The goal is to run and analyze the results from a 100 ps MD simulation of the myoglobin protein at two different temperatures (350 K and 50 K).

I. Setup and run a simulation

1. Download the programs:
- VMD (http://www.ks.uiuc.edu/Research/vmd/). The program needs installation.
- NAMD (http://www.ks.uiuc.edu/Development/Download/download.cgi). Unpack the downloaded file (no installation needed).

2. Generate a protein structure file (PSF) for the myoglobin molecule
- Open the structure of myoglobin in the VMD program (PDB code 1MBO).
- Copy the file "1MBO.pgn" and the topology file "top_all27_prot_lipid_na.inp" in one folder. Description of these files can be found in the NAMD tutorial at http://www.ks.uiuc.edu/Training/Tutorials/index-all.html#namd.
- Open the TkCon window (Choose Extentions → Tk Console). In the TkCon window go to the folder containing "1MBO.pgn" file using commands "cd…" to navigate through the folders.
- When you are in the correct folder write "source 1MBO.pgn" to execute the commands listed in the 1MBO.pgn file. As a result two new files should be created in the folder: "myoglobin.pdb" (a new pdb file with the complete coordinates of all atoms, including hydrogens) and "myoglobin.psf" containing the complete structural information of the protein.
- Close the 1MBO.pdb file

3. Start a simulation at 350 K using the configuration file "myoglobin_350.conf" (details about the configuration file can be found in the NAMD tutorial).
- Open in VMD the file "myoglobin.pdb"
- Load the structural information (select the molecule from the "VMD main" window and then select File → Load data into molecule… and choose "myoglobin.psf". Click "Load").
- Optionally you may open the visualization state saved in the file "visualization" (File → Load visualization state).
- Run NAMD specifying the path to the program e.g. by typing in a terminal window:

```
c:\Programs\NAMD_2.10_Win64-multicore\namd2 myoglobin_350.conf > myoglobin_350.log
```

Now the program should wait for a connection to VMD.
- Within VMD, select the menu item *Extensions* → *Simulation* → *IMD Connect (NAMD)*. In the *IMD Connection* window, enter

Hostname: `localhost` and *Port*: `3000`

Click *Connect*. You should see the molecule jiggling as the simulation is running.

II. Analysis of the data

Here you will use the trajectories (*.dcd files) calculated at 50 K and 350 K for the myoglobin protein. Now we are aiming at a graphical presentation of the time dependence of various parameters.

1. Plot the total energy of the system during the initial minimization versus time (first 100 steps)! Are the curves different for the simulation at 50 K and at 350 K? Why?

Hints:

In the VMD program, load: (1) the structural file "myoglobin.psf", (2) the file with the initial coordinates "myoglobin.pdb", and (3) the corresponding trajectory file with extension "dcd".

From the main VMD window, choose: Extensions > Analysis > NAMD Plot.

In the new window, select: File > Select NAMD Log File, and choose the file corresponding to this calculation with extension "log" (this file might not exist if you have run NAMD without specifying an output file name).

After selecting the file, chose the parameter you want to plot (in this case "total"). Select: File > Plot Selected Data. Save the data choosing: File > Export to ASCII matrix, and plot them using another program, selecting only the first 100 steps (for better visualization omit also the points with very high energies).

2. Investigate how the total energy changes during the equilibration of the protein at 50 K and 350 K! Plot also the kinetic energy, the temperature, and the bond energy (versus time)! Discuss the differences between 50 and 350 K!

3. Create a plot showing the changes in the number of H-bonds during the equilibration at both temperatures! Why does the number of H-bonds change in this way at the two temperatures?

Hint: From the main VMD window, choose: Extensions > Analysis > Hydrogen Bonds. Select the molecule and press the button "Find hydrogen bonds!"

4. Plot the distance between the heme Fe (iron of the heme group) and the nitrogen from the His ligand! Discuss the differences observed when comparing the behavior at 50 K and 350 K!

Hints:

In the main VMD window, choose: Mouse > Label > Bonds. Select first the Fe atom, and then the N ligand from the His residue. In the main VMD window, choose: Graphics > Labels.

From the drop-down menu in the new window, select "Bonds" instead of "Atoms". Mark the bond you want to investigate. (Important for the point 5 is that water molecules are labeled as TIP3).

Select Graph and click on the button "Graph". You should see a graphic showing the selected distance during the simulation and now you can save the data in ASCII format for further analysis.

5. Plot the distance between the heme Fe (iron of the heme group) and the oxygen atoms from two specific neighboring water molecules, namely resid 294 and resid 239! Discuss the differences observed when comparing the behavior at 50 K and 350 K! Why the behavior of the two water molecules is different?

Hint: To identify the two water molecules you may create new presentations (Graphics > Representations > Create Rep) and select, e.g., "resid 294", then change the color of the molecule, e.g., to yellow (Coloring Method – ColorID – 4).

In another plotting program (for example EXCEL), create histograms to visualize the distribution of the selected Fe-O distances for the two temperatures. How many semi-stable states can you identify?

6. Maxwell-Boltzmann energy distribution (optional; additional 10 pt)

Confirm that the kinetic energy distribution of the atoms in the system corresponds to the Maxwell distribution for a given temperature, by plotting the distribution (histogram) of the kinetic energies of the atoms in one moment of simulations and comparing it to the theoretically predicted distribution. When comparing the two curves, assume a normalization (scaling) factor, which can be fitted to the "experimental" data.

Hint: The Maxwell-Boltzmann distribution for the kinetic energy can be written as:

$$P(E_{kin}) = \frac{2}{\sqrt{\pi}} \cdot \frac{1}{(k_B T)^{3/2}} \sqrt{E_k} \cdot \exp\left(- \frac{E_k}{k_B T} \right)$$

Where $P(E_{kin})$ is the probability to have a specific kinetic energy.

The kinetic energies might be calculated for each atom during the simulation. For these exercises however, it is sufficient to calculate the kinetic energy for one point in time.

This can be done, e.g., with *.restart.vel or any velocity file automatically generated during the simulation. In the main VMD window, right-click on the loaded molecule (myoglobin.psf) and choose “Delete Frames”. Delete all frames. This will delete also the initial coordinates, so no molecule will be visible anymore.

Now right-click on the “myoglobin.psf” and choose “Load Data Into Molecule”. Choose a velocity file corresponding to the temperature you want to study.

In the “Determine file type:” windows choose “NAMD Binary Coordinates” and click “Load”. The displayed molecule will look very strange, as VMD program treats the velocities as atomic coordinates, but that is okay. In the main VMD window go to Extensions > TkConsole.

Go to the folder where the files for this exercise are stored (for example typing “cd D:”, or “cd Tutorials”). In this folder you should have a simple script (file called get_energy.txt), downloaded from our webpage, which calculates the kinetic energies for each atom and saves them in a file called “energy.dat”. Take a moment to look at the script and try to understand it. The script can be called from the VMD program, typing in VMD TkConsole: “source get_energy.txt”. The created file (energy.dat) contains the kinetic energies for each atom in the moment of simulation when the velocity file was created.