Structure Check

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Introduction

The purpose of this tutorial is to demonstrate how to use the VMD plugins cispeptide and chirality together with AutoIMD to ensure proper structure of peptide bonds and correct stereochemistry of proteins and nucleic acids. The described plugins are designed to work with proteins and nucleic acids and understand atom names as used by the force fields CHARMM and AMBER as well as the standard PDB naming convention for non-hydrogen atoms.

**WARNING:** The current implementation of the chirality plugin does not support DNA yet.

The following points aim to motivate the necessity of the presented plugins:

- Structures from PDB may contain errors in stereochemistry and peptide bond configuration;
- Errors in peptide bond configuration can be introduced by modeling;
- Poor starting conditions, i.e. atoms being too close to each other, can generate huge forces during the initial optimization of the structure leading to errors in structure;
- Molecular dynamics (MD) protocols which apply forces on the molecule may generate forces large enough to introduce errors.

Since MD force fields are defined to support either type of chirality or peptide bond configuration, optimization of the structure will not correct introduced errors.

At this stage, we would like to give a brief outline of the strategy behind the described plugins. The general strategy to ensure correct peptide bond and stereochemical configuration consists of four steps.

1. **Identify unusual configuration at chiral centers and peptide bonds**
   All cis peptide bonds are regarded being unusual. In case of chiral centers, those are regarded unusual which occur less often in nature, e.g. D-form of amino acids.

2. **Inspect the found irregularities**
   Although it is possible to convert all peptide bonds and chirality centers into their “usual” configuration (this feature will be implemented in the future), it is safer to decide for each irregularity individually. First, different chiral forms of biomolecules and true cis peptide bonds are used in biology. Second, it is not always unambiguous how to correct an unusual configuration; this is especially the case for peptide bonds.

3. **Correct the structure if necessary using structure optimization (and equilibration)**
   The correcting happens in two steps. First one atom is moved to “preset” a proper configuration. In a second step an the structure is relaxed using an MD force field plus restraints enforcing the proper configuration.
4 If proceeding with further MD simulations, apply restraints to preserve chirality and peptide bond configuration. This step may be necessary, if the molecular system is used in a simulation in which large forces may be applied, e.g., MDFF, TMD etc.
1 Chirality in proteins and nucleic acids

1.1 Introduction

All amino acids but glycine have at least one chiral center at \( C_\alpha \) (see Fig. 1). Threonine and isoleucine have an additional chiral center at \( C_\beta \). According to the D-/L- naming convention, naturally occurring amino acids are found in the L-configuration. Note, however, that D-amino acids do occur in biology, e.g., in cell walls of bacteria. Nucleic acids also have chiral centers. For example, in DNA the atoms C1’, C3’, and C4’ are chiral, while RNA has an additional chiral center at C2’. Chirality is central to all molecular interactions in biological systems. A simple experiment demonstrates the principle: try to shake someone’s left hand with your right.

![Figure 1: Chirality at the \( C_\alpha \) carbon atom of amino acids. The two enantiomers are mirror images of each other, like the left and the right hand. Source: http://web99.arc.nasa.gov/~astrochm/chirality.jpg](http://web99.arc.nasa.gov/~astrochm/chirality.jpg)

1.2 Checking stereochemistry of the structure

To demonstrate the usage of the plugin we will start by checking a structure consisting of the protein EF-Tu in complex with a tRNA\(^{Phe}\). The used structure is based on the the PDB structure 1OB2 in which errors have been introduced manually.

Currently, the chirality plugin can be used from the text console and through a GUI. The available commands provided by the plugin can be obtained by typing `chirality` on the Tk Console or the VMD console. The following information should be printed in the console:

**Commands:**

- `check` -- identify chirality errors
- `list` -- list identified chirality errors
- `minimize` -- fix chirality errors using energy minimization
- `move` -- move hydrogen atom to fix chirality
- `reset` -- reinitialize plugin state
- `restrain` -- generate NAMD extrabonds file to prevent chirality changes
- `show` -- visualize identified chirality errors
In the same way, the usage information for any of the provided commands can be obtained. For example, typing `chirality restrain` will show the syntax of the command that generates restraints for the impropers at chiral centers which can be used in NAMD.

In the following, we will describe how to use the `chirality` plugin through its GUI, but all of the tasks can also be accomplished from the console.

1. Load the files `chir_testcase.psf` and `chir_testcase.pdb` into a new session of VMD.

2. Open the `chirality` GUI by selecting Extensions → Modeling → Fix Chirality Errors in the VMD Main menu. In the upper part of the GUI (see Fig. 2) the user can specify the molecule and the atom selection to be tested.

3. The button Check structure initializes the test. Identified unusual chiral center configurations are displayed in the Identified chirality errors form (see Fig. 3). In the present case there are 9 chiral errors identified in both the protein and RNA parts of the loaded structure.

4. By selecting an entry in the Identified chirality errors form and hitting the button Show selected chiral centers it is possible to visually inspect all the individual identified unusual chiral centers. In the created representation (see Fig. 4) the chiral center is highlighted by a transparent purple sphere, while the four bonded atoms are shown in CPK.

![Image of the chirality GUI window]

Figure 2: The chirality GUI window.
1.3 Correcting chirality

If the shown chiral center has the wrong chirality, the user can tag an atom to be moved to flip the chirality at the selected center. This is
done by hitting the button hydrogen. Note, currently only the moving of a hydrogen atom at a chiral center is supported. If the chirality at the displayed center is correct, nothing needs to be done. It is also possible to untag a tagged atom by selecting it and hitting none.

2 Once a chiral center has been inspected, and the atom to be moved has been tagged, the actual moving of the atom can be executed by hitting the button Move tagged atoms for selected chiral centers.

3 Since the described procedure (simple moving of the hydrogen atom) generates an unphysical geometry of the molecule, it is necessary to optimize the structure using an MD force field. The chirality plugin uses the AutoIMD plugin for this purpose. This final step is accomplished by selecting the chiral centers which should be relaxed and hitting the button Minimize/equilibrate selected chiral centers. This will open the AutoIMD Controls window (see Fig. 5).

![AutoIMD Controls](image)

Figure 5: AutoIMD window called by chirality plugin.

4 Select Settings → Minimization Mode in the AutoIMD Controls window and hit then the Submit button. Once the AutoIMD session started, hit the Connect button and a minimization will start. Finish the simulation when the structure relaxed. Usually, it takes up to a few thousand steps until atoms do not move anymore. Is the structure minimized, hit the Finish in the AutoIMD Controls window. For more details about using the AutoIMD plugin, the user is referred to the AutoIMD user’s guide [http://www.ks.uiuc.edu/Research/vmd/plugins/autoimd/ug/](http://www.ks.uiuc.edu/Research/vmd/plugins/autoimd/ug/).

5 WARNING: Depending on the quality of the initial structure, it can happen that not all errors can be fixed in one run. Thus, it is important to check the final result again and correct the remaining errors, if necessary. In order to check the structure after the performed minimization/equilibration using AutoIMD, the chirality plugin should be reset. This can be done by hitting the Reset chirality plugin button at the bottom of the chirality window. Note, in the current implementation it will
be necessary to select the molecule again in the top part of the chirality window. Note also that if the molecule to correct is solvated, it may be necessary to equilibrate the minimized parts of the structure. This can be done by selecting Settings → Equilibration Mode and submitting a new AutoIMD run.

6 Finally, it is a good advice to minimize the structure in the very last step. This should be done by selecting Settings → Minimization Mode in the AutoIMD Controls window, which is still open from the last AutoIMD session, and hit then the Submit button. As mentioned in the introduction, additional restraints during the correction of chirality errors. The restraints will be used always when AutoIMD is started from the chirality window. The equilibrium values for these restraints are very approximate and bring the configuration in the correct region. Thus, in order to obtain a optimized structure, it is necessary NOT to use the restraints defined by chirality. This is achieved by reusing the AutoIMD window from the previous minimization/equilibration; the restraints will not be taken into account.

7 Once all minimization/equilibration steps are performed, the corrected structure should be saved. This can be done in the following way. Select the molecule in the VMD main window. Create a representation containing all atoms in the Graphical Representations window. Select File → Save Coordinates in the VMD main window. Select all in the Selected atoms and hit Save. Finally, specify the file name in the Chose filename to save trajectory and hit OK.

1.4 Preserving stereochemistry during MD simulation

As mentioned in the introduction, structure optimizations and MD protocols applying external forces can introduce chirality errors into the simulated structure. To preserve stereochemistry, the chirality plugin offers the possibility to generate restraints in form of extrabonds which can be used in a NAMD simulations. This feature currently is available only from the console.

1 Assuming the structure of interest is loaded in the top molecule in VMD and one wishes to obtain restraints for all chiral centers in proteins and nucleic acids, the command reads

chirality restrain -o chirality-extrabonds.txt

The file chirality-extrabonds.txt contains now two improper restraints for each chiral center. One includes the chiral center and its non-hydrogen binding partners. In the second restraint, the chiral center is replaced by the hydrogen bound to it. The equilibrium values of the two improper are set to the values present in the used structure.

WARNING: Note, the specified restraints modify the potential energy function of the system, and thus should not be used in equilibrium production runs.
Rather, these restraints are supposed to be used only in simulations in which large forces occur due to poor initial geometry or due to externally applied forces, e.g., MDFF or TMD. Note also that although the final structure resulting from such simulations will be correct, the behavior of the system during the simulation is artificial.
2 cis peptide bonds in proteins

2.1 Introduction

In naturally occurring proteins most peptide bonds are in the trans configuration (see Fig. 6). However, sometimes cis peptide bonds do occur. The vast majority of cis peptides is observed at a proline, Xaa-Pro, Xaa being any amino acid. But non-proline Xaa-non Pro cis bonds are also found in proteins, although they occur much less frequently than Xaa-Pro (see A. Jabs et al., JMB, 286, 291–304 (1999)).

Figure 6: Structurally optimized α-helix containing a cispeptide bond. The cis peptide bond is shown in CPK, while hydrogen bonds within the helix are shown as thick dashed blue lines. The cis configuration of the peptide bond disrupts the hydrogen bond network stabilizing the helix. Note, the hydrogen bond network is broken not only locally.

The configuration of the peptide bond is central to the sort of secondary structure the protein backbone can adopt. It is easy to understand by imagining a normal α helix and converting a trans peptide bond into its cis form. The result is that the hydrogen bond network stabilising the helix is broken (see Fig. [6] and the helix will be unstable in long simulations.

2.2 Checking configuration of peptide bonds

To demonstrate the usage of the plugin we will start by checking a structure consisting of the protein EF-Tu in complex with a tRNA-Phe, although it is clear that in this example only the protein part will contain peptide bonds. The
used structure is based on the PDB structure 1OB2 in which errors have been introduced manually.

Currently, the cispeptide plugin can be used from the text console and through a GUI. The available commands provided by the plugin can be obtained by typing Type cispeptide. The following information should be printed in the console:

Usage: cispeptide <command> [args...]
Commands:
  check -- identify cis peptide bonds
  list -- list identified cis peptide bonds
  minimize -- fix cis peptide bonds using energy minimization
  move -- move specified atom to convert to trans
  reset -- reinitialize plugin state
  restrain -- generate NAMD extrabonds file to restrain peptide bonds
  set -- define how to modify a given cis peptide bond
  show -- visualize identified cis peptide bonds

In the same way the usage information for any of the provided commands can be obtained. For example, typing cispeptide restrain will show the syntax of the command that generates restraints for the dihedral angle at the peptide bond which can be used in NAMD.

In the following, we will describe how to use the cispeptide plugin through its GUI, but all of the tasks can also be accomplished from the console.

1 Load the files pept_testcase.psf and pept_testcase.pdb into a new session of VMD.

2 Open the cispeptide GUI by selecting Extensions → Modeling → Fix Cis Peptide Bonds in the VMD Main menu. In the upper part of the GUI (see Fig. 7) the user can specify the molecule and the atom selection to be tested.

3 The button Check structure initializes the test. Peptides identified to be in cis configuration are displayed in the Identified cis peptide bonds form (see Fig. 8). In the present case there are 4 cis peptide bonds identified in EF-Tu.

4 By selecting an entry in the Identified cis peptide bonds form and hitting the button Show selected cis peptide bond it is possible to visually inspect all the individual identified cis peptides. In the created representation (see Fig. 9) the cis peptide bond is highlighted in CPK representation.

2.3 Converting cis peptides into trans configuration

WARNING: The cispeptide plugin identifies all cis peptide bonds. As noted in the introduction, there are cases in which cis peptides do occur in proteins.
Thus, it should be checked if a given cis peptide is real or is a mistake. This can be done by literature research, by comparing high resolution crystallographic
models, or by comparing crystallographic models of similar proteins.

1. If the shown peptide bond is supposed to have a trans configuration, the user can tag the atom to be moved to flip the configuration from cis to trans. This is done by hitting the button hydrogen or oxygen.

   **WARNING:** The choice which atom to move is crucial and has to be made based on the local structure around the peptide under consideration. The decision is particularly critical for helices and β-sheets: the relaxed backbone should integrate in the existing hydrogen bond network characteristic for the specific secondary structure motif (see Fig. 6).

   If the identified cis peptide bond is supposed to be there, nothing needs to be done. It is also possible to untag a tagged atom by selecting the corresponding cis peptide and hitting none.

2. Once a cis peptide bond has been inspected, and the atom to be moved has been tagged, the actual moving of the atom can be executed by hitting the button **Move tagged atoms for selected cis peptide bonds**.

3. Since the described procedure (simple moving of one atom) generates an unphysical geometry of the molecule, it is necessary to optimize the structure using an MD force field. The cispeptide plugin uses the AutoIMD plugin for this purpose. This final step is accomplished by selecting the cis peptides which should be relaxed and hitting the button **Minimize/equilibrate selected cis peptide bonds**. This will open the AutoIMD Controls window (see Fig. 10).
Figure 10: AutoIMD window called by cispeptide plugin.

4 Select Settings → Minimization Mode in the AutoIMD Controls window and hit then the Submit button. Once the AutoIMD session started, hit the Connect button and a minimization will start. Finish the simulation when the structure relaxed. Usually, it takes up to a few thousand steps until atoms do not move anymore. Is the structure minimized, hit the Finish in the AutoIMD Controls window. For more details about using the AutoIMD plugin, the user is referred to the AutoIMD user’s guide [http://www.ks.uiuc.edu/Research/vmd/plugins/autoimd/ug/].

5 WARNING: Depending on the quality of the initial structure, it can happen that not all errors can be fixed in one run. Thus, it is important to check the final result again and correct the remaining errors, if necessary. In order to check the structure after the performed minimization/equilibration using IMD, the cispeptide plugin should be reset. This can be done by hitting the Reset cispeptide plugin button at the bottom of the cispeptide window. Note, in the current implementation it will be necessary to select the molecule again in the top part of the cispeptide window. Note also that if the molecule to correct is solvated, it may be necessary to equilibrate the minimized parts of the structure. This can be done by selecting Settings → Equilibration Mode and submitting a new AutoIMD run.

6 Finally, it is a good advice to minimize the structure in the very last step. This should be done by selecting Settings → Minimization Mode in the AutoIMD Controls window, which is still open from the last AutoIMD session, and hit then the Submit button. As mentioned in the introduction, additional restraints during the correction of cis peptides. The restraints will be used always when AutoIMD is started from the cispeptide window. The equilibrium values for these restraints are very approximate and bring the configuration in the correct region. Thus, in order to obtain an optimized structure, it is necessary NOT to use the restraints defined by cispeptide. This is achieved by reusing the AutoIMD window from
the previous minimization/equilibration; the restraints will not be taken into account.

7 Once all minimization/equilibration steps are performed, the corrected structure should be saved. This can be done in the following way. Select the molecule in the VMD main window. Create a representation containing all atoms in the Graphical Representations window. Select File → Save Coordinates in the VMD main window. Select all in the Selected atoms and hit Save. Finally, specify the file name in the Chose filename to save trajectory and hit OK.

2.4 Preserving configuration of peptide bonds during MD simulation

As mentioned in the introduction, structure optimizations and MD protocols applying external forces can flip the configuration of a peptide bond in the simulated structure. To preserve peptide configuration, the cispeptide plugin offers the possibility to generate restraints in form of extrabonds which can be used in a NAMD simulations. This feature currently is available only from the console.

1 Assuming the structure of interest is loaded in the top molecule in VMD and one wishes to obtain restraints for all peptide bonds in the system, the command reads

cispeptide restrain -o peptide-extrabonds.txt

The file peptide-extrabonds.txt contains now one dihedral restraint for each peptide bond. The equilibrium value of this dihedral is set to the value present in the used structure.

WARNING: Note, the specified restraints modify the potential energy function of the system, and thus should not be used in equilibrium production runs. Rather, these restraints are supposed to be used only in simulations in which large forces occur due to poor initial geometry or due to externally applied forces, e.g., MDFF or TMD. Note also that although the final structure resulting from such simulations will be correct, the behavior of the system during the simulation is artificial.

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