FLYNN* for fitting ligands into real-space density.

CHAPTER THREE

UTILITY PROGRAMS

The following utility programs are also included in this distribution:

- *AlignGrid*: Utility to align proteins with electron-density maps on a single reference
- *RotFit*: Utility to carry out fast automated refitting of side-chains and peptide flips.
- *Rscc*: Utility to perform real-space correlation coefficient and real-space R-factor calculations.
- *WriteDict*: Refinement dictionary generator that properly handles covalent bond restraints between the ligand and the protein

CHAPTER

FOUR

FLYNN

4.1 FLYNN

4.1.1 Overview

Fitting small molecules to electron density is a challenging problem. Small molecules with few rotatable bonds are amenable to hand placement in density. Add a few rotatable bonds, however, and the problem becomes factorially more complicated. Furthermore, unlike protein modeling, many small molecules are relatively unique entities whose conformation cannot be predicted from previous homologies.

FLYNN uses a high-quality conformational analysis and a fast rigid overlay to position ligands in density in conjunction with a combined forcefield to further position these conformations while, at the same time, minimizing ligand strain. FLYNN can automatically locate ligand density and automatically fit fragment cocktails, or, failing that, **FLYNN** can use a supplied bounding box in which to place the ligands of interest.

Ligands are fit to density, however the placements are scored and ranked based on the real space correlation coefficient (RSCC) and optionally protein based docking scores: Piecewise-Linear-Potential (PLP) and Chemscore.

FLYNN reads common map formats such as CCP4, CNX/CNS (XPLOR) and MTZ as well as OpenEye's grid formats and a plethora of small molecule, protein and connection table formats.

4.1.2 Example Commands

The example commands in this section can be run with files found in the `data/1nhu` directory under the top-level installation directory.

Basic Usage

```
> flynn -in 1nhu.ism -map 1nhu_prot.mtz -prot 1nhu_prot.pdb \
          -out 1nhu-poses.pdb
```

Use the ligand specified by **1nhu.ism** and fit it against the density in the mtz file **1nhu_prot.mtz**. Since this is an mtz file and **-autoMTZ** is defaulting to true, the Fdelwt map will automatically be generated and used as the density target. The protein atoms specified in 1nhu_prot.pdb will be used to mask the density before the map is searched for appropriately sized ligand volumes.

Generating Reports

```
> flynn -in 1nhu.ism -map 1nhu_prot.mtz -prot 1nhu_prot.pdb \
          -out 1nhu-poses.pdb -precheck -reporthtml 1nhu.html
```

Performs a preliminary check of the data, ensuring that the ligand can be processed and that the mtz file can be opened. An html file is generated **1nhu.html** with a corresponding image file **1nhu.html.gif**. The html file can be opened with a web browser to view the ligand being fit and any corresponding issues.

The output will be written to 1nhu-poses.pdb in pdb format.

Choosing box regions of density

```
> flynn -in 1nhu.ism -map 1nhu_sigma.mtz -prot 1nhu_prot.ent \
           -out 1nhu_poses.pdb -box 1nhu.pdb -boxpad 2.0
```

This is the same as above except that the density is pruned down to the bounding box specified by **1nhu.pdb** padded by 2.0 Ångströms. Furthermore, if a suitable blob is not found, then the pruned density will be used as is. This is quite effective for regions of density with a high degrees of disorder. It is also useful to select this option when using the supplied interface to **coot**.

Choosing density close to residues

Use the *-residues* and *-distance* flags to indicate residues close near the desired density. Only blobs that are found within *-distance* to the selected residues will be selected. Residues are specified by <residuenumber><chain> separated by commas.

```
> flynn -in 1nhu.ism -map 1nhu_prot.mtz -prot 1nhu_prot.pdb \
           -out 1nhu-poses.pdb -residues 528B,477B
```

Choosing MTZ columns

```
> flynn -in 1nhu.ism -map 1nhu_sigma.mtz -prot 1nhu_prot.ent \
           -out 1nhu_poses.pdb -box 1nhu.pdb -boxpad 2.0 \
           -boxThenBlobs -autoMTZ false -Fobs Frefined1 -Fc FC \
           -Phic PHIC -mtype 2Fo-Fc -autoMTZ false
```

This example shows how to choose a non-standard column from the MTZ file. the map is generated using **Frefined1** as the observed amplitude column, **FC** as the calculated amplitude column and **PHIC** as the calculated phase column. Furthermore, the regular map **2Fo-Fc** is used. Note that **-autoMTZ** must be set to false for these settings to take effect.

Fragment usage

The **-fragment** flag may be included with any of the above commands. There are three main differences when running in fragment mode:

1. Multiple ligands are fit simultaneously.
2. If the blobs detected for difference ligands overlap, the ligands are assigned to the same blob. (This is for reporting purposes)
3. Ligands are sorted according to the Real Space Correlated Coefficient.

```
> flynn -fragment -in 1nhu.ism -map 1nhu_prot.mtz -prot 1nhu_prot.pdb \
           -out 1nhu-poses.sdf
```

When in fragment mode, it is better to output to a format that supports SD style data (.sdf, .oeb). This makes it much easier to analyze the results in a molecular viewer that supports a sortable spreadsheet. (**VIDA**, **MOE** and so on)

To sort the results using another scoring function, use the **-sortBy** flag.

```
> flynn -sortBy chemscore -in 1nhu.smi ...
```

4.1.3 Command Line Help

A description of the command line interface can be obtained by executing FLYNN with the `--help` option.

```
> flynn --help
```

will generate the following output:

```
Help functions:
  flynn --help simple      : Get a list of simple parameters
  flynn --help all         : Get a complete list of parameters
  flynn --help <parameter> : Get detailed help on a parameter
  flynn --help html        : Create an html help file for this program
```

4.1.4 Required Parameters

-in `<filename>`

File containing a molecule to be fit to density.

File type	Extension
SMILES	.smi .ism .smi.gz .ism.gz
OEBinary	.oeb .oeb.gz
SDF	.sdf .mol .sdf.gz .mol.gz
MOL2	.mol2 .mol2.gz
PDB	.pdb .ent .pdb.gz .ent.gz
MacroModel	.mmod .mmod.gz

-out `<filename>`

File containing resulting conformations exported in the following formats:

File type	Extension
OEBinary	.oeb .oeb.gz
SDF	.sdf .mol .sdf.gz .mol.gz
MOL2	.mol2 .mol2.gz
PDB	.pdb .ent .pdb.gz .ent.gz
MacroModel	.mmod .mmod.gz

-map `<filename>`

Input density grid used to fit ligand. Note that **mtz** files have additional settings, see *MTZ File Options*

Grid File type	Extension
MTZ	.mtz
OpenEye	.grd
Grasp	.phi
CCP4	.map .ccp4
XPLOR	.xplor xplmap
ASCII Grid	.agd

4.1.5 Optional Parameters

Input Options

-prot <filename>

Optional protein used to mask density. This model is used to mask away density where the ligand should not be placed. While the protein is not required, it is highly recommended.

File type	Extension
OEBinary	.oeb .oeb.gz
SDF	.sdf .mol .sdf.gz .mol.gz
MOL2	.mol2 .mol2.gz
PDB	.pdb .ent .pdb.gz .ent.gz
MacroModel	.mmod .mmod.gz

-fragment

Fragments are fit taking the input fragment cocktail and, one at a time, fitting each fragment against each region of detected density. Once a fragment has been placed, it is further analyzed to ensure that all possible orientations of the fragment have been sampled. In poor density, several orientations may fit equally well. To break ties, FLYNN scores each pose with the following scores:

o RSCC (real space correlation coefficient) This is a measure of fit to electron density. [AFITT-CL Jones-1991]_

o PLP Piecewise-linear potential. [AFITT-CL Verkhivker-2000]_

o Chemscore [AFITT-CL Eldridge-1997]_

The docking scores are not used to fit the molecule, they are only used to rank the output. Unless highly symmetric molecules are being input, the real space correlation coefficient (RSCC) is the preferred method of ranking results to density.

All scores are annotated to the output ligands using SD data when appropriate (.SD and .OEB output) or using the PDB remark field:

```
Example PDB output

REMARK OpenEye Flynn MMFF/Shape v 2.1.0
REMARK Stereo Variant:      1
REMARK Blob:                1
REMARK Conformer:           1
REMARK Tanimoto Shape:        0.2004
REMARK Tanimoto MMFF/Shape:   0.2034
REMARK Local Strain:          4.9316
REMARK RSCC: 0.529438
REMARK PLP: -51.395416
REMARK Chemscore: -16.288113

Example SDF output

> <Tanimoto MMFF/SHAPE>
 0.2034

> <Tanimoto Shape>
 0.2004

> <Overlap>
364.767659122018
```

```
> <Fit Overlap>
781.301114117834

> <Local Strain>
 4.9316

> <Lambda>
1.600000023842

> <Ref Overlap>
1371.829467773438

> <Stereo>
1

> <Blob>
1

> <Conformer>
1

> <RSCC>
0.529438

> <PLP>
-51.395416

> <Chemscore>
-16.288113
```

To use fragment mode, please add the "-fragment" option to the command line. In future versions of FLYNN, this will most likely become the default setting.

The output of the fragment fitting process is a file for each density region that includes the fragments fit to the region sorted from best-fit to worst fit. For example:

```
> flynn -in fragments.smi -out 2IKO_cocktail.sdf ...
```

would result in the files:

```
2IKO_cocktail_blob001.sdf
2IKO_cocktail_blob002.sdf
2IKO_cocktail_blob003.sdf
```

one for each blob found. The first molecule in the file is the best fit to the density.

To sort using another measure, for instance, PLP, use the sort flag:

```
> flynn -sortBy plp -in fragments.smi -out 2IKO_cocktail.sdf ...
```

When oeb format is used, the blob is also stored in the OEB file. When using VIDA, simply expand the molecule to investigate the blobs density.

-param `<parameter filename>`

A parameter file is a text file that lists parameter settings to be used during a run. If a parameter is specified both on the command line and in the parameter file, the value specified on the command line is used.

The format of the parameter file is as follows:

- One parameter per line

- For non-list parameters one key-value pair per line.
- For list parameters a key followed by all the values.
- Boolean parameters must be listed as a key followed by true or false.
- The parameter file may not contain the `-param` parameter.
- Lines beginning with # are considered comments

-prefix <file prefix>

Define the default prefix to use for loggging and report file generation.

This -prefix is overridden when using the *-reporthtml* or *-reportfile* command line options.

[default=flynn]

-manualSearch <filename>

Use the input molecule to generate a box volume. All density inside this box will be used to place the ligand.

-box <filename>

Alias for `-manualSearch`

-boxpad <number>

Pad the box created with the *-manualSearch* flag by an amount in Ångströms.

[default=0.0]

-autoThenManualSearch

Automatically search for blobs first, if now suitable blobs are found, use the supplied box to bound density.

[default=false]

-blobsThenBox

See `-autoThenManualSearch`

-densityAsIs

Use the supplied density as it is; do not try to find suitable blobs inside the density. Note that the supplied density has to be pretty close to the actual ligand density for this to work

[default=false]

-distance <value>

Reject blobs whose average distance to the protein is greater than this value.

[default=4.0]

-ligandAsIs

Do not generate conformations for the ligand; use only the supplied conformations.

-mmff94s

Use the MMFF94s variant of the MMFF94 forcefield (this uses planar aniline nitrogens).

[default=false]

-reportfile <filename>

File location for writing the report file. This is a comma separated file containing specifics about the results.

```
Smiles,Blob,Stereo Variant,Conformer,Tanimoto Shape,Tanimoto MMFF/SHAPE,Local Strain
CC(=O)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CCC4=CC(=O)CC[C@]34C)C,1,,,0.4110,0.4296,1.342
CC(=O)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CCC4=CC(=O)CC[C@]34C)C,1,,,0.313,0.3704,4.553
CC(=O)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CCC4=CC(=O)CC[C@]34C)C,1,,,0.3265,0.3628,5.223
```

-resname `<name>`
Set the output residue name to **<name>**. **<name>** must be less than or equal to three characters in length. This forces the output residue to be named <name> even if the residues structure does not match a known deposited residue.

-chainid `<chainid>`
Forces the ligand to be placed in the chain <chainid>

-sortAllChiral `<true/false>`
If set to true, when multiple chiralities are enumerated, sort all chiralities from best to worst per blob, otherwise sort chiral structures independently. For example, if two blobs are found, then the output will have all chiral structures sorted from best fit to worst fit for the first blob, and then all chiral structures sorted from best fit to worst fit for the second blobs, etc Note that the second blob may contain better fits than the first. (Note: This may interleave chiral structures).

[default=true]

-verbose

This is a boolean flag that controls the level of detail written to the log file. By default FLYNN will only write minimal information to the console. Verbose logging will cause more information to be written to the log file in order to follow behavior during program execution.

[default=false]

MTZ File Options

-autoMTZ
Automatically try to open the mtz file using the DELWT and FDELWT columns from REFMAC5 mtz files.

[default=true]

-Fc `<columnname>`
Column to use for Fc. *Note, to load arbitrary columns, you can use the* *-Fc** and **-Phic** columns and the **Fc** maptype.*

[default=FC]

-Fdelwt `<columnname>`
Column to use for Fdelwt or difference map amplitudes

[default=F]

-Fobs `<columnname>`
Column to use for FObs.

[default=F]

-Fwt `<columnname>`
Column to use for Fwt or regular map amplitudes.

[default=FWT]

-Phic `<columnname>`
Column to use for Phic.

[default=PHIC]

-Phidelwt `<columnname>`
Column to use for Phdelwt or difference map phases.

-Phiwt `<columnname>`
Column to use for Phwt or regular map phases.

-mtype

The map type to use for fitting. Fo-Fc, Fc, 2Fo-Fc, 3Fo-Fc, Fwt, Fdelwt...

[default=2Fo-Fc]

Advanced Options

-ciftype
Specify the format of the refinement dictionary. Legal values for refinement dictionaries are 'refmac', 'phenix' and 'buster'. Refinement dictionaries are always written out using the designation specified in the '-split' command. For best results, use -split when writing out cif files when using FLYNN.

-flipper
Force enumeration of all stereochemical centers. Otherwise, only missing stereo chemistry will be enumerated.

[default=false]

-overlays
Set the number of initial rigidly fit overlays to optimize into density.

[default=10]

-precheck
Perform only a preliminary check of the data to see if fitting is possible. (This flag is meaningless without the -reporthtml flag).

[default=false]

-reporthtml
Generate an html report of the fitting process. This is a useful first step to verify the data. It also includes a 2D image of the ligand that is being fit in order to verify bond orders. All output of FLYNN will be captured in the specified html file.

-residues
Comma separated list of residues to use as distance constraints. If residues are specified, the *-distance* flag will use the residues to reject blobs that are too far away. Note that with a residue list, the distance computed is a minimum distance, not an average distance. Residues must be specified as <residue number><chain id>. For example: 120C,134C. On Windows, double quotes must be added to specify two or more residues, i.e. -residues "120C,134C".

-rms
If the input ligand has 3D coordinates, this calculates the RMS distance to the ligand and records it in the molecule. If -split is on, it also records it in the file name.

[default=false]

-split
Split the result into different files. Each filename is annotated with the resulting shape score so that when the output directory is listed alphabetically, the best scores will be displayed in order. In the case where the output format is pdb the ligand fitting results are written in REMARK statements at the top of the file.

The splits are labeled as follows outputname_n###_b###_s###_c### where n### is the number of the ordered results, b### is the blob for the result, s### is the stereovariant and c### is the number of the conformer fit as ordered by MMFF/Shape.

[default=false]

-suppressH
By default FLYNN outputs hydrogens of the resulting poses. Setting this to true will not output hydrogens. It is

always recommended to output hydrogrens for ligands in order to keep their chemistry the same as flynn's internal optimizer. Only suppress hydrogens if your refinement package doesn't handle hydrogen names correctly.

[default=false]

`-fix_serial_numbers`

Force serial numbers to be unique when writing out the resulting pdb file for use in the refinement package. This is usually caused by adding hydrogens to the ligand outside of the context of the protein and then using it in conjunction with the original PDB structure.

[default=false]

Fragment Fitting Options

`-blobTanimoto`

Density is searched for each input ligand. This flag sets the minimum tanimoto overlap for which to consider two blobs the same. When pruning similar blobs, the larger is taken.

[default = 0.45]

`-sortBy`

Choose the fragment sorting function, tanimoto, rscc (real space correlation coefficient), plp (piecewise linear potential) or chemscore.

The fragments are fit in the normal fashion (tanimoto), but then re-scored and sorted using the chosen metric.

[default = rscc]

3D Construction Options

These are advanced conformer generation parameters. Normally, they do not need to be adjusted. Please consult the OMEGA manual for assistance:

`-ewindow`

Controls the MMFF energy window when generating initial conformations. Normally, this setting does not need to be adjusted, however in some cases large floppy molcules may require an adjustment. This setting is in kcals/mol.

[default = 15.0]

4.1.6 Usage Notes

Minimum Requirements

At the minimum, FLYNN requires a density file and a the connection table of the ligand - usually derived from a supplied molecule file e.g. .pdb, .sdf, .mol2, .smi. FLYNN automatically searches the density for volumes of unmodeled density that are similar in size to the input ligand. Because of this it is highly recommended that a protein is also input to FLYNN. This makes the job of locating the ligand density less prone to false positives.

Based on analyzing several hundred protein/ligand combinations, the best way to use FLYNN for ligand fitting is described as follows (of course your mileage may vary):

- **Always use a protein:**

  The protein is used to identify modeled density where the ligand cannot be placed. Without this, FLYNN will identify many candidate locations for placing the ligand that will need to be analyzed.

- **Use difference maps when appropriate:**

  The reflection data should be refined without a previous ligand conformation or generated without the ligand present. While in many cases the same result will be generated, if the ligand is fit to data refined with a previous ligand conformation, FLYNN may be biased towards the density modified by the pre-existing ligand conformation.

- **use fragment mode for fitting fragments**

  If you have an input cocktail, use the *-fragment* flag. This option simultaneously fits all the cocktails against density and sorts them by best fit.

Types of input ligands

Not all ligands can be successfully fit by FLYNN. Such types include large polypeptides or proteins, very flexible molecules, or simply molecules that contain atoms that are not present in the MMFF94s force-field.

Perhaps the most important property is rotatable bond count. Although FLYNN may be able to generate conformers for molecules with more than 20 rotatable bonds, the results of such an exercise would be dubious at best and should be taken with a grain of salt.

FLYNN will automatically check for unhandled atom types, but will gladly try and fit large molecules or proteins that contain many rotatable bonds usually with disappointing results.

Note: Dangers of PDB format for ligand input Many crystallography packages do not enforce writing out connection table or bond order information when outputting pdb files, or worse, strip them out. If bond distances are not correct FLYNN's attempt to automatically assign bond orders and atom types can be suspect. To facilitate this, FLYNN includes a *-precheck* flag that can be used to verify that the input ligand is identified correctly and for validation of the automatic bond order assignments.

Stereochemistry Enumeration

Compounds that contain unspecified or ambiguous definitions of stereochemistry will be preprocessed before conformational sampling to explicitly enumerate stereochemistry. Input molecules that have three dimensional coordinates inherently have stereochemistry specified, but SMILES or two dimensional SD files may have atoms (R/S) or bonds (E/Z) for which the stereochemistry is unknown or unspecified. In the case where the stereochemistry supplied is suspect, FLYNN provides an option that will completely enumerate all stereochemical centers.

The resulting ligands will be sorted based on their best fit to density regardless of the stereochemistry. That is, the best solutions will percolate to the top of the output. See *sortAllChiral* below for more details.

Refinement Dictionaries

Both FLYNN and its companion program WRITEDICT can generate refinement dictionaries. A refinement dictionary is needed to maintain proper ligand geometry when using external reciprocal space refinement programs such as REFMAC5 or CNS X/PLOR.

Refinement dictionaries are a list of geometrical constraints encoding used chemical bonding and molecular conformation information. They are used by various refinement packages to describe standard geometries and constraints that are used during the refinement process. The quality of the post refinement ligand conformations are directly related to the quality of the constraints [AFITT-CL Vagin-2004]_.

FLYNN comes bundled with a refinement dictionary writer named WRITEDICT. WRITEDICT uses the MMFF94 forcefield to derive geometrical constraints for the input ligands or ligand-protein complexes. The output dictionaries enforce, as closely as possible, the input ligand's geometries while allowing the refinement programs to modify the geometry as needed.

WRITEDICT also automatically detects covalent bonds in pdb files and inserts the appropriate PDB LINK records and covalent bond entries in the output refinement dictionary.

REFMAC5 refinement dictionaries

When outputting REFMAC5 refinement dictionaries, WRITEDICT writes out a **.cif** file and a **.pdb** file. The **.pdb** file is written out for two reasons:

- **Inconsistent Hydrogen Naming**

  Some applications write out hydrogen names incorrectly in ways that cause REFMAC5 or visualization programs like coot or WinCoot to be unable to associate the hydrogens in the refinement dictionary with hydrogens in the pdb file. In the worst case, REFMAC5 will crash entirely.

  When necessary, *WRITEDICT* renames and renumbers hydrogens, if they exist, so that REFMAC5 won't crash and so the **.cif** file is consistent with the **.pdb** file.

- **Covalent Bonds**

  WRITEDICT detects covalent bonds and outputs proper LINK records in the **.pdb** file and proper covalent constraints in the **.cif** file. Without using both outputs, REFMAC5 will not detect covalent bonds during refinement.

- **Known residues**

  When WRITEDICT detects a known residue, it may remap the atom names to be canonical with the known residue (this prevents REFMAC5 from failing to refine). These new atom names are saved in the **.pdb** file that WRITEDICT outputs.

- **Input Ligands with no residue information**

  When WRITEDICT analyzes a ligand with no residue information, it assigns the whole ligand to a single residue (by default UNL). If this is not the desired outcome, the ligand must be put into **PDB** or **MOL2** format and the residues must be manually assigned.

It is highly recommended to use WRITEDICT's generated **.pdb** file in conjunction with the **.cif**.

Looking up known residues

WRITEDICT has an internal dictionary of known residues. By default, known residues names are retained when the graph of the known residue is exactly the same as the input residue. When this occurs, writedict can replace REFMAC5's dictionary with the MMFF94 generated dictionary. If the graphs do not match then WRITEDICT relabels the residue with a new residue name since a different dictionary needs to be created.

Options available for residue matching is as follows:

Option	Meaning
exact	Known residue and input residue graphs must match exactly.
substructure	Input residue may be a substructure of the known residue. **Cannot be used with the exact flag.**
fuzzy-bonds	Bond orders do not need to match
atomname	Atom names must match

Options are set using the *-strict* flag and joined with colons (:).

The default is:

-strict substructure:fuzzybonds

To reject dictionaries that do not match exactly, use:

> -strict exact

However, the more permissive default setting has been known to generate invalid dictionaries on occasion. It is always safe to force exact matches.

To have WRITEDICT attempt to see if the ligand's residue has already been deposited in RCSB simply add the *-lookup* switch. This forces WRITEDICT to compare (based on the current *-strict* settings) the input residue to all known residues. This is useful if the input is from a 2D connection table, such as smiles or does not contain residue information.

MMFF94 versus MMFF94s forcefields

The MMFF94s forcefield is a variant of MMFF94 that emulates time-averaged structures typically observed during crystallographic structure determination, mainly planar geometries at unstrained delocalized trigonal nitrogen centers. However, there are many theoretical studies that show nitrogen centers are puckered [AFITT-CL Halgren-VI-1999]_.

That being said, due to the prevalence of crystallographic examples where time averaging has occurred, many chemists erroneously consider the time-averaged structure to be correct, hence this variant is available for use.

WRITEDICT approximates the MMFF94s forcefield by enforcing planar aniline nitrogen configurations using the out of plane atom types and parameters from the MMFF94s. See the *-planarAniline* parameter to specify planar constraints.

FLYNN and writing CIF Files

When FLYNN is run, CIF dictionary files are always written as output. Because the dictionary files are not conformation independent, they are written as if the molecules were split into separate files whether or not the user specifies FLYNN's *-split* flag. (See the split flag in *Advanced Options* below for more details). These files are numbered, however, so that the file with the lowest number corresponds to the best scored fit ligand.

For example, consider using the *-sortBy plp* flag and getting the following output:

> 1nhu-poses_n001_b001_s01_c003.pdb 1nhu-poses_n002_b001_s01_c000.pdb 1nhu-poses_n003_b001_s01_c001.pdb 1nhu-poses_n004_b001_s01_c002.pdb

This indicates that the best fit to density (c000) has the second best PLP score (n001). By default the files are sorted by RSCC.

FLYNN CIF output consists of the following files:

1. a CIF file
2. a PDB file (in case atom names were remapped) or links were added.
3. an OEB file that contains the original molecule with annotations

It is safest, however, to run WRITEDICT on the output ligand and protein complex. The CIF files generated by FLYNN are primarily useful for adjusting torsions and geometries in various ligand building programs, such as coot.

Results on The Gold Test Set

Included in the FLYNN distribution is a copy of the protein+ligand complexes that have structures factors available from the *Electron Density Server* [AFITT-CL EDS]_.

Along with this data set is a simple python script that can be used to fit ligands to the test data set. OpenEye has prepared the input test set as follows:

1. The Structure Factors and protein complexes were downloaded from **EDS**.
2. The ligands were separated from the proteins.
3. The protein was rerefined using the original mtz file to remove ligand bias.

A simple directory structure was created as follows:

- **pdbcode/protein.pdb** - the re-refined protein
- **pdbcode/ligand.pdb** - the original ligand
- **pdbcode/protein.mtz** - the re-refined difference map density.

The following python script was used to run FLYNN on all of the proteins. (This script is also included in the FLYNN distribution):

```
##########################################################################################
## Copyright (C) OpenEye Scientific 2007,2008,2009,2010,2011
##########################################################################################
## This script analyzes the Gold dataset shipped with the flynn distribution
import os,sys
command = "../../../bin/flynn -in %(CODE)s/ligand.pdb -map %(CODE)s/protein.mtz " \
          "-prot %(CODE)s/protein.pdb -out %(CODE)s_results.sdf " \
          "-verbose -rms -reportfile %(CODE)s.log %(REDIRECT)s %(CODE)s.out"
if sys.platform == "win32":
  REDIRECT = "2>"
else:
  REDIRECT = "&>"
RMS = []
for file in os.listdir("."):
   print file
   if os.path.isdir(file) and len(file) == 4:
       code = file
       CMD = command % {"CODE":code, "REDIRECT": REDIRECT}
       print CMD
       os.system(CMD)
       index = None
       rmses = []
       for line in open("%s.log"%code):
          if index is None:
             index = line.strip().split(",").index("RMSD")
          else:
             rmses.append( float(line.strip().split(",")[index]) )
       if rmses:
           print "\t===>Lowest RMS", min(rmses)
```

The results of FLYNN are shown in Figure *Gold Test Set Results*. The mean RMS to the crystal structure is 0.676 Angstroms.

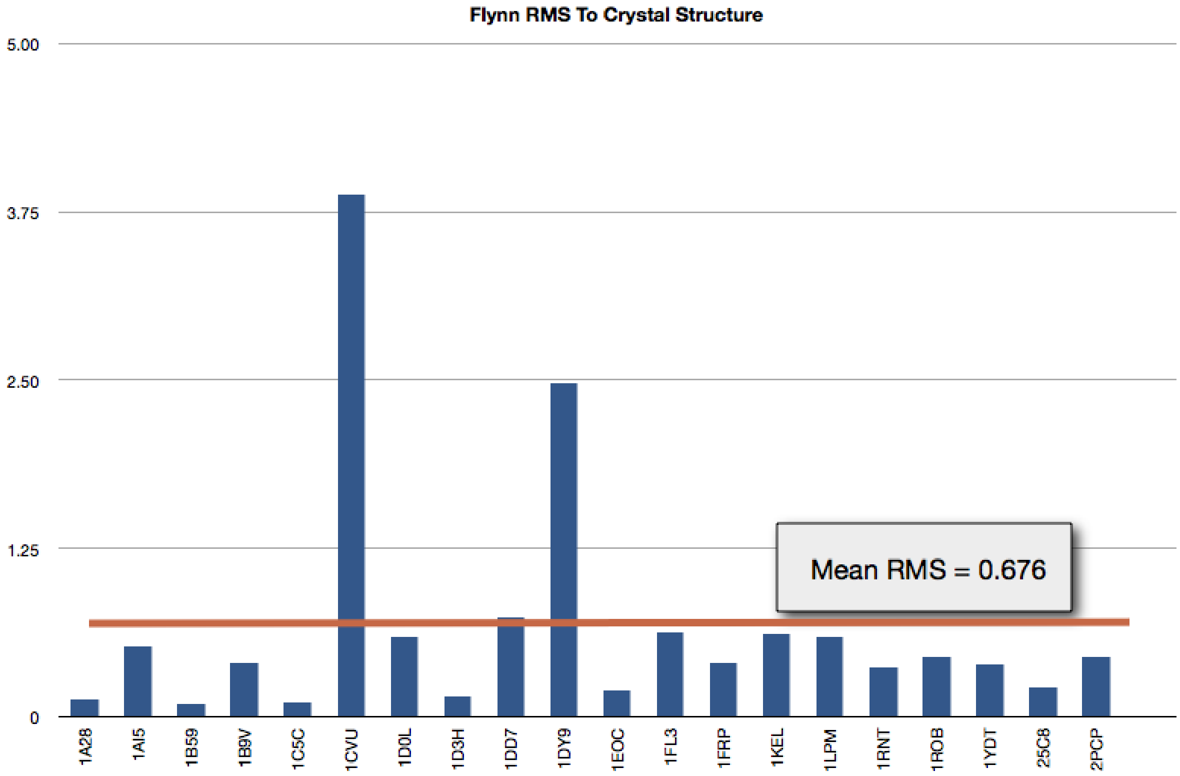


Figure 4.1: **Gold Test Set Results**

CHAPTER

FIVE

COOT INTEGRATION

5.1 COOT Integration

5.1.1 Overview

Due to its command line nature, AFITT-CL tools (such as FLYNN) can be integrated with other crystallographic applications such as Coot (and WinCoot). OpenEye supplies a simple script that allows users of Coot to use FLYNN directly from the Coot GUI.

This script adds “Fit Ligand...” option, a “Make cif...” and a “Fit sidechains...” options to the extensions menu.

5.1.2 Using FLYNN

Activating the “Fit Ligand...” menu option from the Extensions menu opens up the FLYNN dialog:

To use the FLYNN dialog, select the Ligand to fit from the pull-down menu. Next select the Protein and then the desired map file.

If the map is a difference map, click on the “Difference Map” to optimize the analysis. Then click on Fit.

The output of FLYNN will appear in the console window used to start coot. When FLYNN is complete, coot will automatically load the resulting ligands.

FLYNN Advanced Options

FLYNN’s coot interface includes access to some of the advanced options contained in the application.

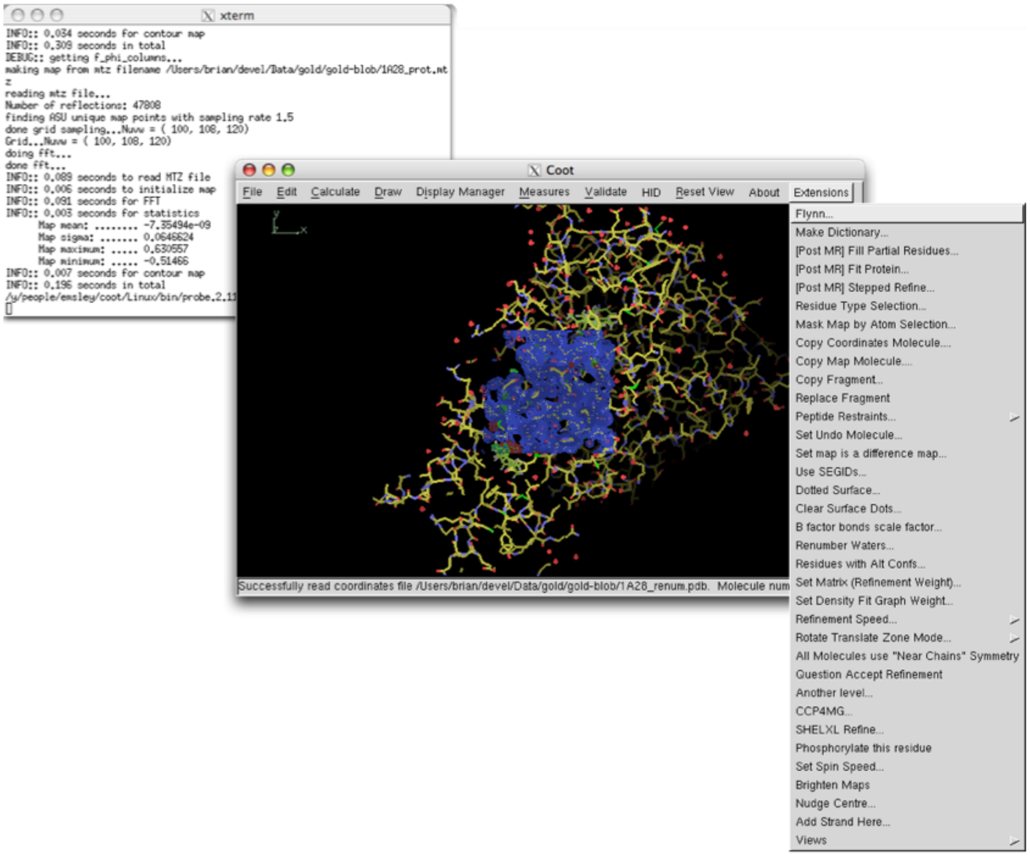
Box option

Unlike the AFITT GUI interface, coot does not have a method to select density blobs to be used for fitting. If the density is poor, flynn can use the input ligand as a bounding box to fit to the surrounding density.

Check option

Running check runs flynn without performing any fitting operation. When the check is completed, an html report is generated and opened in a webbrowser (if available). Since coot only display’s single bonds, the report includes a depiction of the ligand being fit in order to verify that the ligand being analyzed is actually correct.

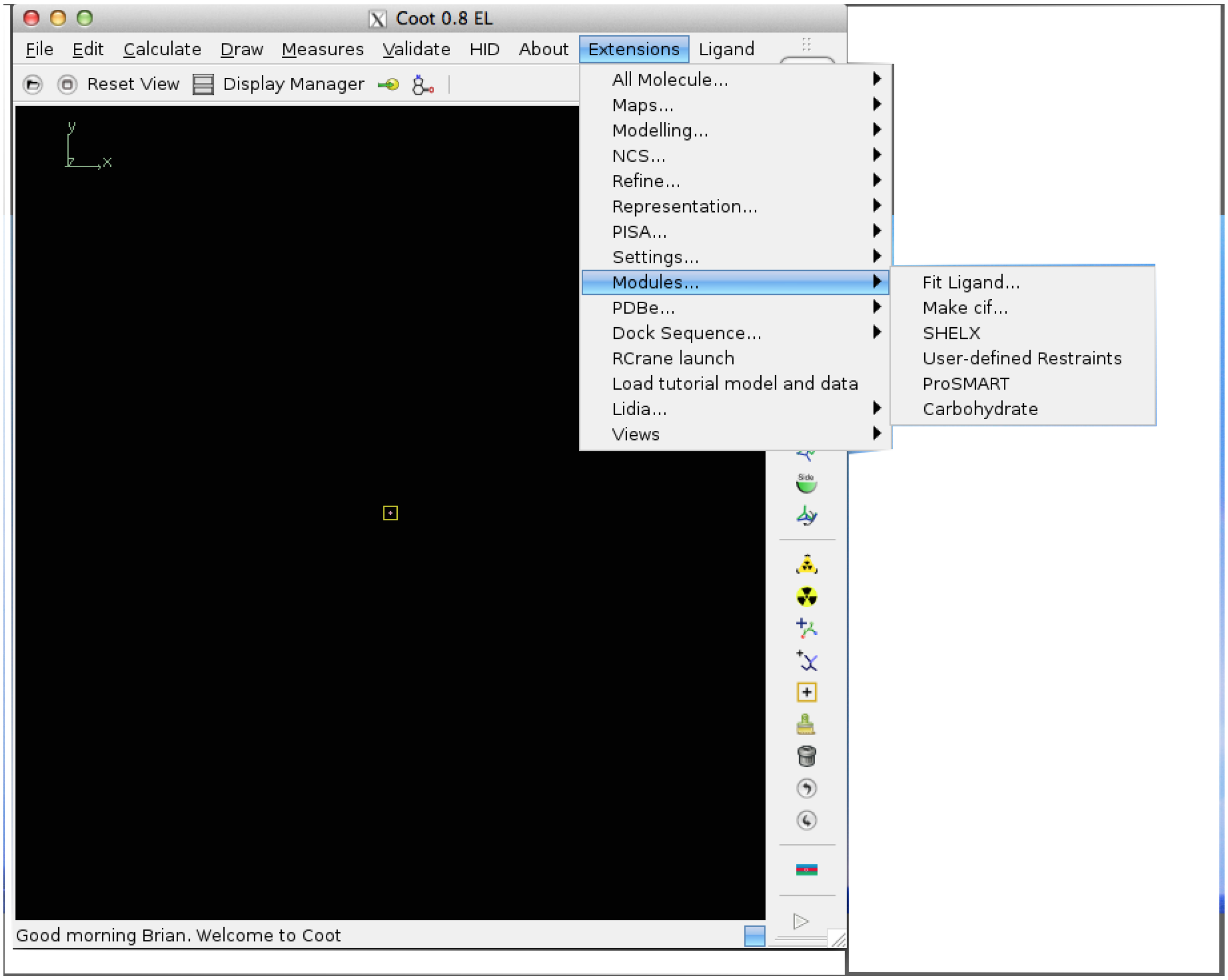
The report also indicates any problems or issues with the ligand or the map being fit against.



```
INFO:: 0.034 seconds for contour map
INFO:: 0.309 seconds in total
DEBUG:: getting f_phi_columns...
making map from mtz filename /Users/brian/devel/Data/gold/gold-blob/1A28_prot.mt
z
reading mtz file...
Number of reflections: 47808
finding ASU unique map points with sampling rate 1.5
done grid sampling...Nuvw = ( 100, 108, 120)
Grid...Nuvw = ( 100, 108, 120)
doing fft...
done fft...
INFO:: 0.089 seconds to read MTZ file
INFO:: 0.006 seconds to initialize map
INFO:: 0.091 seconds for FFT
INFO:: 0.003 seconds for statistics
      Map mean: ........ -7.35494e-09
      Map sigma: ....... 0.0646624
      Map maximum: ..... 0.630557
      Map minimum: ..... -0.51466
INFO:: 0.007 seconds for contour map
INFO:: 0.196 seconds in total
/y/people/emsley/coot/Linux/bin/probe.2.11
```

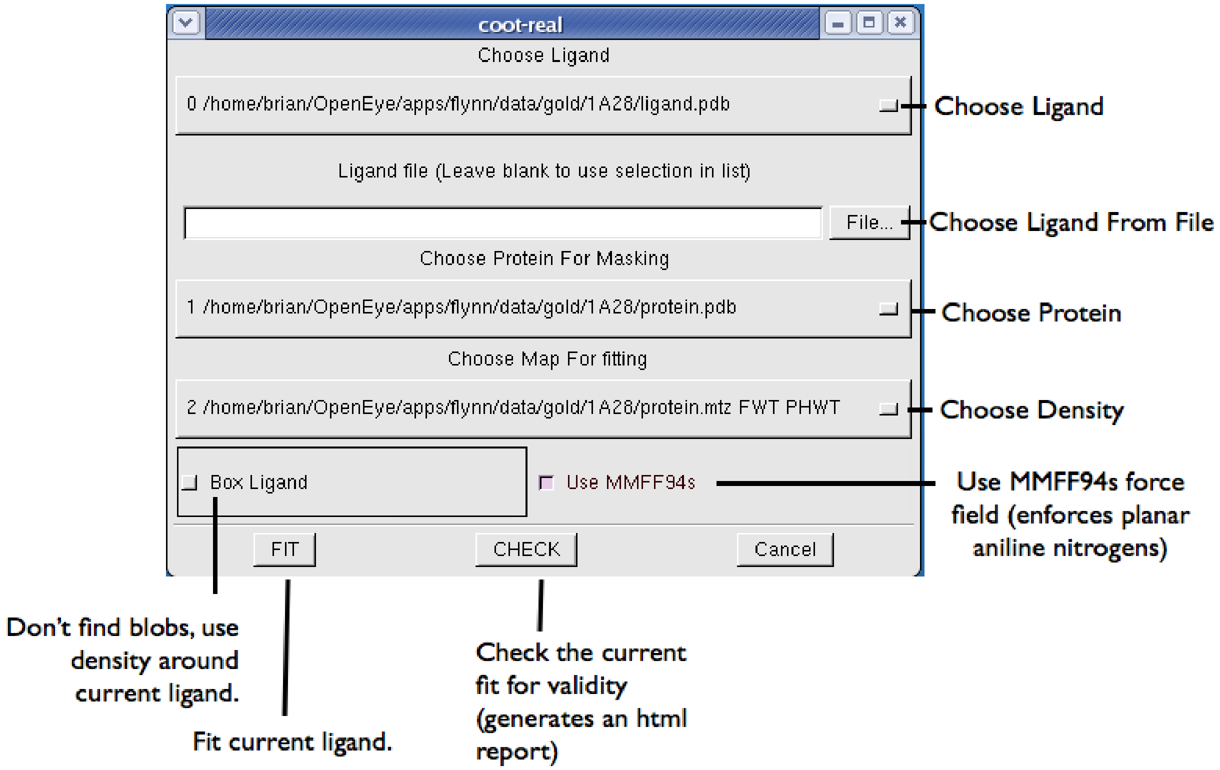
Successfully read coordinates file /Users/brian/devel/Data/gold/gold-blob/1A28_renum.pdb. Molecule num

Figure 5.1: **Coot Integration**



Good morning Brian. Welcome to Coot

Figure 5.2: **COOT 0.8 Integration**



Choose Ligand: 0 /home/brian/OpenEye/apps/flynn/data/gold/1A28/ligand.pdb

Choose Protein For Masking: 1 /home/brian/OpenEye/apps/flynn/data/gold/1A28/protein.pdb

Choose Map For fitting: 2 /home/brian/OpenEye/apps/flynn/data/gold/1A28/protein.mtz FWT PHWT

Choose Ligand

Choose Ligand From File

Choose Protein

Choose Density

Use MMFF94s force field (enforces planar aniline nitrogens)

Don't find blobs, use density around current ligand.

Fit current ligand.

Check the current fit for validity (generates an html report)

Figure 5.3: **FLYNN Dialog to fit a ligand**

MMFF94s option

This option uses the MMFF94s variant of the MMFF94 forcefield to enforce planar aniline configurations. See the FLYNN *Advanced Options* command line flags for more details

5.1.3 Using WriteDict

Activating the "Make Dictionary..." menu option opens up the WriteDict dialog:

To use the WriteDict dialog, simply select the protein or ligand from the pull-down menu and click "ok". *Coot* will automatically open up the resulting dictionary and pdb files generated by WriteDict.

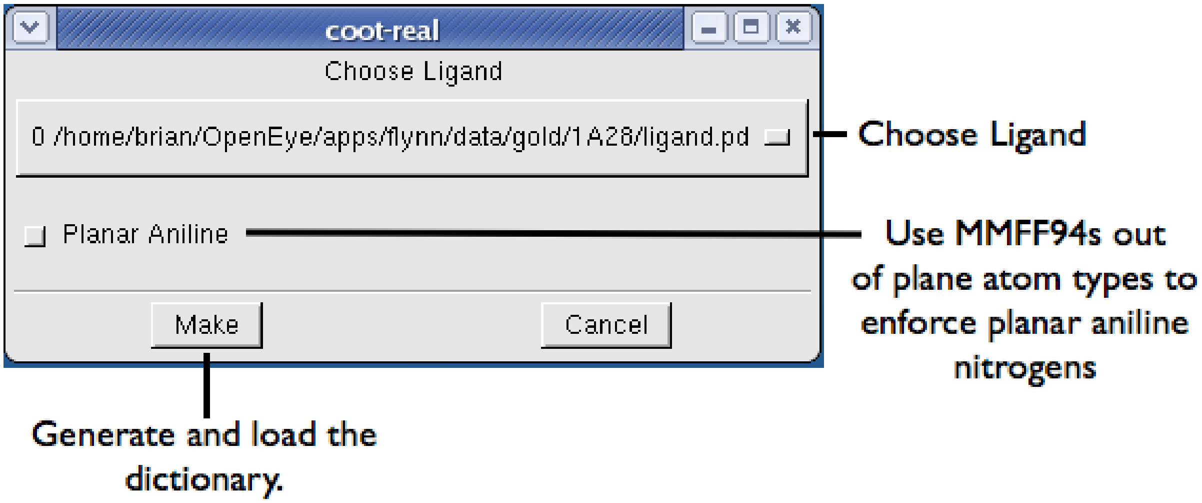
Note that new ".pdb" files are generated when using writedict, this is because hydrogen may have been relabeled and covalent *LINK* records may have been inserted into the *pdb* file.

WriteDict Advanced Options

FLYNN's coot interface includes access to some of the advanced options contained in the application.

planarAniline option

Similar to FLYNN's *MMFF94s* option, this enforces planar aniline nitrogen configurations. Note that since this is an approximation of the *MMFF94s* forcefield using planar constraints, it is not called *MMFF94s*. See the WriteDict *writedictOutputOptions* command line flags for more details.



Choose Ligand

Use MMFF94s out of plane atom types to enforce planar aniline nitrogens

Generate and load the dictionary.

Figure 5.4: **Writedict Dialog**

5.1.4 Using RotFit

Activating the “Fit Sidechains...” menu option opens up the ROTFIT dialog:

To use the ROTFIT dialog, a protein and mtz map must be loaded into coot. The ROTFIT interface deduces the appropriate regular and difference map to use and currently cannot handle non standard or unique column names.

ROTFIT options

ROTFIT is a program that fits protein side-chains into the surrounding density. It utilizes both regular and difference density to place side-chains appropriately searching both rotamers and peptide flips in addition to an optional full MMFF94-driven optimization into density.

Fixup Rotamer

Rotamer fixups selects from the probable rotamer sets to find the rotamer that best matches the surrounding density.

Fixup Pep-flip

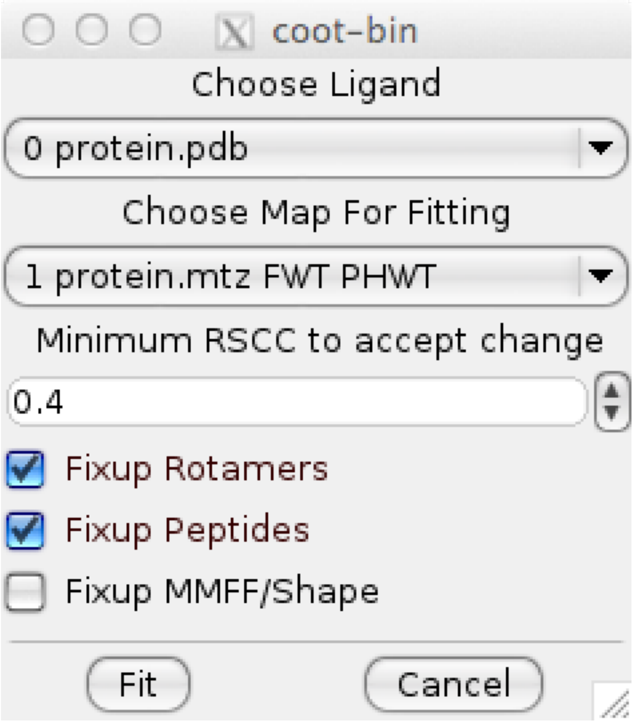
Peptide flips are enumerated and the one that best matches the surrounding density is selected.

Fixup MMFF/Shape

Full MMFF/Shape minimization is performed on compatible residues attempting to fit the residue into the locally surrounding density.

5.1.5 Installing Coot Interface

The coot script file “flynn.scm” is included in your FLYNN distribution in the “etc” directory. The following steps are required to integrate FLYNN with coot.



Choose Ligand

0 protein.pdb

Choose Map For Fitting

1 protein.mtz FWT PHWT

Minimum RSCC to accept change

0.4

☑ Fixup Rotamers

☑ Fixup Peptides

☐ Fixup MMFF/Shape

Figure 5.5: **Writedict Dialog**

The current default version of COOT is 0.7, to install another version please look for the files with the appropriate extension, i.e. "coot-0.8.scm" for coot 0.8.

1. Install coot (at least version 0.3.3)
2. Install flynn and writedict somewhere in your path. If you can't run FLYNN from the command line, neither can Coot.
3. Place *flynn.scm* to the coot extensions directory. This is the directory where the files *extensions.scm* and *coot.scm* are located.

   This directory may be one of the following:

   /usr/local/coot/scheme /usr/local/xtal/coot64/share/coot/scheme/

   on OSX the install for Coot 0.7/0.8 is most likely /Library/Coot/share/coot/scheme/

   Typing **which coot** may help locate these directories.
4. Copy *flynn.scm* to the extension list in *coot.scm* which is in the same directory as *extensions.scm*. Please use the appropriate file for your version of coot.

   To accomplish this, change the following code in *coot.scm* for coot 0.7

```
(define load-all-scheme
  (lambda (use-gui?)
    (let ((pre-list (list "filter.scm"
                          "coot-utils.scm"))
          (post-list (list "coot-lsq.scm"
                           "shelx.scm"
```

   to look like this:

```
(define load-all-scheme
  (lambda (use-gui?)
    (let ((pre-list (list "filter.scm"
           "coot-utils.scm"))
           (post-list (list "coot-lsq."
                            "shelx.scm"
                            "flynn.scm"
```

5. Finally, make the following changes to *extensions.scm*

   For coot 0.3.3 to 0.5 near the top of the file, change:

```
(if (defined? 'coot-main-menubar)
   (let ((menu (coot-menubar-menu "Extensions")))
```

   to become:

```
(if (defined? 'coot-main-menubar)
   (let ((menu (coot-menubar-menu "Extensions")))
     (add-simple-coot-menu-menuitem menu "Flynn..."
       (lambda () (do-flynn-gui)))
     (add-simple-coot-menu-menuitem menu "Make Dictionary..."
       (lambda () (do-flynn-gendict-gui)))
    (add-simple-coot-menu-menuitem menu "Fit Sidechains..."
       (lambda () (do-flynn-fitsidechains-gui)))
```

   For coot 0.6.x, near the top of the file, change:

```
(if (defined? 'coot-main-menubar)
    ;; ---------------------------------------------
    ;;          extensions
    ;; ---------------------------------------------
    ;;
   (let ((menu (coot-menubar-menu "Extensions")))
```

   to become:

```
(if (defined? 'coot-main-menubar)
     ;; ---------------------------------------------
     ;;          extensions
     ;; ---------------------------------------------
     ;;
  (let ((menu (coot-menubar-menu "Extensions")))
     (add-simple-coot-menu-menuitem menu "Flynn..."
       (lambda () (do-flynn-gui)))
     (add-simple-coot-menu-menuitem menu "Make Dictionary..."
       (lambda () (do-flynn-gendict-gui)))
    (add-simple-coot-menu-menuitem menu "Fit Sidechains..."
       (lambda () (do-flynn-fitsidechains-gui)))
```

   For coot 0.8 you should make the change to the “Modules” section as shown below:

```
;; ----------------------------------------------------------------------
;;     Modules
;; ----------------------------------------------------------------------

(add-simple-coot-menu-menuitem
 submenu-modules "Fit Ligand..."
 (lambda ()
   (do-flynn-gui)))
```

```
(add-simple-coot-menu-menuitem
 submenu-modules "Make cif..."
 (lambda ()
   (do-flynn-gendict-gui)))

(add-simple-coot-menu-menuitem
 submenu-modules "Fit sidechains..."
 (lambda ()
   (do-flynn-fitsidechains-gui)))
```

Installing WinCoot Interface

WinCoot does not support writing extensions in GUILE. Extensions may be written in PYTHON, however.

The WinCoot scheme script file *flynn.py* is included in your FLYNN distribution in the *utilities/flynn/wincoot* directory. The following steps are required to integrate FLYNN with *WinCoot*.

1. Install *WinCoot* (at least version 0.3.3)
2. Install flynn and writedict somewhere in your path. If you can't run FLYNN from the command line, neither can *WinCoot*.
3. Place this file *flynn.py* in the coot extensions directory where *extensions.py* is located.

   Typically, this directory is *C:WinCootsharecootscheme*.
4. Add *flynn.py* to the extension list in *coot_load_modules+gui.py*. (same directory as extensions.py) *before extensions.py*

   For example:

   ```
   "coot-gui.py",
   "flynn.py",
   "extensions.py",
   ```
5. Add the following lines to the file named extensions.scm (this will be in your coot distribution)

   The easiest place to put this right after the line

   ```
   menu = coot_menubar_menu("Extensions")
   ```

   here are the lines to add:

   ```
   add_simple_coot_menu_menuitem(menu, "Fit Ligand...", do_flynn_gui)
   add_simple_coot_menu_menuitem(menu, "Make CIF...", do_writedict_gui)
   add_simple_coot_menu_menuitem(menu, "Fit Sidechains...",
     do_fitsidechains_gui)
   ```

CHAPTER

SIX

UTILITIES

6.1 AlignGrid

6.1.1 Overview

The **AlignGrid** utility aligns proteins with electron-density maps on a single reference.

6.1.2 Example Commands

```
> aligngrid -grid input.ccp4 -query protein1.pdb \
            -target protein2.pdb -out output.ccp4
```

Take the input grid in ccp4 format and protein. Align protein1 to protein2 and rotate and translate the input grid using the same alignment outputting the result to *output.ccp4*.

6.1.3 Command Line Help

A description of the command line interface can be obtained by executing **AlignGrid** with the *--help* option.

```
> aligngrid --help
```

will generate the following output:

```
Help functions:
  aligngrid --help simple      : Get a list of simple parameters
  aligngrid --help all         : Get a complete list of parameters
  aligngrid --help <parameter> : Get detailed help on a parameter
  aligngrid --help html        : Create an html help file for this program
```

6.1.4 Required Parameters

-grid
Input density grid. This is the grid that is to be rotated and translated into the new reference frame.

-out
The output grid in any writable format. These include **.ccp4**, **.map** and **.grd**

-protein
The query protein that corresponds to the input grid.

-target

The target protein that the grid and query protein will be aligned toward.

6.1.5 Optional Parameters

-outprot

An optional file to output the query protein after it is translated and rotated into the new reference frame.

-padding

When writing out a **.grd** file, this indicates the added extents (in Ångströms around the protein when outputting the density.

-scale

The final map spacing in Ångströms. (The default is the minimum spacing in X, Y or Z)

-verbose

Triggers copious logging output.

-autoMTZ

Automatically try to open the mtz file using the DELWT and FDELWT columns from REFMAC5 mtz files.

[default=true]

-Fc `<columnname>`

Column to use for Fc. *Note, to load arbitrary columns, you can use the* *-Fc** and **-Phic** columns and the **Fc** maptype.*

[default=FC]

-Fdelwt `<columnname>`

Column to use for Fdelwt or difference map amplitudes

[default=F]

-Fobs `<columnname>`

Column to use for FObs.

[default=F]

-Fwt `<columnname>`

Column to use for Fwt or regular map amplitudes.

[default=FWT]

-Phic `<columnname>`

Column to use for Phic.

[default=PHIC]

-Phidelwt `<columnname>`

Column to use for Phdelwt or difference map phases.

-Phiwt `<columnname>`

Column to use for Phwt or regular map phases.

-mtype

The map type to use for fitting. Fo-Fc, Fc, 2Fo-Fc, 3Fo-Fc, Fwt, Fdelwt...

[default=2Fo-Fc]

6.2 RotFit

6.2.1 Overview

RotFit is a side-chain fitting tool that quickly fits sidechains to the surrounding density. Currently only a MTZ file can be used for density Side chains can be fit using several techniques:

1. Using the most probably rotamers and ranking based on real space correlation (RSCC) to the density.
2. pep-flipping the rotamer and ranking on RSCC.
3. Full MMFF/Shape fitting in to the density.

By default **RotFit** searches the rotamers and pep-flips.

For **RotFit** to apply a changed residue, their has to have an increase in RSCC and be above a minium value (currently 0.4). Additionally, the RSCC must be greater by a minimum amount (0.05).

6.2.2 Example Commands

6.2.3 Command Line Help

A description of the command line interface can be obtained by executing ROTFIT with the *--help* option.

```
> rotfit --help
```

will generate the following output:

```
Help functions:
  rotfit --help simple      : Get a list of simple parameters
  rotfit --help all         : Get a complete list of parameters
  rotfit --help defaults    : List the defaults for all parameters
  rotfit --help <parameter> : Get detailed help on a parameter
  rotfit --help html        : Create an html help file for this program
  rotfit --help versions    : List the toolkits and versions used in the application
```

6.2.4 Required Parameters

-in `<proteinfile>`

Input protein structure. The side chains of this protein will be optimized.

-map `<mtz file>`

The input MTZ file to fit side chains against.

-out `<protein>`

The protein structure with optimized side chains will be outputted to this file.

-param `<parameter filename>`

A parameter file is a text file that lists parameter settings to be used during a run. If a parameter is specified both on the command line and in the parameter file, the value specified on the command line is used.

The format of the parameter file is as follows:

- One parameter per line.
- For non-list parameters one key-value pair per line.
- For list parameters a key followed by all the values.

- Boolean parameters must be listed as a key followed by true or false.
- The parameter file may not contain the `-param` parameter.
- Lines beginning with # are considered comments.

-verbose `<true or false>`

Ouptuts copious extra information.

[Default : false]

6.2.5 Optional Parameters

-fixup_rotamers `<true or false>`

Search the most probable rotamers and choose the one that fits best to the surrounding density.

[Default : true]

-fixup_pepflips `<true or false>`

Search the pep-flips and pick the one that fits best to the surrounding density.

[Default : false]

-fixup_shapefit_residues `<true or false>`

Use MMFF/Shape to fit the residue into the surrounding density. Normally it is best to use **-fixup_rotamers** in conjunction with this flag.

[Default : false]

-minimum_rscc `<number>`

For a give residue if the best tested RSCC of any conformation is below this value the residue will not be modified. This prevents optimization from happenening when the is no density anywhere near the side chain.

[Default : 0.4]

-minimum_rscc_increase `<number>`

For a given residue if the best tested RSCC of any conformation is not this much better than the starting conformation, then the residue will not be modified.

[Default : 0.05]

-autoMTZ `<true or false>`

Automatically try to open the mtz file using the DELWT and FDELWT columns from REFMAC5 mtz files.

[default=true]

-Fc `<columnname>`

Column to use for Fc. *Note, to load arbitrary columns, you can use the* *-Fc** and **-Phic** columns and the **Fc** maptype.*

[default=FC]

-Fdelwt `<columnname>`

Column to use for Fdelwt or difference map amplitudes

[default=F]

-Fobs `<columnname>`

Column to use for FObs.

[default=F]

-Fwt <columnname>
Column to use for Fwt or regular map amplitudes.

[default=FWT]

-Phic <columnname>
Column to use for Phic.

[default=PHIC]

-Phidelwt <columnname>
Column to use for Phdelwt or difference map phases.

-Phiwt <columnname>
Column to use for Phwt or regular map phases.

-mtype <setting>

The map type to use for fitting. Fo-Fc, Fc, 2Fo-Fc, 3Fo-Fc, Fwt, Fdelwt...

[default=2Fo-Fc]

6.3 Rscc

6.3.1 Overview

RSCC is a real-space fit calculation tool both real-space correlation coefficient (rscc) and real-space R-factor (rsr). By default this tool calculates rscc. RSCC was designed to be a standalone tool using the same code that is used for ligand fitting by AFITT.

6.3.2 Example Commands

6.3.3 Command Line Help

6.3.4 Required Parameters

-in <molecule file>
Input molecule(s) to be scored against the density

-mtz <mtz file>
Input MTZ file to be used for calculations. Currently only MTZ format is supported.

6.3.5 Optional Parameters

-clamp_at_level
Set an electron density contour sigma level (positive) to be used for calculating rsr values. The default value is 0.0.

-flatten
Flatten B-factors (20.0) of the input molecule. This flag is to be used with the –scatter flag.

-rsr
Use rsr instead of rscc.

-scatter
Calculate Fc (calculated) density from the input molecule.

-verbose
This triggers copious logging of output to the screen.

6.4 WriteDict

6.4.1 Overview

WriteDict is a comprehensive refinement dictionary generator that properly handles covalent bond restraints between the ligand and the protein.

6.4.2 Example Commands

The basic usage of writedict is to take an input structure and generate the corresponding .pdb and .cif files for input into COOT or REFMAC5.

```
> writedict -in 1nhu.ism -out 1nhu_out
```

This creates the files *1nhu_out.cif* and *1nhu_out.pdb*. The pdb is always created since the atom names may be remapped from the input. Since the input contains no residue information, the ligand is placed in residue “UNL”.

To add your own residue names for unmapped residues:

```
> writedict -in 1nhu.ism -out 1nhu_out -residues 153
```

Note that if the given residue name (153) does not map to the known residue 153 the next residue name in the unmapped residue list will be used. Since none are specified it will go to the standard defaults: “UNL,UN1,UN2...”

Note: When doing whole complexes, renaming residues gets quite complicated since you may not know which residues will map before you run WRITEDICT.

```
> writedict -in 1nhu.ism -out 1nhu_out -residues LIG -nolookup
```

Force the residue to be named LIG. (It will also indicate an invalid dictionary)

```
Warning: LIG   1: Known Residue, but does not map to previous residue: LIG from: RCSB
Warning: LIG   1: previous smiles c1ccc2c(c1)CC3=C(NN=C23)c4ccncc4
Warning: LIG   1: current  smiles c1ccc(cc1)CC(C(=O)[O-])N(Cc2cccc(c2)C(F)(F)F)C(=O)c3ccc(cc3Cl)Cl
Warning: LIG: Keeping residue name (dictionary may be invalid)
Warning: LIG: Adding new residue dictionary
Warning: LIG: already in dictionary from: RCSB but with different structure.
Warning: LIG: c1ccc2c(c1)CC3=C(NN=C23)c4ccncc4
Warning: LIG: c1ccc(cc1)CC(C(=O)[O-])N(Cc2cccc(c2)C(F)(F)F)C(=O)c3ccc(cc3Cl)Cl
Warning: LIG: Overwriting existing residue (dictionary may be invalid)
```

To ensure planarAniline constraints consistent with the MMFF94s forcefield, use the *-planarAniline* flag.

```
> writedict -in 1nhu.ism -out 1nhu_out -planarAniline
```

6.4.3 Command Line Help

A description of the command line interface can be obtained by executing WRITEDICT with the `--help` option.

```
> writedict --help
```

will generate the following output:

```
Help functions:
  writedict --help simple      : Get a list of simple parameters
  writedict --help all         : Get a complete list of parameters
  writedict --help <parameter> : Get detailed help on a parameter
  writedict --help html        : Create an html help file for this program
```

6.4.4 Required Parameters

-in `<filename>`

File containing a molecule to be fit to density.

File type	Extension
SMILES	.smi .ism .smi.gz .ism.gz
OEBinary	.oeb .oeb.gz
SDF	.sdf .mol .sdf.gz .mol.gz
MOL2	.mol2 .mol2.gz
PDB	.pdb .ent .pdb.gz .ent.gz
MacroModel	.mmod .mmod.gz

-out `<filename prefix>`

This specifies a prefix for outputting the refinement dictionary **.cif** or **.xplor** and the **pdb** file. The pdb file (and pdb.oeb) is always output and should be used for purposes of refinement since WRITEDICT sometimes corrects faulty hydrogen atom names and will include LINK records in the pdb if covalent bonds are detected.

The log file will also be written using this prefix as *<filename prefix>.log*.

-prefix `<filename prefix>`

Alias for *-out*

6.4.5 Optional Parameters

-assignCCP4

If true then CCP4 atom types will be assigned to the output pdb file.

[default=true]

-includeRotors

Set to true if the output should include rotor torsion terms. If this is set to false, refinement programs will not be able to modify any rotors.

[default=true]

-suppressH

If true, no hydrogens are output.

[default=false]

-type

set this to 0 for **REFMAC5** refinement dictionaries or 1 for **CNS/CNX (XPLOR)**. By default **REFMAC5** dictionaries are generated.

[default=0]

-planarAniline

Use the MMFF94s variant of the MMFF94 forcefield. This forcefield emulates time-averages structures observed in crystallographic and other structure determination methods.

The MMFF94s forcefield is a variant of MMFF94 that emulates time-averages structures typically observed during crystallographic structure determination, mainly planar geometries at unstrained delocalized trigonal nitrogen centers. However, there are many theoretical studies that show puckering at nitrogen centers. (see MMFF VI. in JCICS)

That being said, due to the prevalence of crystallographic studies, many chemist erroneously consider the time-averaged structure to be correct, hence this variant is available for use.

[default = false]

-strict

Change the known residue matching mode.

Options available for residue matching is as follows:

Option	Meaning
exact	Known residue and input residue graphs must match exactly.
substructure	Input residue may be a substructure of the known residue. **Cannot be used with the exact flag.**
fuzzybonds	Bond orders do not need to match
atomname	Atom names must match

Options are separated by colons. The default settings are set to limit the number of REFMAC5 issues. -strict exact:substructure will find more matches, but increases the risk of REFMAC5 rejecting the dictionary.

[default = exact:fuzzybonds]

-lookup

Attempts to lookup residues from the internal dictionary store using the current -strict status. This is useful to find out if a residue has already been deposited in the RCSB.

If -lookup is not set, mismatching residues will be given new names.

-forceExistingResidueNames

Force WRITEDICT to use the given residues, even if they do not match the deposited residues from the RCSB. Also aliased to *-force*

This flag used to be called -nolookup (which still works) but this was found to be confusing in conjunction with the -lookup flag which does attempts to find the correct residue when there is no match.

-writeFullDict

If true then all refinement dictionaries are created for all residues. If false only residues that are unknown or are in the set of replaceable residues or covalently bound to a ligand are output. Setting this to true can confuse REFMAC5.

{default=false]

-verbose

Output copious information of how writedict operates.

CHAPTER

SEVEN

THEORY

Current methods of ligand fitting that are based on either topological analysis of electron density [AFITT-CL Menendez-2003]_ global optimization of position and conformation of a ligand in a density blob [AFITT-CL Diller-1999]_ interatomic distance matrix [AFITT-CL Koch-1974]_ [AFITT-CL Cascarano-1991]_ or on varying torsion dihedral angles of shape-matched ligand conformations [AFITT-CL Oldfield-2001]_ are unable to prevent creation of high energy, sometimes even chemically unrealistic, ligand models. As a result, there are a number of PDB ligands with unlikely, very high-energy structures [AFITT-CL Perola-2004]_. For example, the PDB structure of an inhibitor of RNA polymerase in *1nhu* has significant repulsion between the two methylene groups.

FLYNN is composed of two main components, location of ligand density and ligand fitting. Location of density generally works well with clean density but there are times when ligand density is unclear or not well resolved. In the latter cases, the user should supply a bounding box or simply just input the ligand density by itself and use the density as is.

By default, FLYNN samples bioactive conformations [AFITT-CL Bostrom-2002]_ [AFITT-CL Bostrom-2003]_ of the input ligand, however, there are times when it might be desirable to input the ligand and use it as is (see section *flynnbasicParameters* for more details).

Once the location of the ligand in the map and the conformations have been selected, FLYNN then adapts the initial conformations to the ligand density using a modern force-field, MMFF94 [AFITT-CL Halgren-I-1996]_ [AFITT-CL Halgren-II-1996]_ [AFITT-CL Halgren-III-1996]_ [AFITT-CL Halgren-IV-1996]_ [AFITT-CL Halgren-V-1996]_ [AFITT-CL Halgren-VI-1999]_ [AFITT-CL Halgren-VII-1999]_

The potential function being used to adapt the ligand is

$$V = V_{ff} + \lambda V_{shape}$$

where V_{ff} represents the internal energy of the ligand and V_{shape} is the overlap between the ligand and the electron density. λ is a mixing parameter that represents the degree to which the shape of the density dominates the combined potential during the current optimization step [AFITT-CL Wlodek-2006]_.

The strain placed on the ligand is bounded while the function is optimized producing high-quality fits with low-strain ligand conformations.

7.1 Fragment Fitting

Fragments are fit taking the input fragment cocktail and, one at a time, fitting each fragment against each region of detected density. Once a fragment has been placed, it is further analyzed to ensure that all possible orientations of the fragment have been sampled. In poor density, several orientations may fit equally well. To break ties, FLYNN scores each pose with the following scores:

o RSCC (real space correlation coefficient) This is a measure of fit to electron density (higher is better). [AFITT-CL Jones-1991]_

o PLP Piecewise-linear potential (lower is better). [AFITT-CL Verkhivker-2000]_

> o Chemscore (lower is better). [AFITT-CL Eldridge-1997]_

The docking scores are not used to fit the molecule, they are only used to rank the output. Unless highly symmetric molecules are being input, the real space correlation coefficient (RSCC) is the preferred method of ranking results to density.

To use fragment mode, please add the "-fragment" option to the command line. In future versions of FLYNN, this will most likely become the default setting.

The output of the fragment fitting process is a file for each density region that includes the fragments fit to the region sorted from best-fit to worst fit. For example:

```
prompt> flynn -in fragments.smi -out 2IKO_cocktail.sdf ...
```

would result in the files:

```
2IKO_cocktail_blob001.sdf
2IKO_cocktail_blob002.sdf
2IKO_cocktail_blob003.sdf
```

one for each blob found. By default, the first molecule in the file is the best fit to the density (RSCC):

> $RSCC = \frac{\Sigma|p_{obs} - <p_{obs}>|\Sigma|p_{calc} - <p_{calc}>|}{(\Sigma|p_{obs} - <p_{obs}>|^2 \Sigma|p_{calc} - <p_{calc}>|^2)^{\frac{1}{2}}}$ [AFITT-CL Jones-1991]_

where obs refers to the experimental electron density and calc refers to the calculated electron density sampled from grid points around the residue being scored. The experimental density is usually the sigma weighted difference map when loading an MTZ map. The calculated density is generated by simulating scattering of the ligand conformation with bfactors set to 20.

To sort using another measure, for instance, PLP, use the sort flag:

```
prompt> flynn -sortBy plp -in fragments.smi -out 2IKO_cocktail.sdf ...
```

Note: The *-sortBy* flag can be used in non-fragment mode as well.

CHAPTER

EIGHT

RELEASE NOTES

8.1 Release History

8.1.1 AFITT 2.5.1

Spring 2020

- This version of **AFITT** has been built using **OEToolkits 2020.0**.
- The **AFITT** GUI is not available as part of the 2020.0 release.

8.1.2 AFITT 2.5.0

Nov 2019

- This is the first inclusion of **AFITT** applications in the **OpenEye Applications** bundle. They have been built using **OEToolkits 2019.Oct**.
- The **AFITT** GUI is not available as part of the **2019.Nov** release.

8.1.3 AFITT-CL 2.4.1

March 2017

Platform Support

- Support has been added for Ubuntu 16, OSX 10.10, OSX 10.11, and OSX 10.12.
- OSX 10.7, OSX 10.8, OSX 10.9, SuSe 11, and Redhat 5 x86 are no longer supported.

Coot Support

- Support has been added for Coot 0.8.
- Coot versions 0.6 and earlier are no longer supported.

Bug Fixes

- A bug in WRITEDICT that caused inappropriate bond lengths to be written for some cyclic systems has been fixed. Originally, WRITEDICT outputted the MMFF bond potential minimum as the bond length. However, for aromatic rings this value is inappropriately large since MMFFs angle and torsion potentials also affect the bond length significantly. In these cases, the equilibrium bond lengths are used by WRITEDICT.
- A bug that caused PDB remark data that was more than 72 characters long to be clipped from the refined output has been fixed. Practically, this only occurred when the input and output file formats were both PDB.
- On Windows, the default "save" location has been changed from C:Program Files (x86)AFITT 2.4.0 to the Users Home directory.
- Previously, if an older version of AFITT was installed on Windows, users could not overwrite the old preferences. This issue has been fixed.
- A crash that occurred on Windows 10 after selecting the Refmac option without a locally installed copy of Refmac has been fixed.
- The AFITT suite ships with a simple Python-based do_gold.py test script, to make sure AFITT-CL (FLYNN) is functioning as intended. This script analyzes the Gold dataset (protein+ligand complexes) available from the Electron Density Server (EDS) and included with the distribution. The do_gold.py test script has been modified to run correctly with either Python 2 or Python 3.

8.1.4 AFITT-CL 2.4.0

November 2014

- Add helper programs to allow ligand forcefields during refinement for PHENIX and BUSTER
- Updated PHENIX support, including covalent bond support
- Alter the protein masking to not remove density that may be created by hydrogen or covalent bonds. This was causing density very close to the protein to be truncated and hard to find automatically.

Platform Support

- Added support for RedHat 7, Ubuntu 14 and OSX 10.9
- Dropped support for RedHat 5 32 bit, Ubuntu 10 and OSX 10.6
- This will be the last release for SuSe 11

FLYNN

- Alter the protein masking to not remove density that may be created by hydrogen or covalent bonds. This was causing density very close to the protein to be truncated and hard to find automatically.
- -box is now called -manualSearch (-box still works)
- -blobsThenBox is now called -autoThenManualSearch for clarity (-blobsThenBox still works)
- -ciftype now follows the new writedict convention (accepts both numeric and more readable names such as "refmac", "xplor")
- -ciftype parameter is now in the correct parameter section (Advanced not Fragment)
- FLYNN always fixes serial numbers when writing CIF output to better enable use in workflow situations. These can be caused when hydrogens are added to ligands outside the context of the protein for instance.

WRITEDICT

- AFITT now Post-processes equilibrium angles to see if they are compatible with geometric forcefields. If they are not, replace them with fully MMFF optimized angles. This mostly affects 6-5 aromatic systems, but is a general fix ensuring the geometric force-field will work properly given the constraints in the CIF dictionary.
- Writedict now properly fail if the input protein has duplicate serial numbers. Most refinement programs cannot handle this state.
- Prevent duplication of CRYST1 records in the writedict output.
- Always output disulfide bridges if input
- Never rename standard residues hydrogens, this was causing Markly naming violations.
- Fix phenix covalent link output when covalently linking two residues of the same residue type.
- WRITEDICT no longers fails if input standard residues have alternate conformations with different graphs.
- WRITEDICT now defaults to MMFF94s
- WRITEDICT now understands human readable names for the dictionary type, i.e. -type refmac as opposed to -type 0
- WRITEDICT now has a -fix_serial_numbers flag that corrects duplicate serial numbers in the resulting pdb output

ROTFIT

- Added rotfit rotamer driving command line program. This used to live entirely in the GUI.

8.1.5 AFITT-CL 2.3.0

July 2013

Other

Note: Last release for SUSE Linux Enterprise 10.

WRITEDICT

- Hydrogen naming is now consistent with RSCB standards.
- added -prefix flag for controlling report and logging generation

FLYNN

- Optimization fixes for molecules with high strain.
- Better default ligand density location.
- Optional PM3 and AM1 ligand minimization (see the -ff flag).
- Fixed symmetry operations on cubic lattices.
- added -prefix flag for controlling report and logging generation.

COOT Integration

- Better error message when the ligand and protein are set to the same coordinates
- Added ability to choose both chain id and residue name for the fit ligand.

8.1.6 AFITT-CL 2.2.0

August 2012

WRITEDICT

- Updated to support REFMAC 5.6 and 5.7 (CCP4 6.3.0)
- Monomers updated to current RCSB and CCP4 releases.
- PDB LINK records are validated to be in the correct order. This validation is required for REFMAC 5.6 and up.

FLYNN

- Bugfixes and general speedups applied to internal optimizer.
- A memory leak in the optimzer was fixed.

8.1.7 AFITT-CL 2.1.1

December 2011

WRITEDICT

- Added PHENIX suppoert
- Monomers in CIF files are given IUPAC names when possible
- Updated dictionaries to support REFMAC5 in CCP4 6.2.0

FLYNN

- Added PHENIX suppoert
- Fixed issues where ligands fit in poor density could be overstrained.

8.1.8 AFITT-CL 2.1.0

July 2011

WRITEDICT

- Updated internal REFMAC and RCSB to current versions.
- When remapping dictionaries, WRITEDICT now indicates where the matching monomer was sourced.
- Moved "lookup" out of the -strict settings to a new flag "-lookup" adding this switch will search for a matching monomer from the internal libraries using the current -strict settings.
- -force options added. WRITEDICT will output the residue name even if it doesn't match the internal list of known monomers.
- Various corrections to aniline nitrogen dictionaries.

FLYNN

- FLYNN now sorts the output fits based on real space correlation coefficient if applicable (RSCC)
- FLYNN now can automatically fit fragment cocktails. (use -fragment flag)
- FLYNN adds additional scores to all ligands besides the default MMFF Tanimoto/Shape, these are RSCC (Real space correlation coefficient), PLP (Piecewise-linear potential, requires protein) and Chemscore (requires protein).
- -resname now forces the ligand to use the given residue name even if it conflicts with the internal monomer library.
- -chainid Output the ligands with the given chain id.

8.1.9 AFITT-CL 2.0.1

September 2010

Package renamed from FLYNN to AFITT-CL. None of the internal programs have been renamed. AFITT and AFITT-CL will be released simultaneously with the same version numbers from this point forward.

WRITEDICT

- Added correct dictionary creation including connection table. This is required for newer versions of *coot*
- CIF dictionaries properly encode all LINK records.
- CIF dictionaries now encode proper planarity for guanine and no longer have duplicate planar atoms when using MMFF94s.
- CIF Dictionaries now look up known non-peptide residues. If the residue is not consistent with the known dictionary, the residue name is changed and a new MMFF94 derived dictionary is generated.
- Updated COOT integration to 0.6+

FLYNN

- Enhanced automatic density location. Blobs are now slightly larger than before making better targets for fitting.
- Added experimental fragment fitting. See the documentation for more information about this command line option. For version 2.0.1 this requires the fragment feature of the FLYNN license. This requirement will be removed when this feature is considered non-experimental. If you are interested in helping us test this feature, please contact business@eyesopen.com for more details.

- Refinement dictionaries are always written out for the ligand. Please note that these are written as if the -split flag is set to true. Refer to the documentation or type “flynn –help” at the command line for more details.
- Updated COOT integration to 0.6+

8.1.10 FLYNN 2.0.0

February 2010

- Internal Release used with collaborators. Used for testing new dictionary formats.

CHAPTER

NINE

BIBLIOGRAPHY

9.1 Bibliography

CHAPTER

TEN

CITATION

10.1 Citation

Note: To cite AFITT or FLYNN please use the following:

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