



Mestrelab Research

# Mnova StereoFitter 1.1.6

---

USER MANUAL



Document Number P/N 301 R8

## COPYRIGHT

©2024 MESTRELAB RESEARCH S.L.

All rights reserved. No parts of this work may be reproduced in any form or by any means - graphic, electronic, or mechanical, including photocopying, recording, taping, or information storage and retrieval systems - without the written permission of the publisher.

Products that are referred to in this document may be either trademarks and/or registered trademarks of the respective owners. The publisher and the author make no claim to these trademarks.

While every precaution has been taken in the preparation of this document, the publisher and the author assume no responsibility for errors or omissions, or for damages resulting from the use of information contained in this document or from the use of programs and source code that may accompany it. In no event shall the publisher and the author be liable for any loss of profit or any other commercial damage caused or alleged to have been caused directly or indirectly by this document.

## TABLE OF CONTENTS

<b>1. STEREOFITTER .....</b>	<b>3</b>
1.1. TECHNICAL BACKGROUND CONSIDERATIONS .....	3
1.1.1. <i>Conformational deconvolution of NMR properties</i> .....	3
1.1.2. <i>Model selection</i> .....	5
1.2. STEREOFITTER RIBBON .....	7
1.2.1. <i>Load section</i> .....	9
1.2.2. <i>3D Structure section</i> .....	10
1.2.3. <i>3D Correlations section</i> .....	28
1.2.4. <i>StereoFitter section</i> .....	30
1.2.5. <i>DFT Prediction section</i> .....	31
1.3. CONFORMATIONAL ANALYSIS: USING NOES TO DETERMINE 3D STRUCTURE FROM A SET OF CONFORMERS.....	36
1.4. CONFIGURATIONAL ANALYSIS: USING RDCs AND PREDICTED CHEMICAL SHIFTS TO DETERMINE 3D STRUCTURE FROM A SET OF STEREOISOMERS .....	49
1.5. DATA BLOCKS AND KEYWORDS.....	67
1.5.1. <i>Fitting to NOESY/ROESY determined distances</i> .....	67
1.5.2. <i>Fitting of scalar J Couplings</i> .....	69
1.5.3. <i>Fitting of chemical shifts</i> .....	72
1.5.4. <i>Fitting of RDCs, RCSAs and PCSs</i> .....	75
1.5.5. <i>Keywords</i> .....	77
1.6. APPENDIX. EMPIRICAL EQUATIONS AND PARAMETERS.....	90
1.7. REFERENCES.....	94
1.8. KEYWORDS .....	98

## 1. StereoFitter

StereoFitter is a multiplatform software tool for the fitting of conformational amplitudes to different NMR observables. It computes the probability of 3D structural configurations and/or conformations, to match various forms of experimental data inputs. Currently, StereoFitter can accept and combine six distinct types of NMR parameters in order to calculate the best 3D structure candidate(s): NOE based distances,  $J$ -couplings, chemical shifts, and chemical shifts, anisotropic data such as Residual Dipolar Couplings (RDCs), Residual Chemical Shift Anisotropies (RCSAs), and pseudocontact shifts (PCSs) obtained in systems with paramagnetic tags.

Information can be supplied from one or more of the techniques in parallel, and it is this potential for combining information from different types of parameters in one calculation that makes StereoFitter uniquely powerful. Predicted NMR observables of configurational/conformational models are compared to experimental ones, each given a score, and results presented in order of best fit.

StereoFitter starts with a set of possible 3D configurations and/or conformations of a molecule. These can be imported from external programs, or generated within StereoFitter using built-in 3D coordinates generation (Balloon) and conformational search (GMMX) utilities. All possible relative configurations can be obtained within StereoFitter from an initial 2D structure if the stereochemical configuration of any chiral centers are not known. Various formats of external structure file formats are compatible: SDF; XYZ; MAE, outputs from quantum chemistry programs (Gaussian09/16, Orca, Jaguar, NwChem, ReSpec) and others. 3D structures can be viewed, manipulated, and their properties measured within StereoFitter using an interactive OpenGL based molecular visor.

Chemical shifts can be used within StereoFitter using results from DFT calculations, either importing users' own computational log files or by using MestReLab computational servers. It is also possible to use DFT computations for the analysis of  $J$ -coupling data in a similar way.

### 1.1. Technical background considerations

#### 1.1.1. Conformational deconvolution of NMR properties

Under fast exchange in solution NMR properties are observed as time-averaged values.<sup>1</sup> In the general case a property  $\langle T \rangle$ , such as chemical shifts, scalar couplings, NOE/ROE integrals or volumes is the result of instantaneous contributions from all the visited region of the potential surface

$$\langle T \rangle = \int w(\mathbf{R}) T(\mathbf{R}) d(\mathbf{R}) \quad (1)$$

where  $\mathbf{R}$  is a vector describing the  $3N$  coordinates of the molecule,  $w(\mathbf{R})$  the weight or probability of point  $\mathbf{R}$  in the phase space and  $T(\mathbf{R})$  the instantaneous value of the property  $T$  at the point  $\mathbf{R}$ .

However, if the basins of the potential surface are deep enough the continuous movement, i.e., the molecular dynamics, of the molecule over the potential surface can be approximated as a discrete collection of instantaneous jumps between the minima in the potential surface and therefore we can approximate the continuous integral in (1) as a summation of terms.

$$T = \hat{A} w_i T_i \quad (2)$$

where  $w_i$  is the population of each visited discrete conformation and  $T_i$  the value of the considered property for the  $i$ -th conformation.

Hence for a particular set of properties  $\{T\}$ , a set which can involve different type of observables such as chemical shifts, scalar couplings, NOE intensities, or anisotropic properties such as residual dipolar couplings or residual chemical shift anisotropies. we can try to determine the individual contributions of each conformation by fitting the data to individual  $T_i$  values obtained through different empirically calibrated or ab initio procedures. I.e., we will deconvolute the spectrum into a series of discrete conformation contributions.

Generally, the set of discrete conformations can be generated using appropriate molecular modeling conformational search techniques such as different Monte Carlo methodologies, simulated annealing procedures, etc. When generating this initial ensemble of conformations we must ensure that the topology and generated geometries are sufficiently accurate. For organic compounds with common functional groups, small-molecule oriented force fields as MMFF94,<sup>2</sup> OPLS3<sup>3,4</sup> or others can provide a sufficient degree of accuracy. This initial ensemble can be employed as it is, but in some circumstances it can be desirable to refine the geometries at a higher level of theory such as modern DFT methods. There are nowadays many excellent introductory books on the subject as well as detailed protocols for the application of isotropic<sup>5-7</sup> and anisotropic data<sup>8</sup> to three-dimensional structural elucidation problems.

If the individual contributions  $T_i^j$  can be predicted for each  $j$ -th conformation the averaged values  $T_i^{calc}$  can be computed as  $T_i^{calc} = \hat{A} w_j T_i^j$ . The agreement between the so-computed values can then be expressed as a sum of quadratic terms.

$$C^2 = \frac{T_i^{exp} - T_i^{calc}}{S_i^2} \quad (3)$$

Where  $\chi^2$  is the associated uncertainty, as a standard error, to the property  $T_i$ . Minimization of  $\chi^2$  respect to the population weights  $w_i$  will provide then a set of best fitting populations.

StereoFitter may currently handle different types of NMR properties and restraints:

- Chemical Shifts  $\delta$  and chemical shifts differences  $\Delta \delta$
- Scalar couplings  $J$  and scalar couplings among groups of not-fully assigned spins ( $J$ -groups)
- Residual Dipolar Couplings (RDCs)  $D$
- Residual Chemical Shift Anisotropies (RCSAs)

- Pseudo contact shifts (PCSs)
- Internuclear distances  $d$  derived from quantitative NOE experiments.

Hence equation (3) can be expressed as

$$C^2 = \sum_{i=1}^{N_d} \left( \frac{d_i^{exp} - d_i^{calc}}{S_d^i} \right)^2 + \sum_{i=1}^{N_J} \left( \frac{J_i^{exp} - J_i^{calc}}{S_J^i} \right)^2 + \sum_{i=1}^{N_d} \left( \frac{d_i^{exp} - d_i^{calc}}{S_d^i} \right)^2 + \dots \quad (4)$$

For instance,  $^1D_{CH}$  RDCs measured in polymeric gels may be attributed a common uncertainty of 1.2 Hz, whereas we can associate to the  $^3J_{HH}$  couplings, predicted with the Haasnoot-Altona Karplus-type equations,<sup>9</sup> an uncertainty of 0.5 Hz. It is very important to note that *changing the uncertainties  $\sigma$  will change the relative weight of the different NMR properties to the fitting.*

A very simple but practical way to estimate the uncertainties  $\sigma$  will be to fit a particular kind of property  $T$  in a known rigid molecule of known molecular structure.<sup>10</sup> Polycyclic molecules such as camphor,  $\beta$ -pinene, strychnine etc. are good targets for this purpose. Once the desired properties have been measured, they can be fit inside StereoFitter. The RMSD between the experimental and computed properties can be used as your uncertainty  $\sigma_T$  to be applied in your particular structural problem

### 1.1.2. Model selection

Like older NMR property conformational deconvolution methods such as NAMFIS,<sup>1</sup> StereoFitter relies in the use of a set of conformations obtained through molecular modelling. I. e., the “jump” approximation is applied where whole molecular dynamics is approximated as a series of instantaneous jumps between discrete conformations and vibrational effects are neglected. This set of conformations is usually obtained through molecular mechanics conformational searches procedures and in some cases can be later refined through DFT optimizations. A key parameter is therefore how many conformations are stored in the ensemble. It is essential that conformations that are present in the experimental “true” ensemble are not missed in the modelled ensemble. Commonly, a  $\sim 5$  kcal/mol window has been recommend in published protocols using the MMFF94 force field.<sup>5</sup> However in molecules which could potentially present hydrogen bonds the intramolecularly bonded conformers are usually over-stabilized by the computational methods and a larger cutoff, up to 10 kcal/mol might be necessary.<sup>11-13</sup>

Since in many cases very large conformer ensembles can be obtained in this way a useful strategy can be to use a second cutoff where DFT energies are used to define a second more strict energy-threshold. The use of functionals which include dispersion effects, either built-in (M062X<sup>14</sup> and many others) or empirical approaches as for instance the popular D3BJ<sup>15</sup> method as well as the use of implicit solvation<sup>16</sup> can be beneficial.

After filtering structures using the MM/DFT cutoffs, StereoFitter will ignore, by default, computed energies and conformational populations will be obtained by fitting to the experimental data. If desired however they can be read from and used in the fitting.

In order to avoid overfitting of the data StereoFitter will perform a model selection using the well-known Akaike Information Criterion (AIC)<sup>17</sup> The AIC is a statistical parameter which tries to find the most statistically parsimonious (“simplest”) model which still explains the data according to the data uncertainties. This criterion has a very simple form

$$\text{AIC} = c^2 + 2k \quad (5)$$

Where  $k$  is the number of independent parameters in the model. In our case  $k$  is just the number of conformations in the model,  $m$ , minus one. I.e.,

$$\text{AIC} = c^2 + 2(m - 1) \quad (6)$$

A well-known criterion in the structural analysis of biomolecules, the AIC has been applied in several occasions to the analysis of RDC data in small organic molecules.<sup>18-20</sup>

To avoid astronomical computation times the AIC is computed for models of increasing number of conformations until a minimum, respect to the number of conformations is found. If the number of conformations is increased and all the ensembles furnish a higher AIC the computation is stopped and the model with the lowest AIC is printed as the solution to the structural problem. The solution with the lowest AIC is the simplest, statically most parsimonious, model that fits the data according to experimental uncertainties. This strategy has been applied to the analysis of anisotropic data (RDCs)<sup>21</sup> as well as multiparametric RDC/chemical-shift computations.<sup>10</sup>

The first step in using the StereoFitter module is to load a conformational ensemble either by using the built-in molecular modelling tools (GMMX) or loading the conformations from the corresponding files in external computations. Once the conformations are read the user must load an input file. The format of this file is very similar to that used in the RDC module. Each field in the input file follows the format,

```
keyword {  
}
```

comments should be preceded of the # sign

```
keyword {  
#this is a comment  
}
```

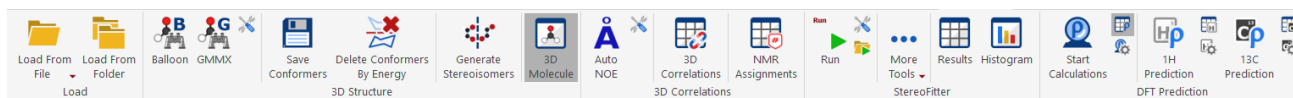
Note that the input file is not case-sensitive.

The following block names for input data are recognized in the **Fitter** module

- noedistance\_data
- jcoupling\_data
- jcouplinggroup\_data
- rdc\_data
- rcsa\_data
- pcs\_data
- chemicalshift\_data
- chemicalshiftdelta\_data

## 1.2. StereoFitter Ribbon

This section gives a brief overview of the tools available in StereoFitter. A license is required to activate the ribbon. Once activated a new menu will appear, inside which are a collection of tools and features that allow the user to follow workflows to determine 3D structure.



Above is a screenshot of the StereoFitter ribbon. What follows is a brief introduction to each of the items on this ribbon

### Load section

The Load section of the ribbon contains two icons:

- Load From File
- Load From Folder

### 3D Structure section

The 3D Structure section of the ribbon contains various tools that handle chemical structures used for the 3D calculations:

- Balloon & GMMX: A set of conformers for any imported 2D configuration can be generated using either Balloon or GMMX programs. More information can be found in 'Using nOes to determine 3D structure' or 'Using RDCs to determine 3D structure' workflows.
- Save Conformers: Conformers generated from Balloon or GMMX within StereoFitter can be saved to disk, possibly for further manipulation by external programs.
- Generate Stereoisomers: All possible and plausible stereoisomers can be generated from a 2D structure. Note that NMR cannot generally distinguish between enantiomers, so it is necessary to either delete half of the resulting



isomers to avoid redundancy in any 3D calculations further downstream or (recommended) start with a molecule with a stereogenic center of known configuration

- Delete Conformers by energy: to delete conformers with energies exceeding the minimum energy conformer plus a delta value.
- 3D Molecule: Toggles the display of the 3D Molecule popup window. More details about the functionality present in the 3D Molecule window can be found [here](#).

### 3D Correlations section

The 3D Correlations section of the ribbon contains tools that interact with NMR spectra and back-calculations of NOE distances:

- Auto NOE: When nOes are being used to generate 3D structure(s), it is necessary to back-calculate the theoretical distances from nOe volumes. The 'Auto NOE' button will carry out those calculations and place the results in the Assignments Table.
- 3D Correlations: Toggles the display of the 3D Correlations popup window. The window has two tabs, RDC & NOE, each of which are used to manage information extracted from spectra (RDC), or to review the distance information calculated from the nOe volumes (NOE). From either tab the information must be saved to the NMR Assignments table before it can be used in the 3D calculations.
- NMR Assignments: Toggles the display of the NMR Assignments table.

### StereoFitter section

The StereoFitter section of the ribbon contains tools to run the 3D calculations, and to display results of those calculations.

- Run: Starts the 3D calculations. Alongside is a button that opens a window to select various options for the 3D calculations. Also, there is a button to import an external input file for the 3D calculations.
- Results: Toggles the display of the Results popup window. After 3D calculations have been completed, results are displayed in the Results popup window.
- Histogram: Toggles the display of the Histogram popup window. After 3D calculations have been completed on a set of configurations, a histogram is displayed in a popup window.
- More Tools: It provides the capability to create and save input files from Mnova.

### DFT Predictions section

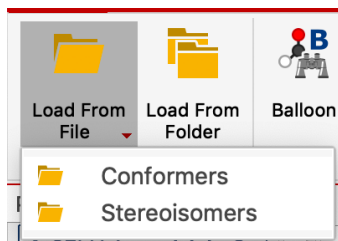
A separated DFT Predictor license is required for running these calculations. The DFT Predictions section of the ribbon contains tools to predict  $^1\text{H}$  or  $^{13}\text{C}$  chemical shifts using results from a server-based DFT program:

- Start Calculations: Start the DFT calculations
- NMR DFT Predictions: Toggles the display of the DFT Predictions Manager table
- Connection Settings: Opens a popup window where settings for the DFT calculations are displayed
- $^1\text{H}$  Prediction: Predict  $^1\text{H}$  chemical shifts from the DFT results
- $^1\text{H}$  Predictions Table: Toggles the display of the  $^1\text{H}$  Predictions table

- $^1\text{H}$  Prediction Options: Opens a popup window where settings for the prediction of  $^1\text{H}$  chemical shifts are displayed
- $^{13}\text{C}$  Prediction: Predict  $^{13}\text{C}$  chemical shifts from the DFT results
- $^{13}\text{C}$  Predictions Table: Toggles the display of the  $^{13}\text{C}$  Predictions table
- $^{13}\text{C}$  Prediction Options: Opens a popup window where settings for the prediction of  $^{13}\text{C}$  chemical shifts are displayed

### 1.2.1. Load section

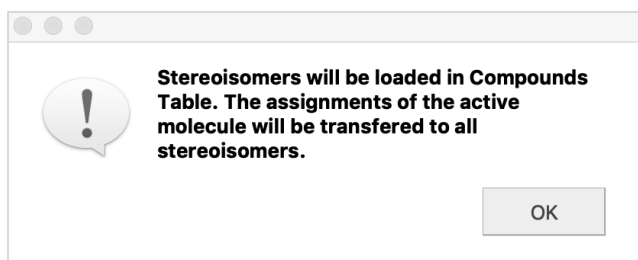
The Load section of the ribbon contains two icons: 'Load From File'; and, 'Load From Folder'.



To import conformations or stereochemical configurations, click on the 'Load From File' icon, which will show a sub-menu. To import a conformer, or a set of conformers, click on 'Conformers'. This will open a file requester, from which files of type MDL V2000 ( \*.sdf, \*.mol), Macromodel/Maestro (\*.mae), XYZ (\*.xyz) and output files from the quantum chemistry programs Gaussian09/16, NWChem, ReSpect, Orca and Jaguar can be opened. To import a stereoisomer, or set of stereoisomers, click on 'Stereoisomers' from the sub-menu. This will open a file browser, from which files of type SDF, MAE, or XYZ can be opened.

To open all conformers or stereoisomers within a folder hierarchy, click on the 'Load From Folder' icon. This will open a file browser, from which a folder can be selected. All compatible files containing conformers or stereoisomers will be loaded together.

If there is an assigned molecule in the Mnova document, then the assignments will be transferred to the incoming conformers and/or stereoisomers:



## 1.2.2. 3D Structure section

The 3D Structure section of the ribbon contains various tools that handle chemical structures used for 3D calculations.

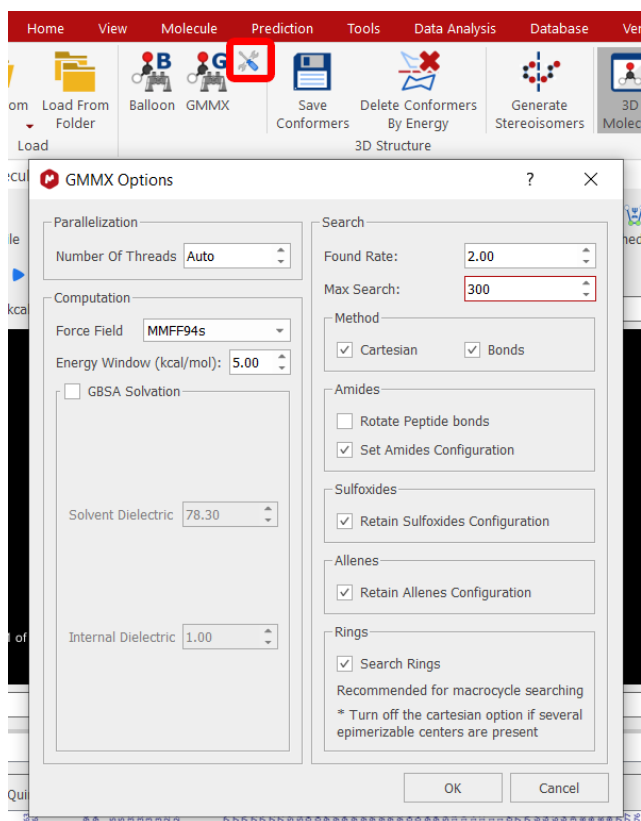


### Balloon and GMMX

A set of conformers for any imported 2D configuration(s) can be generated using either Balloon<sup>22-24</sup> or GMMX<sup>25</sup> algorithms. More information can be found in '[Using nOes to determine 3D structure](#)' or '[Using RDCs to determine 3D structure](#)' workflows. Both Balloon and GMMX use the MMFF94 force field<sup>2</sup> and they can handle a large number of chemical motifs. The MMX force field is also available in GMMX computations.

Balloon it is always used for the 2D to 3D conversion step. It will generate a conformational space using the distance geometry methodology. Note however that we only recommend Balloon as a quick and dirty way to obtain 3D geometries. For production conformational analysis the GMMX engine should be used.

When the GMMX option is applied to a 2D structure, the 3D structure will be first generated through Balloon and then passed to GMMX for the conformational analysis. You can control the most important flags in GMMX computations:

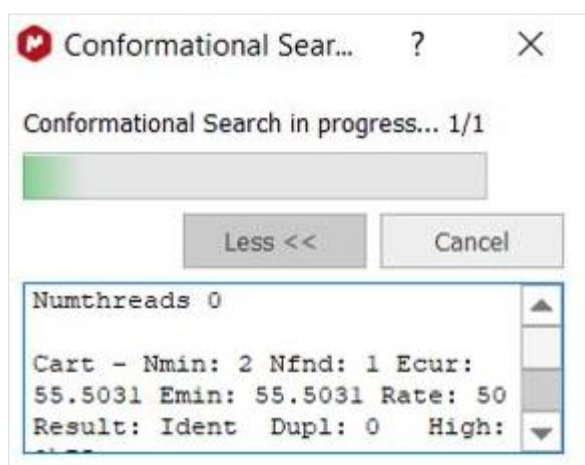


**Number of Threads:** If you set the option to Auto; GMMX will take control of the number of threads which will be equal to the number of logical cores minus two. For instance, in most laptops, which have dual core with hyperthreading two threads will be available.

- A higher value should produce a faster result. Keep in mind that there is a maximum value, according to the cores of each computer. GMMX computations may benefit from hyperthreading so you can use all the available logical cores.
- The spent time can be checked in the Mnova log file, label "GMMX: Elapsed Time".

When running GMMX, the number of threads set is indicated in the Conformational Search progress dialog (at the beginning) and also in the Mnova.log (in <GMMX Control> block).

When the Number of Threads is Auto, the number of threads displayed is 0:



The "ForceField" option can have the MMFF94, MMFF94s and MMX values. Generally, the MMFF94 force field should be chosen except for the case of carbocations where MMX presents a better performance. The MMFF94 force field considers the amides to be pyramidal, while in the MMFF94s force field the amides are considered as flat structures. Additionally the MMFF94s option implements the parametrization of Wahl and coworkers<sup>26</sup> which improves the torsional behavior of phenylpyrroles and *N*-arylamides.

The 'Energy Window' will control the range of energies of the resulting conformers. Conformations below this threshold will be kept in the conformational ensemble.

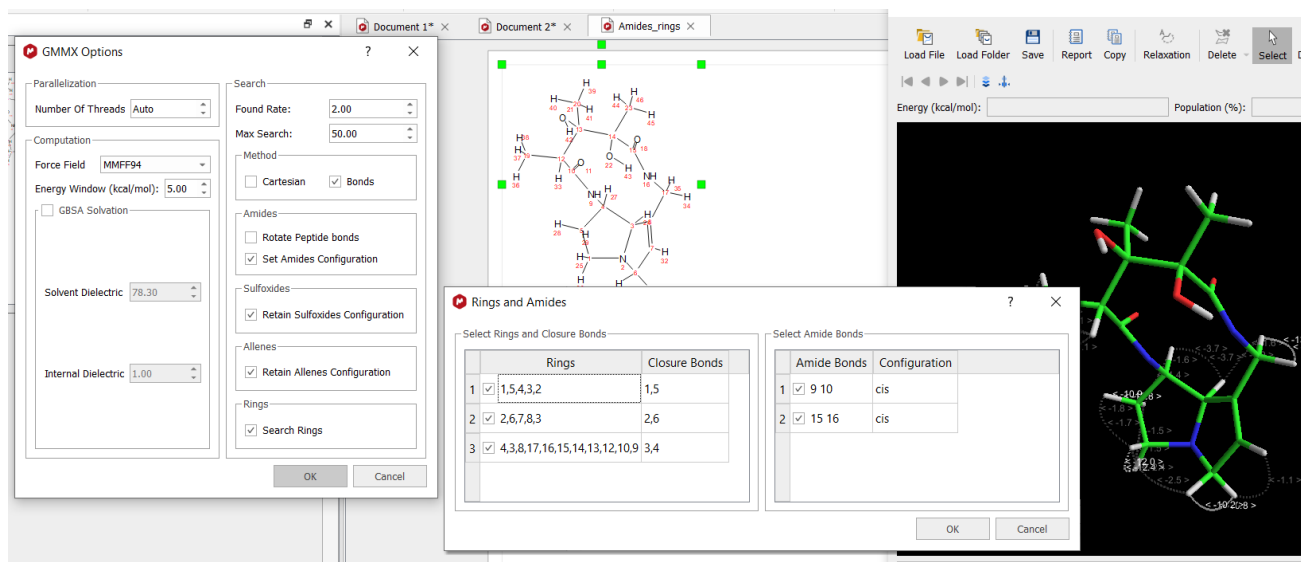
The 'Found Rate' tells the program how low should be the ratio between new found structures and total found structures. For production calculations values between 2.0 (default) and 1.0 are recommended.

The 'Max Search' is the maximum number of steps. Generally, a value of 3000-5000 should suffice for small molecules. Difficult cases such as cyclic peptides or linear molecules with a large number of rotatable bonds might need larger values of up to 20000-30000.

The "GBSA Solvation" box allows to perform computations with inclusion of the Generalized Born implicit solvation. It is possible to define the dielectric constant of the solvent as well as internal cavity one.



It is also possible to perform a so-called ring-search as it can be seen in the screenshot below. In this procedure, one of the ring bonds, the so-called “closure bond” will be broken and a torsional search will be performed on the rotatable bonds of the ring. This option should be used for large rings, at the very least ten-membered ones, If the ring contains epimerizable centers the “Cartesian” option should be checked out in order to prevent may failed steps which will significantly slow down the procedure. For small rings at least up to ten-members the Cartesian option will suffice to explore the conformational landscape of those rings. In any case the user can explore different search protocols to obtain optimal results.



## Save Conformers

Conformers can be saved to disk, in SDF format, possibly for further manipulation by external programs. When several molecules are selected one SDF file is created for each molecule, each of them containing the corresponding conformations.

## Generate Stereoisomers

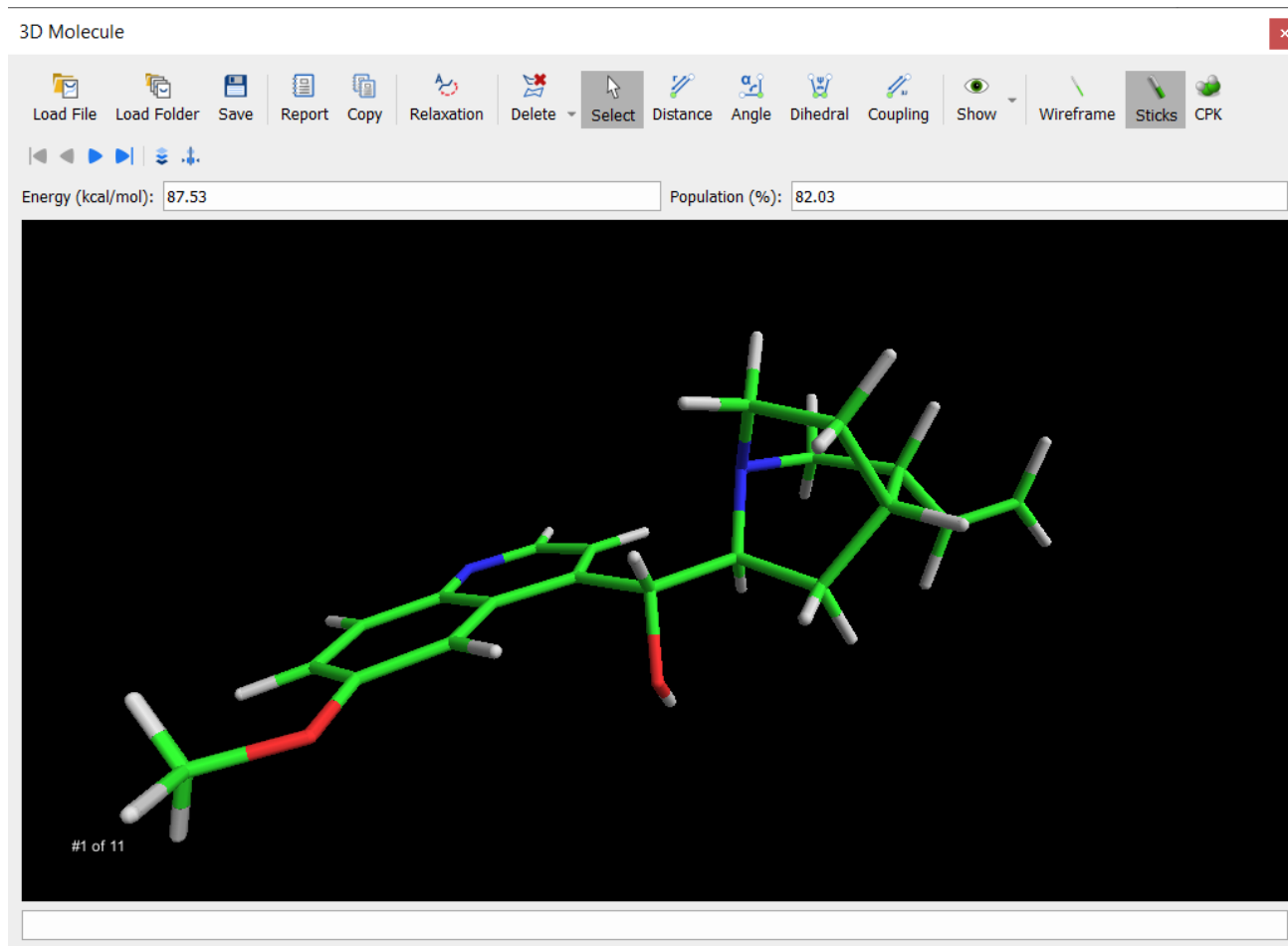
All possible and plausible stereoisomers can be calculated from a 2D structure by clicking this button. Note that NMR cannot generally distinguish between enantiomers, so it is necessary to delete half of the resulting isomers to avoid redundancy in any 3D calculations further downstream. See the 'Using RDCs to determine 3D structure: relative configuration of stereoisomers' section for more details on how this feature is used.

## 3D Molecule

This button toggles the display of the 3D Molecule popup window. More details about the functionality present in the 3D Molecule window can be found in the next section.

### 1.2.2.1. 3D Molecule window

The 3D Molecule popup window is where conformers and/or stereoisomers can be viewed, manipulated, and measured. This window has many functions available on its toolbar:



Click and drag the mouse while in the black display section to rotate the displayed molecule in all three dimensions. Scroll the mouse wheel to grow or shrink the displayed molecule.

#### Save

A SDF file is created for the molecule currently being displayed containing the corresponding conformations.

#### Report

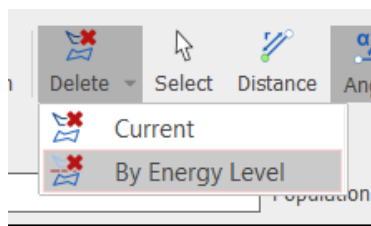
Click the 'Report' icon to place a copy of the black display section of the 3D Molecule window on the currently active page in Mnova.

#### Copy

Click the 'Copy' icon to place a copy of the black display section of the 3D Molecule window on the clipboard. This can be pasted as a raster image into other applications.

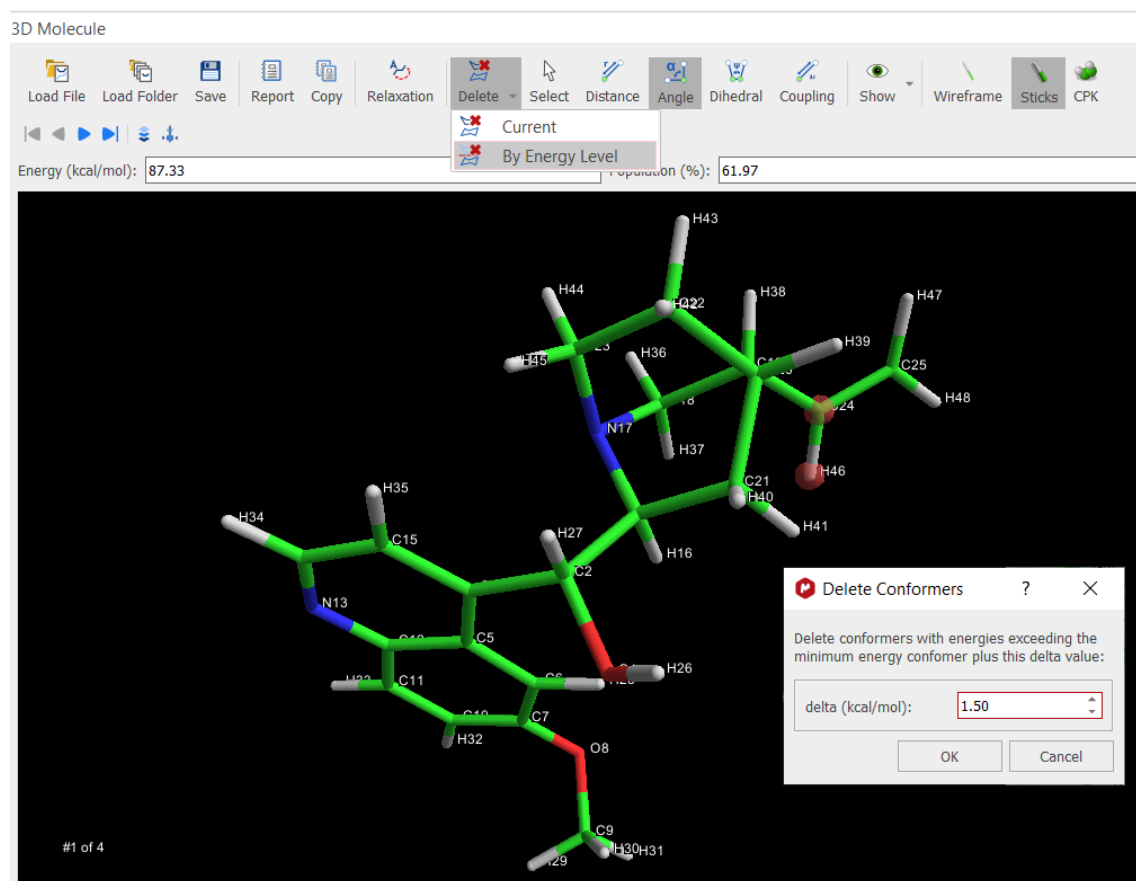
## Delete

The 'Delete' icon is a sub-menu, which allows for deletion of conformers in the set of conformers selected:

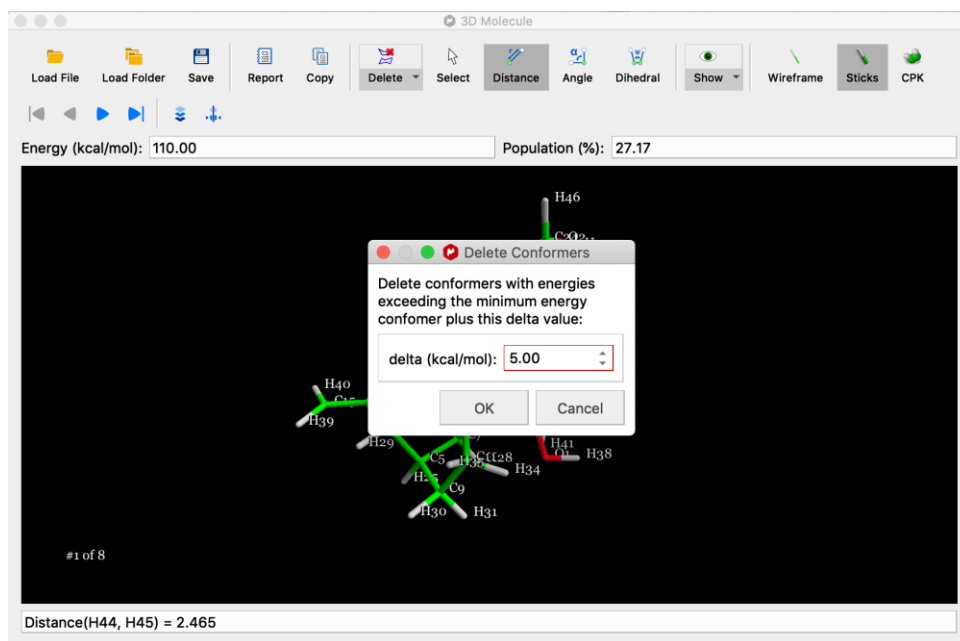


Click 'Delete' → 'Current' to delete the currently displayed conformer. Click 'Undo' to reverse this action.

Clicking 'Delete' → 'By Energy Level' will bring up a pop-up window, where a threshold value above the minimum energy conformer of the set should be entered:







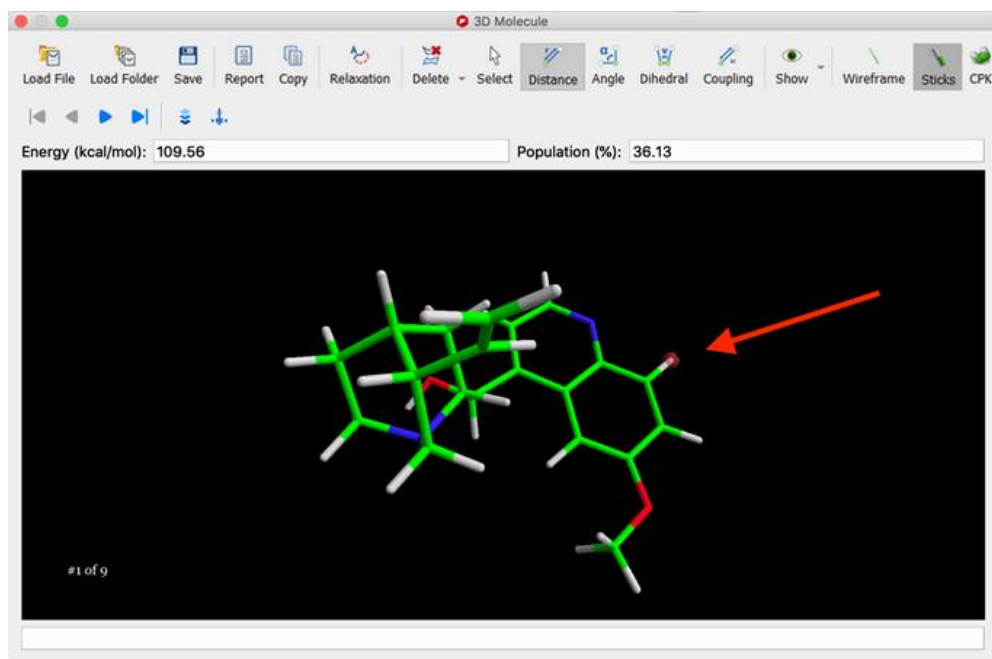
When 'OK' is clicked on the 'Delete Conformers' pop-up window, all conformers with an energy above the value of the minimum-energy conformer + delta will be deleted from the set of loaded conformers. Click 'Undo' to reverse this action.

## Select

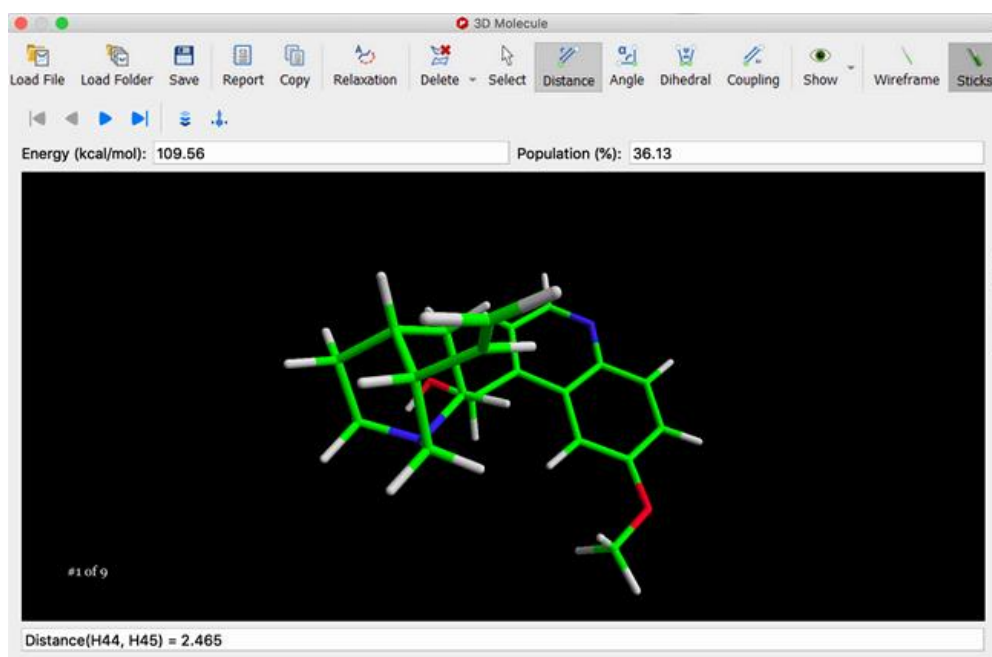
Click the 'Select' icon to disable other modes of action, e.g., Distance, Angle, etc.

## Distance

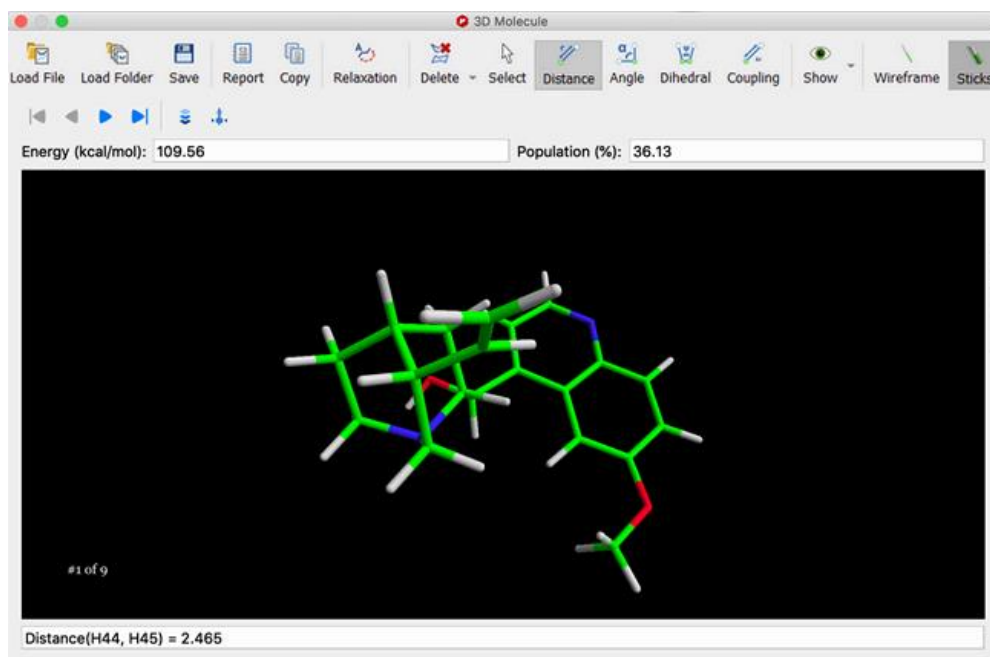
Distances between atoms in a displayed molecule can be measured, in units of Å, using the 'Distance' tool. Load a molecule into the 3D Molecule window, choose the desired display mode (wireframe, sticks, or CPK), then click the 'Distance' icon on the 3D Molecule window toolbar. The 'Distance' icon will be greyed to confirm that it is active. Rotate, move, and/or manipulate the molecule to bring the two desired atoms into clear view. Click on one of the atoms whose distance is to be measured, and a diffuse red ball will highlight the atom:



Click on the second atom whose distance is to be measured from the first. The highlight will disappear from the first atom, and will be replaced with the distance between the two atoms, displayed at the bottom of the 3D Molecule window:



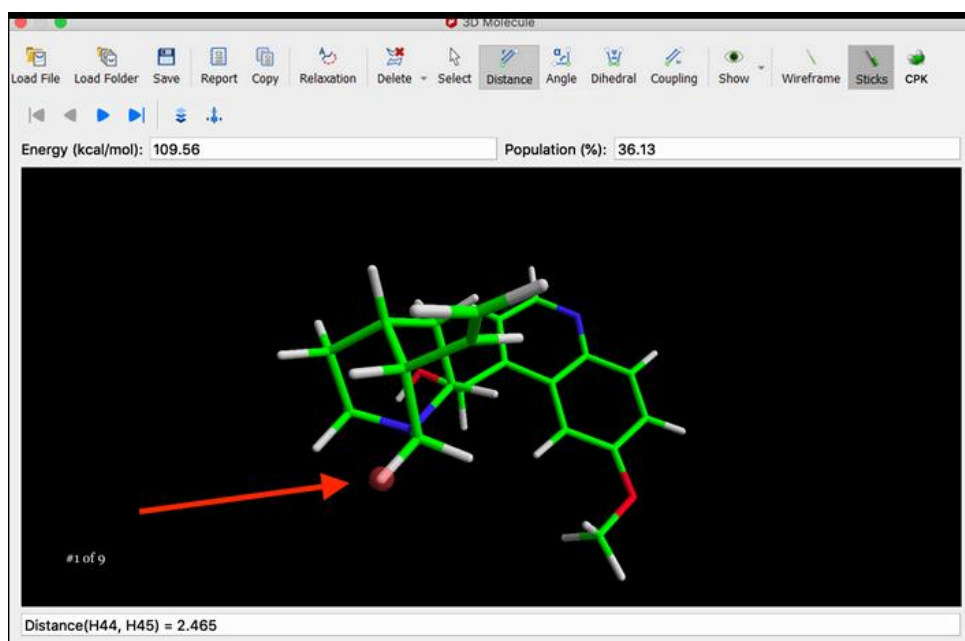
The distance information can be copied to the clipboard by right-clicking on the text, then selecting 'Select All' from the context menu, right-clicking again, then selecting 'Copy' from the context menu. Alternatively, click & drag across as much of the text as desired, then right-click and select 'Copy' from the context menu:



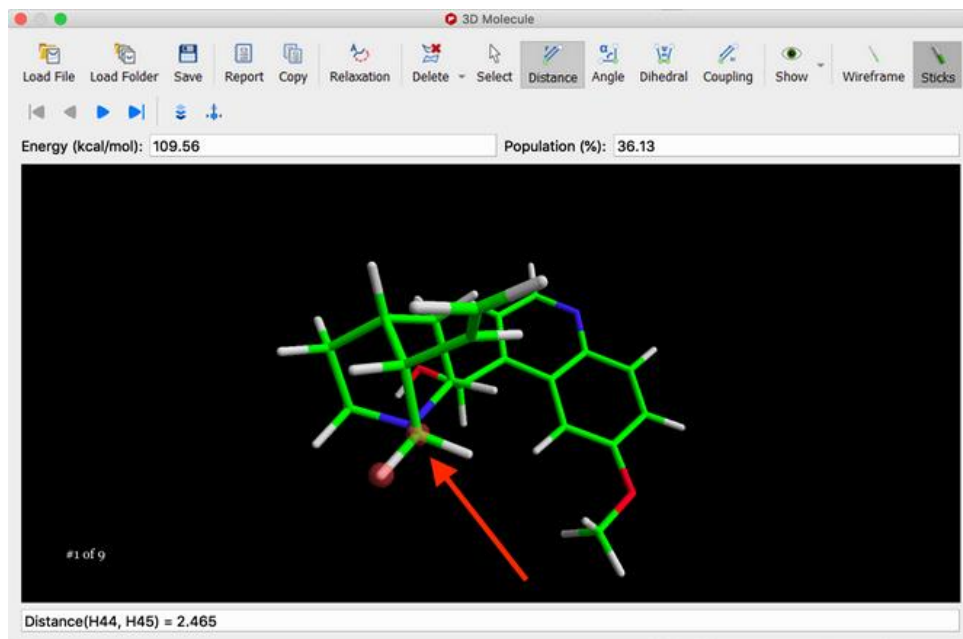
Repeat for another distance measurement by clicking on the first and then the second atoms.

## Angle

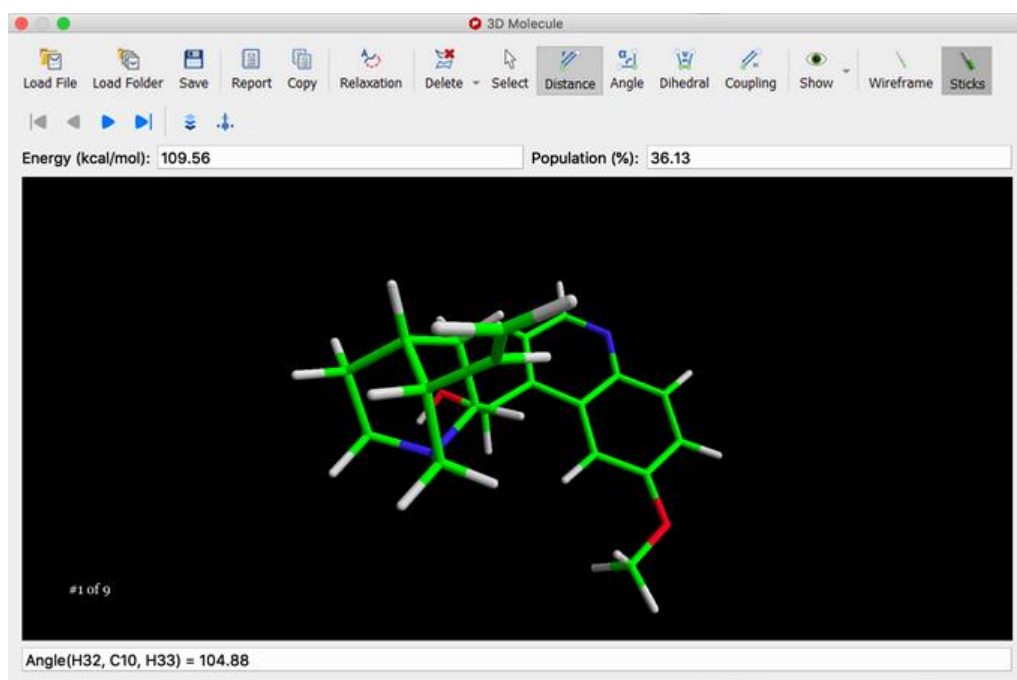
Bond angles in a displayed molecule can be measured using the 'Angle' tool. Load a molecule into the 3D Molecule window, choose the desired display mode (wireframe, sticks, or CPK), then click the 'Angle' icon on the 3D Molecule window toolbar. The 'Angle' icon will be greyed to confirm that it is active. Rotate, move, and/or manipulate the molecule to bring three atoms that make up the bond angle into clear view. Click on one of the atoms that represents one end of the three-atom chain that makes up the bond angle, and a diffuse red ball will highlight the atom:



Click on the second atom at the vertex of the bond angle, and a second diffuse red ball will highlight that atom:



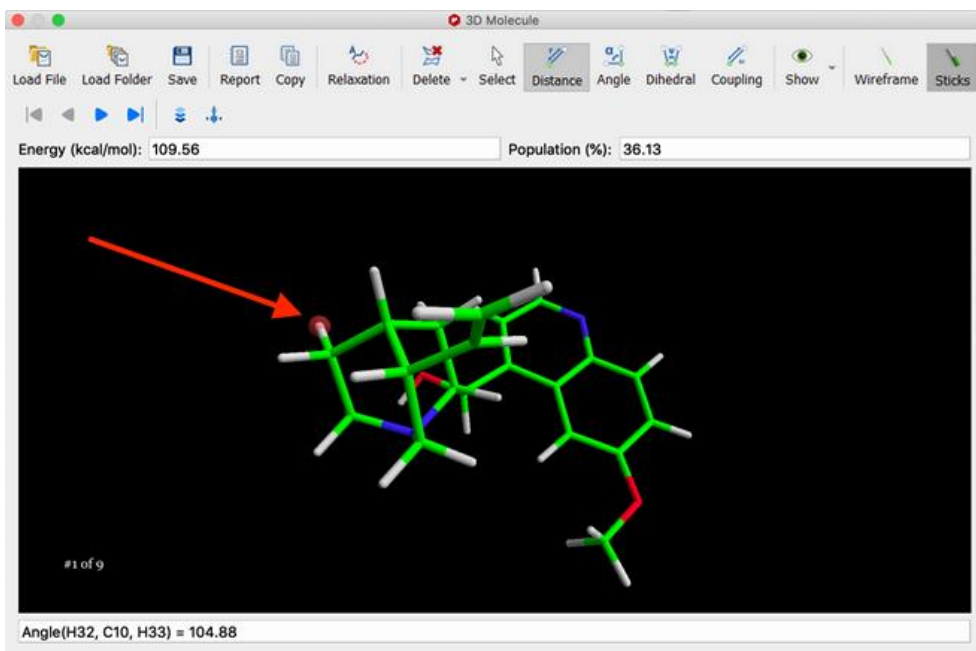
Click on the third atom that makes up the bond angle, which represents the other end of the three-atom chain. The highlight will disappear from the first two selected atoms, and will be replaced with the angle between the three atoms, displayed at the bottom of the 3D Molecule window:



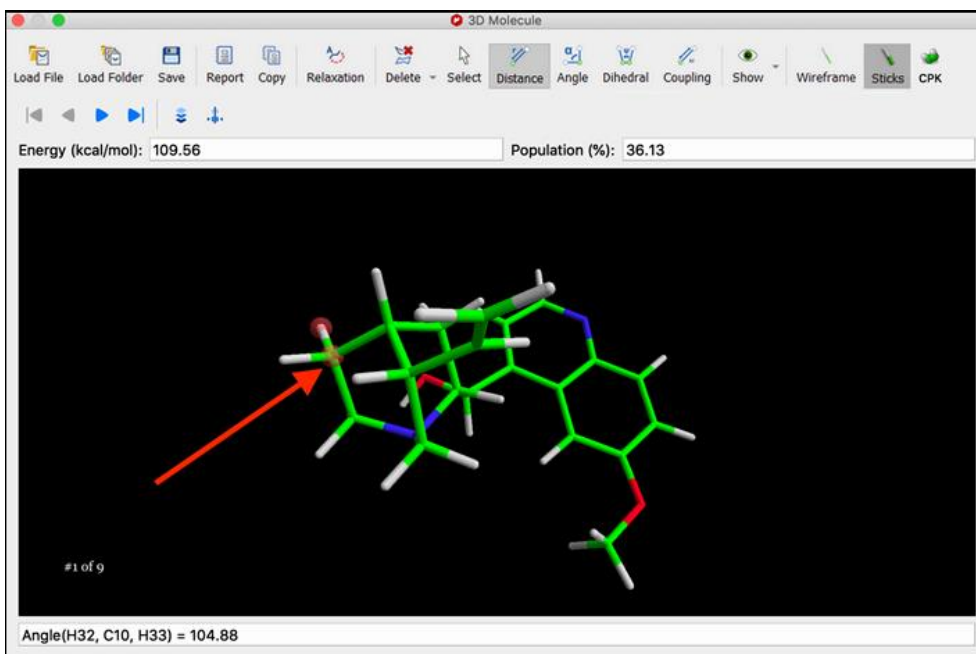
The angle information can be copied to the clipboard by right-clicking on the text, then selecting 'Select All' from the context menu, right-clicking again, then selecting 'Copy' from the context menu. Alternatively, click & drag across as much of the text as desired, then right-click and select 'Copy' from the context menu. Repeat for another bond angle measurement by clicking on three atoms directly connected to each other.

## Dihedral

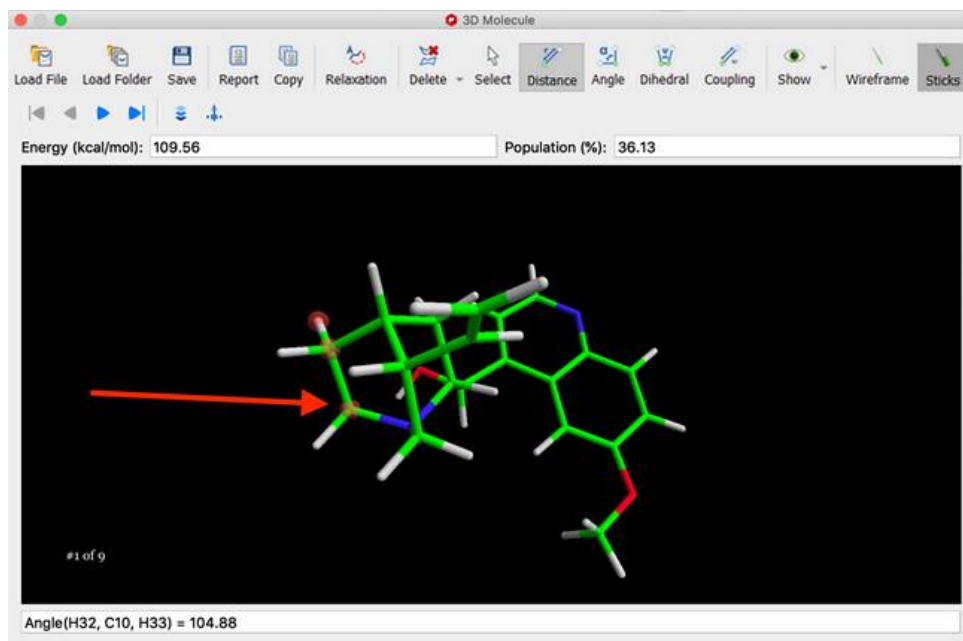
Dihedral angles in a displayed molecule can be measured using the 'Dihedral' tool. Load a molecule into the 3D Molecule window, choose the desired display mode (wireframe, sticks, or CPK), then click the 'Dihedral' icon on the 3D Molecule window toolbar. The 'Dihedral' icon will be greyed to confirm that it is active. Rotate, move, and/or manipulate the molecule to bring four atoms that make up the dihedral angle into clear view. Click on one of the atoms that represents one end of the four-atom chain that makes up the dihedral angle, and a diffuse red ball will highlight the atom:



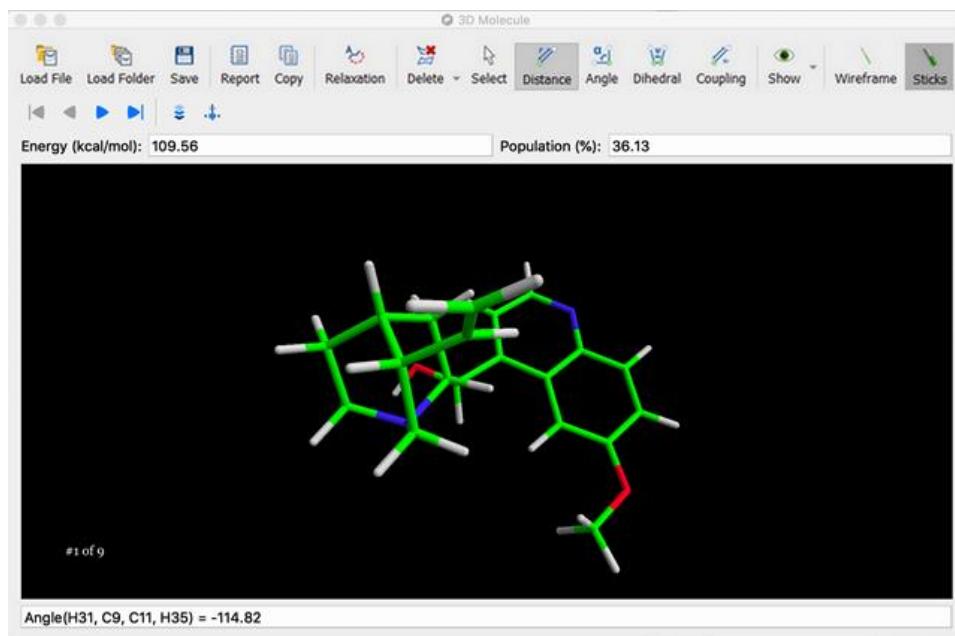
Click on the second atom directly connected to the first, i.e., the second atom in the four-atom chain, and a second diffuse red ball will highlight that atom:



Click on the third atom directly connected to the second, i.e. the third atom in the four-atom chain, and a third diffuse red ball will highlight that atom:



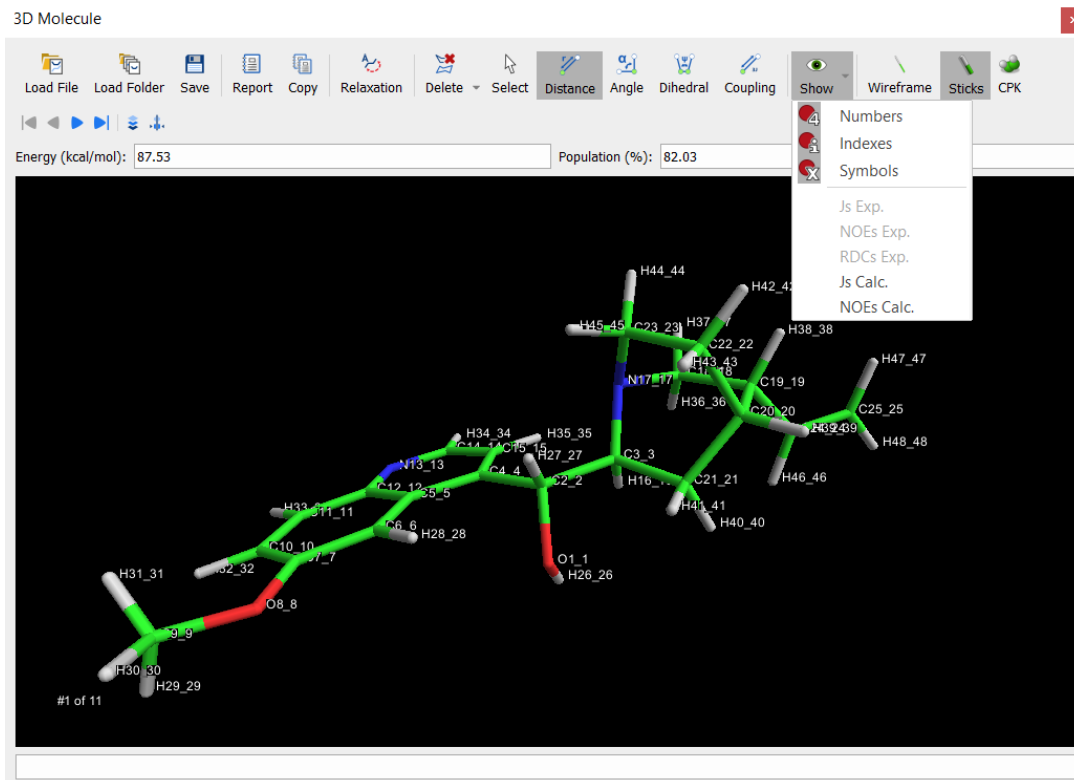
Click on the fourth atom that makes up the dihedral angle, which represents the other end of the four-atom chain. The highlight will disappear from the first three selected atoms, and will be replaced with the angle between the four atoms, displayed at the bottom of the 3D Molecule window:



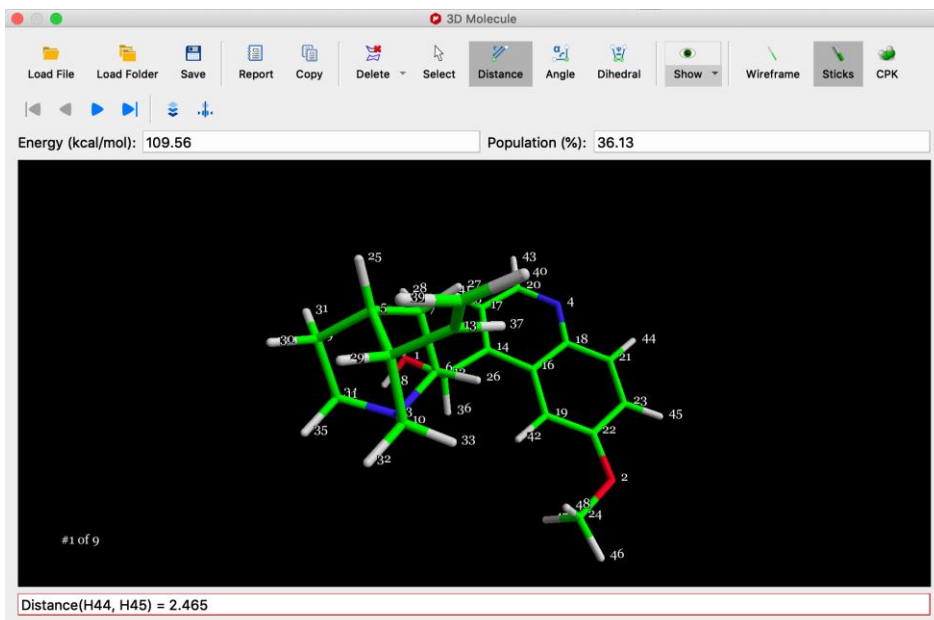
The angle information can be copied to the clipboard by right-clicking on the text, then selecting 'Select All' from the context menu, right-clicking again, then selecting 'Copy' from the context menu. Alternatively, click & drag across as much of the text as desired, then right-click and select 'Copy' from the context menu. Repeat for another dihedral angle measurement by clicking on four atoms directly connected to each other.

## Show

The 'Show' icon is a sub-menu, that allows for display of atom numbers, indexes, symbols as well as experimental or calculated *J*s, NOEs, RDCs.

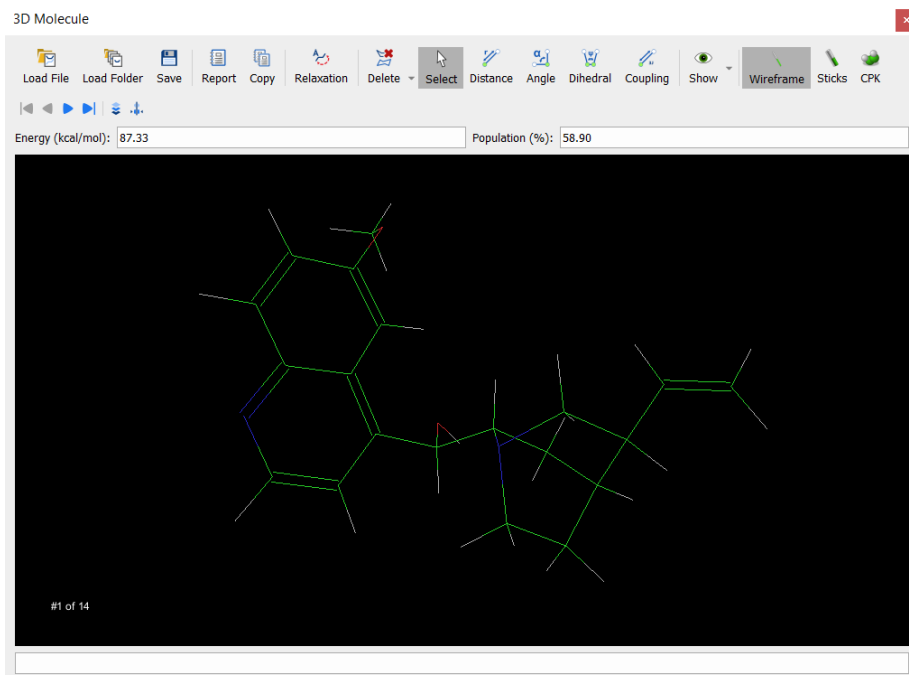


To display atom numbers on the displayed molecule, select the 'Numbers' option on the 'Show' sub-menu:



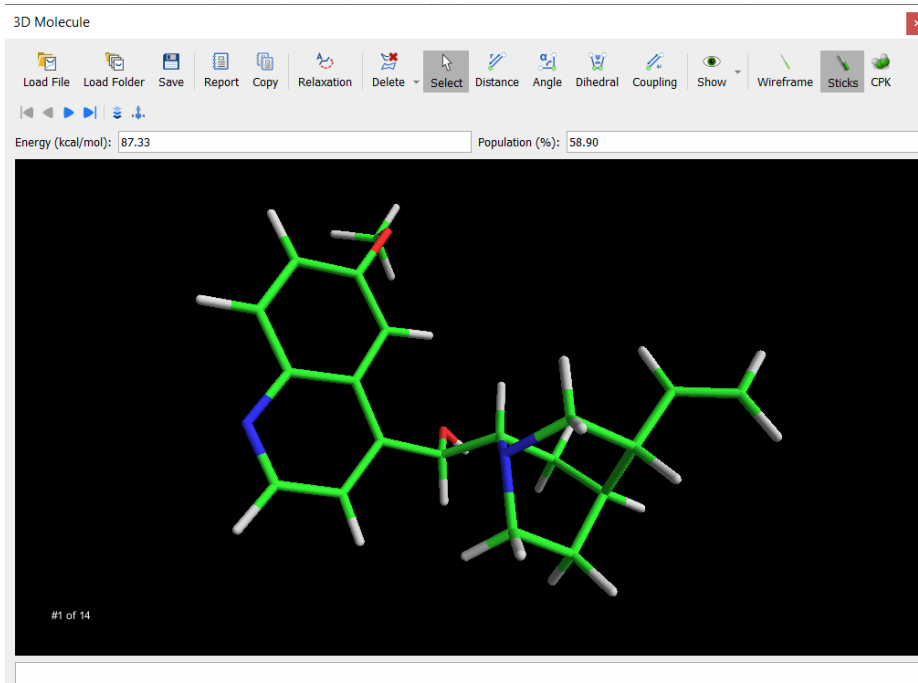
## Wireframe

Click the 'Wireframe' icon to display the molecule in wireframe display mode:



## Sticks

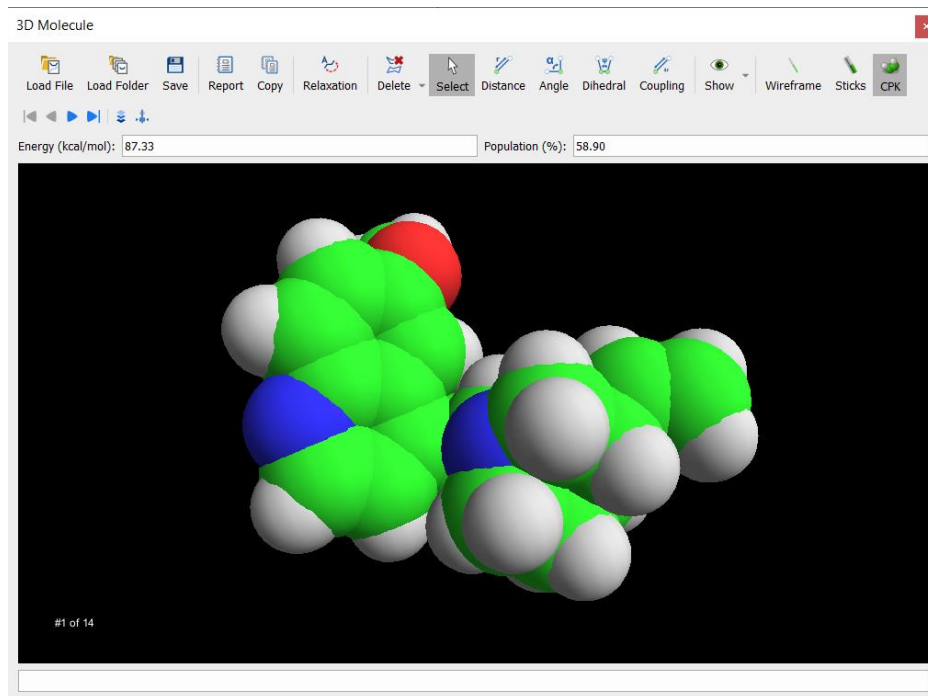
Click the 'Sticks' icon to display the molecule in sticks display mode:



## CPK

Click the 'CPK' icon to display the molecule in space-filling calotte (Corey-Pauling-Koltun) display mode:



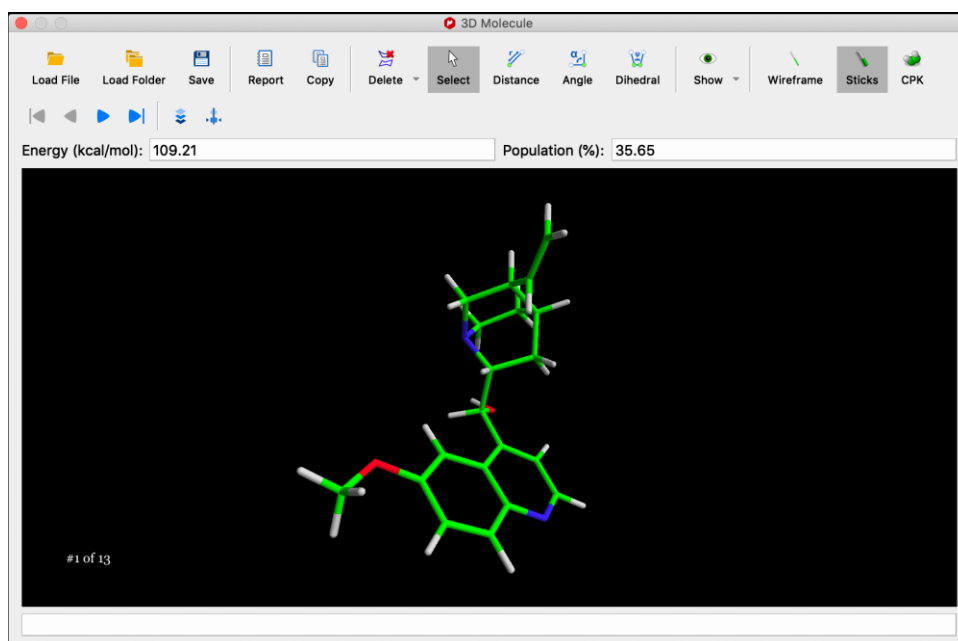


### First/Previous/Next/Last Conformer

When more than one conformer is loaded into the 3D Molecule window, then the four arrow icons will become active to navigate through them:



The total number of conformers, and which one of the set is currently displayed, is shown in the lower left corner of the window, e.g. #1 of 14:

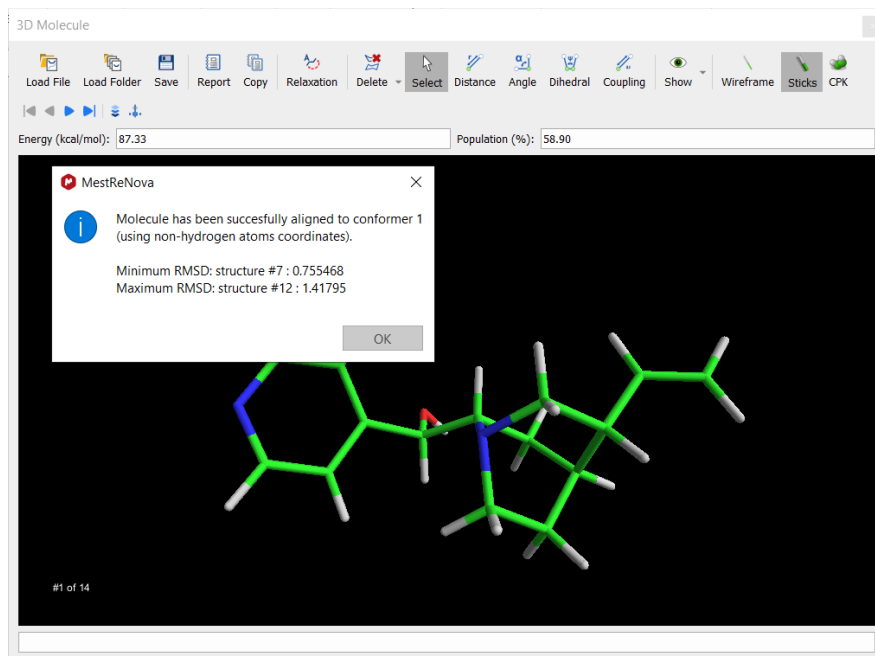


### Align Conformers

To align all the conformers using heavy-atom coordinates click the button:



which will show a message with information about the alignment. Note that this aligning procedure only affects how the conformations are displayed and not the actual coordinates, when for instance exporting to a SDF file.

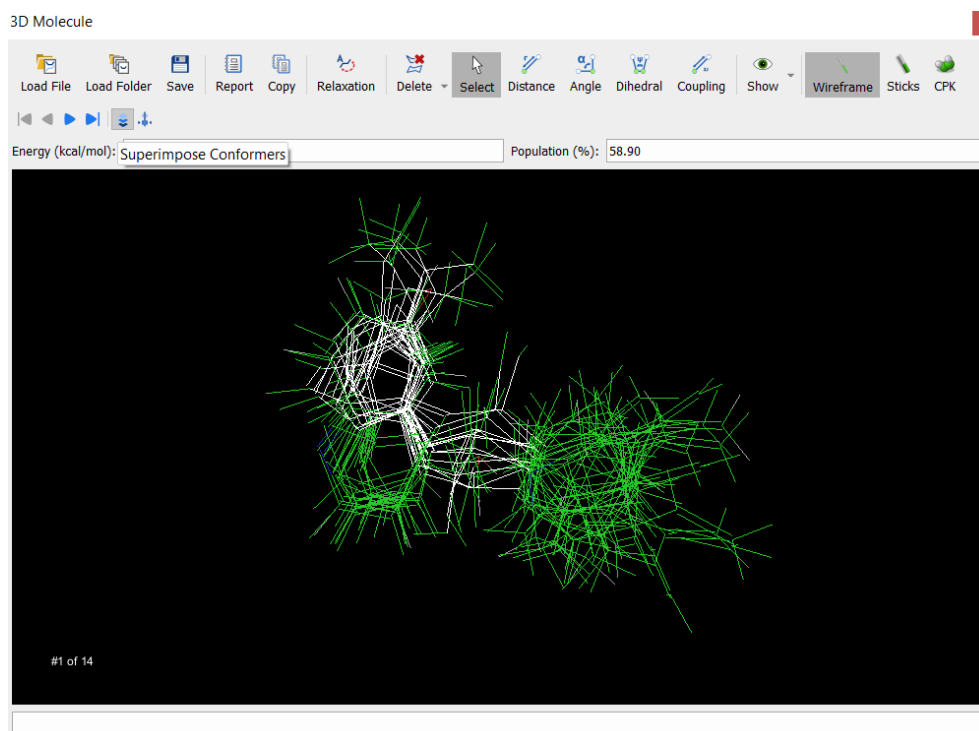


## Superimpose Conformers

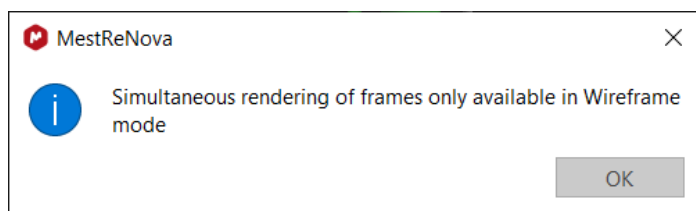
To superimpose all the conformers, displaying all of them at the same time, click the button:



And all the conformations will be rendered simultaneously. The currently selected conformation is displayed in a different color

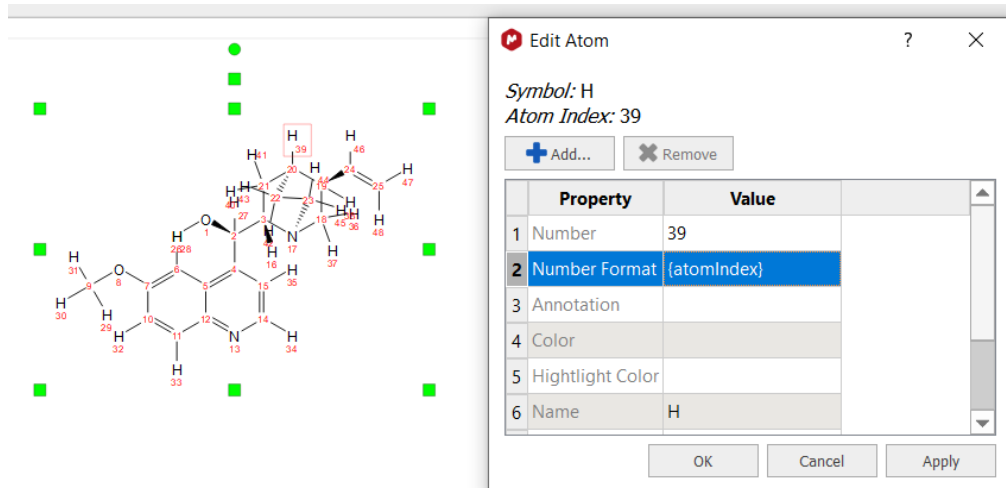


Make sure that the display is in Wireframe mode, otherwise an error message will be displayed:



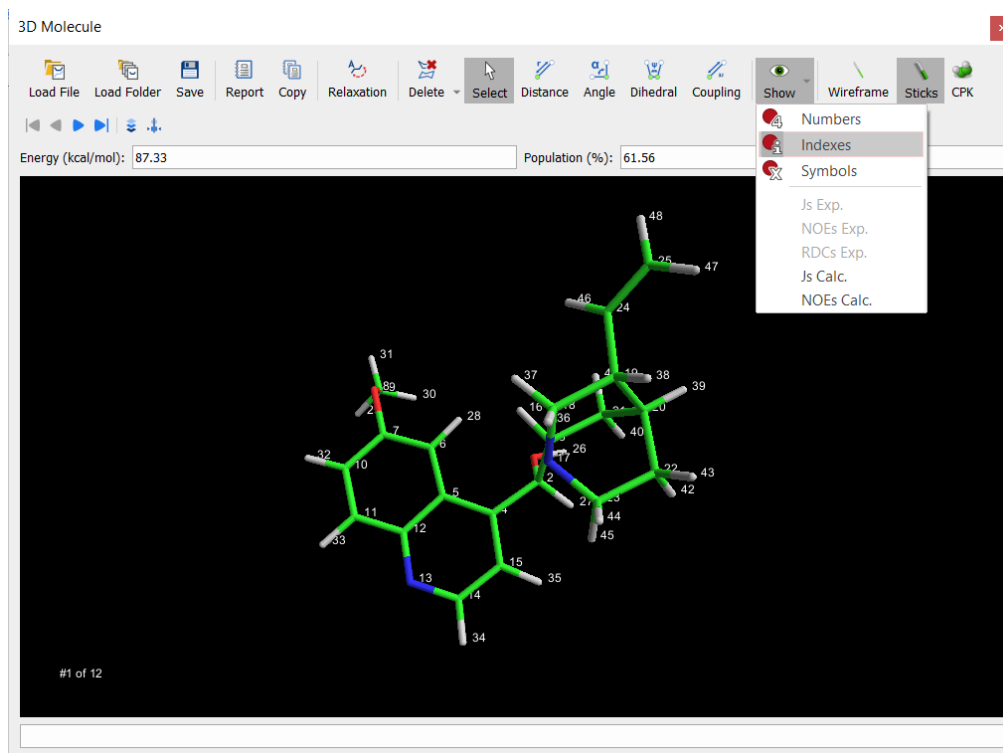
## Numbering atom. Labels vs Indexes

An important thing to note is that Mnova assignment tables use Mnova atom numbers which do not necessarily form a contiguous set. However, internally, the StereoFitter computational engine uses atom indexes (1 to  $N$ ). When generating StereoFitter input files the mapping between numbers and indexes is done automatically. However, if the users generate their own input files they need to take into account that not necessarily numbers will match indexes. Indexes can be displayed in 2D mode by right clicking on an atom, click the **Edit Atom** entry. On the opened window click the **Number Format** cell and add the Atom Index macro.



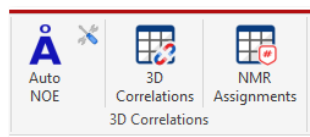
If you load a molecule with differences between visible numbers and atom indexes and you try to run From External Input File, you will get a Warning message with the list of correspondence between Visible Number (atom number) - Index (atom index):

You can also display the atom indexes from the 3D molecule viewer:



### 1.2.3. 3D Correlations section

The 3D Correlations section of the ribbon contains tools that interact with NMR spectra and back-calculations of NOE distances.



#### Auto NOE

When NOEs are being used to generate 3D structure(s), it is necessary to back-calculate the theoretical distances from NOE volumes. The 'Auto NOE' button will carry out those calculations, and place the results in the Assignments Table.

#### NOE Options

Allows to select integration method and reference distance.

#### 3D Correlations

Toggles the display of the 3D Correlations popup window. The window has two tabs, RDC & NOE, each of which are used to manage information extracted from spectra (RDC), or to review the distance information calculated from NOE volumes (NOE).

From either tab the information must be passed to the NMR Assignments table before it will be used in the 3D calculations. This is done by pressing the 'Save' button at the bottom of the 3D Correlations window. More information is available in the '3D Correlations window' section.

**Note:** The 3D Correlations icon will be disabled unless there is a molecule object present on the current Mnova page, or a single compound selected on the Compounds table.

## NMR Assignments

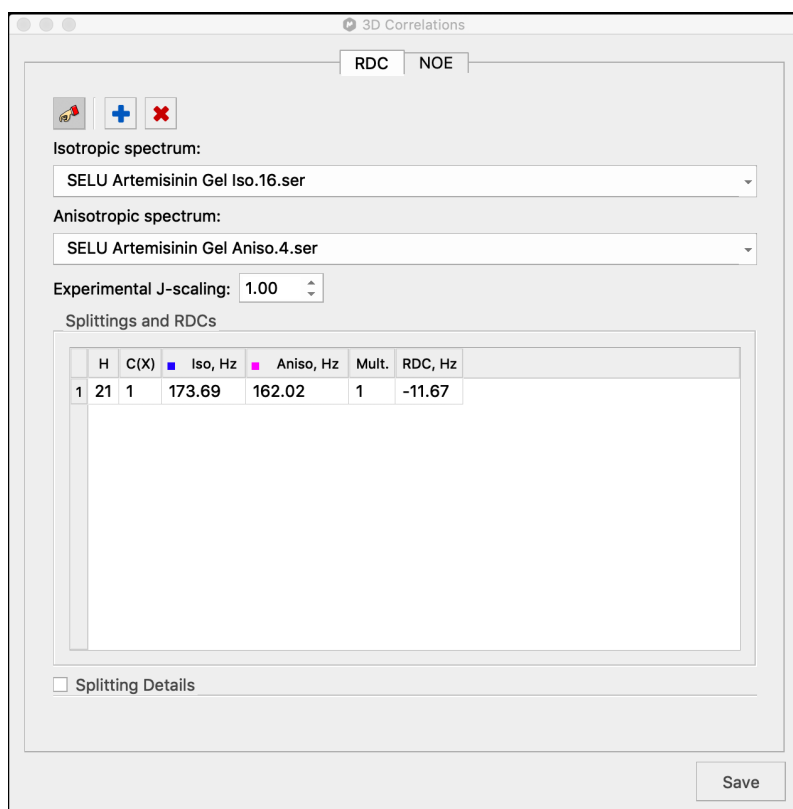
Toggles the display of the Assignments table

### 1.2.3.1.3D Correlations window

The 3D Correlations window contains two tabs: RDC; and NOE. Depending on which type of spectra are being analyzed will determine which of the two tabs are populated with information.

#### RDC tab

Below is a screenshot of the RDC tab with some experimental information displayed:



More information on how RDC information is entered into this tab can be found in the ['Using RDCs to determine 3D structure'](#) section.

## NOE tab

Below is a screenshot of the NOE tab with some experimental information displayed:

3D Correlations ×

RDC **NOE**

Integration method: 2D Peak Volumes

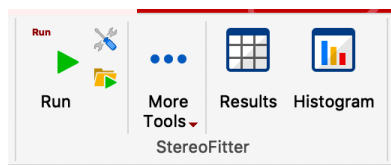
	Pos1	Pos2	#H1	#H2	ppm	Diag1	Diag2	Cross1	Cross2	Using	Dist(Å)
1	30	29	1	1	(1.55, 2.30)	114206	80153.7	-1885.54	-2057.46	c1 d2 c2 d1	2.64
2	27	26	1	1	(1.55, 3.17)	114206	132663	-2607.5	-2617.59	c1 d2 c2 d1	2.63
3	28, 31	34	2	1	(1.77, 3.50)	105821	52053.2	-2101.87	-2045.32	c1 d2 c2 d1	2.66
4	28, 31	36	2	1	(1.77, 5.58)	105821	66845.2	-743.1	-653.638	c1 d2	3.29
5	28, 31	41	2	1	(1.77, 7.51)	105821	83094.6	-780.555	-946.028	c2 d1	3.04
6	25	29	1	1	(1.86, 2.30)	78477.1	80153.7	-1962.84	-1943.85	c1 d2 c2 d1	2.57
7	29	33	1	1	(2.30, 3.09)	80153.7	132663	-2617.89	-2393.08	c1 d2 c2 d1	2.56
8	32	26	1	1	(2.69, 3.17)	115065	132663	-15596	-15696.5	c1 d2 c2 d1	1.95
9	35	34	1	1	(2.69, 3.50)	115065	52053.2	-10434.9	-10259.1	c1 d2 c2 d1	1.91
10	26	36	1	1	(3.17, 5.58)	132663	66845.2	-1408.1	-1379.02	c1 d2 c2 d1	2.77
11	26	42	1	1	(3.17, 7.25)	132663	76031.8	-2187.12	-2235.59	c1 d2 c2 d1	2.60
12	26	41	1	1	(3.17, 7.51)	132663	83094.6	-760.63	-935.067	c2 d1	3.16
13	34	36	1	1	(3.50, 5.58)	52053.2	66845.2	-1343.54	-1295.68	c1 d2 c2 d1	2.61
14	48, 46, 47	42	3	1	(3.90, 7.25)	220974	76031.8	-4034.27	-3402.7	c1 d2	2.71
15	48, 46, 47	45	3	1	(3.90, 7.32)	220974	87937.8	-4034.27	-668.511	c1 d2	2.78
16	36	42	1	1	(5.58, 7.25)	66845.2	76031.8	-4755.12	-5194.08	c1 d2 c2 d1	2.16
17	36	41	1	1	(5.58, 7.51)	66845.2	83094.6	-583.966	-761.265	c2 d1	2.92
18	45	44	1	1	(7.32, 7.97)	87937.8	73331.3	-1732.65	-1807.92	c1 d2 c2 d1	2.61
19	<b>41</b>	<b>43</b>	<b>1</b>	<b>1</b>	<b>(7.51, 8.63)</b>	<b>83094.6</b>	<b>73894.4</b>	<b>-2031.18</b>	<b>-2637.95</b>	<b>c2 d1</b>	<b>2.46</b>

Save

More information on how NOE information is entered into this tab can be found in the ['Using NOEs to determine 3D structure'](#) section.

### 1.2.4. StereoFitter section

The StereoFitter section of the ribbon contains tools to run the 3D calculations, and to display the results of those calculations.



## Run

This button starts the 3D calculations. Alongside is a button that opens a window to select various options for the 3D calculations. Also, there is a button to import an external input file for the 3D calculations.

## Results

After 3D calculations have been completed, results are displayed in the StereoFitter results popup window. This button toggles the display of the results popup window.

## Histogram

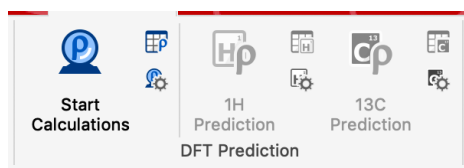
After 3D calculations have been completed, where more than one molecule has been submitted at the same time, a histogram is displayed in a separate popup window. This button toggles the display of the Results Histogram popup window.

## More Tools

It provides the capability to create and save input files from Mnova.

### 1.2.5. DFT Prediction section

A separated DFT Predictor license is required for running these calculations. The DFT Predictions section of the ribbon contains tools to predict  $^1\text{H}$  or  $^{13}\text{C}$  chemical shifts using results from a server-based DFT program.



## Start Calculations

Start the DFT calculations. When started, the DFT Predictions Manager window is displayed, to allow for monitoring of the calculation. This button will be disabled unless a valid 'DFT Predictor' license is installed.

## NMR DFT Predictions

Toggles the display of the DFT Predictions Manager table:



DFT Predictions Manager							
Molecule	Formula	Method	State	Progress	Start Date	End Date	
	C <sub>18</sub> H <sub>22</sub> O <sub>7</sub>	B3LYP-6-31G*-MMFF94	Done	—	Tue Nov 5 13:02:15 2019	Tue Nov 5 13:21:19 2019	

Once the calculation is complete, click the 'Send to Mnova' button to export the DFT results to the Mnova document.

## Connection Settings

Opens a popup window where settings for the DFT calculations are displayed:

**DFT Predictor Options**

Method:

Server

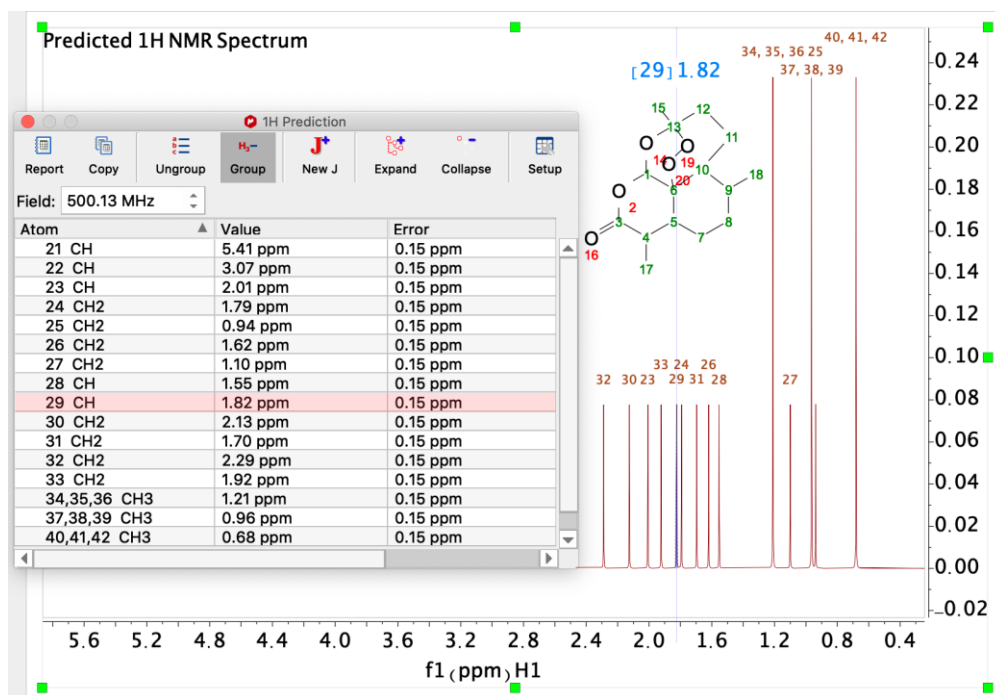
URL:

Username:

## <sup>1</sup>H Prediction

Predict <sup>1</sup>H chemical shifts from the DFT results. This button will be disabled if DFT results are not available.

Once complete, a new predicted <sup>1</sup>H spectrum will be created on a new page in the Mnova document. The <sup>1</sup>H Prediction Table and the predicted spectrum are interactive:



## <sup>1</sup>H Prediction Table

Toggles the display of the <sup>1</sup>H Predictions table:

Atom	Value	Error
21 CH	5.41 ppm	0.15 ppm
22 CH	3.07 ppm	0.15 ppm
23 CH	2.01 ppm	0.15 ppm
24 CH2	1.79 ppm	0.15 ppm
25 CH2	0.94 ppm	0.15 ppm
26 CH2	1.62 ppm	0.15 ppm
27 CH2	1.10 ppm	0.15 ppm
28 CH	1.55 ppm	0.15 ppm
29 CH	1.82 ppm	0.15 ppm
30 CH2	2.13 ppm	0.15 ppm
31 CH2	1.70 ppm	0.15 ppm
32 CH2	2.29 ppm	0.15 ppm
33 CH2	1.92 ppm	0.15 ppm
34,35,36 CH3	1.21 ppm	0.15 ppm
37,38,39 CH3	0.96 ppm	0.15 ppm
40,41,42 CH3	0.68 ppm	0.15 ppm

This button will be disabled if DFT results are not available.

## <sup>1</sup>H Prediction Options

Opens a popup window where settings for the prediction of <sup>1</sup>H chemical shifts are displayed:

1H Prediction Options

From: -2.00 ppm

To: 10.00 ppm

Number of Points: 32 K

Frequency: 500.13 MHz

Line Width: 0.75 Hz

Solvent: Chloroform-d

Regression Parameters:

Slope: -1.02900

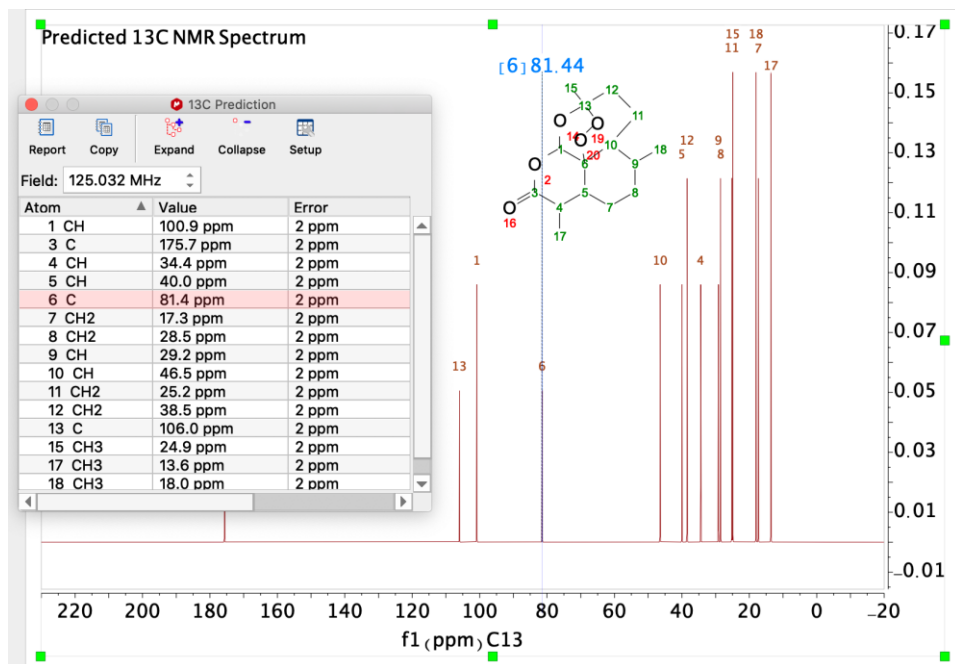
Intercept: 33.000

Error: 0.150 ppm

Buttons: OK, Cancel, Restore Defaults, Reset

## <sup>13</sup>C Prediction

Predict <sup>13</sup>C chemical shifts from the DFT results. This button will be disabled if DFT results are not available. Once complete, a new predicted <sup>13</sup>C spectrum will be created on a new page in the Mnova document. The <sup>13</sup>C Prediction Table and the predicted spectrum are interactive:



### $^{13}\text{C}$ Prediction Table

Toggles the display of the  $^{13}\text{C}$  Predictions table:

Atom	Value	Error
1 CH	100.9 ppm	2 ppm
3 C	175.7 ppm	2 ppm
4 CH	34.4 ppm	2 ppm
5 CH	40.0 ppm	2 ppm
6 C	81.4 ppm	2 ppm
7 CH2	17.3 ppm	2 ppm
8 CH2	28.5 ppm	2 ppm
9 CH	29.2 ppm	2 ppm
10 CH	46.5 ppm	2 ppm
11 CH2	25.2 ppm	2 ppm
12 CH2	38.5 ppm	2 ppm
13 C	106.0 ppm	2 ppm
15 CH3	24.9 ppm	2 ppm
17 CH3	13.6 ppm	2 ppm
18 CH3	18.0 ppm	2 ppm

This button will be disabled if DFT results are not available.

### $^{13}\text{C}$ Prediction Options

Opens a popup window where settings for the prediction of  $^{13}\text{C}$  chemical shifts are displayed:



13C Prediction Options

From:	-20.00 ppm	OK
To:	230.00 ppm	Cancel
Number of Points:	128 K	
Frequency:	500.13 MHz	
Line Width:	1.50 Hz	
Solvent:	Chloroform-d	

Regression Parameters:

Slope:	-1.06000	Restore Defaults
Intercept:	197.500	Reset
Error:	2.000 ppm	

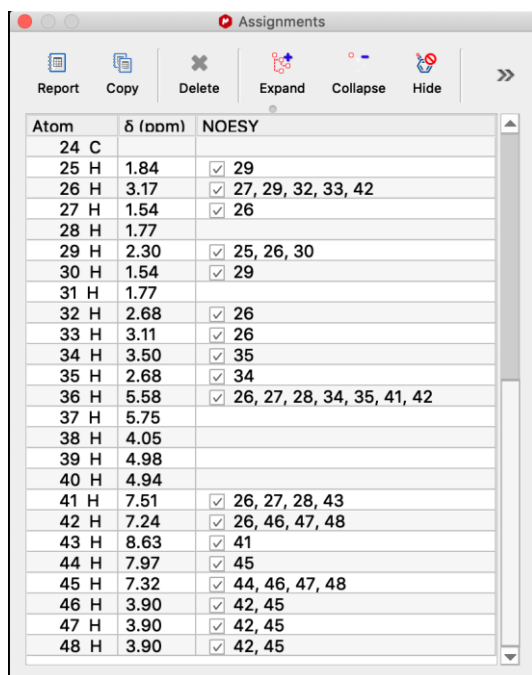
### 1.3. Conformational Analysis: Using NOEs to determine 3D structure from a set of conformers

This section describes a typical workflow using StereoFitter and a NOESY (or ROESY) spectrum to determine the best candidate 3D conformer, or conformers, of a molecule from a supplied set of conformers. Open a NOESY (or ROESY) spectrum in a new Mnova document, along with a 2D or 3D molecular structure (making sure that all the protons are explicit).

Integrate all possible crosspeaks and diagonal peaks in the NOESY spectrum.

From the Analysis ribbon, use the 'Integrate' tool or 'Peak by Peak' tool, depending on the integration method selected in NOE options, to integrate diagonal and cross-peaks associated with atoms in the molecule. The more volumes that can be extracted from the 2D NOESY spectrum, the better the 3D structure result will be.

Make the assignments of the picked NOESY correlations to the molecular structure. The 'Assignments' table will have populated the NOESY column, that summarizes the picked regions:



Atom	δ (ppm)	NOESY
24 C		
25 H	1.84	<input checked="" type="checkbox"/> 29
26 H	3.17	<input checked="" type="checkbox"/> 27, 29, 32, 33, 42
27 H	1.54	<input checked="" type="checkbox"/> 26
28 H	1.77	
29 H	2.30	<input checked="" type="checkbox"/> 25, 26, 30
30 H	1.54	<input checked="" type="checkbox"/> 29
31 H	1.77	
32 H	2.68	<input checked="" type="checkbox"/> 26
33 H	3.11	<input checked="" type="checkbox"/> 26
34 H	3.50	<input checked="" type="checkbox"/> 35
35 H	2.68	<input checked="" type="checkbox"/> 34
36 H	5.58	<input checked="" type="checkbox"/> 26, 27, 28, 34, 35, 41, 42
37 H	5.75	
38 H	4.05	
39 H	4.98	
40 H	4.94	
41 H	7.51	<input checked="" type="checkbox"/> 26, 27, 28, 43
42 H	7.24	<input checked="" type="checkbox"/> 26, 46, 47, 48
43 H	8.63	<input checked="" type="checkbox"/> 41
44 H	7.97	<input checked="" type="checkbox"/> 45
45 H	7.32	<input checked="" type="checkbox"/> 44, 46, 47, 48
46 H	3.90	<input checked="" type="checkbox"/> 42, 45
47 H	3.90	<input checked="" type="checkbox"/> 42, 45
48 H	3.90	<input checked="" type="checkbox"/> 42, 45

#### Back-calculate internuclear distances from the NOESY integral volumes

NOE distances can be derived automatically from the assigned NOESY and peak volumes by just clicking the Auto NOE button in the StereoFitter ribbon. It uses the peak volumes from the integrals or peaks picked in the NOESY spectrum, depending on the integration method selected in NOE options, and automatically sets a reference distance between two vicinal aromatic or geminal aliphatic protons. The 'Assignments' table will now have a newly populated column, 'NOE', with a summary of the distance information.

Atom	$\delta$ (ppm)	NOE	NOESY
24 C			
25 H	1.84	2.43(29), 2.75(41)	<input checked="" type="checkbox"/> 29
26 H	3.17	2.48(27), 2.48(30), 1.85(32), 1.85(35), 2.62(36), 2.46(42), 2.90(41)	<input checked="" type="checkbox"/> 27, 29, 32, 33, 42
27 H	1.54	2.49(29), 2.46(33), 2.48(26)	<input checked="" type="checkbox"/> 26
28 H	1.77	2.35(34), 2.89(36), 2.75(41)	
29 H	2.30	2.49(27), 2.49(30), 2.43(25), 2.77(40), 2.69(39), 2.41(33)	<input checked="" type="checkbox"/> 25, 26, 30
30 H	1.54	2.49(29), 2.46(33), 2.48(26)	<input checked="" type="checkbox"/> 29
31 H	1.77	2.35(34), 2.89(36), 2.75(41)	
32 H	2.68	1.85(33), 1.85(26), 1.81(34)	<input checked="" type="checkbox"/> 26
33 H	3.11	2.46(27), 2.46(30), 2.41(29), 1.85(32), 1.85(35), 2.50(36), 2.36(42), 2.76(41)	<input checked="" type="checkbox"/> 26
34 H	3.50	2.35(28), 2.35(31), 1.81(32), 1.81(35), 2.46(36)	<input checked="" type="checkbox"/> 35
35 H	2.68	1.85(33), 1.85(26), 1.81(34)	<input checked="" type="checkbox"/> 34
36 H	5.58	2.89(28), 2.89(31), 2.50(33), 2.62(26), 2.46(34), 2.04(42), 2.03(45), 2.86(41)	<input checked="" type="checkbox"/> 26, 27, 28, 34, 35, 41, 42
37 H	5.75	2.86(41)	
38 H	4.05		
39 H	4.98	2.69(29)	
40 H	4.94	2.77(29)	
41 H	7.51	2.75(28), 2.75(31), 2.75(25), 2.76(33), 2.90(26), 2.86(36), 2.86(37), 2.35(43)	<input checked="" type="checkbox"/> 26, 27, 28, 43
42 H	7.24	2.36(33), 2.46(26), 2.33(48), 2.33(46), 2.33(47), 2.04(36), 2.43(44)	<input checked="" type="checkbox"/> 26, 46, 47, 48
43 H	8.63	2.35(41)	<input checked="" type="checkbox"/> 41
44 H	7.97	2.43(42), 2.47(45)	<input checked="" type="checkbox"/> 45
45 H	7.32	2.55(48), 2.55(46), 2.55(47), 2.03(36), <b>2.47(44)</b>	<input checked="" type="checkbox"/> 44, 46, 47, 48
46 H	3.90	2.33(42), 2.55(45)	<input checked="" type="checkbox"/> 42, 45
47 H	3.90	2.33(42), 2.55(45)	<input checked="" type="checkbox"/> 42, 45
48 H	3.90	2.33(42), 2.55(45)	<input checked="" type="checkbox"/> 42, 45

This process can be also made manually or semi-automatically following these steps:

1. From the StereoFitter ribbon click the '3D Correlations' icon, which will display the '3D Correlations' window. If not already selected, select the 'NOE' tab.
2. Click the 'Fill NOE Table automatically' icon in the '3D Correlations' window:

3D Correlations											
RDC NOE											
Integration method: 2D Peak Volumes											
	Pos1	Pos2	#H1	#H2	Ppm	Diag1	Diag2	Cross1	Cross2	Using	Dist(Å)
1	27, 30	29	2	1	(1.55, 2.30)	116006	81536.7	-1912.62	-2112.41	d1 d2 c1 c2	2.49
2	27, 30	33	2	1	(1.55, 3.09)	116006	134883	-2656.2	0	d1 d2 c1	2.46
3	27, 30	26	2	1	(1.55, 3.17)	116006	134883	-2656.2	-2682.64	d1 d2 c1 c2	2.48
4	28, 31	34	2	1	(1.77, 3.50)	107569	52970.8	-2145.81	-2093.6	d1 d2 c1 c2	2.35
5	28, 31	36	2	1	(1.77, 5.58)	107569	67879.5	-763.796	-681.592	d1 d2 c1 c2	2.89
6	28, 31	41	2	1	(1.77, 7.51)	107569	84507	-799.175	-981.358	d1 d2 c2	2.75
7	25	29	1	1	(1.86, 2.30)	79734.5	81536.7	-1995.63	-1985.99	d1 d2 c1 c2	2.43
8	25	41	1	1	(1.86, 7.51)	79734.5	84507	0	-981.358	d1 d2 c2	2.75
9	29	33	1	1	(2.30, 3.09)	81536.7	134883	-2669.04	-2445.59	d1 d2 c1 c2	2.41
10	29	40	1	1	(2.30, 4.94)	81536.7	115659	-904.61	0	d1 c1	2.77
11	29	39	1	1	(2.30, 4.98)	81536.7	61905.5	-904.61	-941.536	d1 d2 c1 c2	2.69
12	32, 35	33	2	1	(2.69, 3.09)	116875	134883	-15893.4	-16030.1	d1 d2 c1 c2	1.85
13	32, 35	26	2	1	(2.69, 3.17)	116875	134883	-15893.4	-16030.1	d1 d2 c1 c2	1.85
14	32, 35	34	2	1	(2.69, 3.50)	116875	52970.8	-10603.2	-10472.1	d1 d2 c1 c2	1.81
15	33	36	1	1	(3.09, 5.58)	134883	67879.5	0	-1410.82	d1 d2 c2	2.50
16	33	42	1	1	(3.09, 7.25)	134883	77679.2	0	-2285.29	d1 d2 c2	2.36
17	33	41	1	1	(3.09, 7.51)	134883	84507	0	-954.968	d1 d2 c2	2.76
18	26	36	1	1	(3.17, 5.58)	134883	67879.5	-1434.14	-1410.82	d1 d2 c1 c2	2.62
19	26	42	1	1	(3.17, 7.25)	134883	77679.2	-2223.27	-2285.29	d1 d2 c1 c2	2.46
20	26	41	1	1	(3.17, 7.51)	134883	84507	-774.561	-954.968	d1 d2 c1 c2	2.90
21	34	36	1	1	(3.50, 5.58)	52970.8	67879.5	-1369.89	-1323.68	d1 d2 c1 c2	2.46
22	48, 46, 47	42	3	1	(3.90, 7.25)	224323	77679.2	-4128.96	-3480.58	d1 d2 c1 c2	2.33

- In the '3D Correlations' window, information in the 'Pos1', 'Pos2', '#H1', '#H2', 'Ppm', 'Diag1', 'Diag2', 'Cross1', and 'Cross2' columns is sourced from the 'Integrals/Peaks' and 'Assignments' tables. Nothing new has been calculated in these columns, it is just the same information collated from the individual tables. The 'Using' column is initially populated by looking at the information in the diagonal and cross-peaks for the same row. 'd1' means that the value from 'Diag1' column will be used for the distance calculation. 'd2' refers to the 'Diag2' column, 'c1' to the 'Cross1' column, and 'c2' to the 'Cross2' column. Hover over any value in the 'Using' column to see a tooltip that explains the selection of which cross-peak it used for the distance calculation.

	Pos1	Pos2	#H1	#H2	Ppm	Diag1	Diag2	Cross1	Cross2	Using	Dist(Å)
1	27, 30	29	2	1	(1.55, 2.30)	114206	80153.7	-1885.54	-2057.46	d1 d2 c1 c2	2.69
2	27, 30	33	2	1	(1.55, 3.09)	114206	132663	-2607.5	0	d1 d2 c1	2.65
3	27, 30	26	2	1	(1.55, 3.17)	114206	132663	-2607.5	-2617.59	d1 d2 c1 c2	2.68
4	28, 31	34	2	1	(1.77, 3.50)	105821	52053.2	-2101.87	-2045.32	d1* 2 diagonal and 2 cross-peaks integrals found Cross integrals have been averaged	
5	28, 31	36	2	1	(1.77, 5.58)	105821	66845.2	-743.1	-653.638	d1* PANIC correction has been applied	
6	28, 31	41	2	1	(1.77, 7.51)	105821	83094.6	-780.555	-946.028	d1 d2 c2	2.98
7	25	29	1	1	(1.86, 2.30)	78477.1	80153.7	-1962.84	-1943.85	d1 d2 c1 c2	2.62
8	25	41	1	1	(1.86, 7.51)	78477.1	83094.6	0	-946.028	d1 d2 c2	2.98
9	29	33	1	1	(2.30, 3.09)	80153.7	132663	-2617.89	-2393.08	d1 d2 c1 c2	2.60
10	29	40	1	1	(2.30, 4.94)	80153.7	112796	-886.011	0	d1 d2 c1	2.99
11	29	39	1	1	(2.30, 4.98)	80153.7	61431.3	-886.011	-918.377	d1 d2 c1 c2	2.91
12	32, 35	33	2	1	(2.69, 3.09)	115065	132663	-15596	-15696.5	d1 d2 c1 c2	1.99
13	32, 35	26	2	1	(2.69, 3.17)	115065	132663	-15596	-15696.5	d1 d2 c1 c2	1.99

Finally, the 'Dist(Å)' column is the calculated inter-nuclear distance between the atoms specified in the 'Pos1' & 'Pos2' columns, using the information specified in the 'Using' column.

PANIC correction is applied when diagonal peaks are selected. Cross-peak volumes are normalized by the volume of diagonal peaks, so more robust values are obtained for NOE distances. When the difference between cross-peak volumes is high, only the most intense is considered.

The 'Pos1', 'Pos2', '#H1', '#H2', and 'Using' columns are editable, if it is necessary to alter any of the values. The values in the row will be automatically updated.

In this window, use the hand tool to select graphically integrals/peaks from the NOESY spectrum by click&drag around the signal. Diagonal and cross peaks belonging to the same correlation are automatically detected and added in the same row of the NOE table. If integrals/peaks are already present they will be used, if no integrals/peaks are found, new ones will be added to the spectrum.




Use the Delete button to remove the selected rows in the table. If any changes are made, click the 'Save' button to push the newly calculated distances to the 'Assignments' table. The 'Assignments' table will now have populated the NOE column with a summary of the distance information taken from the '3D Correlations' window.

When adding manually a new correlation using the manual tool to get NOESY integrals, for example by selecting the cross and diagonal integrals for atoms 42 and 36, a command "NOE data altered" appears in the Audit Trail. The new correlation

is not automatically transferred to the Assignments table, so the Warning button gets enabled. This is indicated in the message obtained when clicking the Warning button:


3D Correlations ☰ x

RDC NOE

**Integration method:** 2D Peak Volumes

	Pos1	Pos2	#H1	#H2	Ppm	Diag1	Diag2	Cross1	Cross2	Using	Dist(Å)
1	45	44	1	1	(7.32, 7.97)	72718.3	73331.3	-1732.65	-1740.13	c1 d2 c2 d1	2.58
2	<b>41</b>	<b>43</b>	<b>1</b>	<b>1</b>	<b>(7.51, 8.63)</b>	<b>83094.6</b>	<b>73894.4</b>	<b>-2031.18</b>	<b>-2637.95</b>	<b>c2 d1</b>	<b>2.46</b>
3	42	36	1	1	(7.24, 5.58)	74511.9	65565.2	-5048.7	-4662.85	c1 d2 c2 d1	2.16

 **3D Correlations - NOE** X

These NOE values differ from the current assignments, displayed in the Assignments Table. Do you want to transfer here the Assignments Table NOE data?

You can keep working with these auxiliary data pressing 'No'.  
(Keep in mind that 'Save' button should be pressed to make the assignments effective).



## NOE options

It allows to choose the integration method and reference distance to be used in the calculation of NOE distances.

Choose Peaks Integration Method to measure integration volumes using peaks, or choose Volumes Integration method to measure integration volumes using 2D integrals.

The NOE distance calculation can use either specified integrals from the NOESY spectrum or just the peaks. For integrals everything is simple – their volume will be used for the distance calculations.

If peaks are used, then the routine will use a trace along F2 and calculate the 1D sum integral around the peak. The integration limits are determined by the peak linewidth, found by the peak picking.

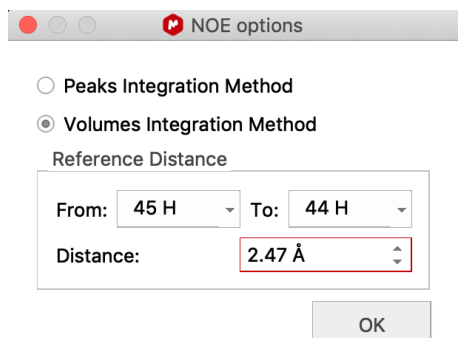
For each correlation, the 'Peak integration method':

- takes the list of peaks in the area formed by the cross of the assignment ranges.
- calculates the f2 interval which contains all peaks with their widths
- takes the f1 point corresponding to the maximum intensity peak.
- takes the horizontal trace at this MaxIntensity point and sum all the trace points contained in the calculated interval.

This sum will be the value of the 'integrated area'.

If neither integrals or peaks are present on a particular NOESY spectrum, the automatic peak picking will be applied. If both, integrals and peaks are present, then again peaks will be used. So, peaks always have a priority –as trace integration is more robust than 2D volumes.

Enter the relevant internuclear distance between two specified atom numbers in the 'NOE options' window. You can use the '3D Molecule' window to measure a known reference distance between two protons in the molecule. Generally, vicinal aromatic or geminal aliphatic protons are good choices. This distance will be used to calibrate all volumes in the NOESY spectrum when extracted into StereoFitter:



NOE options

Peaks Integration Method

Volumes Integration Method

Reference Distance

From: 45 H To: 44 H

Distance: 2.47 Å

OK

Click 'OK' to save these values. The reference distance shows in bold in the 3D Correlations widget and Assignments table.

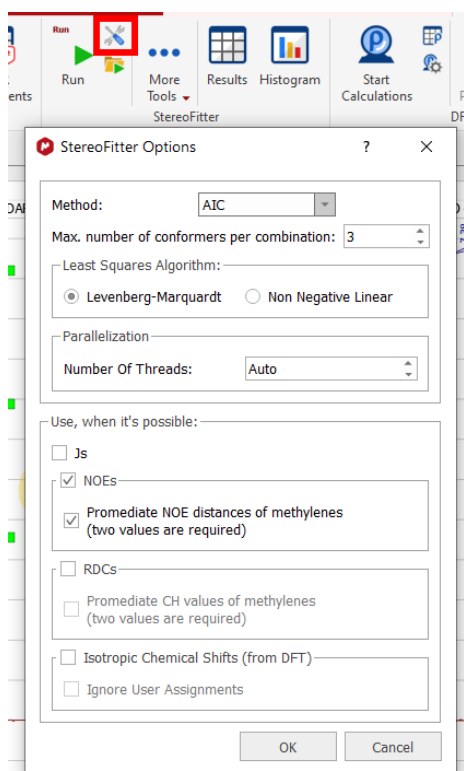
## Import a Set of Conformers, or Generate a Set of Conformers in StereoFitter

If a set of 3D conformers has been computed outside Mnova, import them using the 'Load from File' or 'Load from Folder' options in the StereoFitter ribbon. If only a 2D molecular structure is available, select it in the 'Compounds' table in Mnova, then generate a set of 3D conformers using the 'Balloon' or 'GMMX' icons in the StereoFitter ribbon. Inspect the calculated set of conformers in the '3D Molecule' window, and refine if desired.

## Calculate the best candidate 3D conformer(s) from the set of supplied conformers

StereoFitter can accept four distinct types of input in order to calculate the best 3D structure candidate(s): NOEs, RDCs, RCSAs (from external input files only), *J*-couplings, and/or chemical shifts. Information can be supplied from one or more of the techniques in parallel, and it is this potential for combining information from different techniques in one calculation that makes StereoFitter uniquely powerful.

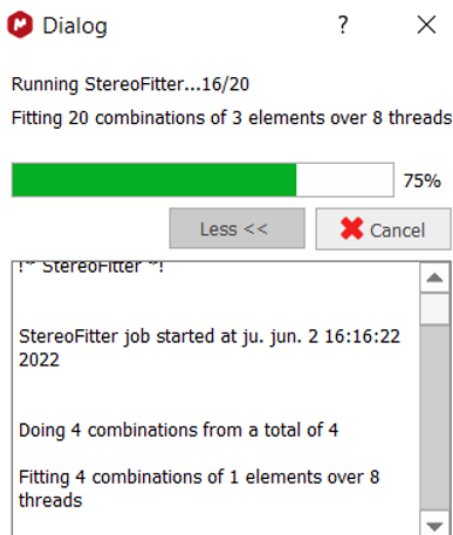
In this example there are only NOEs available so this option should be checked prior to run the calculation. Open the 'StereoFitter options' window by clicking the "wrench & screwdriver" icon in the StereoFitter section of the StereoFitter ribbon:



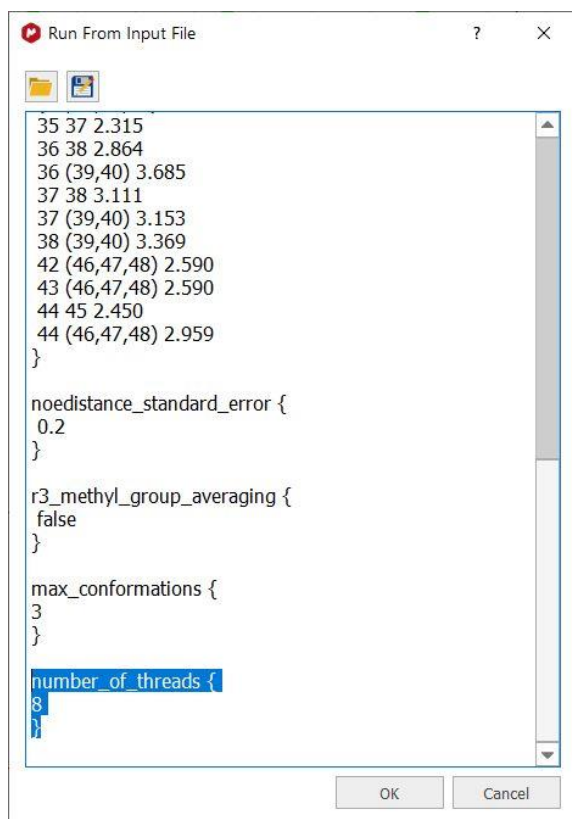
The option to select the maximum number of conformers per combination will allow you to select the upper limit in the combinations (between 0 and 99). You could also select an automatic value which will not impose restrictions to this number; StereoFitter will combine the conformers in groups of 1, 2, ... *N*, being *N* the total number of conformers. This process ends when *N* is reached or when a minimum in the AIC value is found.

You will have the capability to select the 'Least Squares Algorithm'. The 'non negative linear least squares algorithm' is between one and two orders or magnitude faster than the Levenberg-Marquadt algorithm.

Number of Threads: you can choose a numerical value or "Auto" value, which will use all the processors of the machine. The value set by the user can be seen in the StereoFitter progress dialog.



When this value is different from 0 a data block `number_of_threads` with the corresponding value is added to the StereoFitter input file:



If you are using an external input file, you can choose between them by using the commands below:

```
least_squares_method {
#non linear least squares
nnls
}
```

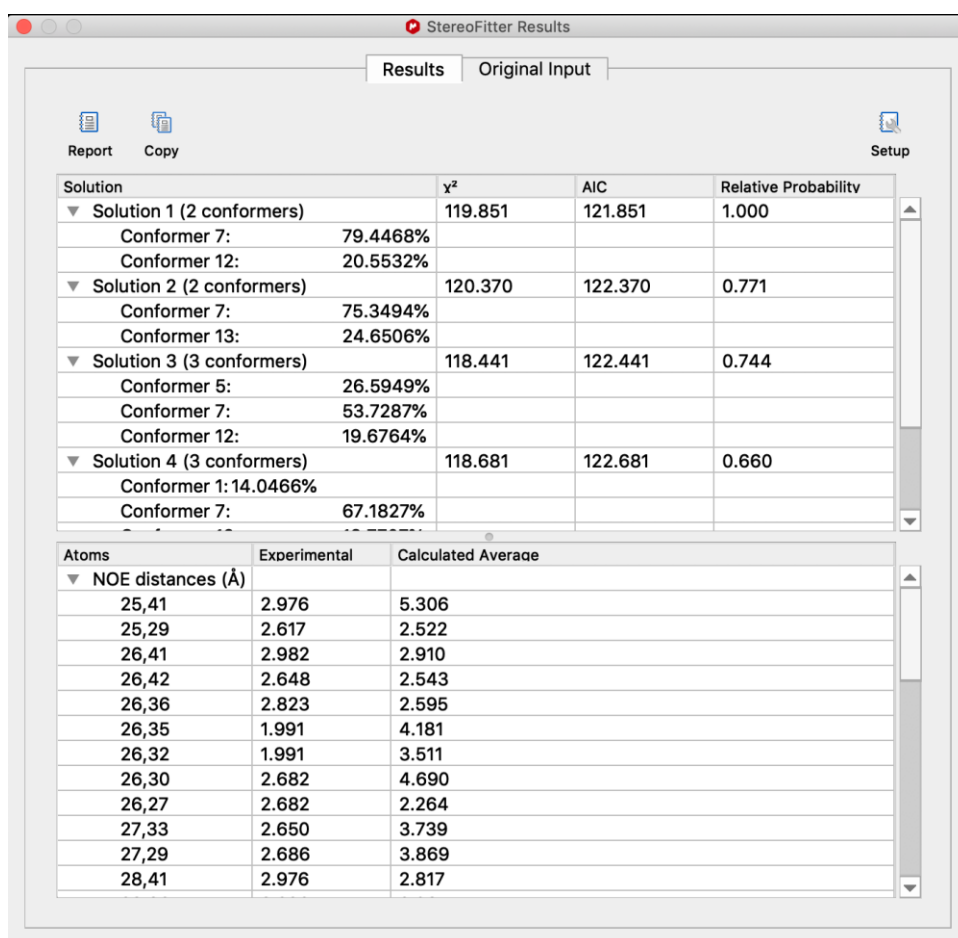
or

```
least_squares_method {
#levenberg-marquardt
lm
}
```

If the 'promediate NOE distances of methylenes' option is checked, all methylene groups of the molecule will be grouped in the input file and the NOE value will be the average calculated from the two individual values. If not, all CH<sub>2</sub> protons are written in separated lines, each one with its NOE value.

## Run the StereoFitter calculation

Click the 'Run' icon on the StereoFitter ribbon. After a few seconds the 'StereoFitter Results' window will be displayed with the results of the calculation:



The screenshot shows the 'StereoFitter Results' window with two tabs: 'Results' and 'Original Input'. The 'Results' tab is active and contains two tables. The first table lists four solutions, each with its conformers,  $\chi^2$ , AIC, and Relative Probability. The second table lists NOE distances for various atoms, comparing experimental values with calculated averages.

Solution	$\chi^2$	AIC	Relative Probability
▼ Solution 1 (2 conformers)	119.851	121.851	1.000
Conformer 7:	79.4468%		
Conformer 12:	20.5532%		
▼ Solution 2 (2 conformers)	120.370	122.370	0.771
Conformer 7:	75.3494%		
Conformer 13:	24.6506%		
▼ Solution 3 (3 conformers)	118.441	122.441	0.744
Conformer 5:	26.5949%		
Conformer 7:	53.7287%		
Conformer 12:	19.6764%		
▼ Solution 4 (3 conformers)	118.681	122.681	0.660
Conformer 1:	14.0466%		
Conformer 7:	67.1827%		

Atoms	Experimental	Calculated Average
▼ NOE distances (Å)		
25,41	2.976	5.306
25,29	2.617	2.522
26,41	2.982	2.910
26,42	2.648	2.543
26,36	2.823	2.595
26,35	1.991	4.181
26,32	1.991	3.511
26,30	2.682	4.690
26,27	2.682	2.264
27,33	2.650	3.739
27,29	2.686	3.869
28,41	2.976	2.817

The result of this calculation suggests that Conformer #7 & Conformer #12, at a ratio of 79.5% & 20.5%, are the most likely 3D structures from a set of thirteen supplied conformers. Solutions 2-5 are less likely ( 'Relative Probability' column ), and are listed so that the differences between possible solutions can be viewed. The most probable solution has a normalized relative probability of 1.000. All other relative probabilities will be less than 1.000. The further from 1.000 the other solutions are, the more confident one can be that the first solution is an accurate representation of the result. If more than one solution has a relative probability near to 1.000, then there is less confidence that the first solution is the best result.

The 'StereoFitter Results' and '3D Molecule' windows are interactive. Select a conformer in the upper part of the 'StereoFitter Results' window, and that conformer will be displayed in the '3D Molecule' window:

Solution	$\chi^2$	AIC	Relative Probability
▼ Solution 1 (2 conformers)	119.851	121.851	1.000
Conformer 7:	79.4468%		
Conformer 12:	20.5532%		
▼ Solution 2 (2 conformers)	120.370	122.370	0.771
Conformer 7:	75.3494%		
Conformer 13:	24.6506%		
▼ Solution 3 (3 conformers)	118.441	122.441	0.744
Conformer 5:	26.5949%		
Conformer 7:	53.7287%		
Conformer 12:	19.6764%		
▼ Solution 4 (3 conformers)	118.681	122.681	0.660
Conformer 1: 14.0466%			
Conformer 7:	67.1827%		

Atoms	Experimental	Calculated Average
▼ NOE distances (Å)		
25,41	2.976	5.191
25,29	2.617	2.518
26,41	2.982	4.129
26,42	2.648	2.450
26,36	2.823	2.533
26,35	1.991	4.175
26,32	1.991	3.499
26,30	2.682	4.667
26,27	2.682	2.272
27,33	2.650	3.833
27,29	2.686	3.873
28,41	2.976	2.715

With the same conformer selected, hover over one of the rows in the lower table in the 'StereoFitter Results' window. Two red diffuse balls will highlight the corresponding atoms in the '3D Molecule' window:

Solution	$\chi^2$	AIC	Relative Probability
▼ Solution 1 (2 conformers)	119.851	121.851	1.000
Conformer 7:	79.4468%		
Conformer 12:	20.5532%		
▼ Solution 2 (2 conformers)	120.370	122.370	0.771
Conformer 7:	75.3494%		
Conformer 13:	24.6506%		
▼ Solution 3 (3 conformers)	118.441	122.441	0.744
Conformer 5:	26.5949%		
Conformer 7:	53.7287%		
Conformer 12:	19.6764%		
▼ Solution 4 (3 conformers)	118.681	122.681	0.660
Conformer 1: 14.0466%			
Conformer 7:	67.1827%		

Atoms	Experimental	Calculated Average
▼ NOE distances (Å)		
25,41	2.976	5.191
25,29	2.617	2.518
26,41	2.982	4.129
26,42	2.648	2.450
26,36	2.823	2.533
26,35	1.991	4.175
26,32	1.991	3.499
26,30	2.682	4.667
26,27	2.682	2.272
27,33	2.650	3.833
27,29	2.686	3.873
28,41	2.976	2.715

The molecule can be rotated at any time in the '3D Molecule' window to get a clearer view of the highlighted atoms. Hovering over any row in the lower table of the 'StereoFitter Results' window will move the highlights around the molecule.

Clicking on a row will add a yellow annotation to the '3D Molecule' window, which shows the two atoms selected, the calculated average internuclear distance, and the experimentally derived internuclear distance in '<>' brackets:

The screenshot shows two windows. The '3D Molecule' window on the left displays a 3D ball-and-stick model of a molecule with a yellow annotation '<2.6>' between two atoms. The 'StereoFitter Results' window on the right shows a table of results for four solutions and a table of NOE distances.

Solution	$\chi^2$	AIC	Relative Probability
▼ Solution 1 (2 conformers)	119.851	121.851	1.000
Conformer 7:	79.4468%		
Conformer 12:	20.5532%		
▼ Solution 2 (2 conformers)	120.370	122.370	0.771
Conformer 7:	75.3494%		
Conformer 13:	24.6506%		
▼ Solution 3 (3 conformers)	118.441	122.441	0.744
Conformer 5:	26.5949%		
Conformer 7:	53.7287%		
Conformer 12:	19.6764%		
▼ Solution 4 (3 conformers)	118.681	122.681	0.660
Conformer 1: 14.0466%			
Conformer 7:	67.1827%		

Atoms	Experimental	Calculated Average
▼ NOE distances (Å)		
25,41	2.976	5.191
25,29	2.617	2.518
26,41	2.982	4.129
26,42	2.648	2.450
26,36	2.823	2.533
26,35	1.991	4.175
26,32	1.991	3.499
26,30	2.682	4.667
26,27	2.682	2.272
27,33	2.650	3.833
27,29	2.686	3.873
28,41	2.976	2.715

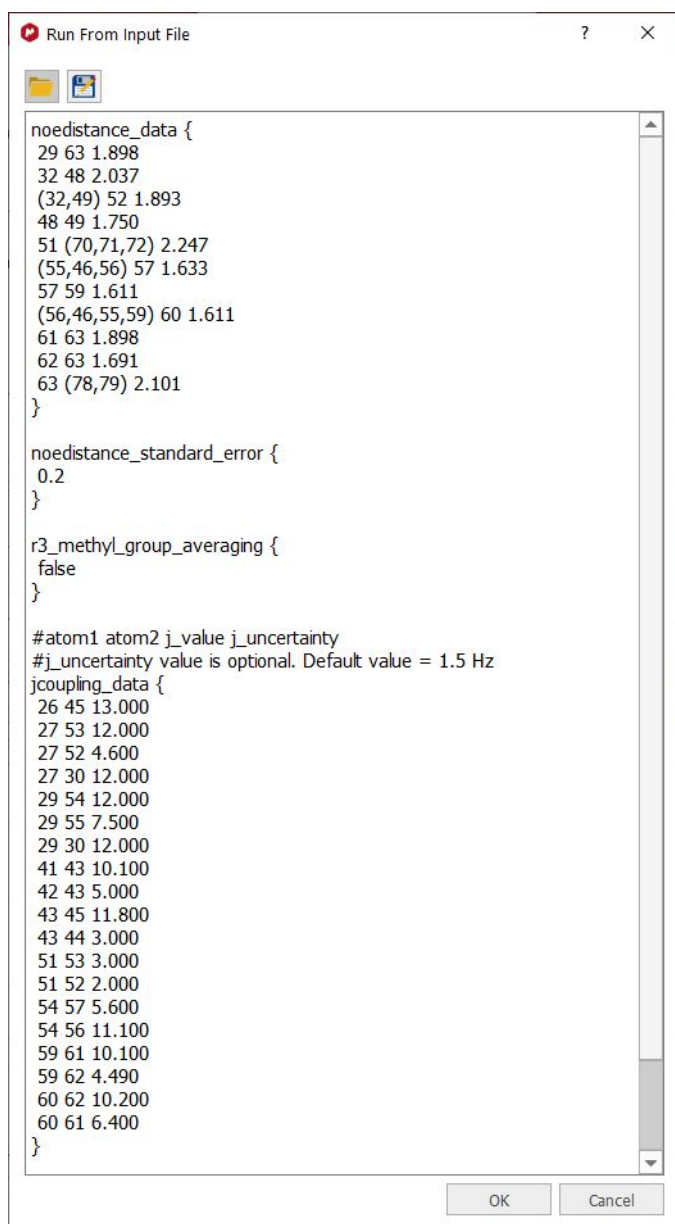
All NOE derived distances can be displayed simultaneously in the 3D Molecule window by selecting 'NOEs Exp.' from the 'Show' menu:

The screenshot shows the '3D Molecule' window with a 3D ball-and-stick model of a molecule. The 'Show' menu is open, and 'NOEs Exp.' is selected. The model is overlaid with a network of dashed lines representing NOE distances, each labeled with a value in '<>' brackets, such as '<2.0>', '<2.4>', '<2.6>', '<2.8>', and '<3.0>'.

You can run StereoFitter analysis from an external input file containing the information of your NOE distances (with the capability to support nonoe values).



When the action 'Run From Input File' is pressed a new dialog is displayed, where the input file can be loaded/saved, shown and edited:



By default, this dialog will show in the TextEdit widget the input file created from the contents of the Assignments Table, according to the StereoFitter settings. If there is no available data in Assignments Table, this TextEdit widget will appear empty.

**Here you can find an example of an external input file:**

```
noedistance_data {
#MRC numbering
#H2 H3 reference of 2.42 Angstrom
20 19 2.42

##When we do not know a priori the assignment of peaks. we just sum up the scaled volumes
and get a
# r^-6 distance from that volume
#H2 H10a e H10beta
20 (25,26) 2.39

#NOE from a methyl group to a single proton
#H2 Me14
20 (28,29,30) 2.71
#H7 H11
21 27 2.19
#H7 Me14
21 (28,29,30) 2.39

#Any NOE is observed between 8 and Me13, we use the nonoe keyword
#8 Me13
22 (34,35,36) 2.82
#7 Me13
21 (34,35,36) nonoe
}

r3_methyl_group_averaging {
false
}
```

You could also combine chemical shift and *J*-couplings information as in the example below:

```
chemicalshiftdelta_data {
22 (27,28,29) 0.62
23 (27,28,29) 0.62
(30,31) (27,28,29) 1.95
20 (27,28,29) 2.13
21 (27,28,29) 2.47
```



```
24 (27,28,29) 4.32
```

```
}
```

```
max_conformations {
```

```
4
```

```
}
```

```
chemicalshiftdelta_standard_error {
```

```
1 0.1
```

```
6 0.25
```

```
}
```

```
jcoupling_group_data {
```

```
#21 (20,22,23) (5.6,6.6,6.6) 0.5 altona
```

```
20 (30,31) (3.5,4.6) 0.5 altona
```

```
}
```

```
jcoupling_data {
```

```
20 21 5.4 0.5 altona
```

```
}
```

```
least_squares_method {
```

```
#nnls
```

```
lm
```

```
}
```

```
noedistance_data {
```

```
26 24 2.67
```

```
26 (22,23) 2.99
```

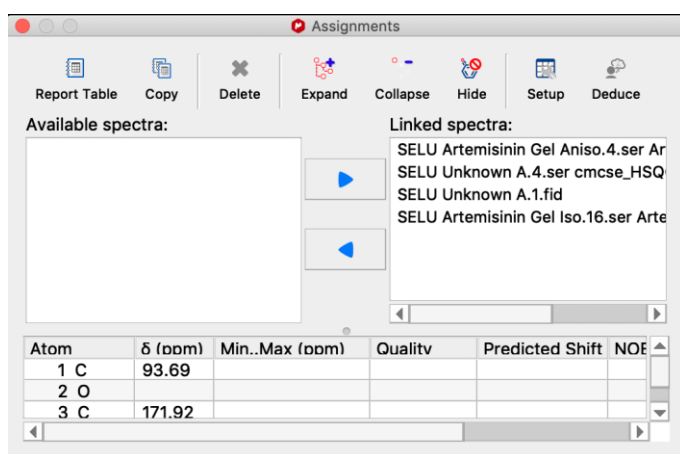
```
26 (30,31) 3.67
```

```
#26 (27,28,29) 2.9
```

```
}
```

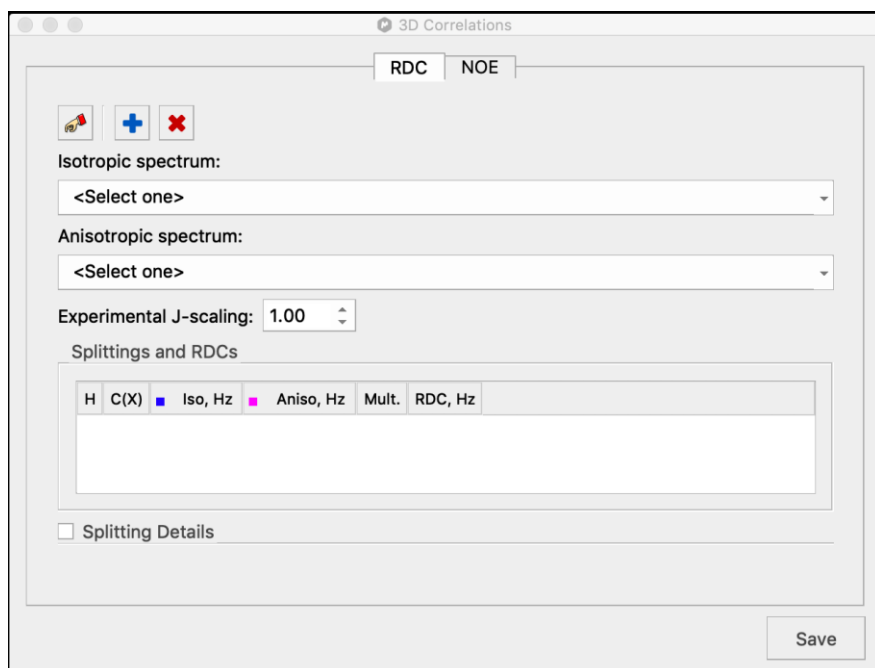
## 1.4. Configurational Analysis: Using RDCs and predicted chemical shifts to determine 3D structure from a set of stereoisomers

This section describes a typical workflow in StereoFitter using predicted  $^{13}\text{C}$  chemical shifts and F1-coupled HSQC spectra together to determine the best candidate 3D stereoisomer of a molecule from a supplied set of stereoisomers. For each stereoisomer, a 3D calculation will be performed to determine the best conformer, or conformers. Open a pair of F1-coupled HSQC spectra, in uncompressed (or unstretched) and compressed (or stretched) media into the same Mnova document, along with a 2D or 3D molecular structure. If the spectra have not already been assigned to the molecular structure, then this will need doing first. Make sure that all spectra are linked in the 'Assignments' table before going to the next section:

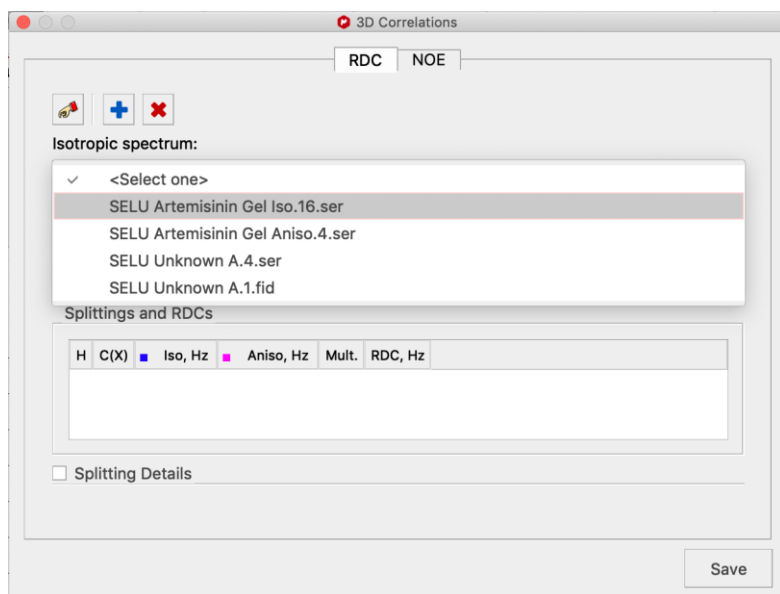


### Extract RDCs from experimental spectra

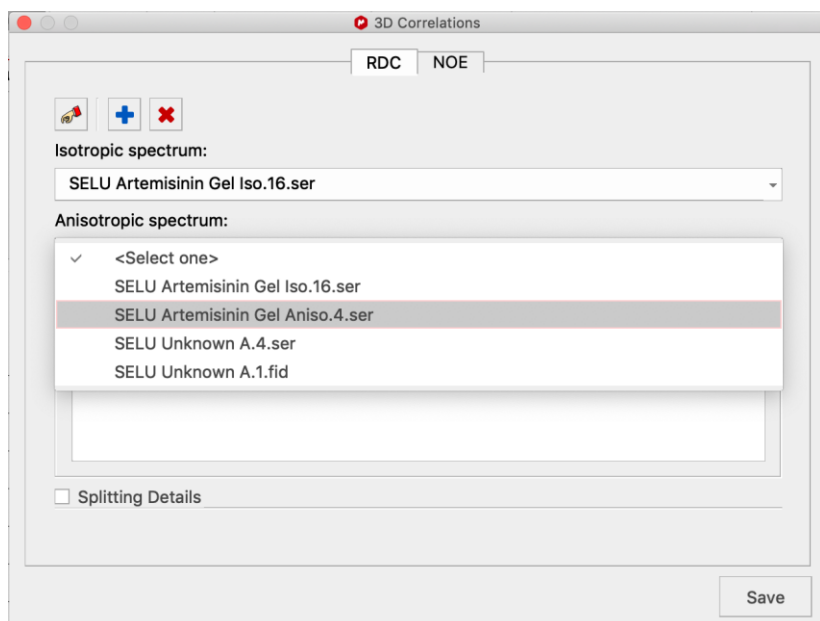
Open the '3D Correlations' window, and select the 'RDC' tab:



Open the 'Isotropic spectrum' menu, and select the uncompressed/unstretched F1-coupled HSQC spectrum from the list of loaded spectra in the Mnova document:

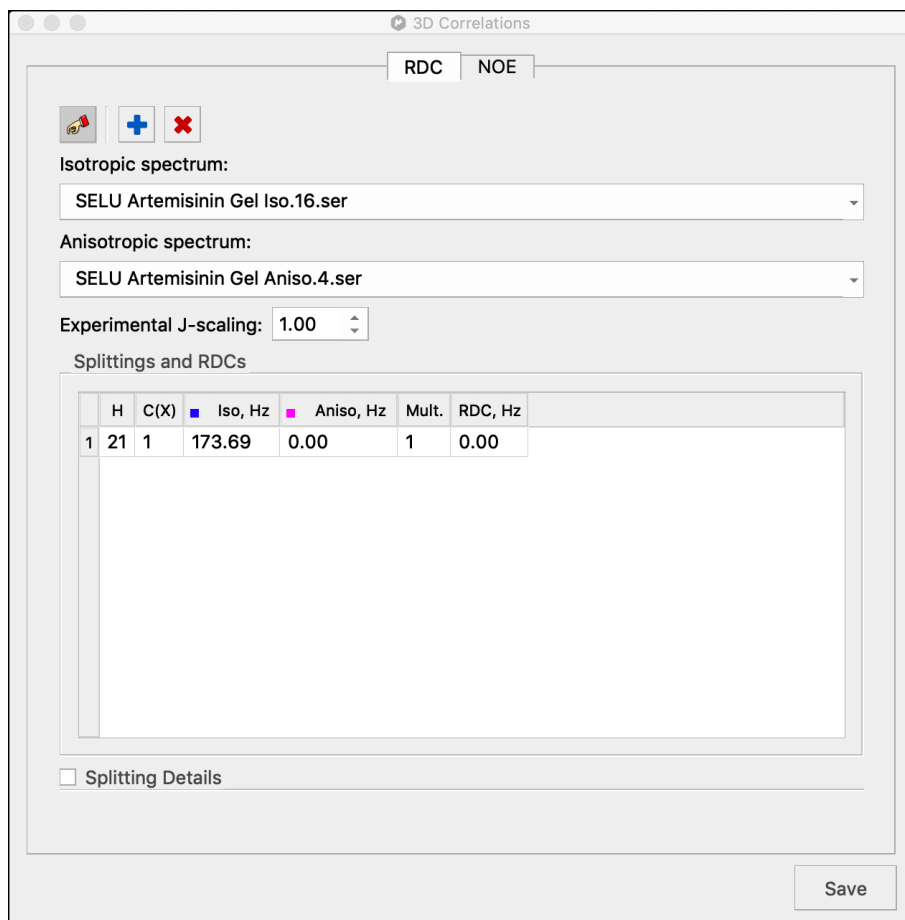


Open the 'Anisotropic spectrum' menu, and select the compressed/stretched F1-coupled HSQC spectrum from the list of loaded spectra in the Mnova document:

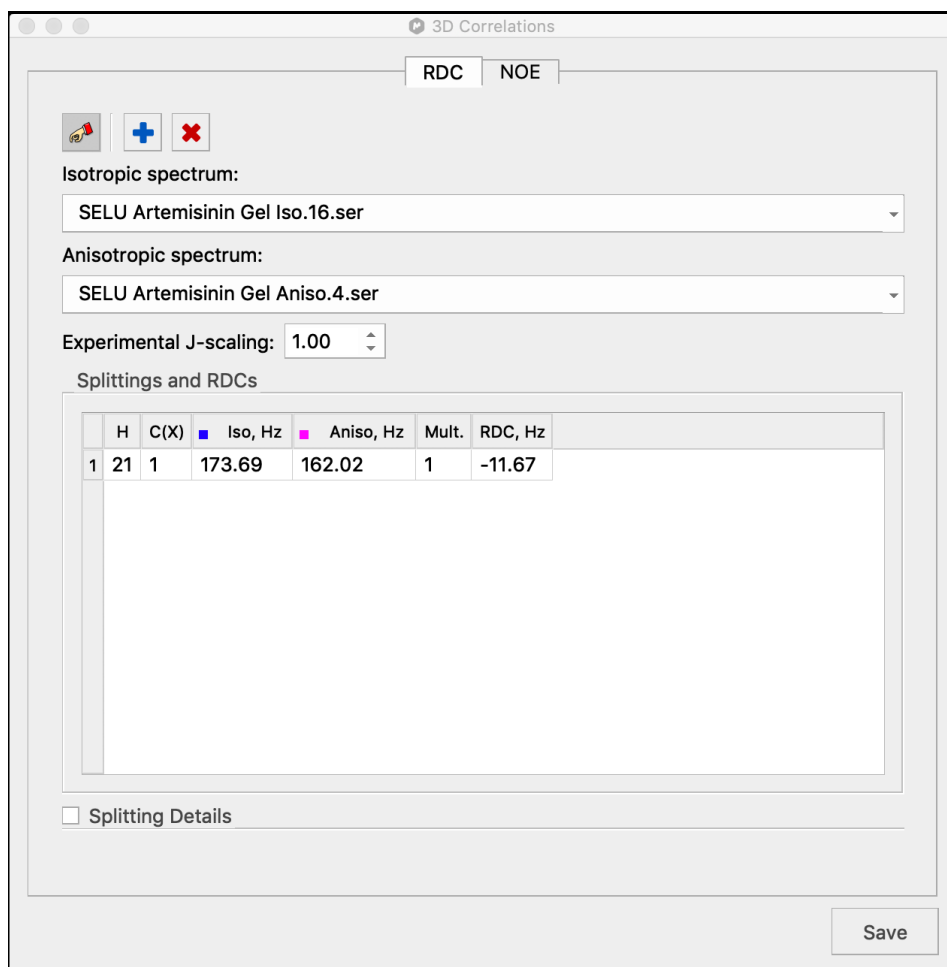




After dragging across a pair of F1 splittings, an entry will appear in the 'Splittings and RDCs' section of the 'RDC' tab':



Now select the anisotropic spectrum and click & drag across the same pair of F1 splittings. The splitting information will be added to the same row, and the RDC will be computed from the difference between the splitting in the isotropic and anisotropic spectra:



Repeat this for all F1 splittings. The more RDCs that can be extracted from the F1-coupled spectra, the better the 3D structure will be.

Individual splitting details can be viewed in the 'RDC' tab by checking the 'Splitting details' checkbox, then selecting any one of the entries in the 'Splittings and RDCs' section:



3D Correlations
RDC
NOE

Isotropic spectrum:

Anisotropic spectrum:

Experimental J-scaling:

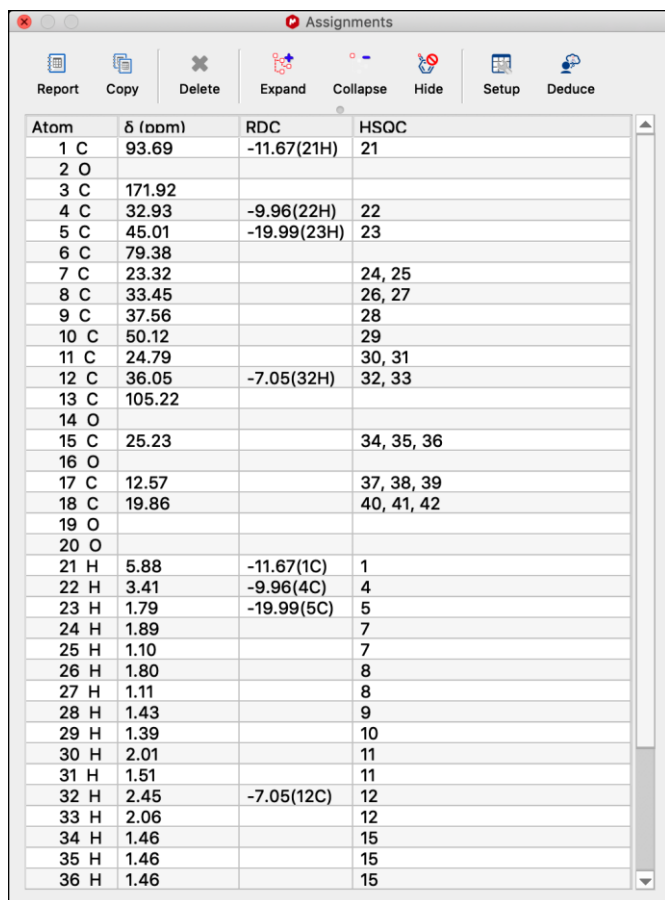
Splittings and RDCs

	H	C(X)	Iso, Hz	Aniso, Hz	Mult.	RDC, Hz
1	21	1	173.69	162.02	1	-11.67
2	22	4	125.44	115.48	1	-9.96
3	32	12	255.06	240.96	2	-7.05
4	23	5	130.12	110.13	1	-19.99

Splitting Details
 

	Property	Value
1	Spectrum:	SELU Artemisinin Gel Iso.16.ser
2	Iso/Aniso:	Isotropic
3	H:	21
4	C(X):	1
5	Peak1 (ppm,ppm):	(5.889, 0.696)
6	Peak2 (ppm,ppm):	(5.889, -0.685)
7	Splitting (Hz):	173.69

Once all RDCs have been extracted into the 'RDC' tab of the '3D Correlations' window, click the 'Save' button to push the RDC information into the 'Assignments' table:



Atom	δ (ppm)	RDC	HSQC
1 C	93.69	-11.67(21H)	21
2 O			
3 C	171.92		
4 C	32.93	-9.96(22H)	22
5 C	45.01	-19.99(23H)	23
6 C	79.38		
7 C	23.32		24, 25
8 C	33.45		26, 27
9 C	37.56		28
10 C	50.12		29
11 C	24.79		30, 31
12 C	36.05	-7.05(32H)	32, 33
13 C	105.22		
14 O			
15 C	25.23		34, 35, 36
16 O			
17 C	12.57		37, 38, 39
18 C	19.86		40, 41, 42
19 O			
20 O			
21 H	5.88	-11.67(1C)	1
22 H	3.41	-9.96(4C)	4
23 H	1.79	-19.99(5C)	5
24 H	1.89		7
25 H	1.10		7
26 H	1.80		8
27 H	1.11		8
28 H	1.43		9
29 H	1.39		10
30 H	2.01		11
31 H	1.51		11
32 H	2.45	-7.05(12C)	12
33 H	2.06		12
34 H	1.46		15
35 H	1.46		15
36 H	1.46		15

## Import a Set of Configurations/Stereoisomers

If a set of 3D stereoisomers has been computed outside Mnova, import them using the 'Load from File' or 'Load from Folder' options in the StereoFitter ribbon. They will be loaded into the 'Compounds' table. Each stereoisomer may have multiple conformers available.

If DFT results are included with the set of stereoisomers, then they will be imported at the same time. Otherwise, compute the DFT results using the 'DFT Predictor' in StereoFitter for each of the stereoisomers.

Imported stereoisomers can be inspected using the '3D Molecule' window.

If only a 2D molecular structure is available, select it in the 'Compounds' table, then generate the complete set of stereoisomers using the 'Generate Stereoisomers' icon in the StereoFitter ribbon:



	Structure	Stereo
1		SRRSRSS
2		RRRSRSS
3		SRSSRSS
4		RRRSRSS
5		SSRSRSS
6		RSRSRSS

If you have a 2D assigned molecule and you import the 3D stereoisomers, the assignments will be automatically transferred to all the stereoisomers.

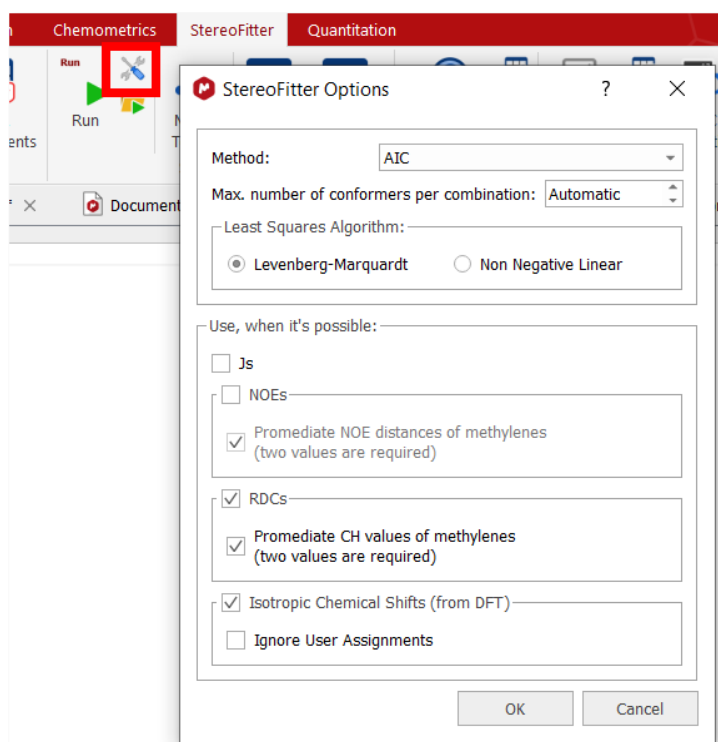
If the 2D structure already contains chiral centers, then the number of computed stereoisomers will be constrained.

Note that NMR cannot generally distinguish between enantiomers, so it is necessary to delete some of the resulting stereoisomers to avoid redundancy in any 3D calculations further downstream. Delete any unwanted stereoisomers from the 'List of Stereoisomers' window by selecting one, or selecting a range by shift-clicking, then right-clicking to reveal a context menu, from which 'Delete' should be chosen. Once only the desired set of stereoisomers remain in the 'List of Stereoisomers' window, choose either 'Copy to Table' or 'Copy to Document and Table' icons at the top of the window. The stereoisomers will be copied into the 'Compounds' table.

## Calculate the best candidate 3D configuration from the set of supplied stereoisomers

StereoFitter can accept four distinct types of input in order to calculate the best 3D structure candidate(s): NOEs, RDCs, *J*-couplings, and chemical shifts.

In this example RDCs and predicted chemical shifts are available so these options should be checked prior to run the calculation. Open the 'StereoFitter options' window by clicking the "wrench & screwdriver" icon in the StereoFitter section of the StereoFitter ribbon:



The option to select the maximum number of conformers per combination will allow you to select the upper limit in the combinations (between 0 and 99). You could also select an automatic value which will not impose restrictions to this number; StereoFitter will combine the conformers in groups of 1, 2, ... *N*, being *N* the total number of conformers. This process ends when *N* is reached or when a minimum in the AIC value is found.

You will have the capability to select the 'Least Squares Algorithm'. The 'non negative linear least squares algorithm' is between one and two orders or magnitude faster than the Levenberg-Marquadt algorithm.

If you are using an external input file, you can choose between them by using the commands below:

```
least_squares_method {  
#non linear least squares  
nnls  
}
```

or

```
least_squares_method {  
#levenberg-marquardt  
lm  
}
```

If the 'promediate CH values of methylenes' option is checked, all CH<sub>2</sub> groups of the molecule will be grouped in the input file and the RDC value will be the average calculated from the two individual values. If not, each C-H vector in the CH<sub>2</sub> groups will be written in separated lines, each one with its RDC value.

Click 'OK' to select these options for the calculation.

### Run the StereoFitter calculation

Select all of the stereoisomers in the 'Compounds' table, then click the 'Run' icon on the StereoFitter ribbon. After a few seconds the 'StereoFitter Results' window will be displayed with the results of the calculation:

StereoFitter Results

Results Original Input

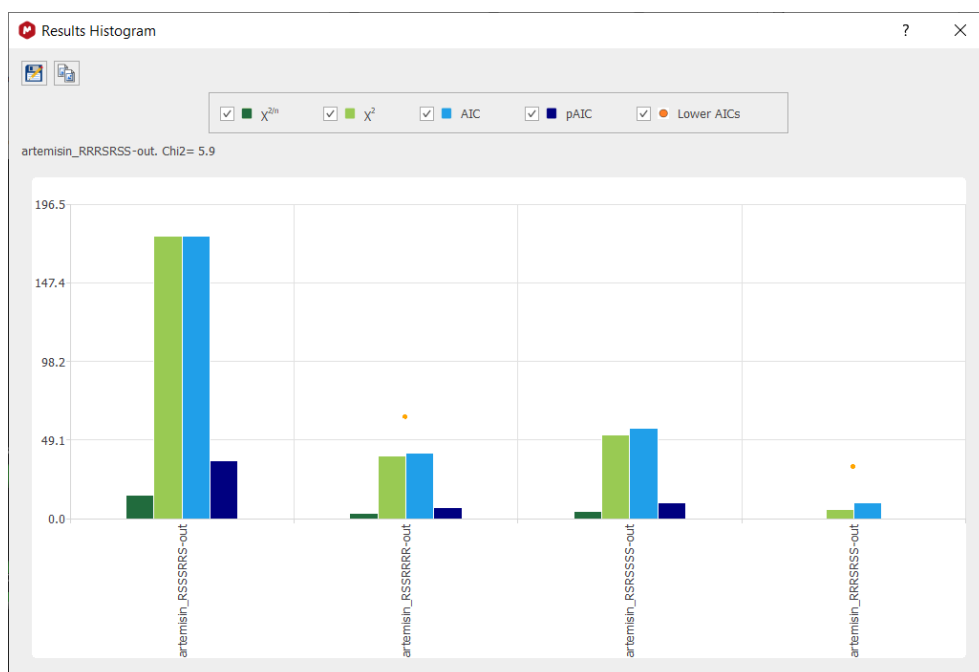
Report Copy Setup

Solution	$\chi^2/n$	$\chi^2$	AIC	Relative Probability
▼ Solution 1 (3 conformers)	0.488	5.860	9.860	1.000
Conformer 2:	53.5205%			
Conformer 3:	13.3119%			
Conformer 6:	33.1676%			
▼ Solution 2 (3 conformers)	0.551	6.616	10.616	0.685
Conformer 2:	44.6195%			
Conformer 5:	21.1238%			
Conformer 6:	34.2567%			
▼ Solution 3 (3 conformers)	0.623	7.477	11.477	0.446
Conformer 2:	54.6063%			
Conformer 3:	11.0258%			
Conformer 4:	34.3679%			
▼ Solution 4 (4 conformers)	0.480	5.762	11.762	0.386

Atoms	Experimental	Calculated Average
▼ RDC couplings (Hz)		
10,24	-12.000	-12.716
14,31	-10.300	-10.309
8,22	-20.800	-21.236
(11,25) (11,26)	-2.400	-2.324
(13,29) (13,30)	-12.500	-12.527
9,23	-21.400	-21.895
7,21	-23.800	-21.866
(12,27) (12,28)	-8.500	-8.852
(16,32) (16,33)	-7.600	-7.582
(20,40) (20,41) (20,42)	-2.400	-3.422
(19,37) (19,38) (19,39)	-4.700	-4.234
(17,34) (17,35) (17,36)	-3.900	-5.436

A 'Results Histogram' will also be displayed, showing the results from the 'StereoFitter Results' in graphical form (including AIC,  $\chi^2$ ,  $\chi^2/n$  and pAIC values). Clicking on the save button will allow you to export the results and a SVG file. You will have also the option to copy the histogram to the clipboard (to be pasted in a third party application).



Hovering the mouse over any bar will display on the upper part of histogram the molecule label, and the value of each bar. Clicking on one of the bars will make the corresponding molecule of the compounds table as the active one.

The more stable stereoisomers will contain an orange mark (Lower AICs). These points can be shown/hidden clicking on the corresponding checkbox of the legend box. Clicking on one of these points will make the applicable molecule as the active one.

You can display the pAIC values<sup>12</sup> (which represents the relative probabilities respect to the best model for each diastereoisomer) when selecting all the compounds from Compounds table. The pAIC values are computed according to the following expression:

$$pAIC_j = -\log_{10} e^{-\frac{AIC_j - AIC_{min}}{2}} \quad (7)$$

Each stereoisomer will have its own results displayed in the StereoFitter Results window, representing the best (set of) conformer(s) for that stereoisomer. Click on any of the 3D compounds in the Compound table or the Results Histogram to display the results for each stereoisomer in the StereoFitter Results window.

The result of this calculation, displayed in the screenshot above, suggests that conformers 2, 3 and 6 from stereoisomer RRRSRSS, at a ratio of 53.5%, 13.3% and 33.2%, are the most likely 3D structures from the set of three supplied conformers. Solutions 2 & 3 are less likely ('Relative Probability' column), and are listed so that the differences between possible solutions can be viewed. The most probable solution has a normalized relative probability of 1.000. All other relative probabilities will be less than 1.000. The further from 1.000 the other solutions are, the more confident one can be that the first solution is an accurate representation of the result. If more than one solution has a relative probability near to 1.000, then there is less confidence that the first solution is the best result.

The 'StereoFitter Results' and '3D Molecule' windows are interactive. The molecule can be rotated at any time in the '3D Molecule' window to get a clearer view of the highlighted atoms. Select a conformer in the upper section of the 'StereoFitter Results' window, and that conformer will be displayed in the '3D Molecule' window:

Solution	$\chi^2/n$	$\chi^2$
Solution 1 (3 conformers) <ul style="list-style-type: none"> <li>Conformer 2: 53.5205%</li> <li>Conformer 3: 13.3119%</li> <li>Conformer 6: 33.1676%</li> </ul>	0.488	5.860
Solution 2 (3 conformers) <ul style="list-style-type: none"> <li>Conformer 2: 44.6195%</li> <li>Conformer 5: 21.1238%</li> <li>Conformer 6: 34.2567%</li> </ul>	0.551	6.616
Solution 3 (3 conformers) <ul style="list-style-type: none"> <li>Conformer 2: 54.6063%</li> <li>Conformer 3: 11.0258%</li> <li>Conformer 4: 34.3679%</li> </ul>	0.623	7.477

Atoms	Experimental	Calculated Average
RDC couplings (Hz) <ul style="list-style-type: none"> <li>10,24</li> <li>14,31</li> <li>8,22</li> <li>(11,25) (11,26)</li> <li>(13,29) (13,30)</li> <li>9,23</li> <li>7,21</li> <li>(12,27) (12,28)</li> <li>(16,32) (16,33)</li> <li>(20,40) (20,41) (20,42)</li> <li>(19,37) (19,38) (19,39)</li> <li>(17,34) (17,35) (17,36)</li> </ul>	-12,000 -10,300 -20,800 -2,400 -12,500 -21,400 -23,800 -8,500 -7,600 -2,400 -4,700 -3,900	-12,716 -10,309 -21,236 -2,324 -12,527 -21,895 -21,866 -8,852 -7,582 -3,422 -4,234 -5,436

With the same conformer selected, hover over one of the 'RDC couplings (Hz)' rows in the lower table in the 'StereoFitter Results' window. Red diffuse balls will highlight the corresponding atoms in the '3D Molecule' window:

3D Molecule

Energy (kcal/mol): 1.02      Population (%): 13.73

#2 of 6

StereoFitter Results

Results    Original Input

Report    Copy    Setup

Solution	$\chi^2/n$	$\chi^2$
<ul style="list-style-type: none"> <li>▼ Solution 1 (3 conformers)               <ul style="list-style-type: none"> <li>Conformer 2: 53.5205%</li> <li>Conformer 3: 13.3119%</li> <li>Conformer 6: 33.1676%</li> </ul> </li> </ul>	0.488	5.860
<ul style="list-style-type: none"> <li>▼ Solution 2 (3 conformers)               <ul style="list-style-type: none"> <li>Conformer 2: 44.6195%</li> <li>Conformer 5: 21.1238%</li> <li>Conformer 6: 34.2567%</li> </ul> </li> </ul>	0.551	6.616
<ul style="list-style-type: none"> <li>▼ Solution 3 (3 conformers)               <ul style="list-style-type: none"> <li>Conformer 2: 54.6063%</li> <li>Conformer 3: 11.0258%</li> <li>Conformer 4: 34.3679%</li> </ul> </li> </ul>	0.623	7.477

Atoms	Experimental	Calculated Average
<ul style="list-style-type: none"> <li>▼ RDC couplings (Hz)               <ul style="list-style-type: none"> <li>10,24</li> <li>14,31</li> <li>8,22</li> <li>(11,25) (11,26)</li> <li style="background-color: #f0f0f0;">(13,29) (13,30)</li> <li>9,23</li> <li>7,21</li> <li>(12,27) (12,28)</li> <li>(16,32) (16,33)</li> <li>(20,40) (20,41) (20,42)</li> <li>(19,37) (19,38) (19,39)</li> <li>(17,34) (17,35) (17,36)</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>-12.000</li> <li>-10.300</li> <li>-20.800</li> <li>-2.400</li> <li style="background-color: #f0f0f0;">-12.500</li> <li>-21.400</li> <li>-23.800</li> <li>-8.500</li> <li>-7.600</li> <li>-2.400</li> <li>-4.700</li> <li>-3.900</li> </ul>	<ul style="list-style-type: none"> <li>-12.716</li> <li>-10.309</li> <li>-21.236</li> <li>-2.324</li> <li style="background-color: #f0f0f0;">-12.527</li> <li>-21.895</li> <li>-21.866</li> <li>-8.852</li> <li>-7.582</li> <li>-3.422</li> <li>-4.234</li> <li>-5.436</li> </ul>

RDCs values can be displayed in the 3D Molecule widget by selecting each solution in the upper part of the table and clicking each RDC row in the bottom part:

The 'StereoFitter Results' window displays the following data:

Solution	$\chi^2/n$	$\chi^2$
▼ Solution 1 (3 conformers)	0.488	5.860
Conformer 2:	53.5205%	
Conformer 3:	13.3119%	
Conformer 6:	33.1676%	
▼ Solution 2 (3 conformers)	0.551	6.616
Conformer 2:	44.6195%	
Conformer 5:	21.1238%	
Conformer 6:	34.2567%	
▼ Solution 3 (3 conformers)	0.623	7.477
Conformer 2:	54.6063%	
Conformer 3:	11.0258%	
Conformer 4:	34.3679%	

Atoms	Experimental	Calculated Average
▼ RDC couplings (Hz)		
10,24	-12.000	-12.716
14,31	-10.300	-10.309
8,22	-20.800	-21.236
(11,25) (11,26)	-2.400	-2.324
(13,29) (13,30)	-12.500	-12.527
9,23	-21.400	-21.895
7,21	-23.800	-21.866
(12,27) (12,28)	-8.500	-8.852
(16,32) (16,33)	-7.600	-7.582
(20,40) (20,41) (20,42)	-2.400	-3.422
(19,37) (19,38) (19,39)	-4.700	-4.234
(17,34) (17,35) (17,36)	-3.900	-5.436

Hover over one of the Chemical Shifts (ppm)' rows in the lower table in the 'StereoFitter Results' window. A red diffuse ball will highlight the corresponding atom in the '3D Molecule' window:

3D Molecule

Energy (kcal/mol): 0.00      Population (%): 77.22

#1 of 6

StereoFitter Results

Results    Original Input

Report    Copy    Setup

Solution	$\chi^2/n$	$\chi^2$
Solution 1 (3 conformers) <ul style="list-style-type: none"> <li>Conformer 2: 49.1813%</li> <li>Conformer 3: 14.4943%</li> <li>Conformer 6: 36.3244%</li> </ul>	6.093	262.013
Solution 2 (4 conformers) <ul style="list-style-type: none"> <li>Conformer 2: 49.0597%</li> <li>Conformer 3: 13.9226%</li> <li>Conformer 4: 6.57268%</li> <li>Conformer 6: 30.445%</li> </ul>	6.082	261.508
Solution 3 (4 conformers) <ul style="list-style-type: none"> <li>Conformer 1: 0.581595%</li> <li>Conformer 2: 48.8907%</li> </ul>	6.094	262.023

Atoms	Experimental	Calculated Average
(20,40) (20,41) (20,42)	-2.400	-3.473
Chemical shifts (ppm)		
6	79.380	83.036
7	50.118	46.076
8	45.014	42.121
9	37.557	30.326
10	93.686	99.686
11	23.320	24.527
12	24.790	23.463
13	33.450	29.084
14	32.928	38.765
15	105.220	106.286
16	36.054	37.522



You can run StereoFitter analysis from an external input file containing the information of your RDC values (with the capability to support RCSA's values).



Here you can find an example of an external input file containing RDC values:

```
rdc_data {
  1 21 -12.000
  4 22 -10.300
  5 23 -20.800
  7 24 -2.400
  8 26 -12.500
  9 28 -21.400
  10 29 -23.800
  11 30 -8.500
  (12,32) (12,33) -7.600
  (15,34) (15,35) (15,36) -2.400
  (17,37) (17,38) (17,39) -4.700
  (18,40) (18,41) (18,42) -3.900
}
rdc_standard_error {
  1.2
}
superimpose {
  true
}
superimpose_atoms {
  auto
}
```

and here containing RCSA values:

```
rcsa_data {
#C1
4 0.282 0.015
#C2
3 0.183 0.012
```

```
#C3
2 0.249 0.012
#C4
1 0.293 0.013
#C5
6 0.154 0.011
#C6
5 0.196 0.015
#C7
9 -0.004 0.011
#C8
8 -0.088 0.025
#C11
19 -0.022 0.011
#C12
20 0.037 0.014
#C13
16 0.013 0.011
#C14
15 0.029 0.012
#C15
14 -0.025 0.011
#C16
13 0.010 0.025
#C17
10 0.022 0.011
#C18
11 0.043 0.012
#C20
25 0.001 0.012
#C22
23 0.192 0.011
#C23
22 -0.043 0.011
}
```

Syntax for Pseudocontact shift restraints are similar to that of RCSAs. The **pcs\_data** keyword is needed to define a PCS block. The molecule should contain a paramagnetic center (tag) where the index of the tag is specified using the **tags** keyword. The tags block may contain more than one index however the presence of multiple paramagnetic centers it is not yet supported in StereoFitter.

```
pcs_data {
#13C pcs
  13      -13.04
```

```
15      -9.54
19      -8.09
21      -7.88
20     -10.50
17     -12.28
18      -8.84
22      -8.62
23      -6.44
}

tags {
49
}

temperature {
298.15
}

spectrometer_frequency {
600
}

pcs_standard_error {
6 0.2
}

least_squares_method {
nnls
}
```

Additionally, the **spectrometer\_frequency** and **temperature** keywords are needed in order to be able to define the scale factor between the alignment tensor and the anisotropy of the magnetic susceptibility one. The **pcs\_standard\_error** keyword is used in order to define a global error (ppm). Individual errors are given after the PCS values similarly to RDC or RCSA data.

## 1.5. Data Blocks and keywords

In this section we will describe the NOESY/ROESY, *J*-couplings, chemical shifts and RDCs and RCSAs data blocks with the applicable keywords.

### 1.5.1. Fitting to NOESY/ROESY determined distances

#### The noedistance\_data block

The noedistance\_data block is used to enter distance restrictions obtained by the means of quantitative NOE experiments. The requirements and protocols for quantitative NOE measurement, based on the Macura/PANIC correction,<sup>27</sup> have been carefully tailored by the group of Craig Butt's in several publications.<sup>28,29</sup> Note that determination of distances from ROESY experiments require the compensation of offset dependence. This can be easily obtained through the use of adiabatic pulses as for instance in the popular EASY-ROESY<sup>30</sup> experiment.

The noedistance\_data block follows the following format

```
noedistance_data {
#a distance of 3 A between protons 15 and 25
#the default uncertainty for NOE computations will be used
15 25

#a distance of 1.9 A between protons 26 and 36
#experimental uncertainty is 0.3 A
16 26 0.3
}
```

When having equivalent by symmetry or synchronous group of nuclei it is possible to directly use them in the fitting by grouping them inside parentheses.

```
noedistance_data {
#a noe distance between a CH2 and a CH3 group
(10,11) (20,21,22) 3.10
#a noe distance between a CH and the two methyl
#groups of an i-propyl group
30 (40,41,42,43,44,45) 2.95
}
```

A useful feature is the ability to use NOE restraints in unassigned groups. Let us consider the case when a CH have a NOESY cross-peak with asynchronous diastereotopic protons. The assignment of these peaks can be avoided by determination of a  $r^{-6}$  averaged distance by summing up the NOESY volumes for the two cross-peaks.

```
noedistance_data {
#an averaged noe distance between a CH two unassigned
#diastereotopic CH2 protons
10 (20,21) 2.85
}
```

It is also possible in StereoFitter to use the absence of a NOESY/ROESY cross peak as a restraint. To do that the indexes of the spins of group of spins must be followed by the nonoe keyword.

```
noedistance_data {

#lack of noe between two protons
30 31 nonoe
#lack of noe between two methyl groups
(20,21,22) (23,24,25) nonoe
}
```

Note that is possible to use a  $\langle r^{-3} \rangle^2$  model for NOEs involving methyl groups. This feature can be invoked using the `r3_methyl_average` block keyword to true or false.

In order to be able to linearize the fitting of NOE distances when using non-negative least-squares algorithm (see below) StereoFitter internally converts the distances in the input to volumes using a  $V \propto \langle R^{-6} \rangle$  relationship. It is the case that if we consider a fixed distance uncertainty  $\sigma_d$  the associated uncertainty for the volumes will grow as  $\sigma_v = 6R^{-7}\sigma_d$  which will be absurdly small for protons located far away. To compensate for that we looked for a weighting function which penalizes noesy restraints between protons located far away. We chose a hyperbolic tangent function of the following form

$$w_{NOE} = \frac{e^x - e^{-x}}{e^x + e^{-x}} \quad (8)$$

$$x = (d/3)^{-3}$$

Where  $w_{NOE}$  is the weighting factor associated to the NOE restraint with distance  $d$ . For the nonoe restraints they are associated with a zero experimental volume and the uncertainty is computed using the following relationship

$$S_d = 6 \times 2.0^{-7} S_d \quad (9)$$

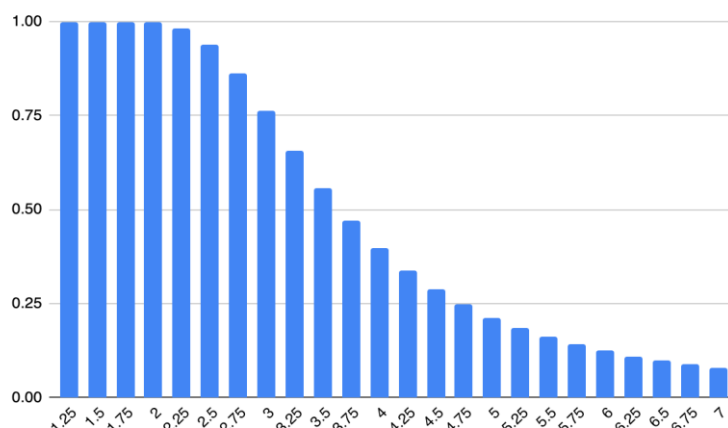


Figure 1. Weighing vs distance in NOESY/ROESY restraints

## 1.5.2. Fitting of scalar J Couplings

### The `jcoupling_data` block

A useful feature in StereoFitter is the possibility to compute couplings through any a large variety of Karplus-type equations. The interface used by StereoFitter is fully compatible with the MSpin program as previously described.<sup>31</sup> Currently, only  $^3J_{\text{HH}}$  proton-proton couplings can be introduced through the assignment table but any kind of coupling can be used in the input file provided that an empirical equation is available or they have been computed at the DFT level.

Experimental  $J$ -couplings are introduced through the `jcoupling_data` block

```

jcoupling_data {
#a proton proton coupling of 8.2 Hz between nuclei 15 and 25
#with an uncertainty of 1.0 Hz
#and computed using the karplus equation
15 25 8.2 1.0 karplus
#a proton proton coupling of 6.0 Hz
#using default uncertainty of 1.0 Hz
#and the default altona equation
16 26 6.0
#a proton proton 3JH-Calpha-N-H coupling using the Pardi equation (for oncoming versions)
#with an uncertainty of 1.0 Hz
17 27 4.0 1.0 Pardi-[H-N-Ca-H]
}

```

Note that in the current version of the module in the case of equations with different sets of parameters associated the first set listed in the **JCoupling** program<sup>17</sup> is loaded. However, in the case of the Altona equation different parameters sets are used for different substitution patterns according to the original publication.

The program will check if the given couplings can be computed using the specified equations. If not the computation will be aborted.

It is also possible to use *J*-couplings obtained in DFT computations. In order to do that the **dft** keyword is used instead of the equation name.

```
jcoupling_data {  
#a coupling between nuclei 10 and 11 the computed values will  
#be retrieved from the original outputs  
10 11 2.0 0.1 dft  
}
```

You can also use unsigned couplings as in the example below:

```
jcoupling_group_data {  
#21 (20,22,23) (5.6,6.6,6.6) 0.5 altona  
20 (30,31) (3.5,4.6) 0.5 altona unsigned  
}
```

```
max_conformations {  
4  
}
```

```
jcoupling_data {  
20 21 5.4 0.5 altona unsigned  
}
```

The experimental values displayed in the results table under the J couplings section will appear as absolute numbers when they are marked as "unsigned" in the input file. These absolute values are shown in Combinations and also in the Conformers items.

Solution	$\chi^2$	AIC	Relative Probabi
Solution 1 (3 conformers) Conformer 1: 28.9981% Conformer 5: 46.4279% Conformer 9: 24.5741%	23.025	27.025	1.000
Solution 2 (3 conformers) Conformer 1: 27.5374% Conformer 5: 49.1959% Conformer 8: 23.2667%	28.718	32.718	0.058
Solution 3 (3 conformers) Conformer 1: 28.6188% Conformer 4: 45.9564%	31.787	35.787	0.013

Atoms	Experimental	Calculated Average
NOE distances (Å)		
24,26	2.670	2.059
(22,23),26	2.990	3.862
26,(30,31)	3.670	6.152
J couplings (Hz)		
20,21	5.400	0.962
20,(30,31)	13.500,14.600	5.372,11.189
Chemical shifts deltas (ppm)		
22,(27,28,29)	0.620	0.375
23,(27,28,29)	0.620	1.043
(30,31),(27,28,29)	1.950	1.860
20,(27,28,29)	2.130	2.100

If the computational burden to compute  $J$ -couplings at the desired DFT level is too high it is possible to fit a Karplus curve in a proper model system, by computing  $J$ -couplings at different values of the chosen dihedral angle, and to fit the DFT so obtained DFT couplings to a cosine series or cosine/sine series depending if the curve is symmetrical or not around a  $0^\circ$  dihedral value. See the **jcoupling\_data** block entry for more details.

When several parameter sets are available for a particular empirical equation it is possible to choose a particular set by appending the name of the parameter set to the equation name. See the **jcoupling\_data** block entry for more details.

## Relativistic force field computations

We have also implemented inside StereoFitter the relativistic force field method of Kutateladze and coworkers. In this method proton-proton couplings<sup>32</sup> are computed at the DFT level. In this protocol molecular structures are optimized at the DFT B3LYP/6-31G\* level and NBO parameters are computed on the optimized structure. A single point computation of the proton-proton couplings, using only the Fermi-Contact term is then done on the optimized structures. The couplings are then scaled according to the expression

$$J = c_1 HF + c_2 F + c_3 H + c_4 \quad (10)$$

Where  $H = h_a h_b$  is the product of the NBO computed  $p$  to  $s$  hybridization ratios in the carbon attached to the coupled protons and  $F$  is the computed Fermi Contact term. The parameters  $c_1$ ,  $c_2$  and  $c_4$  depend on the type of coupling according to the below table.



Presently we only support the RFF parametrization of proton-proton couplings as well as Gaussian09/16 computations. Instructions for the preparation of Gaussian input can be found in the supporting information of the original article describing the method.

**Table 1. Coefficients for the relativistic force field computation of proton-proton  $J$ -couplings**

Type	$c_1$	$c_2$	$c_3$	$c_4$
$^2J$ . H-C-H	-0.020	1.676	-0.151	0.300
$^3J$ . H-C-C-H	-0.004	1.550	0.030	-0.204
$^4J$ . H-C-X-C-H	----	1.505	-0.027	0.381
$^nJ$ . $n > 4$	----	0.950	---	0.200

### The `jcoupling_group_data` block

A potential problem when using  $J$ -coupling restraints is the presence of unassigned diastereotopic protons. Instead of just averaging the values, as in the case of NOE restraints, which will lead to too much information lost we created a new type of restriction called a  $J$ -coupling group. For instance in a AMX spin system, where M and X are unassigned diastereotopic protons it is possible to create such a group in the following way

```
jcoupling_group_data {
#proton 1 is coupled to 10 and 11 with couplings values of 1.3 and 11.0
#but we do not know who is 10 and who is 11
1 (10,11) (11.0,1.3) 0.2 altona
#couplings to three unassigned protons
20 (21,30,31) (3.5,4.6,5.4) 0.5 altona
}
```

The same options available for the `jcoupling_data` block can be applied to the `jcoupling_group_data` block.

### 1.5.3. Fitting of chemical shifts

Chemical shift data<sup>10</sup> can be used in StereoFitter as long chemical shieldings can be computed, using ab initio or DFT methods, for the nuclei of interest.<sup>5,33</sup> The chemical shift restrictions should be entered in the `chemicalshift_data` block

```
chemicalshift_data {
#some carbon shifts
1 23.0
# a t-butyl group
(30,31,32) 11.1
}
```

Equivalent nuclei must be enclosed in parentheses and separated by commas. In the case of diastereotopic groups assignment can be avoided by using the average shift as the chemical shift restriction.

```
chemicalshift_data {
#an averaged ch2 group
(20,21) 2.1
# 1H shifts for averaged diasteretopic methyl groups
(30,31,32,33,34,35) 1.5
}
```

The uncertainties can be given for each datum or using the `chemicalshift_standard_error` block. The atomic number of the nuclei must be given before the value of the uncertainty in ppm

```
chemicalshift_data {
#protn shift, 0.10 ppm uncertainty
40 1.1 0.1
#13C t-butyl group 0.05 ppm uncertainty
(30,31,32) 1.10 0.05
}
```

```
chemicalshift_standard_error {
#13C uncertainty of 2.0 ppm
6 2.0
#1H uncertainty of 0.15 ppm
1 0.15
}
```

## Referencing

Computed shieldings must be transformed into shifts by proper referencing. StereoFitter uses a linear scaling model where computed shifts are calculated from isotropic shieldings as follows

$$d = aS + b \quad (11)$$

The intercept  $b$  and slope  $a$  are obtained by calibration against a molecule or set of molecules of known structure and assigned spectra. These values depend on the particular levels of theory employed for the optimization and computation of shielding tensors. A large collection of linear scaling factors can be found in the CHESHIRE web page.<sup>34</sup>

The linear scaling parameters must be specified for each type of nucleus through the `chemicalshift_references` block

```
chemicalshift_references {
#13C b3lyp/6-31G**//mmff94
#1H b3lyp/6-31G**//mmff94
}
```

Instead of employing the linear scaling method for transformation of shieldings into shifts it is also possible to use a multistandard (double referencing) approach. For instance, a reference can be used for carbons of  $sp^3$  hybridization whereas another one is used for the  $sp^2/sp$  ones. This is done by appending a group number after each linear scaling entry. Note that the slope would be in this method normally set to -1.0

```
chemicalshift_references {
#double reference for sp3 and sp2/sp carbons
6 182.7 -1.0 1
6 184.9 -1.0 2
}
```

Then a **refgroup** label must be appended to each entry on the `chemicalshift_data` block

```
chemicalshift_data {
8      211.9 2.8 refgroup=2
9      42.2  1.7 refgroup=1
10     31.4  1.7 refgroup=1
2      86.6  1.7 refgroup=1
}
```

### The `chemicalshiftdelta_data` block

In some cases, particularly if data belong to nuclei of the same hybridization type or in the case of  $^1H$  nuclei attached to carbons with the same hybridization it is possible to avoid the `chemicalshift_references` block. This block has a similar syntax to the `chemicalshift_delta` block but a chemical shift difference would be used between two groups of spins. The first index or group of indexes correspond to the most deshielded group.

```
chemicalshiftdelta_data {
#difference between two carbons
5 6 46.0
#difference between two methylene groups
(21,22) (17,18) 0.93
}
```

The standard uncertainty for the different nuclei can be controlled by the `chemicalshiftdelta_data_standard_error` block. See corresponding keyword for more details.

### The `chemicalshift_group_data` block

In previous versions of StereoFitter the difference in chemical shift between unassigned spin groups, for instance two unassigned diastereotopic protons, two unassigned methyl groups in an isopropyl group and similar situations could be managed by providing the mean value in the `chemicalshift_data` block followed by their difference in chemical shift using the `chemicalshiftdelta_data` block and the **unsigned** keyword. Following 1.1.5 version it is possible to use a simplified method by using the new `chemicalshift_group_data` block where a list of groups of equivalent spins is followed by a list

of the measured chemical shift values. During the fitting a ordered list of chemical shift values is compared against the ordered experimental values and the corresponding squared differences added to the penalty term. Note that this restriction is not compatible with the non-negative least\_squares\_method (**npls**) in **least\_squares\_method** keyword.

The uncertainty is again managed by the **chemicalshift\_standard\_error** keyword and reference groups can be managed in the same way as for the **chemicalshift\_data** block.

For example, using two unassigned diastereotopic protons in a methylene group, as well as two unassigned methyl groups from an isopropyl group will be managed in the following way

```
chemicalshift_data {
#two diastereotopic protons
[24 25] (2.04,2.53)
#two diastereotopic methyl groups
[(20,21,22) (23,24,25)] (1.05,1.23)
}
```

#### 1.5.4. Fitting of RDCs, RCSAs and PCSs

Current version of StereoFitter can manage anisotropic NMR data such as residual dipolar couplings (RDCs) or residual chemical shift anisotropies (RCSAs). The alignment tensor components are computed through the singular value decomposition (SVD) methodology.<sup>35,36</sup>

Note that use of RDCs or RCSAs turns the fitting problem non linear and computations can be slower than those involving only isotropic data as several trial solutions must be tested to ensure not be trapped in local minima.

RDC from any pair of nuclei can be used. StereoFitter uses the  $D=T-J$  convention where the difference in splitting between the total coupling and the scalar coupling directly corresponds to the dipolar coupling instead of the  $D=(T-J)/2$  convention.

The RDC data, given in units of Hz, are introduced through the `rdc_data` block. Equivalent or averaged groups are given by consecutive pairs of indexes enclosed in parentheses.

```
rdc_data {
#1DCH coupling index1 index2 value uncertainty
20 41 -20.3 0.2
#1DCH coupling, methylene group
(10,40) (11,41) -3.2 0.2
#H-H coupling methyl group
(50,51) (51,52) (50,52) -1.5 0.1
}
```

The global uncertainty of RDCs is controlled by the `rdc_standard_error` keyword. RCSA data are introduced in ppm units. Equivalent nuclei must be enclosed in parentheses and separated by commas

```
rca_data {  
#a carbon rca index value error  
10 0.0076 0.0005  
#RCSA of a t-butyl group  
(50,51,52) -0.0010 0.0005  
}
```

Most commonly RCSAs use an internal reference,<sup>8</sup> in many cases the most shielded atom rather than an external one. The atom used as reference should be indicated using the `rca_reference` keyword. Currently using groups of chemically equivalent spins such as *t*-butyl ones or others are not supported

```
rca_reference {  
#carbon 12 is the internal reference  
12  
}
```

RCSAs measured using compressible gels need of a correction of the changes introduced by solvation changes between the relaxed and compressed states of the polymer gel.<sup>37</sup> This solvation influence is assumed to be a fraction *c* of the observed chemical shift differences (after proper internal or external referencing) between the isotropic layer surrounding the gel and the signals of the analyte inside the relaxed polymer. The factor can be either estimated from the molecular structure, assuming that there is enough sp<sup>3</sup> carbons whose chemical shielding tensors span the 5D space of the alignment tensor<sup>38</sup> or alternatively it can be a free parameter which will be optimized along with the ensemble populations. Isotropic correction is activated by setting the `include_rca_solvent_shift` to *true*.

Estimation of the factor is chosen by setting the `estimate_rca_solvent_shift_scale` keyword to *true* and optimization of the factor is called via the `optimize_rca_solvent_shift_scale`. The scaling factor can be also manually set through the `rca_solvent_shift_scale` keyword.

Another set of important keywords are related to the so called single tensor approximation which is used by default in StereoFitter. Within this approximation a single common alignment tensor is used for all conformations. This requires the definition of a common frame for all conformations which is defined by spatial superimposition of atoms through molecular rotation. StereoFitter implements a robust quaternion based algorithm for this purpose. By setting the `superimpose` keyword to *true* conformations will be superimposed to the coordinates of the first frame in the ensemble. The list of atoms to be superimposed is defined by the `superimpose_atom` keyword. By default we apply an automatic algorithm where heavy atoms are drop from the list of superimposable atoms until deviation between atomic coordinates reaches a defined threshold.<sup>21</sup> This option is called by setting the `superimpose_atoms` keyword to **auto**. Setting the keyword to **heavy** will use all non-hydrogen atoms for molecular superimposition. Finally, atom numbers (1 to *N*) can be specified through a comma separated list (1,2,3...). This list can contain also ranges of atoms ( 1-4,8,9,...).

It is also possible to use pseudocontact shifts (PCSs) obtained in molecules tagged with paramagnetic centers. In this case two experiments are necessary, one with the molecule tagged with a non-paramagnetic metal, Lanthanum in most of the times, and the one with a paramagnetic center.

```
pcs_data {  
#data go here  
  
}
```

The `pcs_data` block has the same syntax as the `RCSA` block. Units are ppm and you can group equivalent spins in the same way.

```
pcs_standard_error {  
#13C standard error  
6 0.8  
#1H standard error  
1 0.1  
}
```

Global pcs errors are controlled using the `pcs_standard_error` keyword. Individual errors for each measurement can be specified for every nucleus following the same syntax as for the `chemicalshift_standard_error` block.

Finally, the index of the paramagnetic tag must be indicated through the keyword

```
tags {  
29  
}
```

which will hold the index of the paramagnetic center. In principle you can define several tags but in the current version only the first one is considered.

### 1.5.5. Keywords

Keywords and data blocks obey the following syntax in StereoFitter. The keyword must be followed by a opening brace, separated by tab/spaces in the same line. Then the actual content on the block must be written in new lines. After that the block must be closed with a closing brace in a new line. Comments must begin with a # symbol.

```
keyword {  
#this is a comment  
data/keywords value lines  
...  
}
```

### List of Keywords/Blocks

#### AIC

- `aic {`  
    `true|false`

```
}

```

default: true

The default mode of computation in StereoFitter is the AIC mode where subensembles of 1 to  $N$  conformers are generated until the AIC does not longer decrease by increasing the number of conformations on the generated subensembles. The default value in StereoFitter is true. If **aic** is set to false the program will simultaneously optimize the conformational amplitudes for all the conformers using the chosen algorithm in the **least\_squares\_method** option in a similar way to computations performed in the NAMFIS program.

### CHEMICALSHIFT\_DATA

```
• chemicalshift_data {
atom1(integer) chemicalshift_value(real) chemicalshift_uncertainty(real)
}
```

In this block the user must input the experimental chemical shift data by given the indexes (1 to  $N$ ) of the nuclei and the experimental value in ppm. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed (see the **chemicalshift\_standard\_error** keyword).

Equivalent nuclei can be given a single experimental datum by grouping them in the input

```
chemicalshift_data {
#equivalent carbons of a t-butyl group
(30,31,32) 15
#the nine equivalent protons
(50,51,52,53,54,55,56,57,58) 0.8
}
```

### CHEMICALSHIFT\_REFERENCES

```
• chemicalshift_references {
nucleus1(integer) intercept(real) slope(real)
}
```

In this block parameters to convert ab initio computed shieldings into chemical shifts are provided. Shifts are computed from shieldings according to equation

$$d = \text{slope} * S + \text{intercept} \quad (12)$$

Slope and intercept must be precalledibrated on known compounds. A pair intercept/slope must be provided for each type of nucleus used in the computation. For instance:

```
chemicalshift_references {
6 169.79 -1.001
1 32.22 -0.995
}
```

It is possible to define several calibration groups (multistandard approach) by appending an integer number starting from 1 after the linear fitting parameters

```
chemicalshift_references {
6 182.7 -1.0 1
6 184.9 -1.0 2
}
```

When using this method the group to be used for a particular datum should be specified in the **chemicalshift\_data** block:

```
chemicalshift_data {
8 211.9 2.8 refgroup=2
9 42.2 1.7 refgroup=1
10 31.4 1.7 refgroup=1
2 86.6 1.7 refgroup=1
}
```

### CHEMICALSHIFTDELTA\_DATA

- ```
chemicalshiftdelta_data {
atom1(integer) atom2(integer) chemicalshift_value(real) chemicalshift_uncertainty(real)
#groups of synchronous or averaged atoms can also be used
group1(integer, integer...) group2(integer, integer...)
}
```

In this block the user must input the difference in experimental chemical shift data by given the indexes (1 to  $N$ ) of two nuclei or groups of nuclei and the experimental value in ppm. If an additional real value is given in the line this value is interpreted as the uncertainty. The global uncertainty is managed through the **chemicalshift\_standard\_error** keyword.

### CHEMICALSHIFT\_GROUP\_DATA

- ```
chemicalshiftdelta_data {
[groups of atoms](integer) (list of values (real) chemicalshift_value(real)
chemicalshift_uncertainty(real)
}
```

This block is intended to be used for groups of unassigned spin sets. Between brackets [] the user should include the  $N$  unassigned spin sets followed by, between parentheses, the  $N$  chemical shift values. The global uncertainty is again managed through the **chemicalshift\_standard\_error** keyword. Reference groups can be also used, similarly to the **chemicalshift\_data** keyword.

```
chemicalshift_data {
#two diastereotopic protons
[24 25] (2.04, 2.53)
#two diastereotopic methyl groups
[(20, 21, 22) (23, 24, 25)] (1.05, 1.23)
}
```



## CHEMICALSHIFT\_STANDARD\_ERROR

```
• chemicalshift_standard_error {  
nucleus1(integer) error(real)  
}
```

This keyword allows to assign general errors for each type of nucleus in the molecule. For example, this entry will assign general errors of 2 ppm and 0.2 ppm to carbon and proton

```
chemicalshift_standard_error {  
6      2.0  
1      0.2  
}
```

## COMPUTE\_BOLTZMANN\_POPULATIONS

```
• compute_boltzmann_populations {  
true|false  
}
```

default:false

If **true** the program will compute Boltzmann populations according to the molecular energies present in the loaded molecular files. This keyword is intended to be use along with the **single\_point** and **temperature** keywords.

## ENERGIES

```
• energies {  
energy1(real)  
energy2(real)  
energy3(real)  
...  
energyN(real)  
}
```

default: not used

A list of relative energies, in kcal/mol, to be employed in

## ENERGY\_PENALTY

```
• energy_penalty {  
penanlty(bool)  
...  
}
```

default: not used

If true, a penalty will be added to the  $\chi^2$  term which takes into account the difference in relative energies between conformers between those provided by the molecular modelling files, or the energies block, and those calculated taking

into account the optimized populations. This keyword should be used along with the **compute\_boltzmann\_populations** keyword set to true.

### ENERGY\_PENALTY\_STANDARD\_ERROR

```
• energy_penalty_standard_error {
error(real)
...
```

default: 1.0 kcal/mol

The uncertainty, in the form of standard error, associated to the **energy\_penalty** term.

### ESTIMATE\_RCSA\_SOLVENT\_SHIFT\_SCALE

```
• estimate_rcsa_solvent_shfit_scale {
true|false
}
```

default: false

If **true** the scale for the isotropic shift correction in RCSA fitting will be estimated from the computed shielding tensors using the method described in Nath *et al.*<sup>38</sup>

### INCLUDE\_RCSA\_SOLVENT\_SHIFT

```
• include_rcsa_solvent_shfit_scale {
true|false
}
```

default: false

If **true** RCSAs will be corrected according to the solvent shift indicated along with the RCSA value and the computed/estimated scale factor.

### JCOUPLING\_DATA

```
• jcoupling_data {
atom1(integer) atom2(integer) jcoupling(real) jcoupling_uncertainty(real)...
...equation_name(string) unsigned(string) nocheck(string) scale=value(real) (
}
```

In this block the user must input the experimental scalar couplings by given the indexes (1 to  $N$ ) of the coupled atoms and the experimental  $J$ -coupling value in Hz. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed (see the **jcoupling\_standard\_error** keyword).

The equation name must match the ones in the table. Only exception are  $^3J_{HH}$  couplings where the Haasnoot-Altona equation is used by default if no equation name is used. StereoFitter follows the same name convention for equation names

as the MSpin program.<sup>31</sup> If the equation name is given as *dft* then *J*-couplings will be retrieved from the DFT computations. Note that DFT and empirical predictions can be retrieved in the same input.

It is possible to use a certain set of parameters by using the `jequation:parametername` syntax which should also match the MSpin convention. For instance one can choose the Plavec set of parameters in the  ${}^3J_{\text{HF}}$  Govil equation by specifying this set of parameters as `Govil-[H-C-C-F]:Plavec-1998`. Most of the empirical equations implement some degree of chemical intelligence and will check if a coupled pair is computable or not with the equation. This can be bypassed by using the *nocheck* option. As an example, to use the original 1963 parameters for the Karplus equation the corresponding like in the `jcoupling_group_data` should look like

```
jcoupling_data {
#indexes value equation:parameters
10 11 6.0 karplus:Karplus-1963
}
```

It is also possible to specify the Fourier Coefficients of a Fourier series of the type

$$J = \sum_{j=1}^n \overset{\circ}{A} c_{n-j} \cos jq + \sum_{j=1}^n \overset{\circ}{A} s_{n-j} \sin jq + w$$

where *n* is the order of the Fourier Series. By just specifying the order of the equation and the cosine and sine parameters one can a Karplus curve that has been derived from fitting a Karplus equation to a DFT curve. The more general expression would look as

```
jcoupling_data {
#indexes value fourierseries:(n,c(n),c(n-1),...c(1),s(n),s(n-1)...,s(1),w)
#n is the order of the Fourier Series
#there will be 2n+1 terms in the expansion
10 11 6.0 fourierseries:(2,4.0,2.0,-1.0,3.0,0.5,2.0,0.8)
}
```

If the Fourier series contains only cosine terms only *n*+1 terms need to be given in the expression. All sine coefficients will be set to zero.

```
jcoupling_data {
#indexes value fourierseries:(n,c(n),c(n-1),...c(1), w)
#n is the order of the Fourier Series
#there will be n+1 cosineterms in the expansion
10 11 6.0 fourierseries:(2,4.0,2.0,-0.8)
}
```

The *unsigned* keyword is used at the end of the line then the program will assume that the sign of the coupling could not be retrieved, and the fitting will be done using absolute values of the couplings. Note that the unsigned keyword is not compatible with the use of the NNLS fitting algorithm (see `least_squares_method` keyword)

If the *scale=value(real)* token is used the computed value will be scaled by the value factor. This is mostly used for DFT computations.

### JCOUPLING\_GROUP\_DATA

```

• jcoupling_group_data {
#only valid for altona equation
atom(integer) group_of_atoms(list of integers) group of _values(list of reals)
#onlyvalid for altona equation
atom(integer) group_of_atoms(list of integers) group of _values(list of
reals)uncertainty(real)
#onlyvalid for altona equation
group_of_atoms(list of integers) group_of_atoms(list of integers) group of _values(list of
reals)uncertainty(real)
#any equation
atom(integer) group_of_atoms(list of integers) group of _values(list of
reals)equation_name(string)
#any equation
atom(integer) group_of_atoms(list of integers) group of _values(list of reals)
uncertainty(real) \ equation_name(string)
#any equation
group_of_atoms(list integer) group_of_atoms(list of integers) group of _values(list of
reals) uncertainty(real) \ equation_name(string)
}

```

This block is similar to the **jcoupling\_data** one but it defines the coupling between a nuclei and an unassigned group of nuclei. For example,

```

jcoupling_group_data {
#Couplings between a proton and an unassigned methylene group these couplings will be
#computed using the altona equation
1 (20,21) (4.5,2.3)
#Couplings between two unassigned methylene groups. The predicted values will be retrieved
#from the DFT computations
(30,31) (40,41) (11.0,0.5,2.0,0.8) dft
}

```

This restraint is mostly used in situations such as CH-CH<sub>2</sub> and CH<sub>2</sub>-CH<sub>2</sub> groups where the diastereotopic methylene groups have not been assigned Note that jcoupling\_group\_data restrains cannot be used along with the NNLS optimizer.

Similarly to the **jcoupling\_data** block **jcoupling\_group\_data** restrains can use DFT computed values by specifying DFT as the equation name. The **nocheck** and **scale** options are also available using the same syntax.

Different parameter sets as well as general Fourier series can be used in the same form as for the **jcoupling\_data** block.

## LEAST\_SQUARES\_METHOD

```

• noedistance_data {
levenberg-marquardt | nnls
}

```

This block allows to switch between the Levenberg-Marquardt least-squares optimization (default) or the newest and much faster non-negative linear least squares method. **lm** can be used as a synonym of Levenberg-Marquardt. Note that not some restraints cannot such as *J*-coupling groups or unsigned values cannot be used with the NNLS method.

## MAX\_CONFORMATIONS

```

• max_conformations {
integer
}

```

default: not used

If a maximum number of conformations is provided in the input the program will stop the model search at the given number of conformers otherwise it will proceed until a minimum is found for the AIC criterion respect to the number of conformers. Consider to use this keyword for very large ensembles where the necessary computational time or memory demands can be too high.

## NNLS\_CONVERGENCE\_CRITERION

```

• nnls_convergence_criterion {
nnlsconv(real)
}

```

default:  $10^{-6}$

Set the threshold for convergence of the non linear least square equations. For the fitting of anisotropic data tighter values of  $10^{-7}$  or  $10^{-8}$  could be necessary to reach good convergence of the alignment tensor.

## NUMBER\_OF\_THREADS

```

• number_of_threads {
nthreads(integer)
}

```

default: auto

Set the number of threads for OpenMP parallelization if set to **auto** the default number of threads will be set to the number of logical cores or to the environment variable OMP\_NUM\_THREADS if set.

## NOEDISTANCE\_DATA

```

• noedistance_data {
atom1(integer) atom2(integer) distance_value(real)
atom1(integer) atom2(integer) distance_value(real) uncertainty(real)
}

```

```
}
```

In this block internuclei distances, as determined from quantitative NOE experiments, are given and the experimentally determined value in Å. The two indexes (from 1 to  $N$ ) of the two coupled nuclei must be given. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed ( see the **noedistance\_standard\_error** keyword).

Equivalent (or averaged nuclei) can be grouped as follow

```
• noedistance_data {  
#distance between two methyl groups  
(30,31,32) (20,24,25) 2.57  
#average distance from a CH to a CH2 group  
40 (50,51) 2.32  
}
```

StereoFitter allows to use the lack of observation of an NOE using the **nonoe** keyword.

```
• noedistance_data {  
#lack of noe between protons 25 and 26  
25 26 nonoe  
}
```

### NOEDISTANCE\_STANDARD\_ERROR

```
• noedistance_standard_error {  
error(real)  
}
```

This keyword allows to assign a general standard error for all NOE distances. Default value is 0.1 Å.

### OPTIMIZE\_RCSA\_SOLVENT\_SHIFT\_SCALE

```
• optimize_rcsa_solvent_shift_scale {  
true|false  
}
```

default: false

If **true** the scaling factor for the solvent contribution to the isotropic shift would be optimized simultaneously with the populations. The method is described in Hallwass et al.<sup>37</sup>

### PCS\_STANDARD\_ERROR

```
• pcs_standard_error {  
nucleus1(integer) error(real)  
}
```

This keyword allows to assign general errors for each type of nucleus in the molecule. For example, this entry will assign general errors of 0.5 ppm and 0.1 ppm to carbon and proton

```
pcs_standard_error {
6      0.5
1      0.001
}
```

There are no default values for this keyword.

### R3\_METHYLGROUP\_AVERAGING

```
• r3_methylgroup_averaging {
true|false
}
```

If true a  $\langle r^{-3} \rangle^2$  average model instead of  $\langle r^{-6} \rangle$  will be applied for computation NOE volumes involving methyl groups.

### PCS\_DATA

```
• pcs_data {
atom1(integer) pcs_value(real)
atom1(integer) pcs_value(real) pcs_uncertainty(real)
}
```

In this block the user must input the experimental data by given the indexes (1 to  $N$ ) of the atom and the experimental pseudocontact shift value in ppm. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed (see the **pcs\_standard\_error** keyword).

PCSs from equivalent sites are entered in such a way that equivalent nuclei are listed enclosed in parentheses. For instance:

```
• pcs_data {
#PCS from a t-butyl group methyl group RDC
(10,11,12) rcsavalue rcsaerror
#PCS from a rotating phenyl group, ortho carbons
(25,26) rcsavalue rcsaerror
}
```

### RDC\_DATA

```
• rdc_data {
atom1(integer) atom2(integer) rdc_value(real)
atom1(integer) atom2(integer) rdc_value(real) rdc_uncertainty(real)
}
```

In this block the user must input the experimental data by given the indexes (1 to  $N$ ) of the coupled atoms and the experimental RDC value in Hz. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed (see the **rdc\_error** keyword).

Couplings between equivalent sites are entered in such a way that equivalent pairs are listed as pairs enclosed in parentheses. For instance:

```

• rdc_data {
#1DCH methyl group RDC
(20,40) (20,41) (20,42) rdcvalue rdcerror
#1DCH RDC for a rotating phenyl group, ortho protons
(25,50) (27,51) rdcvalue rdcerror
#1DCH rdc for equivalent couplings in a CH2 group
(30,60) (30,61) rdcvalue rdcerror
}

```

Hence any symmetric group can be handled despite its complexity. Note that RDCs for methylene groups coming from conventional F1-coupled HSQCs should be input as the half-sum  $(^1D_{\text{CHa}} + ^1D_{\text{CHb}})/2$  since only this information is available from the experiment.

### RCSA\_DATA

```

• rcsa_data {
atom1(integer) rcsa_value(real)
atom1(integer) rcsa_value(real) rcsa_uncertainty(real)
}

```

In this block the user must input the experimental data by given the indexes (1 to  $N$ ) of the atom and the experimental RCSA value in ppm. If an additional real value is given in the line this value is interpreted as the uncertainty, otherwise a general error is employed (see the **rcsa\_standard\_error** keyword).

RCSAs from equivalent sites are entered in such a way that indexes of equivalent nuclei are listed enclosed in parentheses. For instance:

```

• rcsa_data {
#RCSA from a t-butyl group methyl group RDC
(10,11,12) rcsavalue rcsaerror
#RCSA from a rotating phenyl group, ortho carbons
(25,26) rcsavalue rcsaerror
}

```

### RCSA\_REFERENCE

```

• rcsa_reference {
integer
}

```



The index of the atom to be used as internal reference for RCSA fitting. The shielding tensor of this atom would be subtracted from all other atoms.

### RCSA\_SOLVENT\_SHIFT\_SCALE

```
• rcsa_solvent_shift_scale {  
real  
}
```

Set the value of the scale to be used for the contributions of solvent shifts in RCSA measurements in polymer gels.

### RCSA\_STANDARD\_ERROR

```
• rcsa_standard_error {  
nucleus1(integer) error(real)  
}
```

This keyword allows to assign general errors for each type of nucleus in the molecule. For example, this entry will assign general errors of 2 ppm and 0.2 ppm to carbon and proton

```
rcsa_standard_error {  
6      0.005  
1      0.001  
}
```

Default value of is defined only for carbon (0.005 ppm)

### RELATIVISTIC\_FORCE\_FIELD

```
• relativistic_force_field {  
real  
}
```

default: false

Activate the relativistic force field method of Kutateladze and coworkers.<sup>32</sup>

### SINGLE\_POINT

```
• single_point {  
true|false  
}
```

default:false

If **true** the best  $\chi^2$  value will be computed using the actual Boltzmann populations, usually computed through molecular modelling relative energies, rather than fitting and selecting conformational subensembles through the Akaike Information Criterion.

## SUPERIMPOSE

```
• superimpose {  
true|false  
}
```

If **true** the molecular frames will be superimposed in order to define a common frame for the single tensor approximation. The list of atoms is defined in the **superimpose\_atoms** block.

## SUPERIMPOSE\_ATOMS

```
• superimpose_atoms {  
(integer, integer, integer, integer...)  
}
```

When using the single-tensor approximation in the fitting of anisotropic NMR data this block can be used to control the atoms which will be superimposed in order to define a common tensor. The keyword **auto** will select an automated procedure where all non-hydrogen atoms are initially selected and then dropped until the maximum RMSD between frames is lower than a determined threshold. The **heavy** and **all** keywords can be used instead of an atom list to select all heavy atoms or all atoms in the molecule

## TAGS

```
• tag {  
index(integer)  
}
```

default:298.15

Set the index (1 to  $N$ ) of the paramagnetic tag when fitting pseudocontact shifts. **Tag** is a synonym of this keyword.

## TEMPERATURE

```
• temperature {  
temperature(real)  
}
```

default:298.15

Set the temperature for computation of Boltzmann populations. This temperature will also be used for conversion between alignment and magnetic susceptibility tensors when pseudocontact shifts are employed.

## USE\_UNASSIGNED\_CHEMICALSHIFTS

```
• use_unassigned_chemicalshifts {  
true|false  
}
```

default: false

If **true** the program will assign the experimental shifts to each nuclei according to the predicted values. The user can just enter the shifts in any particular order as far as they belong to the same type of nuclei.

## 1.6. Appendix. Empirical equations and parameters

Valid equations for the `jcoupling_data` and `jcoupling_group_data` blocks.

The valid syntax for choosing a particular equation is as follows. Note that equation and parameter names are case insensitive.

```
jcoupling_data {
#choose equation and parametername
#index1 index2 value error equationname=parametername
#index1 index2 value equationname=parametername
20 21 3.2 0.5 karplus=karplus-1963
}
```

if the **parametername** is omitted the first listed parameter set will be used. If **equationname** is missed the program will chose `altona` as the default equation.

```
jcoupling_data {
#choose equation and select default (first ) parameter set
#index1 index2 value error equationname
#this will choose the default Altona-1980 parameter set.
20 21 3.2 0.5 karplus
#this coupling will be computed using the Altona equation.
30 31 4.3
}
```

**Empirical Equation names and parameters sets for `jcoupling_data` and `jcoupling_group_data` blocks.**

Type	X	A	B	Y	Name & Parameters
<b>General</b>					
$^3J_{\text{HCCH}}$	H	C <sub>sp3</sub>	C <sub>sp3</sub>	H	<b>Karplus</b> Altona-1980 <sup>39</sup> Karplus-1963 <sup>40</sup>

$^3J_{\text{HCCH}}$	H	C <sub>sp3</sub>	C <sub>sp3</sub>	H	<b>Altona</b> <sup>39</sup> SetB <sup>i</sup> SetD SetE
$^3J_{\text{HCCH}}$	H	C <sub>sp3</sub>	C <sub>sp3</sub>	H	<b>Diez-Donders</b> <sup>9</sup> Altona-2007
$^3J_{\text{HCCH}}$	H	C <sub>sp2</sub>	C <sub>sp2</sub>	H	<b>Karplus-[C=CH-HC=C]</b> <sup>ii</sup> B3LYP/6-31G*
$^3J_{\text{HCCH}}$	H	C <sub>sp2</sub>	C <sub>sp2</sub>	H	<b>Karplus-[C=CH-HCR]</b> <sup>iii</sup> B3LYP-6-31G*
$^3J_{\text{CCCH}}$	C <sub>sp3</sub>	C <sub>sp3</sub>	C <sub>sp3</sub>	H	<b>Bifulco-[C-C-C-H]</b> <sup>41</sup> Bifulco-2010
$^3J_{\text{CCCH}}$	C <sub>sp3</sub>	C <sub>sp3</sub>	C <sub>sp3</sub>	H	<b>Kalinowsky-[C-C-C-H]</b> Kalinowsky-1984 <sup>42</sup> Guenther-1990 <sup>43</sup>
$^3J_{\text{CCCH}}$	C(=O)	C <sub>sp2</sub>	C <sub>sp2</sub>	H	<b>Tormena-[C-C-C-H]</b> <sup>44</sup> E-geometry-aldehydes Z-geometry-aldehydes E-geometry-a-ciano-aldehydes Z-geometry-a-ciano-aldehydes E-geometry-ketones Z-geometry-ketones E-geometry-a-ciano-ketones Z-geometry-a-ciano-ketones E-geometry-amides Z-geometry-amides E-geometry-a-ciano-amides Z-geometry-a-ciano-amides E-geometry-esters Z-geometry-esters E-geometry-esters-a-ciano Z-geometry-esters-a-ciano
$^3J_{\text{HCNH}}$	H	C <sub>sp3</sub>	N <sub>sp3</sub>	H	<b>General-[H-C-N-H]</b> Govil-1971 <sup>45</sup> Fukui-1997 <sup>46</sup> Fukui-2000 <sup>47</sup> Alkorta-2004 <sup>48</sup>
$^3J_{\text{HCOH}}$	H	C <sub>sp3</sub>	O	H	<b>General-[H-C-O-H]</b> Govil-1969 <sup>49</sup> Govil-1971 <sup>45</sup>

					Fukui-1997 <sup>46</sup> Fukui-2000 <sup>47</sup> Alkorta-2004 <sup>48</sup> Serianni-2007 <sup>50</sup>
$^3J_{\text{HCCF}}$	H	C <sub>sp3</sub>	C <sub>sp3</sub>	F	<b>Govil-[H-C-C-F]</b> Govil-1971 <sup>45</sup> Plavec-1998 <sup>51</sup>
$^3J_{\text{CNCP}}$	C	N	C	P(O)OR <sub>3</sub>	<b>Rabiller-[C-N-C-P]</b> <sup>52</sup> Rabiller-1982
$^3J_{\text{CCCP}}$	C	C	C	P(O)OR <sub>3</sub>	<b>Thiem-[C-C-C-P]</b> <sup>53</sup> Thiem-Meyer-1978
$^2J_{\text{HCN}}$	H	C	---	N	<b>Azide2JHN-[H-C-N]</b> <sup>54</sup> B3LYP/pcJ-2
$^3J_{\text{HCNN}}$	H	C	N	N	<b>Azide3JHN-[H-C-N-N]</b> <sup>54</sup> B3LYP/pcJ-2
<b>PEPTIDES</b>					
$^3J_{\text{CC}}$					
$^3J_{\text{C(=O),N, C}\alpha, \text{C}\beta}$	C(=O)	N	C <sub>α</sub>	C <sub>β</sub>	<b>Barfield-[C(O)-N-Ca-Cb]</b> <sup>55</sup> Barfield-1985
$^3J_{\text{C(=O),N, C}\alpha, \text{C(=O)}}$	C(=O)	N	C <sub>α</sub>	C(=O)	<b>Bax-[C(O)-N-Ca-C(O)]</b> <sup>56</sup> Bax-1996
$^3J_{\text{C(=O), C}\alpha, \text{C}\beta \text{ C}\gamma}$	C(=O)	C <sub>α</sub>	C <sub>β</sub>	C <sub>γ</sub>	<b>Schmidt-[C(O)-Ca-Cb-Cg]</b> <sup>57</sup> Schmidt-2001
$^3J_{\text{CH}}$					
$^3J_{\text{C(=O),N, C}\alpha, \text{H}}$	C(=O)	N	C <sub>α</sub>	H	<b>Barfield-[C(O)-N-Ca-H]</b> Barfield-1985 <sup>55</sup> Mohanakrishnan-1979 <sup>58</sup>
$^3J_{\text{C(=O),N, C}\alpha, \text{H}}$	C(=O)	N	C <sub>α</sub>	H	<b>Bax [C(O)-N-Ca-H]</b> <sup>59</sup> Bax-1995
$^3J_{\text{C}\beta, \text{C}\alpha, \text{N, H}}$	C <sub>β</sub>	C <sub>α</sub>	N	H	<b>Bax-[Cb-Ca-N-H]</b> <sup>60</sup> Bax-1997-x-ray Bax-1997-solution
$^3J_{\text{CH}_3, \text{N, C}\alpha, \text{H}}$	C(H <sub>3</sub> )	N	C <sub>α</sub>	H	<b>Cung-[CH<sub>3</sub>-N-Ca-H]</b> <sup>61</sup> Cung-1980
$^3J_{\text{C=O, C}\alpha, \text{C}\beta, \text{H}}$	C(=O)	C <sub>α</sub>	C <sub>β</sub>	H	<b>Schmidt-[C(O)-Ca-Cb-H]</b> <sup>57</sup> Schmidt-2001

$^3J_{C\gamma, C\beta, C\alpha, N, H}$	C $\gamma$	C $\beta$	C $\alpha$	H	<b>Schmidt-[H-Ca-Cb-Cg]<sup>57</sup></b> Schmidt-2001
$^3J_{NH}$					
$^3J_{H, C\alpha, C(=O), N}$	H	C $\alpha$	C(=O) )	N	<b>Bax-[H-Ca-C(O)-N]<sup>59</sup></b> Bax-1995
$^3J_{N, C\alpha, C\beta, H}$	N	C $\alpha$	C $\beta$	H	<b>Schmidt-[N-Ca-Cb-H]<sup>57</sup></b> Schmidt-2001
$^3J_{FH}$					
$^3J_{F, N, C\alpha, H}$	F	N	C $\alpha$	H	<b>Hammer-[F-N-Ca-H]<sup>50</sup></b> Hammer-1984
$^3J_{HH}$					
$^3J_{HN, C\alpha, H}$	H	N	C $\alpha$	H	<b>Pardi-[H-N-Ca-H]<sup>62</sup></b> Pardi-1980
$^3J_{HC\alpha, C\beta, H}$	H	C $\alpha$	C $\beta$	H	<b>Schmidt-[H-Ca-Cb-H]<sup>57</sup></b> Schmidt-2001
$^3J_{CN}$					
	N	C $\alpha$	C $\beta$	C $\gamma$	<b>Schmidt-[N-Ca-Cb-Cg]<sup>57</sup></b> Schmidt-2001
<b>Glycosides</b>					
$^2J_{C, O, H}$	C	O	---	H	<b>Carlomagno-[C-O-H]<sup>63</sup></b>
$^3J_{C, O, C, C}$	C	O	C	C	<b>Milton-[C-O-C-C]<sup>64</sup></b> Milton-1998
$^3J_{C, C, O, H}$	C	C	O	H	<b>Serianni-[C1-C2-O-H]<sup>65</sup></b> Serianni-2007
$^3J_{C, O, C, H}$	C	O	C	H	<b>Serianni-[C-O-C-H]<sup>66</sup></b> Serianni-1999
$^3J_{C, O, C, H}$	C	O	C	H	<b>Tvaroska-[C-O-C-H]<sup>67</sup></b> Tvaroska-1989

i The parameters for the Altona equation are chosen as a function of the substitution pattern and are automatically chosen by the program.

ii A five term Karplus equation of the form  $-0.24 \cos 4f + 0.35 \cos 3f + 2.57 \cos 2f - 1.82 \cos f + 5.59$ . Parameters were obtained by fitting a B3LYP/6-31G\* (four Ramsey terms) curve.

iii A four term Karplus equation of the form:  $-0.18 \cos 3f + 3.74 \cos 2f - 1.36 \cos f + 5.71$ . Parameters were obtained by fitting a B3LYP/6-31G\* (four Ramsey terms) curve.

## 1.7. References

- (1) Cicero, D. O.; Barbato, G.; Bazzo, R. NMR Analysis of Molecular Flexibility in Solution: A New Method for the Study of Complex Distributions of Rapidly Exchanging Conformations. Application to a 13-Residue Peptide with an 8-Residue Loop. *J. Am. Chem. Soc.* **1995**, *117* (3), 1027–1033. <https://doi.org/10.1021/ja00108a019>.
- (2) Halgren, T. A. Merck Molecular Force Field. I. Basis, Form, Scope, Parameterization, and Performance of MMFF94. *J. Comput. Chem.* **1996**, *17* (5–6), 490–519. [https://doi.org/10.1002/\(SICI\)1096-987X\(199604\)17:5/6<490::AID-JCC1>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6<490::AID-JCC1>3.0.CO;2-P).
- (3) Harder, E.; Damm, W.; Maple, J.; Wu, C.; Reboul, M.; Xiang, J. Y.; Wang, L.; Lupyan, D.; Dahlgren, M. K.; Knight, J. L.; Kaus, J. W.; Cerutti, D. S.; Krilov, G.; Jorgensen, W. L.; Abel, R.; Friesner, R. A. OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins. *J. Chem. Theory Comput.* **2016**, *12* (1), 281–296. <https://doi.org/10.1021/acs.jctc.5b00864>.
- (4) Roos, K.; Wu, C.; Damm, W.; Reboul, M.; Stevenson, J. M.; Lu, C.; Dahlgren, M. K.; Mondal, S.; Chen, W.; Wang, L.; Abel, R.; Friesner, R. A.; Harder, E. D. OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules. *J. Chem. Theory Comput.* **2019**, *15* (3), 1863–1874. <https://doi.org/10.1021/acs.jctc.8b01026>.
- (5) Willoughby, P. H.; Jansma, M. J.; Hoye, T. R. A Guide to Small-Molecule Structure Assignment through Computation of (<sup>1</sup>H and <sup>13</sup>C) NMR Chemical Shifts. *Nat. Protoc.* **2014**, *9* (3), 643–660. <https://doi.org/10.1038/nprot.2014.042>.
- (6) Willoughby, P. H.; Jansma, M. J.; Hoye, T. R. Addendum: A Guide to Small-Molecule Structure Assignment through Computation of (<sup>1</sup>H and <sup>13</sup>C) NMR Chemical Shifts. *Nat. Protoc.* **2020**, *15* (7), 2277–2277. <https://doi.org/10.1038/s41596-020-0293-9>.
- (7) Hehre, W.; Klunzinger, P.; Deppmeier, B.; Driessen, A.; Uchida, N.; Hashimoto, M.; Fukushi, E.; Takata, Y. Efficient Protocol for Accurately Calculating <sup>13</sup>C Chemical Shifts of Conformationally Flexible Natural Products: Scope, Assessment, and Limitations. *J. Nat. Prod.* **2019**, *82* (8), 2299–2306. <https://doi.org/10.1021/acs.jnatprod.9b00603>.
- (8) Liu, Y.; Navarro-Vázquez, A.; Gil, R. R.; Griesinger, C.; Martin, G. E.; Williamson, R. T. Application of Anisotropic NMR Parameters to the Confirmation of Molecular Structure. *Nat. Protoc.* **2019**, *14* (1), 217–247. <https://doi.org/10.1038/s41596-018-0091-9>.
- (9) Altona, C. Vicinal Coupling Constants and Conformation of Biomolecules. In *eMagRes*; American Cancer Society, 2007. <https://doi.org/10.1002/9780470034590.emrstm0587>.
- (10) Navarro-Vázquez, A.; Gil, R. R.; Blinov, K. Computer-Assisted 3D Structure Elucidation (CASE-3D) of Natural Products Combining Isotropic and Anisotropic NMR Parameters. *J. Nat. Prod.* **2018**, *81* (1), 203–210. <https://doi.org/10.1021/acs.jnatprod.7b00926>.
- (11) Zanardi, M. M.; Sortino, M. A.; Sarotti, A. M. On the Effect of Intramolecular H-Bonding in the Configurational Assessment of Polyhydroxylated Compounds with Computational Methods. The Hyacinthacines Case. *Carbohydr. Res.* **2019**, *474*, 72–79. <https://doi.org/10.1016/j.carres.2019.01.011>.
- (12) Navarro-Vázquez, A. When Not to Rely on Boltzmann Populations. Automated CASE-3D Structure Elucidation of Hyacinthacines through Chemical Shift Differences. *Magn. Reson. Chem.* **2020**, *58* (2), 139–144. <https://doi.org/10.1002/mrc.4951>.
- (13) Zanardi, M. M.; Marcarino, M. O.; Sarotti, A. M. Redefining the Impact of Boltzmann Analysis in the Stereochemical Assignment of Polar and Flexible Molecules by NMR Calculations. *Org. Lett.* **2020**, *22* (1), 52–56. <https://doi.org/10.1021/acs.orglett.9b03866>.
- (14) Zhao, Y.; Truhlar, D. G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Functionals. *Theor. Chem. Acc.* **2008**, *120* (1), 215–241. <https://doi.org/10.1007/s00214-007-0310-x>.

- (15) Grimme, S.; Ehrlich, S.; Goerigk, L. Effect of the Damping Function in Dispersion Corrected Density Functional Theory. *J. Comput. Chem.* **2011**, *32* (7), 1456–1465. <https://doi.org/10.1002/jcc.21759>.
- (16) Tomasi, J.; Mennucci, B.; Cammi, R. Quantum Mechanical Continuum Solvation Models. *Chem. Rev.* **2005**, *105* (8), 2999–3094. <https://doi.org/10.1021/cr9904009>.
- (17) Akaike, H. A New Look at the Statistical Model Identification. *IEEE Trans. Autom. Control* **1974**, *19* (6), 716–723. <https://doi.org/10.1109/TAC.1974.1100705>.
- (18) Erdélyi, M.; d’Auvergne, E.; Navarro-Vázquez, A.; Leonov, A.; Griesinger, C. Dynamics of the Glycosidic Bond: Conformational Space of Lactose. *Chem. – Eur. J.* **2011**, *17* (34), 9368–9376. <https://doi.org/10.1002/chem.201100854>.
- (19) Trigo-Mouriño, P.; de la Fuente, M. C.; Gil, R. R.; Sánchez-Pedregal, V. M.; Navarro-Vázquez, A. Conformational Analysis of the Anti-Obesity Drug Lorcaserin in Water: How To Take Advantage of Long-Range Residual Dipolar Couplings. *Chem. – Eur. J.* **2013**, *19* (44), 14989–14997. <https://doi.org/10.1002/chem.201202509>.
- (20) Lancefield, C. S.; Slawin, A. M. Z.; Westwood, N. J.; Lebl, T. The Use of Residual Dipolar Coupling for Conformational Analysis of Structurally Related Natural Products. *Magn. Reson. Chem.* **2015**, *53* (6), 467–475. <https://doi.org/10.1002/mrc.4213>.
- (21) Troche-Pesqueira, E.; Anklin, C.; Gil, R. R.; Navarro-Vázquez, A. Computer-Assisted 3D Structure Elucidation of Natural Products Using Residual Dipolar Couplings. *Angew. Chem. Int. Ed.* **2017**, *56* (13), 3660–3664. <https://doi.org/10.1002/anie.201612454>.
- (22) Vainio, M. J. Balloon. <http://users.abo.fi/mivainio/balloon/>
- (23) Vainio, M. J.; Johnson, M. S. Generating Conformer Ensembles Using a Multiobjective Genetic Algorithm. *J. Chem. Inf. Model.* **2007**, *47* (6), 2462–2474. <https://doi.org/10.1021/ci6005646>.
- (24) Puranen, J. S.; Vainio, M. J.; Johnson, M. S. Accurate Conformation-Dependent Molecular Electrostatic Potentials for High-Throughput in Silico Drug Discovery. *J. Comput. Chem.* **2010**, *31* (8), 1722–1732. <https://doi.org/10.1002/jcc.21460>.
- (25) Gilbert, K. E. GMMX. <http://www.serenasoft.com/gmmx.html>
- (26) Wahl, J.; Freyss, J.; von Korff, M.; Sander, T. Accuracy Evaluation and Addition of Improved Dihedral Parameters for the MMFF94s. *J. Cheminformatics* **2019**, *11* (1), 53. <https://doi.org/10.1186/s13321-019-0371-6>.
- (27) Hu, H.; Krishnamurthy, K. Revisiting the Initial Rate Approximation in Kinetic NOE Measurements. *J. Magn. Reson.* **2006**, *182* (1), 173–177. <https://doi.org/10.1016/j.jmr.2006.06.009>.
- (28) Butts, C. P.; Jones, C. R.; Towers, E. C.; Flynn, J. L.; Appleby, L.; Barron, N. J. Interproton Distance Determinations by NOE – Surprising Accuracy and Precision in a Rigid Organic Molecule. *Org. Biomol. Chem.* **2010**, *9* (1), 177–184. <https://doi.org/10.1039/C0OB00479K>.
- (29) Butts, C. P.; Jones, C. R.; Harvey, J. N. High Precision NOEs as a Probe for Low Level Conformers—a Second Conformation of Strychnine. *Chem. Commun.* **2011**, *47* (4), 1193–1195. <https://doi.org/10.1039/C0CC04114A>.
- (30) Thiele, C. M.; Petzold, K.; Schleucher, J. EASY ROESY: Reliable Cross-Peak Integration in Adiabatic Symmetrized ROESY. *Chem. – Eur. J.* **2009**, *15* (3), 585–588. <https://doi.org/10.1002/chem.200802027>.
- (31) Navarro-Vázquez, A.; Santamaría-Fernández, R.; Sardina, F. J. MSpin-JCoupling. A Modular Program for Prediction of Scalar Couplings and Fast Implementation of Karplus Relationships. *Magn. Reson. Chem.* **2018**, *56* (6), 505–512. <https://doi.org/10.1002/mrc.4667>.
- (32) Kutateladze, A. G.; Mukhina, O. A. Minimalist Relativistic Force Field: Prediction of Proton–Proton Coupling Constants in <sup>1</sup>H NMR Spectra Is Perfected with NBO Hybridization Parameters. *J. Org. Chem.* **2015**, *80* (10), 5218–5225. <https://doi.org/10.1021/acs.joc.5b00619>.
- (33) Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. Computational Prediction of <sup>1</sup>H and <sup>13</sup>C Chemical Shifts: A Useful Tool for Natural Product, Mechanistic, and Synthetic Organic Chemistry. *Chem. Rev.* **2012**, *112* (3), 1839–1862. <https://doi.org/10.1021/cr200106v>.



- (34) *CHESHIRE Chemical Shift Repository*. <http://cheshirenmr.info/> (accessed 2021-05-09).
- (35) Losonczi, J. A.; Andrec, M.; Fischer, M. W.; Prestegard, J. H. Order Matrix Analysis of Residual Dipolar Couplings Using Singular Value Decomposition. *J. Magn. Reson. San Diego Calif 1997* **1999**, *138* (2), 334–342. <https://doi.org/10.1006/jmre.1999.1754>.
- (36) Sánchez-Pedregal, V. M.; Santamaría-Fernández, R.; Navarro-Vázquez, A. Residual Dipolar Couplings of Freely Rotating Groups in Small Molecules. Stereochemical Assignment and Side-Chain Conformation of 8-Phenylmenthol. *Org. Lett.* **2009**, *11* (7), 1471–1474. <https://doi.org/10.1021/ol8029565>.
- (37) Hallwass, F.; Teles, R. R.; Hellemann, E.; Griesinger, C.; Gil, R. R.; Navarro-Vázquez, A. Measurement of Residual Chemical Shift Anisotropies in Compressed Polymethylmethacrylate Gels. Automatic Compensation of Gel Isotropic Shift Contribution. *Magn. Reson. Chem.* **2018**, *56* (5), 321–328. <https://doi.org/10.1002/mrc.4711>.
- (38) Nath, N.; Schmidt, M.; Gil, R. R.; Williamson, R. T.; Martin, G. E.; Navarro-Vázquez, A.; Griesinger, C.; Liu, Y. Determination of Relative Configuration from Residual Chemical Shift Anisotropy. *J. Am. Chem. Soc.* **2016**, *138* (30), 9548–9556. <https://doi.org/10.1021/jacs.6b04082>.
- (39) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. The Relationship between Proton-Proton NMR Coupling Constants and Substituent Electronegativities—I: An Empirical Generalization of the Karplus Equation. *Tetrahedron* **1980**, *36* (19), 2783–2792. [https://doi.org/10.1016/0040-4020\(80\)80155-4](https://doi.org/10.1016/0040-4020(80)80155-4).
- (40) Karplus, Martin. Vicinal Proton Coupling in Nuclear Magnetic Resonance. *J. Am. Chem. Soc.* **1963**, *85* (18), 2870–2871. <https://doi.org/10.1021/ja00901a059>.
- (41) Palermo, G.; Riccio, R.; Bifulco, G. Effect of Electronegative Substituents and Angular Dependence on the Heteronuclear Spin-Spin Coupling Constant  $3J_{C-H}$ : An Empirical Prediction Equation Derived by Density Functional Theory Calculations. *J. Org. Chem.* **2010**, *75* (6), 1982–1991. <https://doi.org/10.1021/jo902704u>.
- (42) Kalinowski, H.-O.; Berger, S.; Braun, S.  *$^{13}C$ -NMR-Spektroskopie*; Wiley, 1988.
- (43) Aydin, R.; Günther, H.  $^{13}C$ ,  $^1H$  Spin-Spin Coupling. X—Norbornane: A Reinvestigation of the Karplus Curve for  $3J(^{13}C, ^1H)$ . *Magn. Reson. Chem.* **1990**, *28* (5), 448–457. <https://doi.org/10.1002/mrc.1260280513>.
- (44) Couto, U. R.; Navarro-Vázquez, A.; Tormena, C. F. Unexpected Behavior of the  $3J_{CH}$  Coupling Constant in Unsaturated Compounds. *Magn. Reson. Chem.* **2019**, *57* (11), 939–945. <https://doi.org/10.1002/mrc.4885>.
- (45) Govil, G. *Indian J. Chem.* **1971**, *9* (824).
- (46) FUKUI, H.; BABA, T.; INOMATA, H.; MIURA, K.; MATSUDA, H. Calculation of Nuclear Spin-Spin Couplings IX. Vicinal Proton-Proton Coupling Constants in Methanol and Methylamine. *Mol. Phys.* **1997**, *92* (1), 161–165. <https://doi.org/10.1080/002689797170725>.
- (47) Fukui, H.; Baba, T.; Kurogi, Y. Calculation of Nuclear Spin-Spin Couplings. X. Analytical Derivative Method of Perturbation Energy. *J. Chem. Phys.* **2000**, *112* (8), 3532–3539. <https://doi.org/10.1063/1.480508>.
- (48) Alkorta, I.; Elguero, J. Karplus-Type Relationships between Scalar Coupling Constants:  $3J_{HH}$  Molecular versus  $4hJ_{HH}$  Supramolecular Coupling Constants. *Theor. Chem. Acc.* **2004**, *111* (1), 31–35. <https://doi.org/10.1007/s00214-003-0486-7>.
- (49) Fraser, R. R.; Kaufman, M.; Morand, P.; Govil, G. Stereochemical Dependence of Vicinal H—C—O—H Coupling Constants. *Can. J. Chem.* **2011**. <https://doi.org/10.1139/v69-058>.
- (50) Zhao, H.; Pan, Q.; Zhang, W.; Carmichael, I.; Serianni, A. S. DFT and NMR Studies of  $2J_{COH}$ ,  $3J_{HCOH}$ , and  $3J_{CCOH}$  Spin-Couplings in Saccharides: C—O Torsional Bias and H-Bonding in Aqueous Solution. *J. Org. Chem.* **2007**, *72* (19), 7071–7082. <https://doi.org/10.1021/jo0619884>.
- (51) Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. A New Generalized Karplus-Type Equation Relating Vicinal Proton-Fluorine Coupling Constants to H—C—C—F Torsion Angles. *J. Org. Chem.* **1998**, *63* (15), 4967–4984. <https://doi.org/10.1021/jo980144k>.
- (52) Rabiller, C.; Dehnel, A.; Lavielle, G. Stéréochimie de diéthylphosphono carbéthoxy-4 pyrrolidines. Étude par rmn du proton à haut champ (250 MHz) et du carbone ( $^{13}C$ ). *Can. J. Chem.* **2011**. <https://doi.org/10.1139/v82-139>.

- (53) Thiem, J.; Meyer, B. A Karplus-Type Relationship for the Vicinal Coupling  $3J(\text{CCCP})$  in Phosphonates. *Org. Magn. Reson.* **1978**, *11* (1), 50–51. <https://doi.org/10.1002/mrc.1270110115>.
- (54) Navarro-Vázquez, A.; Pennestri, M. Karplus Relationships of the  $2J\text{HN}\alpha$  and  $3J\text{HN}\beta$  Couplings in Organic Azides. *Magn. Reson. Chem.* **2021**, *59* (2), 187–194. <https://doi.org/10.1002/mrc.5101>.
- (55) Kao, L. F.; Barfield, M. Conformational Dependencies of Vicinal Carbonyl-Nitrogen-Carbon-Carbon ( $13\text{C}(\text{O})\text{-N-C}\alpha\text{-}13\text{C}$ ) and Carbonyl-Nitrogen-Carbon-Hydrogen ( $13\text{C}(\text{O})\text{-N-C}\alpha\text{-}1\text{H}$ ) Coupling Constants in Compounds Which Model the Peptide Backbone. *J. Am. Chem. Soc.* **1985**, *107* (8), 2323–2330. <https://doi.org/10.1021/ja00294a022>.
- (56) Hu, J.-S.; Bax, A. Measurement of Three-Bond  $13\text{C}\text{-}13\text{C}$  J Couplings between Carbonyl and Carbonyl/Carboxyl Carbons in Isotopically Enriched Proteins. *J. Am. Chem. Soc.* **1996**, *118* (34), 8170–8171. <https://doi.org/10.1021/ja9616239>.
- (57) Pérez, C.; Löhr, F.; Rüterjans, H.; Schmidt, J. M. Self-Consistent Karplus Parametrization of  $3J$  Couplings Depending on the Polypeptide Side-Chain Torsion  $X_1$ . *J. Am. Chem. Soc.* **2001**, *123* (29), 7081–7093. <https://doi.org/10.1021/ja003724j>.
- (58) Mohanakrishnan, P.; Easwaran, K. R. K. Conformational Dependence of Vicinal  $13\text{C}'\text{NC}\alpha\text{H}$  Coupling Constants in Peptides: A Dirac Vector Model Investigation. *Biopolymers* **1979**, *18* (7), 1769–1774. <https://doi.org/10.1002/bip.1979.360180714>.
- (59) Wang, A. C.; Bax, A. Reparametrization of the Karplus Relation for  $3J(\text{H}\alpha\text{-N})$  and  $3J(\text{HN-C}')$  in Peptides from Uniformly  $13\text{C}/15\text{N}$ -Enriched Human Ubiquitin. *J. Am. Chem. Soc.* **1995**, *117* (6), 1810–1813. <https://doi.org/10.1021/ja00111a021>.
- (60) Hu, J.-S.; Bax, A. Determination of  $\phi$  and  $X_1$  Angles in Proteins from  $13\text{C}\text{-}13\text{C}$  Three-Bond J Couplings Measured by Three-Dimensional Heteronuclear NMR. How Planar Is the Peptide Bond? *J. Am. Chem. Soc.* **1997**, *119* (27), 6360–6368. <https://doi.org/10.1021/ja970067v>.
- (61) Cung, T. M.; Boussard, G.; Vitoux, B.; Marraud, M. *C R Acad Sc Paris Ser. C* **1980**, 291–294.
- (62) Pardi, A.; Billeter, M.; Wüthrich, K. Calibration of the Angular Dependence of the Amide Proton- $\text{C}\alpha$  Proton Coupling Constants,  $3J\text{HN}\alpha$ , in a Globular Protein: Use of  $3J\text{HN}\alpha$  for Identification of Helical Secondary Structure. *J. Mol. Biol.* **1984**, *180* (3), 741–751. [https://doi.org/10.1016/0022-2836\(84\)90035-4](https://doi.org/10.1016/0022-2836(84)90035-4).
- (63) Fohrer, J.; Reinscheid, U.; Hennig, M.; Carlomagno, T. Calculation of the Dependence of Homo- and Heteronuclear  $3J$  and  $2J$  Scalar Couplings for the Determination of the  $2'$ -Hydroxy Conformation in RNA. *Angew. Chem. Int. Ed.* **2006**, *45* (42), 7033–7036. <https://doi.org/10.1002/anie.200602583>.
- (64) Milton, M. J.; Harris, R.; Probert, M. A.; Field, R. A.; Homans, S. W. New Conformational Constraints in Isotopically ( $13\text{C}$ ) Enriched Oligosaccharides. *Glycobiology* **1998**, *8* (2), 147–153. <https://doi.org/10.1093/glycob/8.2.147>.
- (65) Zhao, H.; Pan, Q.; Zhang, W.; Carmichael, I.; Serianni, A. S. DFT and NMR Studies of  $2J\text{COH}$ ,  $3J\text{HCOH}$ , and  $3J\text{CCOH}$  Spin-Couplings in Saccharides: C–O Torsional Bias and H-Bonding in Aqueous Solution. *J. Org. Chem.* **2007**, *72* (19), 7071–7082. <https://doi.org/10.1021/jo0619884>.
- (66) Cloran, F.; Carmichael, I.; Serianni, A. S. Density Functional Calculations on Disaccharide Mimics: Studies of Molecular Geometries and Trans-O-Glycosidic  $3J\text{COCH}$  and  $3J\text{COCC}$  Spin-Couplings. *J. Am. Chem. Soc.* **1999**, *121* (42), 9843–9851. <https://doi.org/10.1021/ja984384t>.
- (67) Tvaroška, I.; Hricovíni, M.; Petráková, E. An Attempt to Derive a New Karplus-Type Equation of Vicinal Proton-Carbon Coupling Constants for  $\text{C}\square\text{O}\square\text{C}\square\text{H}$  Segments of Bonded Atoms. *Carbohydr. Res.* **1989**, *189*, 359–362. [https://doi.org/10.1016/0008-6215\(89\)84112-6](https://doi.org/10.1016/0008-6215(89)84112-6).

## 1.8. Keywords

AIC	77
CHEMICALSHIFT_DATA .....	78
CHEMICALSHIFT_REFERENCES .....	78
CHEMICALSHIFTDELTA_DATA .....	79
CHEMICALSHIFT_GROUP_DATA.....	79
CHEMICALSHIFT_STANDARD_ERROR.....	80
COMPUTE_BOLTZMANN_POPULATIONS .....	80
ENERGIES.....	80
ENERGY_PENALTY .....	80
ENERGY_PENALTY_STANDARD_ERROR .....	81
ESTIMATE_RCSA_SOLVENT_SHIFT_SCALE.....	81
INCLUDE_RCSA_SOLVENT_SHIFT .....	81
JCOUPLING_DATA .....	81
JCOUPLING_GROUP_DATA .....	83
LEAST_SQUARES_METHOD.....	84
MAX_CONFORMATIONS.....	84
NNLS_CONVERGENCE_CRITERION .....	84
NUMBER_OF_THREADS.....	84
NOEDISTANCE_DATA.....	84
OPTIMIZE_RCSA_SOLVENT_SHIFT_SCALE .....	85
R3_METHYLGROUP_AVERAGING.....	86
PCS_DATA.....	86
RDC_DATA.....	86
RCSA_DATA .....	87
RCSA_REFERENCE .....	87
RCSA_SOLVENT_SHIFT_SCALE .....	88
RELATIVISTIC_FORCE_FIELD.....	88
SINGLE_POINT .....	88
SUPERIMPOSE .....	89
SUPERIMPOSE_ATOMS.....	89
TAGS.....	89
TEMPERATURE .....	89
USE_UNASSIGNED_CHEMICALSHIFTS .....	89

