Possible Homework Exercises in BIOENG 455

See the User Guide for how to execute commands in a DPD simulations.

1) Simulation of an entropic spring - apply a stretching force to both ends of a single polymer in a DPD simulation and measure the end-end length as a function of the force and extract the spring constant for the *entropic spring* (i.e., at low stretching force).

Create a single polymer in a box of water with distinct beads on the two ends. A possible molecular architecture is: " (BH (18 B) BT) ". Apply a constant stretching force (F) to the ends (i.e., oppositely directed force on beads BH and BT). Let the system equilibrate for some time (how do you know when it is stable?), then measure the end-to-end length (L). Start with a small force, and repeat the simulations with increasing the force until the polymer is strongly stretched.

How do you choose a sensible range for F?

Plot the mean end-to-end length against applied force. Repeat each case 3 times with different random number seeds to get a mean and standard deviation for <L> for a given F. Include error bars of the statistical errors in your plot.

Quantities in the simulation are dimensionless: what are L and F quantified in terms of? What would you need to know before you can convert your results to physical units?

Now make some of the beads in the middle of the polymer sticky (so that the polymer tends to stick to itself) and see how this changes the F(L) curve. You will need to vary the number of sticky beads to find an interesting regime (too few and nothing will happen, too many and the polymer will just collapse into a tight ball). *Interesting* means that the system shows some unusual, non-linear behaviour. (For a biological example, see Graeter et al. JACS 13:11578 2008 on moodle in Lecture 4.)

Output: Plot of L(F) against F for the normal polymer over a range that shows the complete response of the polymer to the force from entropic spring to highly stretched. Measure the slopes of the curve for the regime where F ~ 0 and for large F where it should be linear. Second plot showing the effects of the sticky domain on the L(F) curve from the first part. How many regions where the curve has a different shape (or slope) do you observe?

2) Tethered FRET experiment

In a FRET experiment, two parts of a molecule (or two molecules) have half a fluorescent group attached so that when the molecules are close enough, the fluorescent group emits light: it is a proximity sensor.

Here we use a linear molecule with two *sticky* endcaps (E), and we measure the average separation of the endcaps as their stickiness (i.e., conservative parameter aEE) and backbone stiffness is varied. The minimum value for the conservative interaction parameter is the same as the water-water parameter (25). In this case the endcaps are attracted as strongly to each other as they are to water, so the polymer should behave as a random walk. Reducing the conservative parameter makes the endcaps more likely to stick to each other, and so reduces their mean separation (NB all DPD forces are repulsive, so a smaller conservative parameter means a less repulsive force). Conversely, increasing the stiffness of the backbone tends to extend the molecule and should push the endcaps farther apart.

Note Adjust the number fractions to ensure that you only have one FRET molecule in the box.

The command SavePolymerBeadRDF should be used as described in the User Guide.

Output: A plot of the radial distribution function (RDF) of the endcaps for several values of aEE including 25 as the null case. Integrate the RDF to get the mean separation as a mean and standard deviation. How does this compare to the mean polymer length in the dmpcas file?

Plot the RDF for several values of the backbone stiffness (in the BondPair lines). Note that 5 kBT is the usual value. Try 0 kBT (random walk) and higher values (stiffer molecules).

3) Polymer in good/bad solvent - measure a polymer's mean end-end length as a function of the interaction between the polymer and the solvent and determine if there is a phase transition as the polymer is increasingly repelled from the solvent.

- Simulate a single polymer of beads "B" with length N = 20 in bulk solvent using aBW=10 (highly solvated)
- Increase the aBW parameter from 10 - 50 systematically and measure Lee for each case. NB Lee = $\sqrt{<R_{ee}^2>}$ here, where <…> indicates a time average throughout a simulation.
- Plot the polymer's end-end length as a time series from the dmpchs file and check for equilibration. How long do you need to simulate to ensure it is equilibrated? Then measure the mean end-end length from the dmpcas file and plot it as a function of the solvent quality.
- Describe the resulting curve.
- Does the polymer change abruptly from expanded to collapsed as the solvent quality is changed?

Output: Graphs of the above quantities.

4) Integrate a Langevin equation and compare to simulations: Consider a molecular motor dragging a vesicle along a microtubule. Model it as a Langevin equation with a constant force, use the RNG from the lectures, and integrate it to find the distance moved as a function of time, and so get the speed and see how speed varies with the applied force.

Then create a rigid nanoparticle in a simulation box of water and apply a constant force to it. Measure its position as a function of the applied force. From its position as a function of time, calculate its speed. Compare with the Langevin solution.
Hint. What size and/or mass of the nanoparticle should you use?

Output: The Langevin equation you created; a graph of the force against the speed of motor from the Langevin equation and the same graph from the simulation.

5) Molecular force spectroscopy I - calculate the work done in pulling a lipid out of a membrane at different rates. Create a membrane from one lipid type that also contains a single lipid with a different type (so it can be monitored individually) but with the same interactions as the other lipids. Then apply a force perpendicular to the plane of the membrane to the labelled lipid and simulate it long enough to pull the lipid out of the membrane. Repeat for several values of the force.

Note. Because the DPD integrator conserves momentum, pulling on the lipid will also pull the whole membrane and it will start to translate in the direction of the force. You will need to prevent this. How?

You will need to create an initial membrane state from a given number of lipids plus the one labelled one. Let it equilibrate (how will you know when this is?) and measure the end-to-end length of the labelled lipid, and its time-averaged value, and compare to the unlabelled lipids.

Note. For efficiency, each member of a group can simulate the membrane with different pulling force values and you can share the results.

Once you have several values of the force that pull the lipid out of the membrane, repeat the runs with a distance moved and work done decorator applied to the force. Measure the work done during the pulling experiment, and plot it as a function of the force. What force minimises the work done in pulling out the lipid?

Output: Example graph showing the position of the lipid as a function of time for one case. Graph of the work done against the pulling force for a range of forces.

6) **Molecular force spectroscopy II -** calculate the work done in pulling an intrinsically disordered protein out of a phase separated droplet of proteins. You will need the restart state of an equilibrated droplet saved from a previous run (ask me). Select the (unique) labelled polymer by one end and apply a force to pull it out of the droplet. Repeat for several values of the force.

Observe the motion of the polymer as you pull. Apply the force in two directions, e.g., the direction in which the polymer has to move least and the opposite direction where it has to move through the whole droplet.

Note. Because the DPD integrator conserves momentum, pulling on the polymer will also pull the whole droplet and it will start to translate in the direction of the force. If this is a big effect, you will need to prevent this. How?

Once you have several values of the force that pulls the polymer out of the droplet, repeat the runs with a distance moved and work done decorator applied to the force (see User Guide). Measure the work done during the pulling experiment, and plot it as a function of the force. What force minimises the work done in pulling out the polymer?

Output: Example graph showing the position of the polymer as a function of time for the two cases of shortest and longest distance moved. Graphs of the work done against the pulling force for a range of forces for the two cases.

7) **Construct a thermodynamic model of a phase separated polymer droplet in solvent -** Construct the free energy F = U - TS for a model of a spherical droplet immersed in a solvent composed of hydrophilic polymers with several distributed sticky patches that make them bind weakly. You must include at least the following effects/parameters:

- polymer volume fraction (or concentration)
- the variation in the binding energy of all the polymers with the droplet radius, i.e., how the number of accessible bound sites on the poltymers varies with droplet size and polymer conformation
- the variation in a polymer's conformational entropy with droplet radius

Output: Provide written justifications for the terms in your model; state whether it predicts a preferred size for the droplets or whether they grow without limit (cp. model of oil droplet growth from Lecture 3.)

Optional: Calculate the pressure of the droplet from your free energy using the relation

$p = \phi^2 \, d/d\phi \, (f / \phi)$

where $f = F / V$ is the free energy per unit volume, ϕ is the volume fraction of the polymers, and p is the pressure.

8) Compare the diffusion constant of a hard sphere with that of a fluctuating polymer with the same radius of gyration in a DPD simulation and next the same hydrodynamic radius. See Lecture 2 for the definitions of the radius of gyration of a polymer, and the hydrodynamic radius of a hard sphere and a polymer. Create a hard sphere in a DPD simulation with a (small) radius R and measure its equilibrium diffusion constant from the dmpchs file (how do you know when it's equilibrated?). In an independent simulation, create a single polymer out of N beads (of type B, say), set the self interaction of the beads (a_{BB}) to a high value so it is self-avoiding, and their interaction with the water beads equal to the water-water interaction ($a_{BB} > a_{BW} = a_{WW}$). Choose the length of the polymer (N) so that its radius of gyration is equal to that of the hard sphere. You will probably have to try several values of N until you find the best fit.

Useful relations: $L_{ee} = a \, N^{0.6}$ and $L_{ee}/R_g = 2.5$ for a self-avoiding polymer, where a is the monomer diameter; $R_g^2 = 3/5 \, R_h^2$ for a hard sphere.

You can take the hydrodynamic radius of the sphere to be equal to its actual radius. If you need to assign a value to a, the bead diameter in the polymer, then simulate a single polymer of N beads until it is equilibrated and use the value of the mean B-B bond length that is in the dmpcas file. The end-to-end length is also in the dmpcas file. You can use the command *SavePolymerBeadRDF* to measure the radius of gyration of the polymer in the simulation (see DPD User Guide).

Output: A graph comparing the diffusion constants (i.e., Mean square displacement / 6*time) of the hard sphere and polymer as a function of time for a) same radius of gyration, and b) same hydrodynamic radius. The values of the radius of gyration of the sphere and the polymer (which should be the same, of course, except for experimental error …) The hydrodynamic radius of both: a) calculated theoretically from the results in lecture 2, and b) measured from the apparent diffusion constant in the simulations using $D = k_B T/6\pi\eta a$, where a is the hydrodynamic radius, η is the viscosity of water, and T is room temperature.

And a comment explaining why you believe your results (or not as the case may be.)

9) The plasma membrane of cells contains a multitude of lipids, some of which have charged headgroups. It would destroy the membrane's stability if too many lipids were highly charged. Here, the charge state on a fraction of the lipids in a membrane is varied to see at what strength the membrane is destroyed.

a) Single lipid type: create a single-component lipid bilayer whose headgroup can be charged by command. Simulate the membrane with no charge to ensure it is stable. Then execute the command "**ChargeBeadByType time beadType charge range**" for the headgroup bead PO4, and vary the **charge** and **range** to find the minimum charge/range at which the membrane disintegrates (in a reasonable time). What types of lipid aggregate replace the membrane?

Note. The command takes the numeric type of the bead not its name. This is just the 0-indexed number of the bead type in the input file.

b) Two-component membrane: create a lipid bilayer containing a majority fraction of one lipid and a minority fraction of a similar type of lipid but with a new bead type for the headgroup. Assign one of the values of the charge/range obtained from a) that you know destroys the membrane to the minority lipid headgroups, then run a series of simulations in which you increase the fraction of the minority phase lipid starting at 0 and find the minimum fraction at which the membrane is no longer stable.

Output: a) Draw up a table with several (~ 5) values of the charge state/range with increasing charge that gives a stable membrane at low charge (how do you know when it's stable?) and a ruptured one at high charge. Repeat some values keeping the charge constant and vary the range. Label the resulting aggregate types for each value in the table (e.g., membrane, micelle, rodlike micelle, ?) Provide snapshots for the intact membrane and each type of aggregate formed from the ruptured membrane.

b) For a pair of values of charge/range chosen from part a), draw up a table showing the fractions of charged lipid you simulated, the membrane state (intact/ruptured) and the type of aggregates formed. Provide snapshots for the ruptured states. Is there a qualitative difference between the ruptured membrane state when all lipids are charged as in a) and only a fraction of them as in b)?

10) What are the dense and dilute phase concentrations of a phase separated droplet of a model IDP? You can use the provided input files, or choose your own molecular structure for the IDP (How? see Lecture 3 or ask me), and a concentration and sticky site affinity at which they self-assemble into a dense aggregate. The provided files give you a pre-assembled dense phase for analysis. Measure the radius of gyration for the system you choose. Then increase / decrease the affinity and repeat the measurements for several affinities to see how the dense phase size varies with affinity.

Output. Measure the radius of gyration of the dense phase droplet either during the run (with the following commands), or offline with the script in llps.zip.

Commands required
Create a command target that selects one bead type (eg endcaps) in the IDP molecules and invoke the radius of gyration command on that target (see User Guide for these commands). Make sure you simulate long enough that the droplet has formed, and only begin calculating the Rg once the droplet is stable, and average over enough samples to get good statistics.

Command SelectBeadTypeInSphere 100 sphere E 0.5 0.5 0.5 0.0. 10.0

Command RgOfBeadTarget 100 sphere rg1 100 200

Hint. Practise with a spherical micelle (dmpci.m1) to ensure the Rg command works as expected.

And make the average over much longer than the 100 steps shown here for test purposes.

Alternatively, you can run an offline analysis script that does a more careful estimation of the density using a Monte Carlo algorithm. Ask me if you want to do this, and see the next problem.

11) How does the structure of the dense phase of a model disordered protein vary with parameters of the molecules, e.g., molecular weight, number of "sticky" sites, their affinity, etc.

Select a molecular structure for the IDP (How? see Lecture 3 or ask me), and a low concentration, and fairly strong sticky site affinity (how?) at which they self-assemble into a dense aggregate. The provided input file has a box of 48**3, but you can use a smaller one to speed up the runs (not too small, why not?) The provided input files give you a restart state where the dense phase has already formed, so you only need to run the restart run and measure observables. If you use this restart state, the only parameters you can vary are the sticky site affinity, the conservative parameters of the IDPs, backbone stifness etc. If you want to explore changing the concentration, or number and location of sticky sites, you have to create your own input file. Talk to me if you want to do this.

NB. You need to create a virtual environment in python in order to run the analysis script stored in the zipfile llps.zip to get the observables of the dense phase.

Output. Graphs of the observables you measured against simulation time (to check equilibration), and against the parameters you varied, e.g., IDP concentration, sticky site affinity, separation of sticky sites, etc. Conclusions about the impact of the parameters on the dense phase properties.