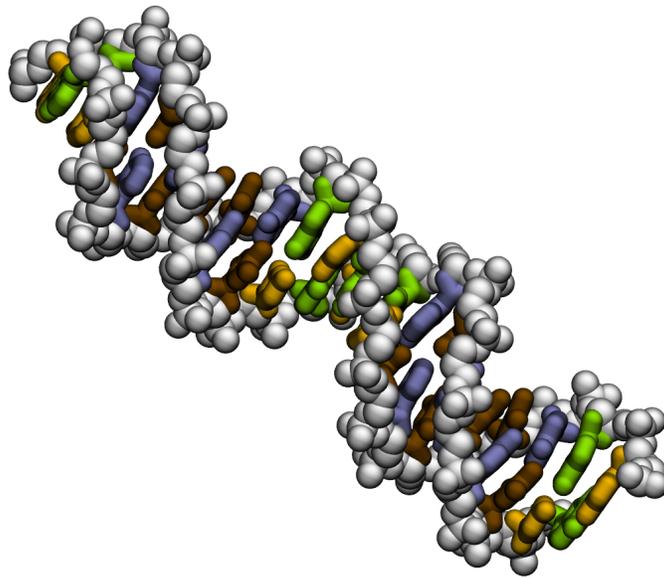**Aksimentiev Group**
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Visualizing MD Results: Mechanical Properties of dsDNA Mini Tutorial

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1 Introduction

In this tutorial, we will use results from the study, "Strain Softening in Stretched DNA" [1], to learn how to visualize data from molecular dynamics (MD) simulations with the Visual Molecular Dynamics (VMD) software [2]. In MD simulations, molecules are treated as collections of point particles which interact via a set of forces; Newton's equation ($\vec{F} = m\vec{a}$) is integrated to describe the temporal evolution of the system. In biophysics settings, it is most common to describe a biomolecule as a collection of atoms which are connected by harmonic bonds (two-body interactions), angles (three-body interactions) and dihedrals (four-body interactions) and interact at long ranges through the Coulomb and van der Waals potentials, Figure 1. The timestep of such a simulation is limited by the highest bond frequency, those due to heavy-atom–hydrogen-atom bonds; for all-atom MD simulations of biomolecules with explicit hydrogens, a time step of 1 femtosecond (fs; 10^{-15} s) is used. With the current generation of supercomputers, MD simulations can span up to microseconds in duration.

The study we focus on in this tutorial, "Strain Softening in Stretched DNA" [1], characterizes the microscopic mechanics of DNA stretching through all-atom MD simulations performed using the NAMD software [3]. This tutorial consists of three sections. In the first section, we will load the starting conformation of the DNA and investigate the structure of DNA and the design of the simulation system. Next, we will investigate an MD simulation of the system which applies a negative pressure to stretch the DNA. Finally, we will compare the results of two simulations where DNA is torsionally restrained or allowed to unwind as it is stretched.

$$U_{\text{total}} \quad = \quad \sum_{\text{bonds } i} k_i^{\text{bond}} \left(r_i - r_{0i}\right)^2 + \sum_{\text{angle } i} k_i^{\text{angle}} \left(\theta_i - \theta_{0i}\right)^2$$

$$+ \sum_{\text{dihedrals } i} k_i^{\text{dihed}} \left\{ \begin{array}{ll} \left[1 + \cos\left(n_i \phi_i - \gamma_i\right)\right] & n_i \neq 0 \\ \left(\phi_i - \gamma_i\right)^2 & n_i = 0 \end{array} \right.$$

$$+ \sum_i \sum_{j>i} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6\right] + \sum_i \sum_{j>i} \frac{q_i q_j}{4\pi\epsilon r_{ij}}$$

Figure 1: The MD potential, where $\vec{F} = -\vec{\nabla} U$.

2 Structure of DNA and the Simulation System

2.1 Load the Files

For this tutorial, we will use VMD through its TCL interface.

1. VMD comes with a convenient console plugin called TK Console. To open the tcl console, start a new VMD session and click on Extensions → Tk Console.

2. We must first move to the directory that we are working in. To do so, enter the following in the console:
   `cd Path_To_Tutorials/Stretching_dsDNA_Tutorial/`
   (All tcl code will be shown in this typeface.)

3. Next, let's load the DNA simulation system. `mol new trajectories/DNA_Stretching.psf`
   `mol addfile trajectories/DNA_Stretching.pdb`
   Now we have loaded a "PDB" file, or Protein Data Bank file, which contains the locations of the atoms, and a "PSF" file, or a Protein Structure File, which contains the atom connectivity (bonds, angles, dihedrals) as well as information necessary to use the system in MD simulations. While these file types have the word "protein" in their title, in practice, they are used for any sort of molecule.

4. When a system is loaded, VMD will use a very simple representation. Here we see the DNA (blue, cyan and red) with a box of water (red and white) surrounding it. We will use specific representations to inspect different aspects of this system. Open the Graphical Representations toolbox through Graphics → Representations.

2.2 The structure of DNA

Now we will inspect the DNA molecule.

1. First, let's look at an individual nucleotide. In the Selected Atoms form, change `all` to `resname ADE and resid 94`. In the Drawing Method drop-down menu, select Licorice, then click on the display window and type an equal sign (=) to recenter the view. Here we have an Adenine nucleotide. It is colored by `name`, meaning that carbons are in cyan, nitrogen in blue, oxygen in red, phosphorus in brown and hydrogen in white. What are the three components of a Nucleotide? What makes this nucleotide an Adenine nucleotide?

2. Now let's look at how this Adenosine nucleotide fits in the structure of the DNA. In the Graphical Representations toolbox, click Create Rep. Change the new representation's selection to `resname THY and resid 18`. Here we see the Adenine nucleotide's complementary Thymine nucleotide. These bases are hydrogen-bonded with one another. Which atoms form these bonds? To answer this question, make a new representation with selection `resname THY ADE and resid 18 94` and Drawing Method `HBonds`. The `HBonds` tool finds hydrogen bonds based on angle and distance criteria for hydrogen-bonding atoms. (Optional: To see why the third hydrogen doesn't bond, change the Coloring Method for both nucleotide representations to Charge.) How long are these bonds? We can use the visual interface to find this out. Click on the display with the mouse and press the "2" key. Now the cursor is a target. Click on one of the hydrogen-bonding atoms. The terminal should show information about the atoms. Now click on its hydrogen-bonding partner. There should now be a dashed line connecting the two atoms, with the distance between the centers-of-mass of the atoms beside it. (If it doesn't appear, click on both again.) You can zoom in to look at it closer. To delete the bonds you have drawn on the screen go to "Graphical Representations::Labels". You can select and delete the labels here. (Advanced users of VMD usually have a tcl script which does this mapped to a keystroke.)

3. Let's look at how these two bases fit into the whole strand of DNA. Delete the last two representations, and change the first representation to `segname ADNA BDNA and backbone`. Change the Drawing Method to VDW and change the Coloring Method to Color ID "8 white". Create a new representation with selection `segname ADNA BDNA and noh and not backbone` Change the Drawing Method to licorice, the Coloring Method to `name` and Bond Radius to `1.0`. Take a look at the DNA. The DNA seen here is in the canonical *B*-DNA structure. Where is the major groove? The minor groove? DNA nucleotides are negatively charged. Where is this charge? Why is a DNA molecule stable in solution?

2.3   The simulation system

The system we have loaded was used to investigate the microscopic mechanics of the DNA when it is stretched. In this section, we take a closer look at how the simulation system was designed.

1. Change the first representation (`segname ADNA BDNA and backbone`) Drawing Method to Licorice. There are two very long bonds from one side of the molecule to the other. In fact, the DNA *is* actually bonded from one side to the other! The MD simulations of this system had periodic boundaries, which made the DNA molecule effectively infinitely long. MD simulations are often performed on spatially periodic systems, as schemes to solve long-range electrostatics, such as the particle mesh Ewald (PME) method, require periodic boundaries. In the Graphical Representations toolbox, click on the `segname ADNA BDNA and backbone` selection, change its Drawing Method back to VDW. Under the Periodic tab, click on +z and -z to show periodic images along z. NAMD will use the shortest distance between two atoms as the bond length, so with periodic boundaries, these two bonds are actually the normal length. The advantage of making the DNA "effectively" infinite through the use of periodic boundaries is that it eliminates effects due to the ends of the DNA and permits simulations of effectively much larger systems using a relatively small number of atoms. What is a disadvantage to this method?

2. A realistic simulation of DNA cannot be performed in a vacuum. Let's look at the rest of the system. Create a new representation with selection `ions`, Coloring Method Name and Drawing Method VDW. Here we see a 1 M concentration of KCl, potassium in brown and chloride in cyan. Add a representation with selection `water`, Coloring Method Name and Drawing Method Licorice. Here we see a box of water surrounding the DNA molecule. Water is necessary for the DNA to be in a biologically relavant conformation, and ions dissolved in water shield the DNA from interacting electrostatically with its neighboring periodic images.

3. Next, we will look at the MD simulations performed with this systems. First, though, delete this current system. In the console, type
   `mol delete all`

3   Stretching dsDNA

Now we will look at MD simulations from the paper "Strain Softening in Stretched DNA" [1]. In these simulations, a negative pressure was applied along the z-

axis (the axis of the DNA) in increasing magnitude. Let's take a look at these simulations.

3.1 Loading the Simulation

1. When analyzing simulation data, it may be necessary to load and reload data, creating new graphical representations each time, which can become repetitive. Advanced users will often create tcl scripts which do the loading and rendering of the simulation systems for them. As an example of such a script, we have prepared a script to load the MD simulation trajectories and render them.

2. Run this script by typing
   `source load_mols_representations.tcl`
   in the console.

3. Optionally, you can look at this script, by typing:
   `less load_mols_representations.tcl`
   The `TK Console` will open the loading script in an editor (it won't actually use the program `less`). This script is written in `tcl`, a simple scripting language which both VMD and NAMD utilize to power their scripting interfaces. More information on `tcl` can be found on the website `http://www.tcl.tk` or by asking your T.A.

3.2 Viewing the Results

1. Take a look at the DNA. Adenosine is shown in blue, Thymidine in ochre, Guanosine in green and Cytidine in yellow. Now let's view the simulation. The motion of the DNA is smoothed here so that it won't appear to be too jerky. To animate the simulation, click on the play button (forward triangle/arrow) in the VMD main toolbox, or type
   `animate forward`
   in the console. To pause, click on the play button again, or type
   `animate pause`
   in the console. To view a specific frame, you can type
   `animate goto 5`
   which would take you to frame 5.

2. The DNA in this simulation is stretched past the elastic limit of *B*-DNA and deforms into the overstreched conformation, *S*-DNA. Earlier, we looked at the structure of *B*-DNA. What is the structure of *S*-DNA and how is it different from *B*-DNA? How do the bases behave when DNA is stretched? Which are the first bases to detach – what part of the DNA

is this? Why do the bases tend to stay together, even when the DNA is stretched from *B*-DNA to *S*-DNA?

3. Before we continue, let's take a look at the entire system. This DNA is stretched by applying a negative pressure in the z direction, but the x and y directions stay at 1 atm. What do you think happens to the water box around the DNA? In the Graphical Representation toolbox, turn on the `water` selection. Now play the simulation by either clicking on the play button (forward triangle/arrow) in the VMD main toolbox, or type:
   `animate forward`
   When you are done, hide the `water` selection again. Now, hide the entire molecule (click on the D next to the molecule name in the VMD main toolbox), but keep it loaded for the next section.

4   Stretching Nicked DNA

In the previous section, we looked at a simulation where DNA was stretched past the elastic limit. In that simulation, the DNA was torsionally constrained, meaning that it could not unwind from its helical structure as it was stretched. In this section, we compare the microscopic mechanics of DNA which is torsionally constrained with DNA which is allowed to unwind as it is stretched.

4.1   Loading and Viewing the Simulation

1. Similar to the last section, we have created a file which will load the trajectory and render it with various representations. To run this script, go to the TK Console and type:
   `source load_mols_representations_unwind.tcl`

2. Here see another strand of DNA, but it is colored differently. How does the sequence differ from the previous simulation? What else is different about this strand of DNA? Change the Drawing Method of the representation `segname ADNA BDNA and backbone` to Licorice. Why is there only one periodic bond? How will this affect the simulation once negative pressure is applied along the DNA strand?

3. View the simulation trajectory. As before, press the play button or type
   `animate forward`
   in the Tk Console.

4.2   Comparing the Simulations

1. Let's look further at the trajectory, and compare it to the previous simulation. Display the first simulation (click on the D next to the molecule name in the VMD main toolbox). Now the two strands of DNA are on top of each other. Fix the first simulation by clicking on the F next to the molecule name in the VMD main toolbox. Click on the main display and type t. Now the mouse will be in `translate` mode. Click the mouse on the main display to drag the unwinding simulation to the side. Try fixing and unfixing both molecules until you have them side-by-side in the main window, then click on the main window and type r. Now you can rotate both molecules at the same time.

2. Now animate both trajectories to compare them. Note that the timescales are different in these two trajectories; you can compare the individual states, but not the time it takes to reach them. How does the simulation in which the DNA strand unwinds differ from the simulation in which the strand is constrained? How do the final states of the DNA backbone compare? The bases? The simulations performed for this paper showed that it takes much less force to stretch the DNA if it is allowed to unwind. Why is this?

References

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