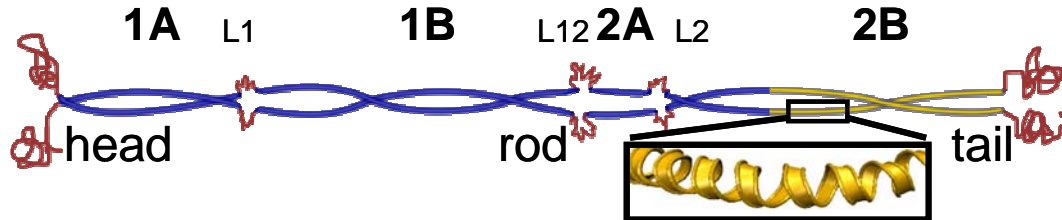## PROBLEM C – Deformation and unfolding of proteins

In this homework we use MD for analyzing and understanding the behaviour of single alpha-helices by performing tensile tests with different pulling velocities, using the CHAHRMM force field implemented in the code NAMD. We use the SMD technique to apply load to the molecule.

Together with beta sheets, alpha helical (AH) structures are the most abundant secondary structures found in proteins. These two patterns are particularly common because they result from hydrogen bonding between the N–H and C=O groups in the polypeptide backbone. An alpha helix is generated when a single polypeptide chain twists around on itself stabilized by hydrogen bonds (H-bond) made between every fourth residue, linking the O of peptide i to the N of peptide $i+4$ in the residue chain. Consequently, at each convolution, three H-bonds are found in parallel arrangement that stabilize the helical configuration.

Here we investigate a single alpha-helical segment of the 2B part of the vimentin intermediate filament (IF) dimer.

A schematic of the vimentin dimer structure is shown bellow. The rod-like structure is 310 residues long and consists of four coiled-coil alpha helices (1A, 1B, 2A, 2B) divided by linkers (L1, L12, L2).

IFs, in addition to microtubules (MTs) and microfilaments (MFs) are one of the three major components of the cytoskeleton in eukaryotic cells. The cytoskeleton plays a critical role in determining the shape and the mechanical properties of the cell, and is vital for numerous additional functions such as cell motility or protein synthesis.

A great diversity of mechanical properties enables the vimentin IFs to satisfy their specific mechanical role in cells, such as to guarantee their structural integrity or their shape. It has been hypothesized that IFs are critical to provide strength to the cell under large deformation, and to absorb large amounts of energy upon a certain load by unfolding. This represents a means to reinforce the cell in extreme deformations so that cells can withstand dramatic loads and deformations.

Note: To create animations of your simulations, you may install the program "videomach" (available free of charge for trial period, http://www.gromada.com/videomach.html). This program can interact directly with

VMD – check the option "MovieMaker" in VMD. Detailed instructions about how to use the NAMD interface are included in the NAMD tutorials posted below.

C.1 Stretching simulation

1. Perform a protein stretching simulation of the vimentin 2A segment (all input files provided on the website), using the SMD method. Use the following parameters:
   ```
   dcdfreq    = 150
   SMDvel.    = 0.002
   Run        = 50000
   Bins       = 100
   ```
   (takes probably approximately 40-50 mins)
2. Plot the force versus extension.
3. Plot stress versus strain. **Hint:** Use Excel or Matlab to process your data and convert displacements to strains and forces to stresses. Discuss challenges of defining the stress for this molecular geometry. You may measure the geometry of the molecule using VMD ("Mouse→Query" function for "Bonds").
4. Repeat this calculation for ½ and ¼ the initially chosen pulling rate, and discuss how the results change.
5. Explain the different regimes of deformation you see in the force-extension plot based on the atomistic mechanisms you can identify in VMD.
   Which of the chemical bonds break first, and why?
6. For the slowest rate, plot the modulus as a function of strain.
7. Considering the DREIDING potential (paper posted on the MIT server, see materials for Lecture 7), estimate the strength of a single hydrogen bond (consider the proper potential function given in the paper).
   How does this result relate to the force-displacement measured above?
   Estimate the theoretical strength of a alpha helix.
8. Estimate Young's modulus for the various deformation rates you have tried. Try to find a way of calculating the modulus at vanishing pulling rates by extrapolation.

C.2 Bending simulation

1. Using modified smd.pdb and fix.pdb files, set up a simulation for bending the molecule by a three-point bending test:

2. Compare the force-displacement curves for bending with that of stretching. Particular points to consider are force levels, strains, shape of curve.
3. Describe mechanisms that occur at large strains.

4. Estimate the bending stiffness based on an analogy between beam bending and the force-displacement data obtained from MD.
5. Estimate the persistence length of the molecule. How would the persistence length change with an increasing number of molecules arranged in a radial packing?
   What scaling behavior do you expect with respect to the number of molecules?
   Hint: Consider changes in area moment of inertia due to molecular assembly.