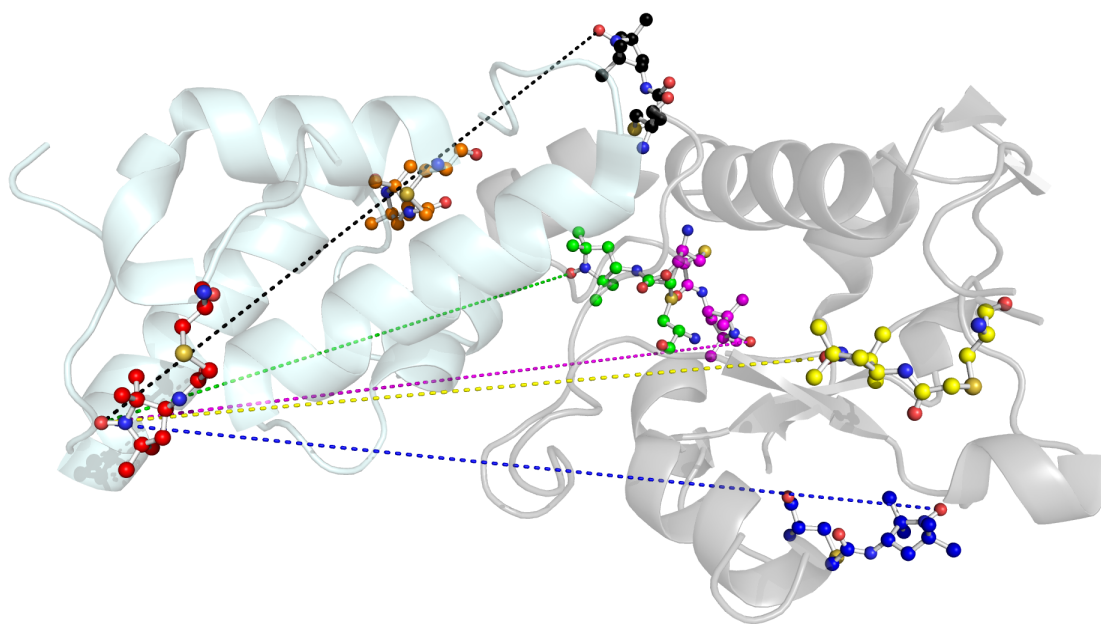# MISHAP

v13.06

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This document was created during study for the degree of Doctor of Philosophy whilst at the University of East Anglia, UK.

11th June 2013

"An expert is a man who has made all the mistakes which can be made, in a narrow field."

- Niels Bohr

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1 General

1.1 Overview

1.1.1 What is MISHAP?

MISHAP is the open-source MMM *in silico* simulated spin label to HADDOCK program. It combines several MatLab scripts, functions, programs and user interfaces as well as a set of additional parameter files for HADDOCK.

MISHAP aims to take spin labelled proteins and the distance distributions between labels from *in silico* (MMM) calculations or experimental data (from pulsed electron-electron double resonance experiments which have been analysed with DeerAnalysis) and pass them to the biomolecular docking program HADDOCK as docking restraints.

1.1.2 Functionality

MISHAP provides the ability to generate HADDOCK compatible PDB files of single chains of amino acids where one or more residues have been substiuted for nitroxide spin label attached cysteine residues.

MISHAP can generate distance based docking restraints from pulsed EPR experiments (PELDOR/DEER) when they have been analysed with DeerAnalysis or MMM.

The generated PDB files can be used in conjunction with or without the MISHAP generated distance based docking restraints to help determine the docking of protein complexes.

Currently, MISHAP only supports the generation of models and restraints in proteins. Though the open-source nature will allow for the expansion of MISHAP to accept non-nitroxide spin labels, DNA, RNA and other common ligands.

1.1.3 Interface

The user interface of MISHAP initially consists of a simple installer that installs MISHAP as well as modifying any existing installations of MMM and HADDOCK.

After installation the user interface largely consists of 2 windows. The first handles the distance restraints whilst the second handles the generation of HADDOCK compatible PDB files.

The changes to HADDOCK allow for the user to continue using HADDOCK normally via the command line with the addition of being able to use the residues R1A (MTSL attached cysteine) and IA1 (IA-PROXYL attached cysteine).

1.1.4 Help

MISHAP is designed to be as intuitive as possible whilst still maintaining user control. With this in mind, all options are clearly labelled as well as extensively documented in this document as well as online at morganbye.net/mishap

1.2 Getting started

1.2.1 Getting MISHAP

MISHAP is available online from 2 locations.

MISHAP general releases (for most users) should be downloaded from:

> https://sourceforge.net/projects/mishap/

MISHAP developer releases (constantly updated and should be considered unstable) can be obtained from the git repositary

> https://github.com/morganbye/MISHAP

Alternatively, you can create a new git folder and pull the repository directly from the command line with:

```
git pull https://github.com/morganbye/MISHAP.git
```

1.2.2 Installing MISHAP

If MISHAP was downloaded from sourceforge then it will come as a ZIP file which includes everything required. Simply unpack the ZIP file into a directory of your choosing. The folder should contain several text files including; `LICENSE.txt`, `README.txt` and `UPDATE.txt`. There will also be `MISHAP.m`; the main MatLab file and 2 directories: `test_files` containing some example files (referenced later) and _private which contains all the files associated with MISHAP.

The extracted folder needs to be added to the MatLab path with subfolders to the; this can be done be navigating to the folder in the *Current folder* view, right clicking and selecting

> *Add to Path → with Selected folders and Subfolders*

or by clicking from the main MatLab menu

> File → Set Path

in the main MatLab window, then click *Add folder with subfolders* then *Save* and *Close*.

The MISHAP installer then needs to be run. This is detailed in §2.2, but simply in the MatLab command window type

> `MISHAP`

This begins the installation of MISHAP.

1.3 Change history

1.3.1 Release v13.6 - BETA

v13.6 is the first publicly available release of MISHAP and marks the first build from alpha. It shows far greater error handling, Windows compatibility and an improved graphical user interface. MMM and MatLab programming is complete but significant errors still occur in HADDOCK/CNS.

1.3.2 Build v13.5 - ALPHA

v13.5 is the first functional build of MISHAP that generates distance distribution constraints and MMM rotamer merged PDBs.

2 User interface

2.1 MMM

MMM is an open-source program for the visualisation, inspection and improvement of models of proteins and protein assemblies based on restraints from multiple experimental techniques, though primarily focussing on experimental techniques from electron paramagnetic resonance.

2.1.1 Save rotamers from MMM

Perhaps MMM's most useful feature is the ability to calculate the rotamer populations of nitroxide spin labels attached to proteins.

For MISHAP we require these spin label attached protein models. To generate a rotamer model first start MMM from the MatLab command window with the command

```
MMM
```

After the splash screen, load a PDB model using the *File* menu - more information available in the MMM manual §2.1.1.
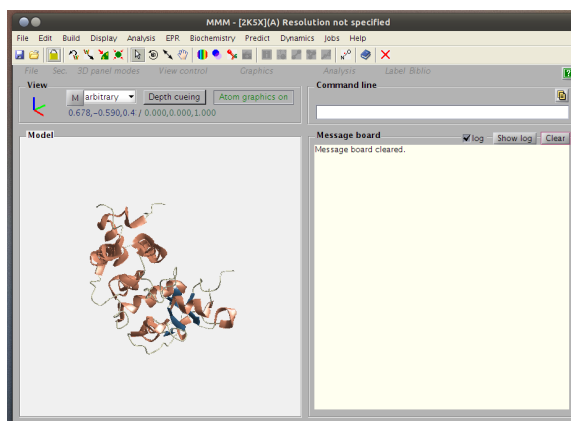
Figure 2.1 Loaded protein model in MMM

The protein model should be visualised in the Model panel whilst the Message board should update.
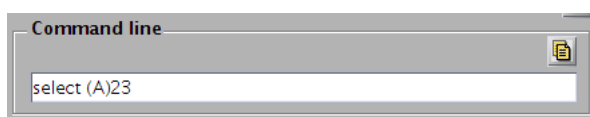
Figure 2.2 Select a residue in the command line

Next select a residue. To do this at the command line level type

```
select [structure](chain)residue
```

the strucutre option is not necessary depending upong the file (MMM §1.6). Residue selection can also be done graphically using:

> Display → Hierarchy (MMM §2.3 and 4.1.2)

However, the command line is superior as it allows the selection of multiple residues at once.

Figure 2.3 *Message board update showing the select residue(s)*

Once a residue is select the message board will update with the selection. If a selection is made incorrectly use:

```
unselect [structure](chain)residue
```

Once happy with the selection we label the selected residues with:

> EPR → Site scan/selected residues (MMM §2.1.7)
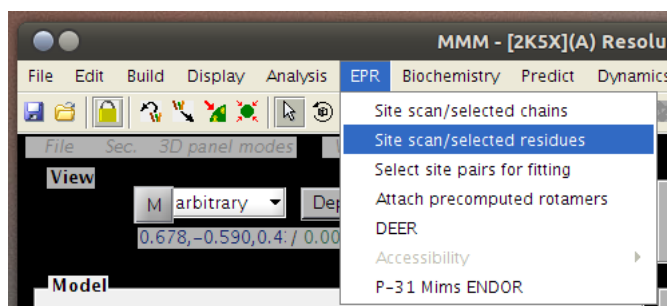
Figure 2.4 *MMM menu showing the site scan option*

This displays the Site scan setup window (MMM §4.1.2). It is imperative that *Save statistics* and *Save PDB rotamers* are selected.
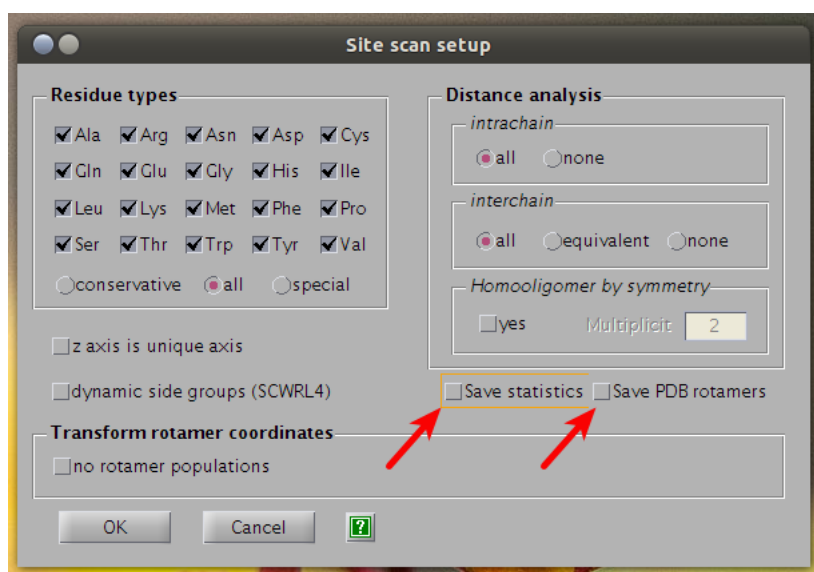
Figure 2.5 *MMM site scan setup window*
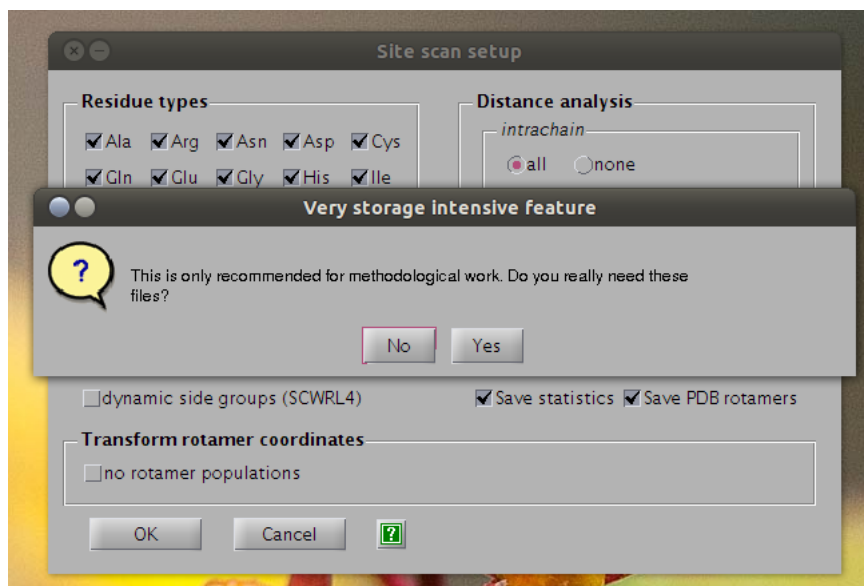
Figure 2.6 *MMM site scan warning dialogue*

Selecting *Save PDB rotamers* will display a warning message. Ensure that you click *Yes*.

Clicking *OK* will display the *Set labeling conditions for selected residues* window. Here select the appropiate nitroxide spin label and temperature.
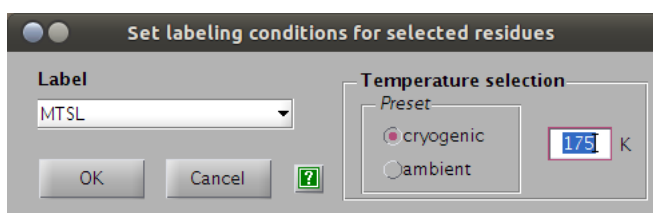
Figure 2.7 *MMM site scan labelling conditions window*

Finally a prompt appears to set the save path of the rotamer analysis files
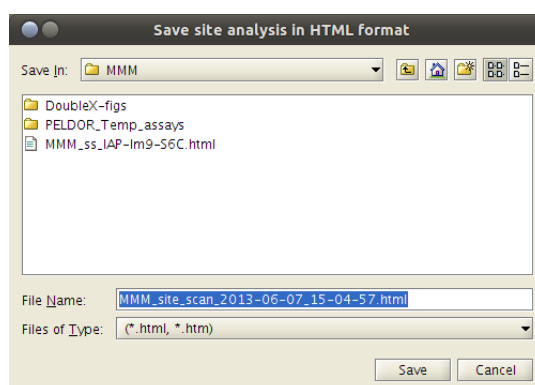
Figure 2.8 *MMM site scan save dialogue window*

The rotamer analysis can be tracked in the message board and once complete a prompt appears to ask if the user wishes to view the results in a browser.

2.2 Installer

Installation of MISHAP is kept as much as possible within the MatLab environment for simplicity.

To install MISHAP, extract the MISHAP zip folder. Add the folder with subfolders to the MatLab path; this can be done be navigating to the folder in the *Current folder* view right clicking and selecting

> *Add to Path → with Selected folders and Subfolders*

or by clicking from the main MatLab menu or by clicking

> File → Set Path

in the main MatLab window, then click *Add folder with subfolders* then *Save* and *Close*.

In the MatLab command window now type

> `MISHAP`

This begins the installation of MISHAP. The installer addresses 3 different programs in turn. First the installer looks to see if MISHAP has previously been installed, if so then the installer aborts and simply loads MISHAP.

If MISHAP has not previously been installed then the installation begins looking first to see if the user has MMM installed (line 36). For safety the installer backs up `MMM_prototype.m` and `MMM_prototype.fig` files to `/.../MMM_path/old_files` before copying across new versions of `MMM_prototype`, these versions allow you to access MISHAP from MMM.

> At a later date this versions will be submitted to MMM and this part of the installer will be removed.

Next the installer asks the user if HADDOCK will be run locally, if so then the local copy of HADDOCK requires some new parameter files. Again exisiting files are backed up first to `/.../HADDOCK_path/old_files` before the new ones are copied across.

> As HADDOCK is outside the MatLab environment a system search is required and this may take some time. As a safety feature, the search for HADDOCK will time out in 10 seconds and ask the user for the location instead.

The command window output from a typical installation should look something like:

```
 1                       __  __ _____  _____ _      _        _____
 2                      |  \/  |_   _|/ ____| |    | |      /\      |   __ \
 3                      | \  / | | | | (___ | |__| |    /  \    | |__) |
 4                      | |\/| | | |  \___ \|  __  |   / /\ \   |  ___/
 5                      | |  | |_| |_ ____) | |    | |/ ____ \| |
 6                      |_|  |_|_____|_____/|_|    |_/_/    \_\_|
 7
 8                                      by
 9                                          _                          _
10                                         | |                        | |
11       _ __ ___    ___   _ __ __ _ _    _ _  _ _ __  | |__   _   _   ___   _ __    ___| |_
12      | '_ ` _ \ / _ \| '__/ _` |/ _` | '_ \| '_ \| | | |/ _ \ | '_ \ / _ \ __|
13      | | | | | | (_) | | | (_| | (_| | | | | |_) | | |_| |  __/_| | | |  __/ |_
14      |_| |_| |_|\___/|_|  \__, |\__,_|_| |_|_.__/ \__, |\___(_)_| |_|\___|\__|
15                            __/ |                   __/ |
16                           |___/                   |___/
17
18
19      ===========================================
20      STARTING INSTALLATION
21      ===========================================
22
23      Installer version - 13.06
24      Build date        - 04th May 2013
25
26      This installer will install
27      MISHAP version    - 13.06
28      Release date      - 04th May 2013
29
30      System            - Linux - 64 bit
31
32      MatLab version    - 7.12.0.635 (R2011a)
33
34      ===========================================
35
36      Finding MMM installation...
37      MMM was located at:
38      /opt/matlab/MyScripts/MMM_2013
39
40      Backing up MMM files to:
41      /opt/matlab/MyScripts/MMM_2013/old_files
42
43      Files backed up!
44
45      Copying across new files...
46      Files successfully copied!
47
48      Installing...
49
50      Configuring...
51      MMM configured!
52
53      ===========================================
54
55      MISHAP parameters are not yet compatible with the HADDOCK webservers,
56      though this should change shortly, MISHAP must be run locally in the mean time
57
58      Are you going to be using HADDOCK locally? Y/N [Y]: y
59
60      Finding HADDOCK installation...
61      detection will abort if nothing is found in 10 seconds...
62      HADDOCK was located at:
63      /opt/haddock
64
65      Backing up HADDOCK files to:
```

```
66  /opt/haddock/old_files
67
68  Files backed up!
69
70  Copying across new files...
71  Files successfully copied!
72
73  Installing...
74
75  Configuring...
76  HADDOCK configured!
77
78  =============================================
79
80  Writing changes to MatLab...
81  MatLab configured!
82
83  =============================================
84
85  I hope you find MISHAP useful.
86
87  Documentation can be found at:
88  morganbye.net/mishap
89
90  Please address any feedback to:
91  morgan.bye@uea.ac.uk
92
93  =============================================
94  INSTALLATION COMPLETE
95  =============================================
```

With MISHAP installed, MISHAP can now be called from MMM using

Predict → Quaternary → HADDOCK → MISHAP
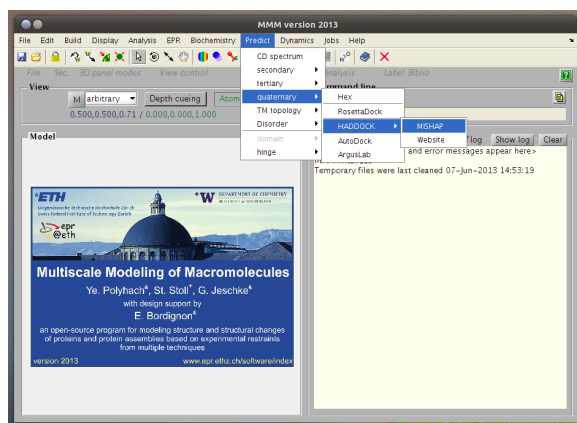
Figure 2.9 *MMM menu showing MISHAP*

Or without MMM from the command window with the command:

```
MISHAP
```

This will launch the primary MISHAP window, the distance distribution window.

2.3 MISHAP distance distribution window

2.3.1 Loading a distance distribution

Calling MISHAP will launch the primary MISHAP window, the distance distribution window.
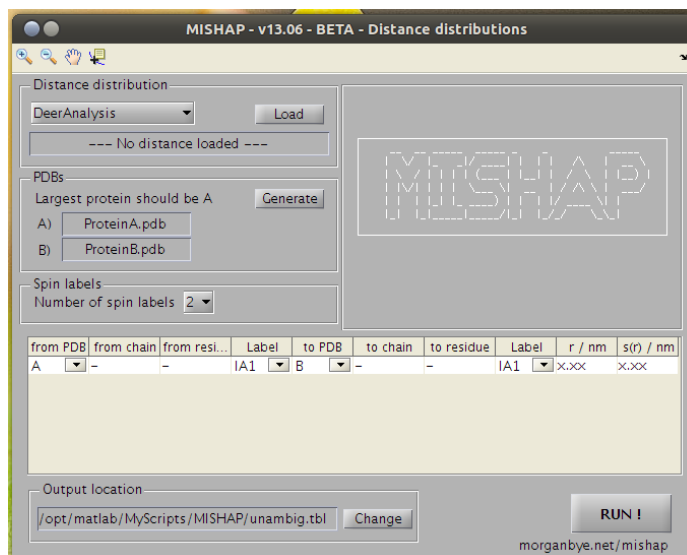
Figure 2.10 MISHAP distance distribution window

From the distance distribution panel select whether the distance distribtion is from Deer-Analysis or MMM and click *Load*.

If a DeerAnalysis or MMM window is open then MISHAP will try to pull the distance distribution information from the open window. If more than one is open then it will load whichever was opened first. If no window can be found then a user interface for the selection of a previously saved file is opened.
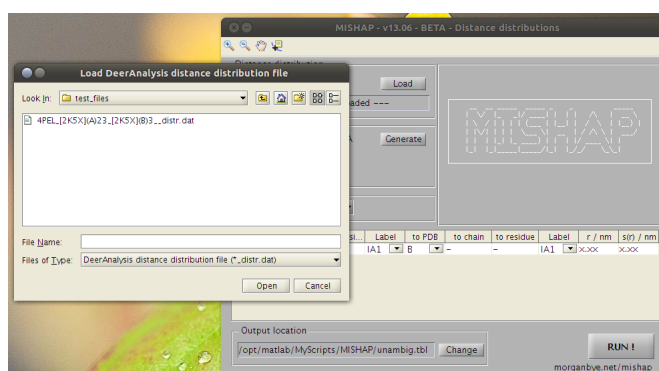
Figure 2.11 MISHAP distance distribution load interface

Once a file is selected MISHAP will automatically plot the distance distribution and search it for any peaks. Peaks are marked with circles. The largest peak is selected as the peak of interest and the peak location is updated into the *r* column of the table.

A *s(r)* of 0.5 nm is automatically set and the boundaries are plotted as about the distance as dotted lines in the same colour as the peak. At a later date the *s(r)* will be detected and calculated from the plot using a half height analysis of a Gaussian fit to the peak.

11
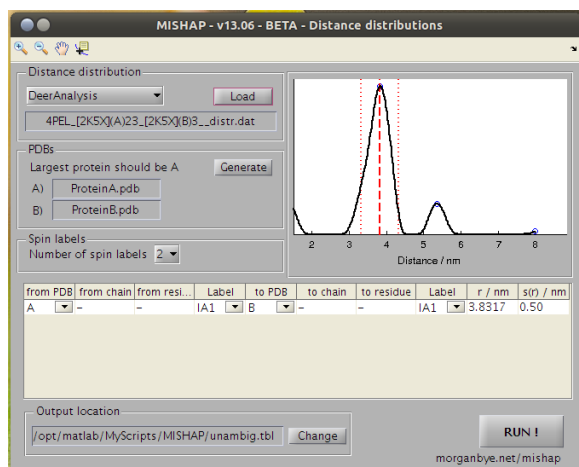
Figure 2.12 MISHAP distance distribution window showing a loaded distance distribution

2.3.2 Multiple spin systems

Currently MISHAP has support for systems with up to 4 nitroxide spin labels. By default it is set for only 2 spins (1 distance), selecting 3 or 4 spin labels adds more distances to the table (3 distances for 3 labels, 6 distances for 4 labels). The distance distribution plot displays each distance in a seperate coloured dashed line with the peak's *s(r)* dotted in the same colour.

2.3.3 Protein information

If the distance distribution tool is being used without the protein PDB creator then the table information needs to be completed with the appropiate information for the chain ID and residue number of the spin label in the PDB.

However, it is recommended to generate the PDBs for HADDOCK with MISHAP before proceeding further. To do so click *Generate* to launch the PDB Creator (see §2.4).

2.3.4 Output location

Once happy with the information displayed in the table finally check the output location box. This is the location and file name that the distance restraints file will be saved as.

By default the file name `unambig.tbl` will be used and the path will be that of the MatLab path when MISHAP was first opened.

If this is not suitable simply click *Change.*

2.3.5 RUN!

When the information in the table is correct and the output location is set click *RUN!*

All information is passed out to the MatLab command window. First the constraint generator checks that the table is complete, then it checks that it can write to the selected location. If

the folder does not exist or the user does not have permission to write to it then they will be prompted.

Finally the file is created. This process should be very fast even on a slow computer, ensure that *RUN!* is only pressed once.
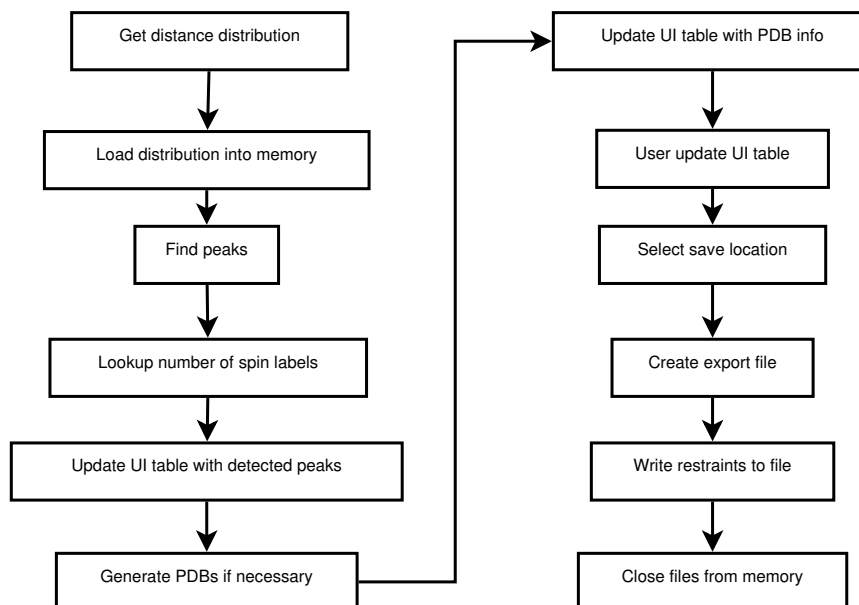
Figure 2.13 *Flowchart of the MISHAP distance distribution window process*

On the next page is an example output from the command window.

```
 1              __  __  _____   _____  _    _           _____
 2             |  \/  |_   _|/ ____| |  | |    /\    |  __ \
 3             | \  / | | | | (___  | |__| |  /  \   | |__) |
 4             | |\/| | | |  \___ \ |  __  | / /\ \  |  ___/
 5             | |  | |_| |_ ____) | |  | |/ ____ \| |
 6             |_|  |_|_____|_____/|_|  |_/_/    \_\_|
 7
 8      _____                  _              _      _
 9     / ____|                | |           (_)     | |
10    | |      ___   _ __   ___| |_ _ __ __ _ _ _ __ | |_
11    | |     / _ \| '_ \ / __| __| '__/ _` | | '_ \| __|
12    | |___| (_) | | | | \__ \ |_| | | (_| | | | | | |_
13     _____/|_| |_|___/\__|_|  \__,_|_|_| |_|\__|
14                                               _
15                                              | |
16                  __ _  ___ _ __   ___ _ __ __ _| |_ ___   _ __
17                 / _` |/ _ \ '_ \ / _ \ '__/ _` | __/ _ \ | '__|
18                | (_| |  __/ | | |  __/ | | (_| | || (_) | |
19                 \__, |\___|_| |_|\___|_|  \__,_|\__\___/|_|
20                  __/ |
21                 |___/
22
23    ==========================================
24    STARTING Distance Constraint Creation
25    ==========================================
26
27    MISHAP version    - 13.06
28    Release date      - 16th May 2013
29
30    System            - Linux - 64 bit
31
32    MatLab version    - 7.12.0.635 (R2011a)
33
34    ==========================================
35
36    Checking the data table...
37    Checking complete
38
39    Setting label variables...
40
41    Saving file...
42    File saved as
43    /opt/matlab/MyScripts/MISHAP/test_files/unambig.tbl
44
45    ==========================================
46
47    MISHAP - distance constraint generator completed in 0.00589 seconds
48    Thank you for using MISHAP
```

2.4 MISHAP PDB creator window

The MISHAP PDB creator window is launched from the MISHAP distance distribution window by clicking *Generate*.
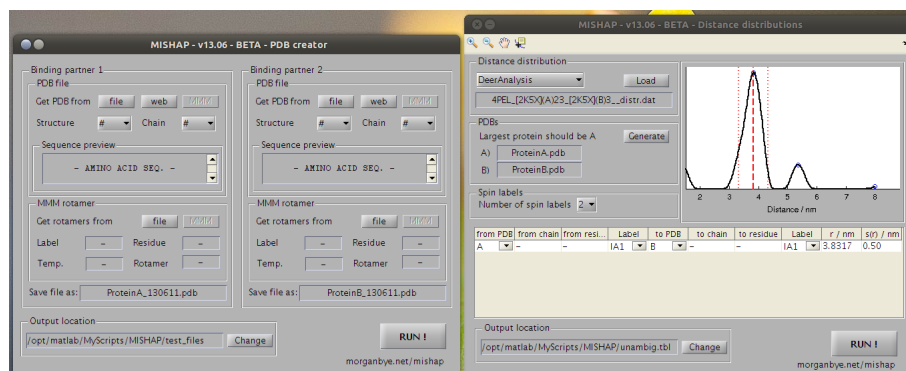

Figure 2.14 MISHAP distance distribution and PDB creator windows

The purpose of the PDB creator is 2 fold. First is to ensure that PDB files are generated that are compaitble with HADDOCK.

Unfortunately, the PDB format has first created in the 1970's and has been extensively developed, often without any strict enforcement of the PDB format meaning that it is quite common for PDB files generated in one program to not be compatible with a different PDB handling program. Previously MMM conformed well with the PDB format, version 2013 after a major rewrite of the PDB writing functions now conforms fully. HADDOCK will only accept PDB files in a very explicit format, which differs significantly from the PDB standard[1].

The second purpose of the PDB creator is to combine the PDB file supplied from a PDB file, directly from the PDB website using an access number or from a loaded MMM model with a MMM rotamer analysis.

MMM provides some basic functionality on this account but we require a single PDB file where an amino acid residue has been mutated to a spin label attached cysteine residue. Alternatively, a set of PDBs and a list file that can be inserted into HADDOCK as an ensemble of starting conformations.

2.4.1 Load a PDB file

For the first protein select the PDB file location. If the PDB is a local file click *file* this will open a user interface for selecting the file.

The PDB can also be obtained directly from rcsb.org in which case select *web* and enter the accession number when prompted.

If MMM is open then the current model can be used by clicking *MMM*.

There will be a slight delay as the file is processed. Once processed the *Structure* box will update with the PDB accession code, the *Chain* dropdown box will update with the chains found in the PDB and the sequence of the selected chain will be shown in the *Sequence preview*.

---

[1] for more information see http://www.nmr.chem.uu.nl/haddock/pdb.html
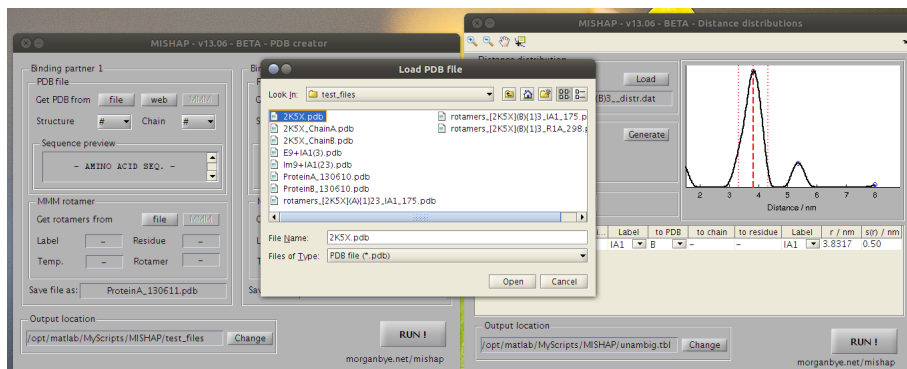
Figure 2.15 MISHAP PDB creator load PDB file selection
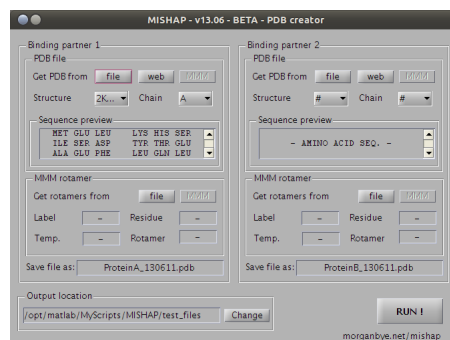
Figure 2.16 MISHAP PDB creator with loaded PDB file

2.4.2 Load a MMM rotamer file

For the first protein select the MMM rotamer file location. If the PDB is a local file saved during the MMM site scan then click *file* this will open a user interface for selecting the file.

In a later release, it will be possible to click *MMM* and pull the rotamer information from MMM.
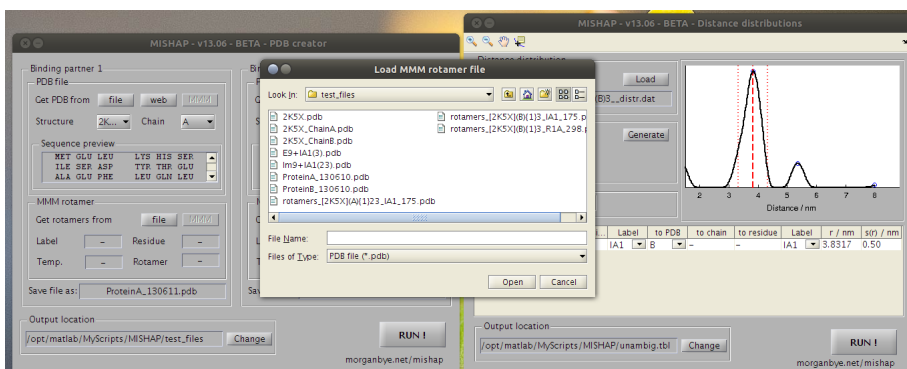
Figure 2.17 MISHAP PDB creator load MMM file selection

It will take a little time to process the rotamer information. Once processed the *Label* box will update with the 3 character amino acid residue code for the spin label, the *Temp.* box will update with the labeling temperature used in the site scan, the *Residue* box will update with the amino acid residue number of the labeling site and the *Rotamer* will update with the selected rotamer number from the MMM rotamer analysis.

By default, the *Rotamer* box will default to rotamer 1 though this can be changed to any rotamer. If a rotamer is selected that is outside the range of avaiable rotamers then the box

16
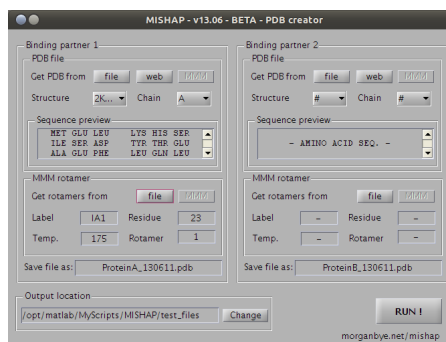
Figure 2.18 MISHAP PDB creator load with PDB and MMM file loaded

will automatically default back to rotamer 1. If an ensemble style rotamer analysis is desired type in this box:

all

2.4.3 Output location

By default the protein PDB will be saved as:

Protein[A/B]_[YYMMDD].pdb

These can be changed freely with any operating system valid name. Do not include *.pdb* in the file name, otherwise you will generate a file called `ProteinA_YYMMDD.pdb.pdb`

The output path is defined in the *Output location* box, the path will be that of the MatLab path when MISHAP was first opened.

If this is not suitable simply click *Change.*

2.4.4 RUN!

When happy with the settings for *Binding Partner 1* the PDB creator can be run or a PDB and MMM rotamer analysis can also be loaded for *Binding Partner 2.*
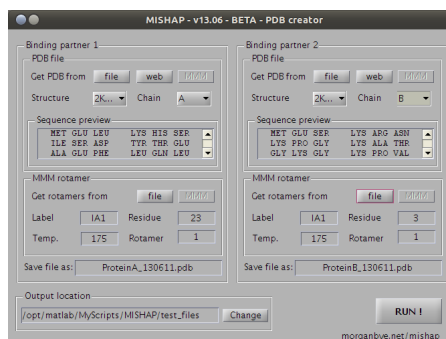
Figure 2.19 MISHAP PDB creator showing 2 proteins loaded with MMM rotamer files

When all the information is loaded and set click *RUN!*

All information is passed out to the MatLab command window. *Binding Partner 1* is addressed before *Binding Partner 2* but in both the creator checks that a PDB and MMM rotamer analysis is loaded, then it checks that it can write to the selected output location. If

17

the folder does not exist or the user does not have permission to write to it then they will be prompted.

Finally the creator merges the selected MMM rotamer(s) with the PDB file is created.

Briefly, the creator essentially splits the PDB file before and after the select residue. It then pastes in the rotamer residue and merges the residues before the residue, the rotamer residue and residies after the rotamer residue with appropiate linkers. All hydrogen atoms are then removed as these are prone to causing problems in HADDOCK and CNS before finally renumbering every atom in the merged PDB so that read sequentially. The file is finally written out line by line.
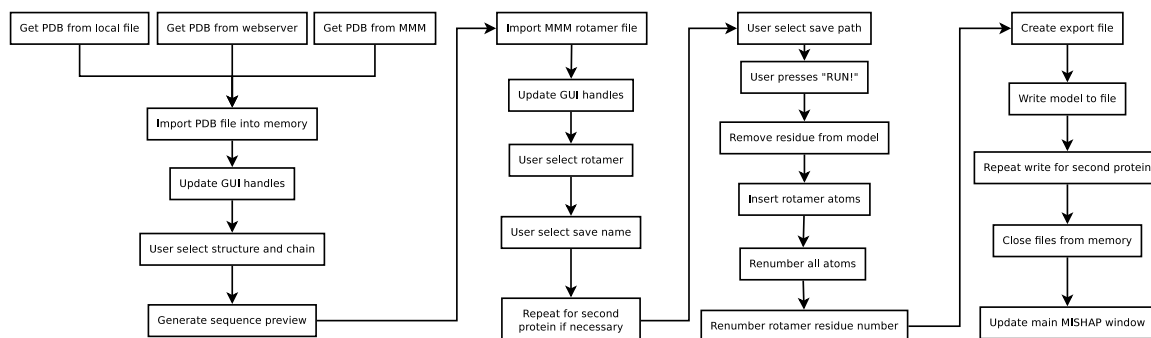
Figure 2.20 *Flowchart of PDB handling within the MISHAP PDB creator*

On the next page is an example output from the command window.

```
 1                     __  __ _____  _____ _    _      _____
 2                    |  \/  |_   _|/ ____| |  | |    /\    |  __ \
 3                    | \  / | | | | (___ | |__| |   /  \   | |__) |
 4                    | |\/| | | |  \___ \|  __  |  / /\ \  |  ___/
 5                    | |  | |_| |_ ____) | |  | | / ____ \| |
 6                    |_|  |_|_____|_____/|_|  |_|/_/    \_\_|
 7
 8        _____  _____  ____     _____                        _
 9       |  __ \|  __ \|  _ \   / ____|                      | |
10       | |__) | |  | | |_) | | |        _ __ ___  __ _| |_ ___  _ __
11       |  ___/| |  | |  _ <  | |       | '__/ _ \/ _` | __/ _ \| '__|
12       | |    | |__| | |_) | | |____   | | |  __/ (_| | || (_) | |
13       |_|    |_____/|____/   \_____|_| \___|\__,_|\__\___/|_|
14
15    ===========================================
16    STARTING PDB Creation
17    ===========================================
18
19    MISHAP version    - 13.06
20    Release date      - 15th May 2013
21
22    System            - Linux - 64 bit
23
24    MatLab version    - 7.12.0.635 (R2011a)
25
26    ===========================================
27    Binding partner 1
28    ===========================================
29
30    Finding the selected PDB...
31    Loading file...
32    /opt/matlab/MyScripts/MISHAP/test_files/2K5X.pdb...
33    Using structure 2K5X  and chain A
34    PDB loaded!
35
36    Finding the selected MMM rotamer...
37    Loading file...
38    /opt/matlab/MyScripts/MISHAP/test_files/rotamers_[2K5X](A){1}23_IA1_175.pdb
39
40    Rotamers loaded!
41
42    Setting labelling conditions:
43    Label               - IA1
44    Temperature         - 175 K
45    Attaching to residue  - 23
46    Using rotamer       - 1
47
48    Adding rotamer to PDB...
49    Rotamer successfully added!
50
51    Saving file...
52    File saved as
53    /opt/matlab/MyScripts/MISHAP/test_files/ProteinA_130610.pdb
54
55    ===========================================
56    Binding partner 2
57    ===========================================
```

```
58
59  Finding the selected PDB!
60  Loading file...
61  /opt/matlab/MyScripts/MISHAP/test_files/2K5X.pdb
62  Using structure 2K5X  and chain B
63  PDB loaded!
64
65  Finding the selected MMM rotamer
66  Loading file...
67  /opt/matlab/MyScripts/MISHAP/test_files/rotamers_[2K5X](B){1}3_IA1_175.pdb
68  Rotamers loaded!
69
70  Setting labelling conditions:
71  Label                 - IA1
72  Temperature           - 175 K
73  Attaching to residue  - 3
74  Using rotamer         - 1
75
76  Attaching rotamer to PDB...
77  Rotamer successfully added!
78
79  Saving file...
80  File saved as
81  /opt/matlab/MyScripts/MISHAP/test_files/ProteinB_130610.pdb
82
83  ==============================================
84
85  MISHAP - PDB Creator completed in 0.79016 seconds
86  Thank you for using MISHAP
```

3 HADDOCK interface

HADDOCK (the high ambiguity data driven biomolecular docking program) is an approach that makes use of biochemical and biophysical interaction data such as chemical shift perturbations from NMR titration experiments, mutagenesis data or bioinformatic predictions to study protein complexes.

Traditionally most approaches to protein complex formation relied upon a combination of energetics and shape complementarity rather than experimental data. The previously mentioned experiments are presented to the HADDOCK program as ambiguous interaction restraints (AIRs) to drive the docking process. More formally these are defined as an ambiguous distance between all residues shown to be involved in the interaction.

Since the original 2003 JACS publication HADDOCK has been extended to deal with a large varity of data and complexes including protein-DNA, protein-RNA, protein-oligosaccharides and protein-ligand complexes. HADDOCK has consistently performed well in the blind protein-protein complex CAPRI competitions[1].

HADDOCK remains a command line tool requiring the generation and configuration of many files before a run can commence. HADDOCK consists primarily as a collection of python scripts derived from ARIA written by Michael Nilges and Jens Linge and makes use of CNS as structure calculation software.

3.1 Spin label definitions

3.2 Protocols

---

[1]http://capri.ebi.ac.uk

4 Other tools

4.1 Naccess

Naccess is a program that calculates the exposed surface area of a protein by running a simulated sphere across the surface of a protein. By default the sphere radius is set to 1.4 $\mathring{A} \approx$ the size of a water molecule.

Within the `MISHAP/_private` folder is the `NACCESS` folder, within this folder is a special `MISHAP.radii` file which allows Naccess to calculate the exposed surface area of a protein which includes the residues R1A/CYM and IA1.

4.2 HADDOCK processing tools

Included is a MatLab script, `HADDOCK_CLUSTERS_PROCESSING.m`, for the processing of HADDOCK clusters. It allows the user to select a cluster or to operate on all the final structures and present them as a figure plotting HADDOCK score against any of the HADDOCK parameters (bsa, dH, Edesolv, rmsd, rmsd-Emin (default), ener (by default this is Einter), ener-Einter, ener-Enb, ener-Evdw, ener-Eelec, ener-Eair, ener-Ecdih, ener-Ecoup, ener-Esani, ener-Evean, ener-Edani)
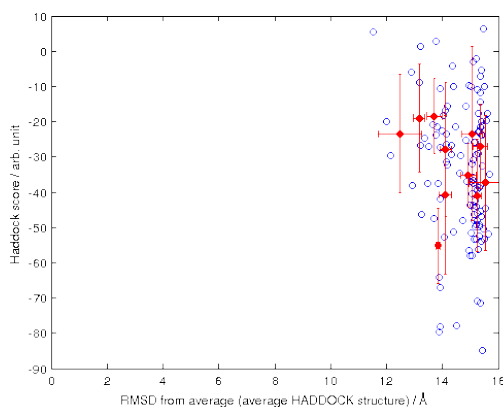
Figure 4.1 *A typical figure from the* `HADDOCK_CLUSTERS_PROCESSING` *script showing HADDOCK score plotted against the RMSD of the structure from the average structure, with structures as blue circles and cluster averages as red filled circles.*

5 Postface

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5.2 Third-party software

5.2.1 Software within MISHAP

MISHAP makes use of the following freely available scripts from MatLab Central

Peak detection

fpeak.m by Geng Jun, 2003

Wrapping of strings

linewrap.m by Steve Eddins, 2006

5.2.2 MMM

MMM (Multiscale Modeling of Macromolecular systems) is an open-source program for the visualization, inspection, and improvement of models of proteins and protein assemblies based on restraints from multiple experimental techniques. Authored by Yevhen Polyhach and Gunnar Jeschke, with design support by Enrica Bordignon from ETH Zurich, Switzerland with additional development by Stefan Stoll, University of Washington, USA.

Available from: http://www.epr.ethz.ch/software

5.2.3 HADDOCK

HADDOCK (High Ambiguity Driven biomolecular DOCKing) is an information-driven flexible docking approach for the modeling of biomolecular complexes. HADDOCK distinguishes

itself from ab-initio docking methods in the fact that it encodes information from identified or predicted protein interfaces in ambiguous interaction restraints (AIRs) to drive the docking process. Authored by Alexandre Bonvin, Utrecht University, Netherlands with support from João Rodrigues.

Available from: http://www.nmr.chem.uu.nl/haddock/

With public webservers available at: http://haddock.science.uu.nl/

5.3 Acknowledgements

Morgan Bye - primary software writer, testing, documentation

Fraser MacMillan - head of Henry Wellcome Unit for Biological EPR

5.4 Hardware and computation time

The vast majority of testing of MISHAP has been conducted upon a 64-bit Linux system (Ubuntu 12.04 LTS), MatLab R2011a.

This system comprises an Intel Core 2 processor @ $2 \times 2.40\,GHz$ with $4\,GB$ of RAM.

With this system generation of a distance restraint file is ~5 ms, PDB file load time is ~3 s (for a ~25 kDa protein with ~2500 atoms), MMM rotamer file load time is ~3 s, the PDB creator running 2 proteins (~ 15 kDa) is ~1 s.

These timings will not vary much with newer machines as MatLab by default will not parallelise tasks. If your version of MatLab is set up to use parrallelisation these times will drop significantly.

A HADDOCK docking run with this setup takes ~4 - 16 h. It is highly recommended to perform the HADDOCK tasks on a computing cluster or server. With a 16 core Intel Xeon server with 16 GB of RAM the HADDOCK runs drop to ~20 min.

The MISHAP MatLab interface has been briefly tested upon a Windows XP machine (MatLab 2010a) and is shown to be functional. Though with the specifications of this machine (Intel Celeron @ $1 \times 2.00\,GHz$ with 1 GB of RAM) loading of PDB files can take as long as 10 s.

5.5 References

S.J. de Vries, M. van Dijk and A.M.J.J. Bonvin "The HADDOCK web server for data-driven biomolecular docking." Nature Protocols, 5, 883-897 (2010).

T.A. Wassenaar, M. van Dijk, N. Loureiro-Ferreira, G. van der Schot, S.J. de Vries, C. Schmitz, J. van der Zwan, R. Boelens, A. Giachetti, L. Ferella, A. Rosato, I. Bertini, T. Herrmann, H.R.A. Jonker, A. Bagaria, V. Jaravine, P. Guntert, H. Schwalbe, W.F. Vranken, J.F. Doreleijers, G. Vriend, G.W. Vuister, D. Franke, A. Kikhney, D.I. Svergun, R. Fogh, J. Ionides, E.D. Laue, C. Spronk, S. Jurka, M. Verlato, S. Badoer, S. Dal Pra, M. Mazzucato, E. Frizziero and A.M.J.J. Bonvin "WeNMR: Structural Biology on the Grid." J. Grid. Comp., 10, 743-767 (2012).