Praktikum Physikalische Chemie

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Molecular Dynamic Simulations

Investigating the Folding and Unfolding of a β-Peptide

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Contents

Summary of the experiment

During this computational experiment you will analyse the results obtained from molecular dynamics (MD) simulations of a β-peptide, which at 340 K shows reversible folding in methanol. The differences in conformations and stability will be investigated.

The aim of this exercise is to: (i) give an introduction to the analysis of computational MD simulations, and (ii) understand the valuable information it can provide on the dynamics of a biomolecular system.

1 Introduction

1.1 Preliminary notes

Questions/assignments are indicated by a cyan box:

Please answer all questions in blue

The actions to perform inside programs like vmd and xmgrace are indicated in green:

```
File > Save
```

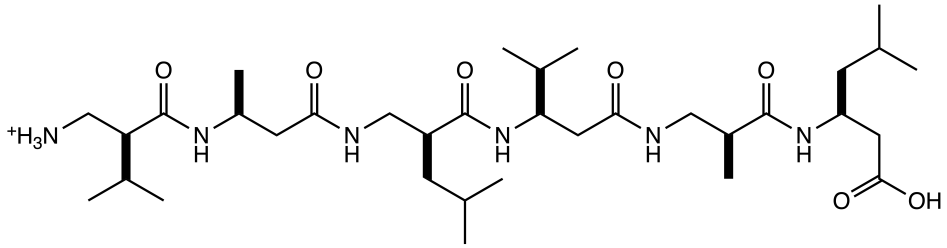
1.2 Biological perspective

β-Peptides are non-natural peptides, which exhibit a strong tendency to form stable, well defined secondary structures, including the 3_{14}-helix, the 2.5_{12}-helix, and the $2.7_{10/12}$-helix as well as the hairpin structure. Especially, the conformation of the peptide can be tuned by altering the side-chain composition.[1] β-Peptides have attracted much attention due to their potential for pharmaceutical use, since they are stable to peptidases. Most importantly, β-peptides offer the possibility to investigate the folding process and folding-unfolding equilibrium in very small systems. They are ideal systems to study the folding process by MD simulation for several reasons. First, although the time scale for protein folding is usually in the milliseconds range, these peptides fold in methanol on a time scale of nanoseconds. This is a very accessible time scale by computer simulations! Second, methanol has a lower density than water, making it a computationally less expensive solvent (by a factor of about three) in which to simulate folding. Third, as already mentioned, β-peptides can adopt a range of secondary-structure elements depending on the side-chain composition and position.

Because the conformation is sensitive to the precise nature of the atomic interactions, the system is a very sensitive test case for the force field used in the simulations. It should be noted that the GROMOS96 force field used in this work has not been developed for β-aminoacids or β-peptides. Therefore,there can be no suspicion that the force field has been artificially adjusted to favor the experimentally observed folds.

In this exercise you will study the conformations of the β-hexapeptide, shown in Figure 1, in explicit methanol solvent at 340 K. The chosen temperature of 340 K is an approxi-

mation to the melting temperature of the helices chosen in order to maximize the number of folding-unfolding events.[2]



H-β^2-HVal-β^3-HAla-β^2-HLeu-β^3-HVal-β^2-HAla-β^3-HLeu-OH

covjsdovj

Figure 1: 2D-structure and sequence of the β-hexapeptide. The numbers in superscript indicate the position of the side-chain.

1.3 Molecular Dynamic Simulations

Here, we will exclusively work with computer simulations. The main difference between experiments and simulations is that in experiments you have a complete description of the chemical or biological system you want to study, but it is very difficult to get structural details. On the other hand, computer simulations are performing a list of operations on models of chemical or biological systems, making the structural information easily available. But this means that the choice of the model is crucial since it contains all the information that will be taken into account. The model depends on consideration such as the level of accuracy needed and the properties of interest. Since we want to measure the extent of conformational changes experienced by β-peptides in an explicit solvent representation, the level of accuracy needed is fitting with atomistic molecular dynamic simulation. This it due to the fact that representing molecules at the quantum level is too costly for large systems, making molecular dynamics a good accuracy/computational cost trade-off. It is nevertheless important to notice that the observation of transition states inducing bond breaking or forming cannot be observed by a classical molecular dynamics description.

In an atomistic description, Newton's equations of motion are integrated in a stepwise manner. The model consists then of three components:

- The initial coordinates from which the forces will be calculated and applied to get the next step
- The topology, which contains all the chemical information on the system: atom types (elements), bonds types, masses, charges, ...
- The system parameters: at which temperature and pressure the system has to be simulated, what type of boundary condition have to be applied, ...

A complete description of the molecular dynamic process is displayed in Figure 2.

The coordinates of the system are combined together with the topology information. The latter contains all the information about which atoms are bound together and the

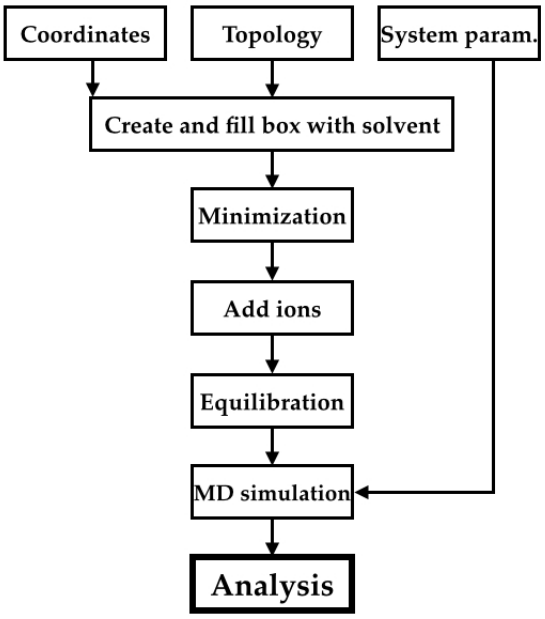


Figure 2: Overview of the computational work-flow that led to the data you are going to analyse

force field parameters. In other words, the coordinates are strictly the positions of the atoms and the topology gives the additional information needed to represent the model of the molecule. Simulations also need a set of adjustable parameters like time-step or temperature, that will depend on what you want to do with the system you are interested in.

There are two ways to obtain the starting coordinates from experiments: X-ray crystal structures and NMR bundles. Crystal structures fail to represent the highly mobile parts of proteins because the data acquisition is made with frozen protein. The coordinates that are obtained by X-ray crystallization are furthermore optimized with a force field that does not include electrostatic interactions. This means that there might be high energy contacts within the protein. Thus the minimization step is of particular importance if the starting structure comes from X-ray crystallography. NMR data can sample accurately mobile parts of the protein in solution, but is limited to rather small proteins as signals can get too noisy for larger ones. Furthermore NMR methods require a bigger amount of the protein for sampling. This is why the combination of the two methods gives the most complete idea on the protein structure[3].

In order to have the most physically meaningful simulation, one uses an explicit solvent representation. This means that one surrounds the protein by a box of water. As opposed to the implicit solvent model, one will be able to see the solvent molecules, they will be all represented explicitly. The box is not exactly the boundary of the system. Indeed, during the simulation you are going to analyse, we used periodic boundary conditions.

This means that the box is mirrored infinitely in every direction. A water molecule that leaves the box on its right side will reenter from the left. This allows to minimize the surface effects that arise from simulating a very small volume. To illustrate this, you can think of how the water behaves when it comes close to the edges of a glass: the surface tension has a strong influence. Bulk water (water that is not at a surface interface) behaves differently than surface one. We generally consider very small volumes, this is why we use this artificial way to increase the size of the simulated system.

Counter-ions are generally added to the system so the complete system is neutral. It matters for the physical handling of the calculations. We will not go into more details here, there are not ions in the systems we are going to investigate.

One does not start the actual simulation immediately but performs first an equilibration period. This is needed because the molecules need first to relax as they were generated using a different force field and at different experimental conditions (crystal structures, solid NMR,...).

After the equilibration, the actual production run can start, which gives the trajectories that we are going to analyse. A trajectory consists of a series of frames at given steps of the simulation.

In this exercise, we will focus on the analysis part and give you hint on how to rationalize what you see in the trajectory visualization with more precise quantification of structural changes.

1.4 Introduction to the Practical Know-How

This section will guide you through the steps that are needed prior to the actual analysis work.

1.4.1 Download the required files

Download the structure files and MD trajectory from Polybox. The link to the Polybox folder has been provided via email.

The PC-Praktikum folder contains three sub-folders: "Trajectory", "H-bond", and "Videos".

1.4.2 Download the required files

Install VMD on you personal computer. Download VMD from this Webpage. To be able to download VMD, you are first requested to create an account. For **Windows** and **Mac**, the installation is straightforward. For **unix** systems, installation instructions are provided in a separate file, InstallVMD_unix.pdf. If you have MAC Catalina, please use this version of VMD.

1.4.3 Basic VMD commands

VMD has some very useful keyboard shortcuts:

- `1`: to select atoms and display the atom name as well as the name of the residue it belongs to (1 atom)
- `2`: to measure and display distances (2 atoms)
- `3`: to measure and display angles (3 atoms)
- `4`: to measure and display dihedral angles (4 atoms)
- `r`: to rotate the system (Default). Use it to also exit from the 1,2,3,4 modes
- `t`: to translate the system
- `s`: to zoom in and out (You can also use scroll wheel)
- `=`: recenter the box

2 Theory

Watch all the videos provided in the Videos sub-folder (about 17 minutes).

3 Experiment: Analysis of the MD Simulation of a β-hexapeptide

3.1 Computational details of the simulations

From NMR experiments, it was found that the β-hexapeptide predominantly forms a right-handed helix in methanol. We used the NMR refined structure as starting structure for the computer simulations. The peptide was solvated by 1123 molecules of methanol. The MD simulation was performed at 340 K with a time step of 0.002 ps. The system was simulated for 500 ns.

3.2 Visualize the system and the trajectory

3.2.1 Visualize the system using VMD

VMD is a tool to visualize the molecule in 3D. Section 1.4.3 lists some useful commands for VMD.

As an example, we will have a look at the β-hexapeptide.

```
Open VMD on you computer
```

Load the peptide structure, which you can find in the PC-Praktikum folder:

PC-Praktikum/Trajectory/peptide_solv.pdb

```
File > New Molecule > Browse > Go to the file path >
select the peptide_solv.pdb file > Open > Load
```

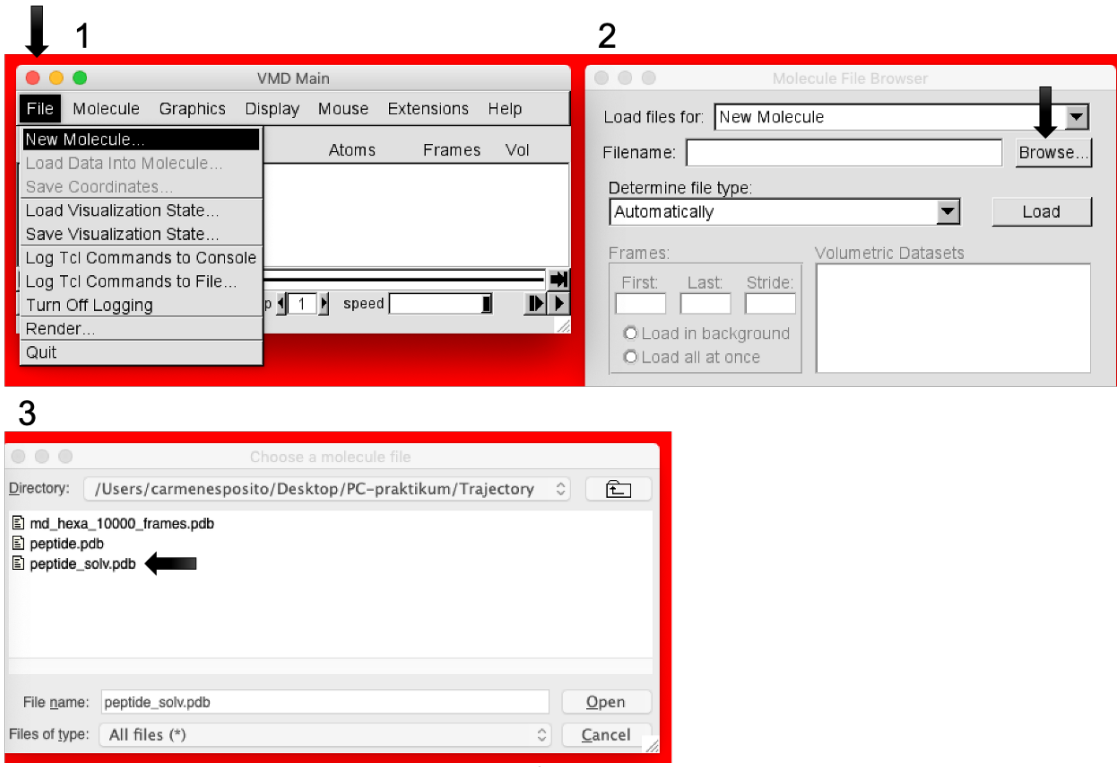


Figure 3: Load structure in VMD

To hide the solvent and only display the peptide, do:

```
Graphics > Representation > Selected Atoms > all and not resname SOLV >
> Enter
```

You should be able to see the peptide in a representation showing the bonds between the protein atoms.

To better visualize it, let's change the representation from Lines to Licorice.

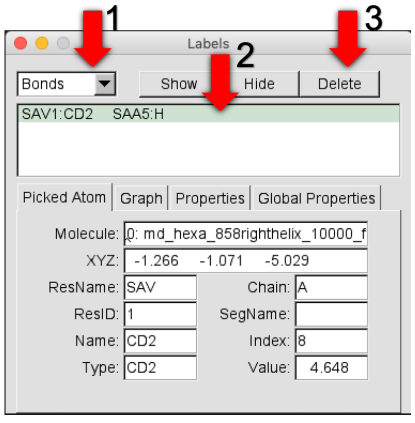
```
Graphics > Representation > Drawing Method > Licorice > Apply
```

Do you agree that the peptide forms a right-handed helix?
The β-peptide forms five intramolecular backbone hydrogen bonds. Highlight all of them as follows:

- rotate the peptide with the mouse to see the two atoms forming one of the hydrogen bonds
- press "2" on the keyboard
- click on the two atoms forming the hydrogen bond (and nothing else)
- press "r" on the keyboard
- rotate again the peptide to visualize the next hydrogen bond and repeat the previous steps until you have displayed all five hydrogen bonds.

If you made a mistake in the selection, you can delete the wrong distance in this way:

```
Graphics > Labels > Bonds > Click on the bond to delete >
Click on the Delete button
```



Write the hydrogen bonds in a table as the one displayed in Figure 4 in the format DONOR_ATOM(RESIDUE) – ACCEPTOR_ATOM(RESIDUE). Write the conformation assumed by the peptide in the column "Conformation". The column "Frequency" will be filled in section 3.3.1

Let's now generate a figure of the peptide with the H-bonds displayed.

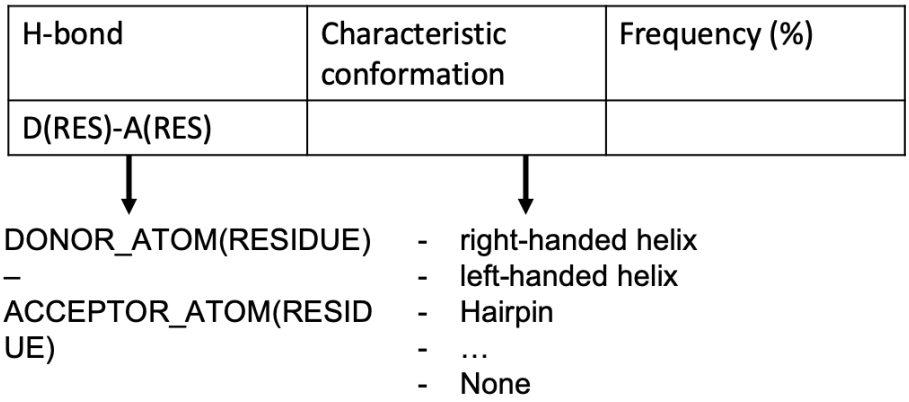


Figure 4: Example of the Table to include in the report

Remove the "fog":

```
Display > "uncheck" Depth Cueing
```

Set the background color to white:

```
Graphics > Colors... > Display > Background > 8 white
```

If you want to change the color of the carbon atoms:

```
Graphics > Colors > Name > C > COLOR > Apply
```

To change the perspective settings:

```
Display > Orthographic
```

To put the visible part of your system in the center:

```
Display > Reset view
```

To save the image:

```
File > Render... > Filename > Browse >
select the folder where to save the figure and specify the figure name:
PATH/YOUR_FILENAME.png > Start Rendering
```

Prepare a picture of the peptide with the hydrogen bonds clearly visible and include it in your report with a short comment.

Display again the methanol solvent:

```
Graphics > Representation > Selected Atoms > all > Enter
```

Which interactions can the β-hexapeptide form with methanol?

Do you expect the peptide to be stable under the simulation conditions?

You need to exit the vmd program to be able to work again in your terminal window:

```
[X]
```

3.2.2 Visualize the trajectory

Open again VMD.

Load the 500 ns trajectory file in VMD. The trajectory contains only the peptide coordinates even if the simulations were run in a methanol box. This saves memory and makes the loading of the trajectory in VMD faster:

```
File > New Molecule > Browse > Go to the file path >
select the md_hexa_10000_frames.pdb file > Open > Load
```

Display the peptide as sticks:

```
Graphics > Representation > Drawing Method > Licorice > Apply
```

```
Play the movie with the buttons at the bottom of the "VMD Main" window.
You can adjust the speed with the speed bar.
```

Write down the behavior of the β-hexapeptide. Is this as you expected?

From the movie, identify a snapshot where the β-hexapeptide forms a left-handed helix and where four backbone hydrogen bonds are formed. To help you in your search, we suggest you to look at the first 1000 snapshots. Once you have found the perfect snapshot, display the hydrogen bonds as done in the previous paragraph.

Save a Figure where the hydrogen bonds are clearly visible:

```
File > Render... > Filename > Browse >
select the folder where to save the figure and specify the figure name:
PATH/YOUR_FILENAME.png > Start Rendering
```

Prepare a picture of the peptide in the left-handed helical conformation with the hydrogen bonds clearly visible and include it in your report with a short comment.

Write the hydrogen bonds in the table as done in section 3.2.1. Write the conformation assumed by the peptide in the column "Conformation".

Compare the right-handed helical to the left-handed helical conformation. Based on the differences in the structural features, make hypothesis on the stability of the two structures.

Exit VMD:

```
[X]
```

3.3 Quantitative Analyses

You will now do the following analysis:

- Hydrogen bonds analysis
- RMSD analysis

- Calculation of free energy differences
- Dihedral Angles analysis
- Clustering analysis
- Comparison between simulation distances and experimental NOE distances (optional)

You can find more information about each of the analysis in the file "MDAnalysis.pdf". First, you will do the hydrogen bonds analysis in the shell. Second, you will run the command to calculate the RMSDs. Then, you will do the rest of the analysis using the online Jupyter notebook provided by the assistants via email.

3.3.1 Hydrogen bonds analysis

Go to the directory that contains the hydrogen bond analysis:

PC-Praktikum/H-bond

Open the output file "hbond.dat" with an Editor, e.g. TextEdit, Notes, emacs, etc. This file is provided as output by GROMOS when running the hbond program.

```
Right click on hbond.dat > Open with > select editor
```

As you can see, this output file contains all the hydrogen bonds that occurred during the simulation together with the frequency of occurrence. We sorted for you the hydrogen bonds in decreasing order based on the frequency. You can find the sorted hydrogen bonds in the file "hbond_sorted.dat".

Close the "hbond.dat" file and open the "hbond_sorted.dat" file with an editor.

Add the frequencies to the table that you have previously generated (Figure 4). For SAV1, whose donor nitrogen can donate indistinctly any of the attached hydrogen atoms (H1, H2, and H3), sum up the frequencies of the three hydrogen atoms. Add to the table also all the hydrogen bonds with a frequency higher than 1%.

Why are there hydrogen bonds with a frequency higher than 1%, which are not characteristic of either the right-handed or the left-handed helical conformation?

Comment on the hydrogen bond frequency Table. Are the results in agreement with what you have observed in the movie?

3.3.2 Open the Jupyter notebook

All following analysis will be carried out in the Jupyter notebook. Open the Jupyter notebook using the link provided in the email.

```
(1) Open link from email > (2) Clone > (3) Sign in > (4) Clone >
(5) Run on Free Compute > (6) Simulation_Analysis.ipynb
```

3.3.3 RMSD

The RMSD (root-mean-square deviation) measures the similarity between the trajectory snapshots and a reference structure. The lower the RMSD the more the trajectory snapshots resembles the reference structure.

We have calculated for you the RMSD over the simulation time using as reference structure either the right-handed or the left-handed helical conformation.

Follow the instructions in the Jupyter Notebook to plot:

1. The RMSD calculated from right-handed conformation as a function of time
2. The RMSD calculated from left-handed conformation as a function of time

Include the RMSD figures in your report and comment on them.
How do you interpret the plots? Which conformation seems to be the most stable?
Can you draw conclusions that correlate with what you observed in the movies?

3.3.4 Calculation of free energy differences

For a two-state process $state1 \rightarrow state2$, the free energy difference can be calculated as:

$$\Delta G = -k_b N_A T ln(p_{state2}/p_{state1}) \tag{1}$$

where, k_b is the Boltzmann constant, N_A is the Avogadro number, T is the temperature, p_{state2} is the probability of $state2$ and p_{state1} is the probability of $state1$.

For the system we are studying, we can define three states: folded right-handed helical state, folded left-handed helical state, and unfolded state. The thermodynamic cycle is in Figure 5.

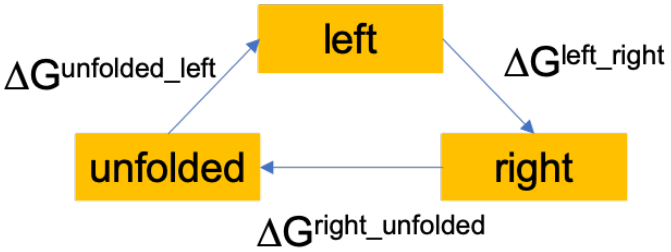


Figure 5: Free energy cycle

You can calculate the probability for each of the states from the MD simulation as the ratio between the number of structures sampling the given state and the total number of snapshots.

The snapshots were classified as follows:

- right-handed helical state: if the RMSD < 0.08 nm from the reference right-handed helical structure
- left-handed helical state: if the RMSD < 0.08 nm from the reference left-handed helical structure
- unfolded state: if both RMSD values > 0.12 nm
- unclassified: snapshots that do not fall into any of the previous criteria (for example, a structure that has a RMSD_right $= 0.15$ and RMSD_left $= 0.10$)

The number of structures sampling each of the states are provided in the Jupyter notebook. Calculate the probability of the states and the free energy differences, as described above. Note that, if the free energy differences do not sum up to zero, you have made a mistake in the calculations.

Report the free energy differences $\Delta G^{unfolded\_left}$, ΔG^{left_right}, $\Delta G^{right\_unfolded}$

Which conformation is more stable?

Is this in agreement in what you observed so far? How do you explain this result?

3.3.5 Dihedral angles analysis

Now you are going to visualize the flexibility of the dihedral angles of the peptide.

The dihedral angle (torsion angle) is defined as the angle between two planes (see Fig. 6).

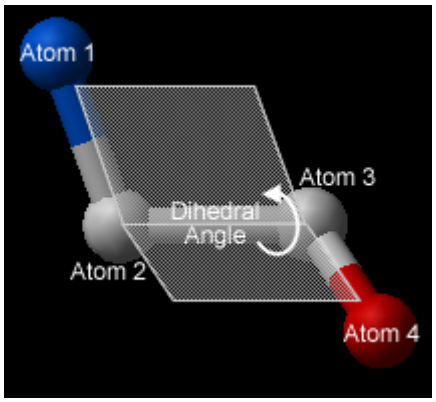


Figure 6: Dihedral angle definition

Three successive chemical bonds form a dihedral angle, which gives an indication on the overall 3D structure of the molecule.

Here, we monitor the ψ and ϕ angles, as shown is Figure 7. β-peptides also contain an additional backbone dihedral (rotation around the C_α - C_β bond) angle compared to α-peptides. However, these are not monitored because they are very flexible.

Plot the time evolution of the dihedral angles 1 to 10 using the Jupyter notebook. Include the figures in you report.

Discuss what you see, e.g. do you see a correlation between the different dihedral angles?

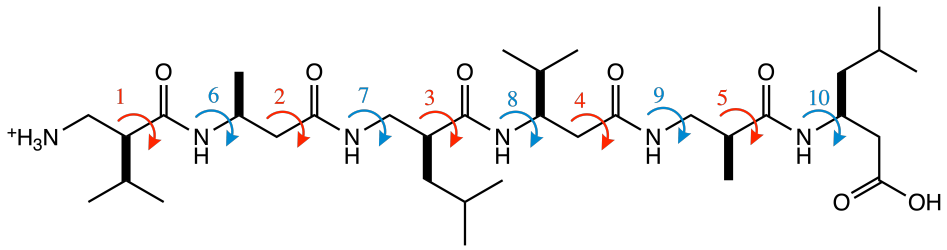


Figure 7: Labelling of the dihedral angles of interest. ψ Angles are shown in red while ϕ angles are shown in blue.

What can you conclude from these plots and is what you observe in agreement with what reported so far?

3.3.6 Clustering

To define the occurrence of a bundle of very similar structures within a trajectory you can perform clustering.

Performing clustering will thus allow you to track down the dominant structures within the trajectory used for analysis.

The clustering procedure is based on the calculation of the similarities between all the conformations within a trajectory and is performed in three steps. We give a cutoff corresponding to a general deviation beyond which two structures have to be placed in different clusters. The clustering procedure takes the first frame into the first cluster, and compare the second frame to the first cluster, decides if they should be placed in the same cluster (in or out of the cutoff), if the next frame is outside of the cutoff, a new cluster is created with this frame, and all similar structures will be grouped together.

We performed the clustering for you already with a cutoff of 0.08 nm. The next step is to look at the results.

Follow the instructions in the Jupyter notebook to:

- Check how many cluster have been generate
- Look at the population of the clusters
- Look at the central structure of the 10 most populated clusters

The central structure of a cluster is defined as the structure with the highest number of neighbours. In other words, the central structure is the one which is the most similar to all the other structures within the same cluster.

Write down: the size (number of structures) of the ten first clusters, the total number of clusters and the cutoff value used and include that data into your report

Include the plot of the cluster populations in your report.

Include the Figure of the most 10 populated clusters in your report.

Discuss both the most populated conformations and the difference in population between the clusters.

Which cluster is more similar to the NMR starting structure?

```
You should now send all the figures (both the ones generated with VMD
and the ones saved from the Jupyter notebook) to yourself via
email or using an USB flash drive.
```

3.3.7 Nuclear Overhauser Effect (NOE) analysis - Optional

This section is optional!

NMR can be used to monitor intra-molecular distances. The NOE distances for the β-hexapeptide are reported in the Supporting Figure 9.

To check the agreement between experiments and simulations, you can compare the NOEs distances to the distances obtained from simulations. It is important to point out that while NOEs distances are averaged over an ensemble of conformations, simulations provide you distances at every time step, and thus, for every sampled conformation.

The interesting part is to determine if certain center conformations of the clusters fulfil the NOE-distances. This will give us information on the structure of the most populated clusters.

We have compared for you the NOE distances to:

- the distances in the central structure of cluster 1
- the distances in the central structure of cluster 2
- the average distances over the ensemble of conformations visited during the simulation

Let's check the violations. A violation occurs when the inconsistency between a restraint (NOE distance) and a structure is higher than 0.1 nm. Essentially, a NOE distance is violated if the distance from the simulated structure is at least 0.1 nm larger than the NOE distance.

Figure 8 contains the plots of the violations for the three comparisons listed above. The table below contains the list of violations as well as the violated distances.[4]

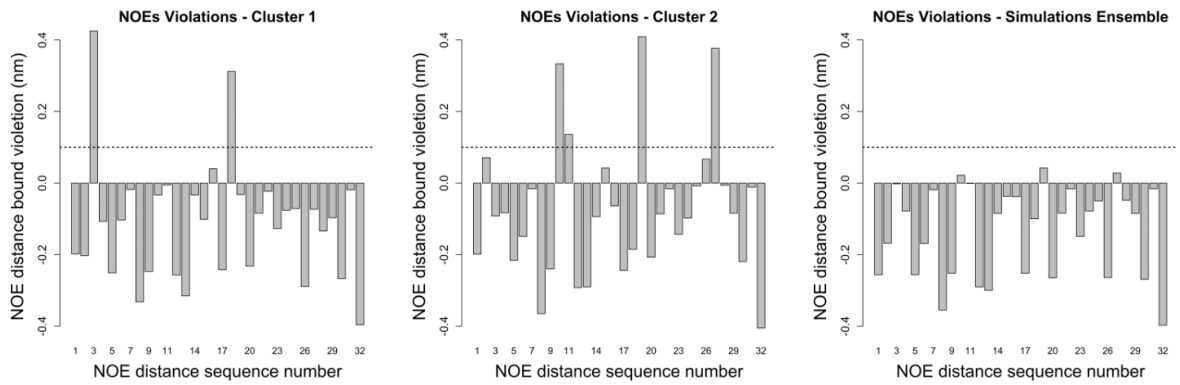


Figure 8: Comparison between the simulated distances and the experimental NOEs

Table: NOE violations for the central structure of cluster1, the central structure of cluster 2 and the ensemble of conformations sampled during the simulation.

	N_Violations	Violated Distance	Violation (nm)
Cluster 1	2	HA(1) – HB(4)	0.425
		HA(3) – HB(6)	0.312
Cluster 2	4	HB(2) – HA(4)	0.333
		H(3) – HB(2)	0.136
		H(4) – HB(2)	0.409
		H(6) – HB(4)	0.377
Ensemble	0		

Looking at the results of the NOE analysis, answer the following BONUS questions:

Compare the violations for cluster 1 and cluster 2. Which cluster fulfils better the experimental NOEs?

Check the violations for the conformational ensemble obtained from MD simulations. Are the average MD distances in agreement with the NOE distances?

SUPER BONUS QUESTION:

Compare the results that you have obtained from the three different comparisons. Why no violation is observed for the simulation ensemble while there are 2 and 4 violations for the central structures of cluster 1 and 2, respectively?

4 Instructions for the report

Just as for experimental approaches, mastering the technique is only one component in the scientific investigation of a given problem. Equally important components - in experiment as well as in simulation - are to:

- Formulate the question(s) clearly
- Design an appropriate experiment to answer the question(s)
- Interpret the results in terms of the question(s)
- Be aware of the shortcomings and approximations of the employed method

The report has to contain:

- **Abstract:** A short summary that gives the motivation of the study, the most important findings of the study and the conclusion. It shouldn't contain more than 150 words.
- **Introduction:** Introduce the system we have been studying (β-hexapeptide).

Highlight why it is important to monitor the conformational changes of the β-hexapeptide.

- **Methods:** short description of what you understood of MD simulations
- **Results and Discussion**: Graphs and tables of the analysis, all numbered and all with a legend. Here you have to answer the guiding questions in the script (lightblue) and explain the results of the previous sections. Graph labels must be readable, captions should clearly describe what is in the picture
- **Conclusions:** the take-home message drawn from analysis or results. This does not mean that you have to repeat the results. Instead, you should mention what you have observed and which conclusions you have drawn from your observations. Conclusions should be formulated clearly and be connected logically to the outcome of experiment. Some example sentences are:
  - "From the XXX and the XXX analysis we have observed that ... . Thus we can conclude that .... "
  - "Similarly, on the basis of the XXX analysis, we have deducted that... ."
  - "On the contrary, the XXX analysis showed that ... . Therefore, ..."
  - "In conclusion, we found that..."
- **References:** list of articles, books, or other sources used to write the report

  You have three weeks to hand-in the report. We would like to have double spacing to favor the corrections. Furthermore, please pay attention to the fact that good scientific work is concise!

When writing a report you should keep in mind following things:

- make sure that you understand the experiment. In case of questions you can always contact the assistants!
- formulate sentences clearly, so that the person reading the report doesn't have to guess what you had in mind
- the report should be understandable for a person who did not perform the experiment
- make sure that statements you present make sense; try to avoid "definite" statements when you don't have enough proof
- conclusions must be drawn basing on results
- don't forget units
- always read the report again after finishing it!

References

[1] Xavier Daura, Bernhard Jaun, Dieter Seebach, Wilfred F van Gunsteren, and Alan E Mark, Mark. Reversible peptide folding in solution by molecular dynamics simulation. *J. Mol. Biol.*, 280(5):925–932, July 1998.

[2] Xavier Daura, Karl Gademann, Bernhard Jaun, Dieter Seebach, Wilfred F Van Gunsteren, and Alan E Mark. Peptide folding: when simulation meets experiment. *Angew. Chem. Int. Ed.*, 38(1-2):236–240, 1999.

[3] Hossam Elgabarty, Peter Schmieder, and Daniel Sebastiani. Unraveling the existence of dynamic water channels in light-harvesting proteins: alpha-C-phycocyanobilin in vitro. *Chem. Sci.*, 4:755–763, 2013.

[4] Zhixiong Lin, Nathan Schmid, and Wilfred F van Gunsteren. The effect of using a polarizable solvent model upon the folding equilibrium of different β-peptides. *Mol. Phys.*, 109(4):493–506, February 2011.

5 Supporting Information

Table S3: NOE, *i.e.* ROESY, distance bounds (in nm) of the peptide **2**[5] and bound violations (in nm) of the r^{-6} averaged distances from the structures in the MD simulations.[a]

NOE seq.	hydrogen atom pair		NOE	simulated bound violations		
	H atom	H atom	bound	2^{298K}_{pol}	2^{340K}_{pol}	2^{340K}_{nonpol}
1	H*-C^{β}(1)	H-CMe_2(1)	0.45(m)	-0.15	-0.16	-0.16
2	H*-C^{β}(1)	H-C^{α}(3)	0.55(w)	-0.12	-0.09	-0.07
3	H-C^{α}(1)	H-C^{β}(4)	0.35(m)	0.35	0.15	0.20
4	H-N(2)	H-C^{α}(1)	0.30(s)	-0.08	-0.08	-0.08
5	H-N(2)	H*-C^{β}(1)	0.55(w)	-0.15	-0.16	-0.16
6	H-N(2)	H-CMe_2(1)	0.45(w)	-0.15	-0.16	-0.16
7	H-N(2)	H-C^{β}(2)	0.30(s)	-0.02	-0.02	-0.02
8	H-N(2)	H*-C^{α}(2)	0.55(w)	-0.25	-0.26	-0.25
9	H-C^{β}(2)	H*-C^{α}(2)	0.40(s)	-0.15	-0.15	-0.15
10	H-C^{β}(2)	H^{Re}-C^{α}(4)	0.30(s)	-0.01	-0.00	-0.02
11	H-N(3)	H-C^{β}(2)	0.30(s)	-0.02	-0.02	-0.02
12	H-N(3)	H*-C^{α}(2)	0.45(m)	-0.18	-0.18	-0.19
13	H-N(3)	H*-C^{β}(3)	0.45(m)	-0.20	-0.20	-0.20
14	H-N(3)	H-C^{α}(3)	0.35(m)	-0.08	-0.08	-0.09
15	H-N(3)	H-C^{α}(5)	0.45(w)	-0.06	-0.05	-0.02
16	H*-C^{β}(3)	H-C^{α}(1)	0.55(w)	0.10	0.10	0.09
17	H*-C^{β}(3)	H-C^{α}(3)	0.40(s)	-0.15	-0.15	-0.15
18	H-C^{α}(3)	H-C^{β}(6)	0.45(w)	0.32	0.03	0.08
19	H-N(4)	H-C^{β}(2)	0.30(s)	0.00	0.01	0.00
20	H-N(4)	H*-C^{β}(3)	0.55(w)	-0.19	-0.17	-0.19
21	H-N(4)	H-C^{α}(3)	0.30(s)	-0.08	-0.09	-0.09
22	H-N(4)	H-C^{β}(4)	0.30(s)	-0.02	-0.02	-0.02
23	H-N(4)	H^{Re}-C^{α}(4)	0.35(m)	-0.09	-0.09	-0.10
24	H-N(4)	H-CMe_2(4)	0.35(m)	-0.08	-0.08	-0.08
25	H-C^{β}(4)	H-CMe_2(4)	0.30(s)	-0.05	-0.05	-0.05
26	H-C^{β}(4)	H*-C^{α}(6)	0.55(w)	-0.20	-0.19	-0.20
27	H-N(6)	H-C^{β}(4)	0.30(s)	-0.00	0.00	0.01
28	H-N(6)	H^{Si}-C^{α}(4)	0.45(w)	-0.00	0.00	0.00
29	H-N(6)	H-C^{α}(5)	0.30(s)	-0.09	-0.09	-0.09
30	H-N(6)	H*-C^{β}(5)	0.55(w)	-0.16	-0.17	-0.16
31	H-N(6)	H-C^{β}(6)	0.30(s)	-0.02	-0.02	-0.02
32	H-C^{β}(6)	H*-C^{γ}(6)	0.55(w)	-0.30	-0.30	-0.30

[a]H-CMe_2 refers to the proton at the tertiary carbon in a valine side chain; H*-C^{α}, H*-C^{β}, H*-C^{γ} refer to the non-stereospecific protons bound to the C^{α}, C^{β}, and C^{γ}

Figure 9: NOE-distances from Ref. [4]