EFFICIENT SIMULATION OF PROTEIN SURFACE ADSORPTION USING
DISSIPATIVE PARTICLE DYNAMICS WITH SPECULAR CHAIN REFLECTION

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by

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ABSTRACT

Understanding interactions within complex biological systems is essential to study protein function and transport, and to enable design of biocompatible devices. Studying such complex systems through experiment encounters many challenges, including availability and resolution of experimental data, and control over system parameters to be studied. Computer models are frequently employed to explore such systems. Many biological systems of interest, such as protein surface adsorption, cannot be effectively simulated at the atomistic level. In order to simulate these large systems for the durations required for the desired behavior to evolve, atomistic structure is often represented approximately by “coarse-grain” techniques. Dissipative particle dynamics is one simulation technique which makes large size- and time-scales accessible. Current DPD simulations typically represent two surfaces for adsorption, even when the second surface merely serves to bound the opposite one end of the simulation box. To eliminate the computational demand of such a redundant system, here we use a specular reflecting boundary condition as an alternative. This boundary inverts bead Z-velocity at the box ceiling to bounce them back into the simulation. We identify requirements for a successful reflecting boundary. This boundary is validated by comparison with results of a reference system with a second surface and no reflective boundary. Simulation results including surface adsorption, fluid bead density and temperature are used to confirm the equivalence of the results with both boundary methods. Simulation data are evaluated to assess the adsorption behavior of model protein chains of varying geometry onto simulated surfaces of varying hydrophilicity. It is found that such efficient systems with precise parameter control can prove ideal to evaluate a wide range of surface adsorption behavior which may otherwise be impractical to study in detail.
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Chapter 1
Introduction

A focus of current materials science research focuses on the design of materials for biological environments. The complex environment and interactions between engineered and natural molecules requires an enormous amount of study. Key to this understanding is the characterization of large proteins at surfaces both man-made and biological. While experimental study has made great inroads into this challenging area, there are significant constraints on current experimental methods where molecular simulations could contribute. Using molecular simulations to study these systems allows for overlapping and parallel study, wherein insights gained from experiment and simulation can inform one another. However, computer simulation methods have their own set of constraints when exploring these complex environments. Appropriate computer models are continuously being developed, and when possible they are expected to match empirical data, in order to study the behavior of these systems in greater detail. An overriding challenge to all simulation methods is computational efficiency for problems of a given level of detail. Current simulation methods designed to explore protein surface adsorption in the mesoscale are presented here. While some problems are more efficiently addressed with a specific technique, it is essential for a problem to be defined precisely and efficiently to maximize the potential of any technique. Of interest of this thesis is the common simulation technique use when studying protein surface adsorption, i.e. the use of two separate adsorbing surfaces to define the simulation volume. The potential for increasing the efficiency of these simulations by replacing one of these bounding surfaces with an analytical boundary is discussed, along with
requirements for such simulations to maintain the strong correlation with experimental results currently achieved with computer simulations.

1.1 Experimental study of biological systems

Many modern applications of soft materials science focus on the study of biological environments. A greater understanding of the interaction of biological agents at surfaces (both natural and man-made) promises to allow advancement of numerous areas. For example, understanding of cellular responses within the body is advanced by new methods in observation of protein surface adsorption \(^1,2\). Improved biocompatibility of engineered products can reduce negative effects of objects temporarily working in biological environments, such as reducing thrombogenic response of blood proteins on catheters used in vascular access \(^3,4,5\). Tailored response of adsorption characteristics is essential for new biosensors used in vivo \(^6,7,8,9,10,11\). Permanently implanting man-made materials in vitro, including replacement joints, tissue scaffold materials, and neural electrode prosthetic devices, also requires biocompatible surfaces both for efficient assimilation into the body and reduced immunological response \(^12,13\). While these design requirements have been long-known, advances in these complex fields remains challenging.

Common to all these design problems are basic properties of the biological environments in which they are used. Biological response is directed by native molecules, particularly proteins found in the bloodstream, recognizing the surface of interest and responding in the desired manner. This recognition relies on characteristics of the surface which are not trivial to monitor or control. To aid the design of materials used in these complicated environments, greater understanding of protein adsorption is needed. Key parameters related to adsorption in biological environments include: the adsorption rate of proteins onto surfaces of interest, the configuration
of the surface itself and how that controls the conformation and layers of adsorbed molecules, conformational changes in adsorbed molecules as they seek reduced surface energy, and the occurrence and frequency of any desorption events.

1.1.1. Characterizing protein adsorption

Characterization of the process of protein adsorption provides insight into the sought parameters controlling protein adsorption. Two characteristics of the adsorption process are essential: Adsorption isotherms and adsorption kinetics. Isotherms measure the amount of protein adsorbed to a surface over time at a constant temperature. Often the amount of adsorbed material (measured as a thickness) is studied as a function of bulk concentration. Adsorption isotherms can effectively provide an understanding of surface adsorption behavior for such fields as: design of biocompatible materials, biosensors, cultivation of animal cells, drug delivery systems, membrane separation, and bio-products manufacturing processes. A basic representation of the adsorption process is described by the Langmuir adsorption isotherm equation:

$$\frac{\Gamma}{\Gamma_m} = \frac{KC}{1+KC}$$

Where $\Gamma$ is the amount of adsorbed protein per unit surface area, $\Gamma_m$ is the maximum adsorption value for a complete monolayer, $C$ is the bulk protein concentration, and $K$ is an equilibrium adsorption/desorption ratio constant. This equation relies on several assumptions: that all adsorption sites are equivalent and allow only one molecule to adsorb, that the surface is homogenous and adsorbed molecules do not interact, that there are no phase transitions, and that adsorption only occurs at the surface, forming only a monolayer. While these constraints do not describe many cases of protein adsorption, the Langmuir adsorption isotherm continues to
describe the adsorption behavior of many proteins well. A modified equation, the Languir-
Freundlich isotherm, is used to describe more complex behaviors observed \(^{16}\):

\[
\frac{\Gamma}{\Gamma_m} = \frac{K C^n}{1 + K C^n}
\]

As in Equation 1, \(\Gamma\) represents the instantaneous adsorbed amount per unit surface area, and \(\Gamma_m\) is
the maximum (saturated) adsorbed amount. \(C\) is the bulk protein concentration, and \(K\) is the
adsorption/desorption ratio constant. This equation adds the empirical parameter \(n\) to describe the
heterogeneity of the protein. The heterogeneity constant is constrained by: \(0 \leq n \leq 1\), with \(n = 1\)
describing a completely homogenous material, for which the formula reduces to Langmuir
isotherm. Unlike \(K\), which depends on both the protein and surface, \(n\) depends only on the
protein, and allows for greater specificity in fitting adsorption isotherms. Examples of isotherms
described by this formula are shown in Figure 1-1.

![Figure 1-1. Example Langmuir-Freundlich isotherms of BSA adsorption on sulfonated polystyrene particles. Reproduced from \(^{16}\).]
Adsorption kinetics tracks the protein adsorption process through time, monitoring: transport to the surface, adsorption, any conformation changes, and possible desorption and transport away from the surface. With respect to understanding protein surface adsorption, many kinetics models have been developed to describe the possible behaviors seen when observing protein surface adsorption. These newer models attempt to describe not only initial adsorption, but any conformation changes and desorption events which may occur. A graphical representation of some of these models is shown in Figure 1-2.

Figure 1-2. Kinetic models applied to protein surface adsorption. Model name and description in left column, pictogram of model behavior in center column, and adsorption isotherm behavior in right column. Reproduced from.
1.1.2. Experimental techniques to observe protein surface adsorption

There are many challenges to overcome to gain insight into the adsorption kinetics and adsorption isotherms of proteins in biological environments. For example, characterization of biological surfaces can be difficult to achieve. *In situ* and real-time characterization may not be possible, limiting study of dynamic systems. Indirect measures of system behavior must be carefully interpreted to assure inferred results accurately portray the unseen phenomena. Additionally, most behaviors studied evolve over microseconds or less, creating a very short window to observe experimentally. There are many experimental methods used to gain understanding of adsorption behavior, each of which provides access to different data about the surfaces being studied.

Imagery of proteins adsorbed on surfaces provides essential insight into biological systems. Several imaging methods are commonly applied to biological systems. Scanning electron microscopy (SEM) may be used to image cellular adhesion to surfaces and structures. Figure 1-3 shows an SEM image of a cell adsorbed to a biomaterial fiber structure. This method requires treatment of the specimen to be scanned by drying the sample and treating the surface to be electrically conductive, and thus cannot be used *in situ* or to monitor dynamic behaviors.
Figure 1-3. Example SEM image. Human dermal fibroblast adhered to PLGA/sP (EO-statPO) fibers. Reproduced from 19.

Another observation technique used to characterize a biosurface is atomic force microscopy, particularly operating in dynamic “tapping mode” (TM-AFM) 21, 22. Figure 1-4 shows an example TM-AFM image, which plots the height of nanotubes adsorbed on Ti plates. A mechanical probe contacts the surface, and the coordinates of the probe at contact are recorded as the probe is moved in an x-y plane. Tapping mode oscillates the probe vertically as it is passed over the surface, preventing damage to the specimen by mechanical contact. AFM does not require treatment of the specimen, but cannot be used to observe transient behavior.
While most characterization techniques have inherent limitations when working with biological systems, two techniques complement one another to provide a unique understanding of macromolecule structure and function. Individually, the Nuclear Magnetic Response (NMR) technique and the Small Angle X-Ray Scattering (SAXS) technique provide unique but constrained insight into biological systems of interest. Using the two techniques in combination allow greater insight and error-correction over either method alone. NMR is a high-resolution technique to gain insight at the residue level. By studying a sample within a high-strength magnetic field which may be nonlinearly varied, the magnetic response of individual nuclei may
be observed. By comparing the resonance frequency of these nuclei to a reference resonance frequency, the position of individual nuclei may be discerned. Studying the “chemical shift” of nuclei in residues over time provides an understanding of the shape changes occurring within these molecules \(^{24}\). An example of the data and related protein unfolding interpretation is shown in Figure 1-5. Thus, this technique excels at identifying local conformational dynamics that control molecular recognition, but is unable to accurately assess changes in global orientations \(^{25}\). While NMR has made strides in identifying transient folding behavior of proteins in real-time, this technique still requires time in the order of seconds to measure conformational changes \(^{26}\).

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Figure 1-5. Example plot of chemical shift NMR data. Secondary \(^{13}\)C\(^\alpha\) chemical shift (\(\delta_{\text{observed}} - \delta_{\text{random coil}}\)) vs. residue number indicates unfolding as solution pH/temp is increased. Reproduced from \(^{24}\).
The SAXS method directs a beam of X-rays through a sample. The X-rays which interact with the sample are scattered and detected. This scattering pattern (once the background scattering pattern is subtracted) can be used to infer structure on the nano- to micro-scale size range, as shown in the example Figure 1-6. As opposed to NMR, the SAXS method provides global insight into the ensembles of conformations which are all represented by proteins in solution, but cannot be used to accurately identify conformation at the residue level to predict molecule interactions. SAXS can characterize intrinsically disordered proteins with their large number of conformations, but this method does require minutes (for lower-resolution line-collimation techniques) or hours (for the more detailed point-collimated method).

Figure 1-6. Example plot of SAXS data. Scattering of two molecular weights of polyacrylic acid (PAA) adsorbed on zirconia surface, as well as scattering of background ZrO2 surface without polymer (to be subtracted from PAA results). Reproduced from 29.
There are other characterization methods which allow dynamic study of samples. Isothermal microcalorimetry (IMC) measures heat changes corresponding to chemical reactions or physical changes in real time without interfering with the processes occurring\textsuperscript{30}. Specimens may include solids and liquids, allowing for biological activity during observation. The gathered data tracks any endothermic or exothermic reactions by monitoring the heat of the specimen within the calorimeter. Figure 1-7 shows a typical thermogram output from IMC observation. This indirect measure cannot be used to determine which processes are occurring, and therefore cannot distinguish between different processes which may occur concurrently, such as protein surface adsorption versus conformation changes of adsorbed proteins. While IMC is very sensitive to small heat changes, this sensitivity requires significant time for the specimen to slowly reach any non-ambient temperature of operation. Thus, while biological processes can be observed at physiological temperatures, the required time (on the order of 30-40 minutes) prevents observation of dynamics occurring on a short time scale.

Figure 1-7. Example isothermal microcalorimetry (IMC) thermogram. Adsorption of lysozyme on iron-silica surface at various concentrations. Reproduced from \textsuperscript{30}.
An additional observation technique to observe transient is dynamic tensiometry. This application of goniometry (measurement of contact angles) results in a very simple test method which provides a depth of understanding of behaviors occurring within solution. Typically, an adsorbing surface is slowly immersed into a solution, which may contain an adsorbent solute. This action is performed periodically into one or more solutions to investigate behavior over time. Measurement of the wetting force required to move the plate allows for determination of the directly-related advancing contact angle ($\theta_a$) and receding contact angle ($\theta_r$)\(^{31}\). The difference in advancing and receding contact angles defines a contact angle hysteresis which correlates to surface homogeneity, and in the presence of an adsorbent, indicates the amount of matter adsorbed to the surface\(^ {32}\). An example of this data is shown in Figure 1-8. The relationship between water at the surface and protein adsorption is captured well within these measurements, which is essential to understand the biological systems where the adsorption of interest occurs\(^ {33}\). Additionally, by utilizing a secondary solution without adsorbent, it is possible to observe any desorption behavior in reversible processes. While these data provide critical insight into adsorption behavior of homogenous systems, this characterization method remains unable to discern between multiple interactions which may occur simultaneously, such as initial protein adsorption to the surface versus conformation changes of adsorbed proteins. This method also does not allow study of behaviors happening in timescales under one second.
Another characterization method capable of measuring surface adsorption in real-time is ellipsometry. This is an optical measure which observed the change in polarity of light source after reflection on a surface. This change in polarity correlates to the thickness of a thin film formed on the surface. Ellipsometry may be favorable for biological systems of adsorbent proteins in solution, as this characterization technique may be a more direct measure of molecule-to-surface interactions than other methods based on rheology\textsuperscript{34}. An example of imaging ellipsometry applied to biosensors is shown in Figure 1-9.
In the case of protein adsorption, one can multiply the thickness measured by the protein density to obtain concentration (Γ). Tracking this data over time provides an adsorption isotherm used to characterize the rate and amount of adsorption a particular surface will adsorb. Figure 1-10 shows an adsorption isotherm obtained by this technique. Ellipsometry can be used to monitor evolving behavior in real-time, but again on the order of seconds. And while the data may be directly analyzed for non-homogeneity in the thickness of adsorbent, information regarding changes in conformation upon adsorption is difficult to discern.
Figure 1-10. Example of adsorption isotherms obtained by ellipsometry. Adsorption of Immunoglobulin G (IgG) onto silicon plates for varying pH systems. Reproduced from 21.

While optical measures report interactions between light and an adsorbed protein layer to understand adsorbed thickness, spectroscopic techniques extend the available information to be gained by photons. The Fourier-transformed attenuated total reflection from infrared adsorption (ATR-FTIR) may be used to understand both the amount of adsorbed proteins, and the conformation of those proteins. A broadband light source passes through an interferometer to amass photon adsorption data from proteins on a surface at many wavelengths. A Fourier transform is applied to generate a spectrogram as a function of waveform. The adsorption of photons by specific regions of proteins can be monitored over time. As adsorbed proteins change conformation, the exposed regions change, modifying the absorbance of incident light waves. By comparison to the initial absorbance (or that of bulk solution proteins), changes in conformation may be deduced. Figure 1-11 compares absorbance data from ATR-FTIR at multiple times to infer conformation change resulting from denaturation. This observation technique can be used...
in situ and in real time to assess general changes in adsorption. However, this technique does not allow fine resolution with respect to time, and may not capture early adsorption or rapid changes.

Figure 1-11. Example of adsorption data from ATR-FTIR studies of bovine insulin a) adsorbed to electrostatically negative surface, b) adsorbed to electrostatically positive surface, and c) in bulk solution. Changes between data from time zero (●) and data after 2 hours (○) show shifts in adsorption, corresponding to conformation changes. Reproduced from 36.
1.2 Computer modeling of complex systems

1.2.1 Capability of computer simulations

The experimental techniques described earlier provide a wide range of data about the adsorption of proteins to biological surfaces. However, each technique has limitations. Even combining the use of multiple techniques may not completely illuminate some adsorption behavior, such as the rapid initial adsorption phase when the concentration on the surface is low. Computer simulations are frequently relied upon to augment understanding of adsorption in biological environments. There are many benefits to using computer simulations to work around challenges inherent to experimental studies. Computer simulations allow for direct control of the parameters to be studied. An accurate simulation can be interrogated for data in many different ways, adding the advantage that it can be revisited long after the simulation was run to explore results that were not initially targeted.

The essential component of any computer simulation is that it accurately represents the intended environment to the desired level of detail. Verifying this can be challenging, as one typically should validate the results given by the simulation against data obtained via another method, such as experiment or ab initio data. Computer simulations, especially models which rely on significant approximation, are best used where the model is correlated with specific experimental results. Figures 1-12 and 1-13 compare computer simulations and experimental data for different types of experiment: Structure factors from WAXS experiment, and gas adsorption isotherms from BET experiment. The close correlation shows the model used for simulation represents the targeted physical phenomena properly.
Figure 1-12. Structure factors for a) polycarbonate, b) polyetherimide and c) PIM-1. Predictive simulation data (red lines) closely matches WAXS experimental data (black lines) for these varied materials. Reproduced from.[38]
Once the model is confirmed to accurately represent the interactions intended, the parameters of the computer simulation can be varied to achieve new understanding. These new results can be reliable, provided the assumptions are understood, and allow for fast iteration of many permutations of the model parameters with more flexibility than typically seen via experimental techniques alone. The benefit of this analytical parameterization can be seen when attempting to analyze the effect of individual parameters. When using physical experimental methods, it is not always possible to vary one parameter without varying another. For example, in a physical study of protein adsorption on superhydrophilic to superhydrophobic surfaces, experimental results weren’t consistent with the expected trend of increasing adsorption as hydrophobicity increased. It was found that the example chemistry used to create a superhydrophobic surface resulted in a surface morphology which negatively affected the
intended adsorption: adsorption was chemically preferred, but sterically hindered. This experimental result is shown in Figure 1-14. When studying adsorption as a function of surface hydrophilicity using computer simulation, it is possible to isolate the hydrophilicity parameter first, to study the sole effect of that parameter. Once the behavior is understood, the experimenter is then able to continue the line of research to focus on specific surface chemistries which represent the intended behavior.

![Adsorption Isotherms](image)

Figure 1-14. Adsorption isotherms for BSA on superhydrophilic (solid line), hydrophilic (dashed line), hydrophobic (dotted line) and superhydrophobic (dashed-dotted line). Lowest adsorption on super-hydrophobic surface, inconsistent with trend otherwise observed, is a function of surface morphology used to create super-hydrophobic surface. Reproduced from 39.

Just as many experimental methods exist, there are many different ways to simulate systems, depending on the desired output. Because simulations are only a model of the real world, the simulation method used must be chosen to accurately represent the physics being explored. Factors to account for include: level of detail required to observe the interactions of interest, and the size- and timescale required to simulate the targeted phenomena within the simulated system. Figure 1-15 diagrams a range of computer simulation methods available, based on the size- and time-scale of the interactions to be studied.
Figure 1-15. Size- and time-scale ranges requiring different computer simulation methods. Simulation technique is chosen to match the behavior observable in the range considered, from continuum-level descriptions (macro-scale, at top) to representing individual electrons (electron structure/quantum mechanics, at bottom). Reproduced from 40.
Simulating protein surface adsorption is typically performed using mesoscale techniques for large proteins, such as Immunoglobulin G (IgG) with a size of 150 kDa. Simulations which represent each of the approximately 20,000 atoms in a single IgG molecule, and include the approximately 300,000 atoms to explicitly define a solvent, are very challenging problems for even advanced hardware available today. Protein surface adsorption simulations require a number of proteins, plus the surface definition and representation of the solvent. To represent each atom in a surface adsorption simulation for these large systems would require simulating millions of atoms. Each simulation frame, called a “time-step,” represents the movement and interactions for each particle in the system at a given time. To accurately represent these interactions, a time-step must be sufficiently small to prevent gross movements from overshooting interactions. The adsorption process (including transport to the surface, adsorption, conformational changes and/or desorption) may require tens of microseconds to evolve. This results in simulations which require millions of time-steps for complex systems, such as protein surface adsorption occurring over a time of 5µs or more\textsuperscript{41}. To simulate such mesoscale systems on an atomistic level is currently infeasible due to the sheer volume of calculations involved. Therefore, simulations at the mesoscale level use alternative techniques which are “coarse-grained” to approximately represent structures using fewer elements of larger size. This increase in size-scale also allows for larger time increments between calculations, again increasing efficiency. Several coarse-grain techniques are available to facilitate simulation of these types of systems, as described in the current work.

1.3 Coarse grain techniques for computer simulations

Studies of materials using computer simulations require the same definition of intended material parameters as physical experiments would. Material choices such as desired proteins, solvents, and interacting surfaces must be made. Study parameters such as physical volume,
temperature, pressure, and time are likewise determined. Desired experimental output must be explicitly defined, to ensure the proper data is available at the end of the experiment. However, the step of identifying the process required to gather the necessary data can involve more input when using computer simulations.

When simulating molecular systems, a determination of the desired interactions and resulting data may dictate which simulation methods may be applied to the problem. Computer simulation methods exist to represent sub-atomic components, individual atoms, or systems containing many molecules of thousands of atoms all interacting. However, despite continued advances in computing power, it is not feasible to study the smallest of particles interacting over the largest distances or timeframes. A specific simulation method must be chosen which yields the desired data, typically at the expense of other data which is necessarily unavailable when using that simulation method. This choice constraint becomes apparent when simulating systems representing adsorption of large molecules in solution onto an even larger surface. To simulate physical dimensions in the hundreds of angstroms over the time duration of microseconds required to observe surface adsorption of protein chains, a simulation method must be chosen which reduces the number of particles simulated to a number significantly lower than the number of atoms in the model. Simulation methods which use assumption techniques to reduce the number of particles in the system are referred to as “coarse-grain” simulations.

There are many different coarse-grain techniques which can be applied to the mesoscale problem of protein surface adsorption. All coarse-grain techniques model atomic structure in an approximate way, representing the volume of multiple atoms with a single element, often called a “bead.” One technique which has been steadily increasing application is dissipative particle dynamics (DPD). This technique, as proposed by Hoogerbrugge and Koelman and later refined for polymer simulation by Groot and Warren, represents all bead interaction using three forces (conservative, dissipative and random) to simply govern the system in a manner which maintains
Figure 1-16 illustrates the volume represented by a DPD bead versus the underlying atomic structure.

Figure 1-16. Visual comparison of coarse-grain “beads” used to approximate underlying atomic structure for efficient simulations of large systems for long times. Reproduced from 44.

The DPD technique, which is used for the simulations in this work, is described in detail in Chapter 2. The DPD simulation technique has been applied to a wide range of mesoscale problems. Figure 1-17 highlights the rapid growth of publications using the DPD technique in general, as well as the specific application of DPD to surface adsorption problems.
A wide range of alternate approaches to coarse-graining are also represented in the literature. One of the earliest techniques, called “united atom” (UA), simplifies an all-atom molecular dynamics simulation by implicitly representing hydrogen atoms as part of the explicitly-defined carbon atom to which it is bonded. This method modifies the force-field used to describe the interaction of electrons for atoms in a molecular dynamics simulation. For systems of hydrocarbons, removing the degrees of freedom for each hydrogen atom offers a reasonable step toward reduced model size, but this approach cannot be scaled further and does not allow access to fundamentally more challenging problems than all-atom molecular dynamics simulations can achieve.
An early attempt to significantly reduce the computing load from an atomistic model is the Flory-type lattice model, proposed by Larson in 1992\textsuperscript{47}. This model consists of beads representing structures, such as modeling surfactants as a hydrophilic head bead attached to hydrophobic tail beads, or individual residues in a protein chain. This technique allows much greater reduction in model degrees-of-freedom to solve than the united atom technique, as each bead can represent many atoms. The beads are arranged on a lattice, with exactly one bead per grid location. Monte Carlo simulations are performed, applying random motions to chain beads and resolving the chain structure, as shown in Figure 1-18. This technique is useful to study protein chain folding to identify lowest energy configurations. However, Monte Carlo simulations do not represent time continuously, and therefore cannot provide insight into the full dynamics of protein surface adsorption.

Brownian dynamics is another simulation technique which allows for significant scaling based on the degree of coarse-graining applied. In this technique, inertia is neglected, so that the
state of the polymer is completely specified by the positions of the beads\textsuperscript{49}. Hydrodynamic interactions between beads are introduced in a pairwise-additive approximation, which connects the mean velocity of one bead to the force on an interacting bead\textsuperscript{50}. An example of a Brownian dynamics simulation of amphiphilic molecules is shown in Figure 1-19. This technique can be applied for the determination of equilibrium states, but is not ideally suited to problems emphasizing specific transient behavior or detailed hydrodynamic interactions.

![Figure 1-19. Brownian dynamics simulation of amphiphilic molecules at various temperatures. At higher temperature, (a) small micelles are formed. Decreasing temperature (b) leads to one or two large threadlike micelles. Lower temperature (c) leads to a single vesicle with bilayer structure. Reproduced from\textsuperscript{51}.](image)

Another coarse-grain simulation technique which explicitly represents all particles in the system is Dynamic mean-field Density Functional Theory (DDFT). DDFT is a generalized, time-dependent Ginzburg-Landau theory technique\textsuperscript{52}. Like DPD, polymer chains in DDFT are represented by beads bonded by harmonic springs. These beads from all chains are allowed to penetrate each other completely. The intermolecular interactions are described by mean-field energetic interaction between bead pairs\textsuperscript{53}. This method is an efficient tool for the study of polymer melts which obey Rouse dynamics, as illustrated in Figure 1-20. While this simulation technique can accurately represent the bead chemical potential and thermodynamic driving force for diffusive dynamics, it does so at the cost of increased solution complexity when compared
with DPD. Therefore, the DPD technique of coarse-grain simulations is used in this work. This method provides one of the simplest descriptions which represents the necessary behavior for protein surface adsorption.

Figure 1-20. DDFT simulation of copolymer melt $A_6B_{10}$. Isosurface representation for level $\rho_a = 0.7$ at time $\tau = 500$. Reproduced from $^{57}$.

1.4.1 Simulating systems using periodic boundary conditions

Simulations require a specific definition of the volume of material to be studied, the “box”, chosen to be a minimal volume in which the desired behavior can be studied. The
simulation box must be defined to include a representative sample volume. Initial DPD simulations focused on interactions within solution. These simulations consider a representative sample within the bulk solution, and impose “periodic boundary conditions” on the sample volume. This assumes that neighboring volumes behave the same as the sample volume, such that particles exiting the sample volume on one face can realistically be replaced by an identical particle entering the volume from the opposite side (which theoretically would have just exited the neighboring volume). This allows for representation of an unbounded solution with a finite number of particles, that mimics bulk behavior. See Figure 1-21 for a representation of periodic boundaries.

Figure 1-21. Schematic representation of periodic boundary. The center volume is simulated, and any particle exiting this volume is replaced by an identical particle at the opposite face of the volume, representing a particle entering from an identical neighboring volume.
Simulations making use of periodic boundary conditions are a simple means of studying bulk systems, with application in many cutting-edge areas of modern research, including phase diagrams of supramolecular diblock copolymers and design of complex particles from self-assembled monolayers. However, to study all types of problems, it is essential to have a robust means of simulating a volume within a bounded box.

1.4.2 Simulating systems including a physical bounding surface

Simulations representing adsorbing surfaces encounter a fundamental challenge in defining the system. The simulation “box” volume can use periodic boundary conditions along the unbounded axes, but must explicitly define the boundary used along the axes bounded by surfaces (because particles cannot exit this boundary and re-enter the system at the opposite boundary in a periodic fashion). There are three requirements for an effective solid wall. First, the wall must be impenetrable to all beads within the system. Second, the wall must impose the correct velocity resulting from the bead-boundary interaction, and not allow the bead to slip along the boundary. Third, the wall must not alter the macroscopic system properties, such as density or temperature.

An initial attempt to include a solid wall within DPD simulations included an existing constraint method, the Lees-Edward boundary condition. This simulated two additional boxes in every bounded direction, with the additional boxes moving to shear the primary box. This shear force resulted in particles leaving the primary box and being re-inserted at a nearby position, with modified velocity and acceleration to redirect it into the primary box. Figure 1-22 illustrates this method. However, this method does not meet the no-slip requirement, and results in density fluctuations at the boundary.
Many simulation groups have addressed solid boundaries by “freezing” particles at the box boundary. The particles with fixed position can interact with fluid particles, redirecting them back into the box. In early simulations with this constraint, Kong, et al. increased the bead density at the surface while maintaining the conservative force of repulsion. These simulations therefore had low fluid density at the surface. While this method does affect the macroscopic properties, it has still seen continued use. Many other attempts at modifying the frozen bead wall boundary condition have been examined. Revenga, et al. and Colmenares, et al. explored a high-density frozen bead surface without including conservative force interactions with the fluid. Jones et al. used a frozen bead surface with density equal to the fluid density, but a high conservative force repelling fluid beads from the wall. Fedosov, et al. and Duong-Hong, et al. use a frozen wall with a back-bouncing condition and modified velocity and position of bouncing beads. Later studies continued to find issues with these boundary
conditions and the maintenance of macroscopic properties near the defined boundary. Willemsen, et al. proposed the definition of additional beads outside the boundary to maintain a continuous velocity profile. Pivkin and Karniadakis worked to separately compute the correct repulsion force of wall beads by first performing simulations of test cases of varying densities, and then applied those determined forces to the actual simulation of interest. Altenhoff, et al. also employed specific forcing using stochastic prediction of boundary forces. All these methods achieve improved maintenance of an impenetrable no-slip boundary condition without affecting macroscopic system properties, at the cost of including both frozen DPD beads and an analytical bead reflection mechanism at each non-periodic boundary. Visser, et al. proposed an alternative method, simulating multiple parallel systems simultaneously, using the image of one to be the reflected boundary of the other. This method, however, doesn’t allow for the inclusion of a surface at any boundary, as each wall must be symmetric.

1.4.3 Using an analytical boundary instead of a bounding surface

The above simulations consider a box with frozen beads forming a boundary on two opposite sides, and periodic boundary conditions on the remaining four sides. This addresses the issue of non-periodic boundary along one axis, by using the same boundary method on the two opposite sides of a symmetric box. This method is employed in DPD simulations of surface adsorption, and has been shown to correlate with various methods of experimental observation. An example result from a simulation using two bounding surfaces is shown in Figure 1-23. However, analyzing surface adsorption on both sides of a symmetric box requires twice the computations. A reliable method of simulating only half of this symmetric box would reduce computational effort significantly.
Figure 1-23. DPD simulation exploring surfactant in solvent of super-critical CO₂. Surfactant amphiphilic molecules are represented in solution of increasing scCO₂ concentration (a-d). Surface adsorption causes density of molecules to fluctuate at each surface, but findings are symmetric, indicating a single-surface simulation of smaller size may be appropriate. Reproduced from ⁷⁵.

It is possible for a DPD simulation of surface adsorption to use periodic boundaries in all three directions. This relies on placing the adsorbing surface in a plane at the box middle, rather than any face of the box. This method is not prevalent in the literature, and is most often seen in
simulations of transport through membranes, where the simulated material passes through the mid-plane surface. An example of such a simulation is shown in Figure 1-24. While this system could logically be applied to straightforward surface adsorption, it then represents two sides of a surface. The surface must be defined (typically through increased thickness) such that beads interacting with one surface face do not inappropriately interact with beads adsorbed on the other surface face through the surface thickness. Therefore, this method bears similar computational requirements to simulations using two separate adsorbing surfaces.

Figure 1-24. DPD simulation of a nanoparticle passing through a membrane. Placement of surface in box middle allows use of periodic boundaries on all box faces. Reproduced from 63.

Three logical possibilities have been explored to define an analytical surface boundary. One method employs specular reflection, conserving momentum parallel to the boundary, while reversing the normal component. A bounce back method, in which both parallel and normal components of velocity are reversed, has also been explored. A third method uses Maxwellian
reflections where exiting particles are re-introduced into the system according to a Maxwellian distribution of velocities centered at the velocity of the wall. These three analytical boundary concepts are illustrated in Figure 1-25.

![Analytical Boundaries](image)

Figure 1-25. Analytical boundaries considered for solid wall boundaries. Specular reflection reverses velocity normal to boundary, but does not change velocity parallel to boundary. Bounce-back reflection reverses both normal and parallel components of velocity. Maxwellian reflection defines reflected velocity based on velocity probability density (\(f_v\)) from Maxwell-Boltzmann distribution.

A critical component of evaluating an analytical reflecting boundary relies on the applicability of the boundary method for DPD simulations of connected chains of DPD beads representing large molecules. One such attempt was made by Patterson, et al., where a symmetric boundary constraint is defined on the ceiling of the simulation box by using a specular reflecting boundary \(^{41}\). These simulations studied linear DPD-bead chains of short and long lengths, to approximate real short and long proteins. Examination of data from those simulations illustrates some unusual behavior in the region of the box ceiling where the specular reflecting condition is defined. While the specular reflecting ceiling appropriately redirects the short length chains into...
the simulation box, the long chains appear to show an affinity for the box ceiling. This behavior indicates that a straightforward definition of a specular reflecting boundary may yield inappropriate results for some large molecules represented in DPD. Any discussion of this issue in literature of DPD simulations of large molecules appears absent. Therefore, specific examination of analytical reflecting boundaries to be used with large-chain DPD simulations is necessarily undertaken in the present work.

The majority of DPD simulations reported in the literature do not emphasize the boundary method used in bounded systems which cannot employ periodic boundary conditions in all directions. A common procedure is to simulate identical adsorbing surfaces on opposite sides of the simulation box. Often, studies are described to use periodic boundary conditions in the x- and y-directions, but simply do not state if multiple bounding surfaces are used, or if a reflecting ceiling is used opposite the adsorbing surface. In most cases, the simulation results are presented with respect to one adsorbing surface only. Even in simulations using two adsorbing surfaces, result images are shown in the context of a single surface, as in Figure 1-26. Studies which represent two adsorbing surfaces may state that the simulation is comparable to two separate simulations, both of which contribute to the overall statistics of adsorption kinetics. However, in some cases (such as systems with low concentration of adsorbate particles), this assumption does not bear out, as discussed in Chapter 3. Most studies referenced here simulate small molecules using several DPD beads. The literature does not detail current DPD simulations which represent large molecules with many DPD beads interacting with a reflecting boundary. It is possible that such a reflecting boundary is not appropriate for such molecules, as is described in Chapter 2.
Figure 1-26. Illustration of DPD simulation of nanoparticle surface adsorption. The simulation represented here uses a box of 24 x 24 x 24 with adsorbing surfaces represented at top and bottom. Note that the authors choose to only show half the box height, and only one surface. The top half of the box and the second surface, while simulated, are not relevant to the surface adsorption result shown in this image. Reproduced from 82.

It is clear that a system definition in computer simulations requires awareness of both the actual experimental environment being simulated, and the realities specific to solving computer simulations. It becomes essential to define systems to avoid computational inefficiency. Even with the latest computing hardware available today, mesoscale simulations using coarse-grain techniques such as DPD are still constrained by computation time. To make simulation of the most complex systems possible, DPD simulations must be designed as efficiently as possible. To this end, one of the main goals of this thesis work is to develop and validate an efficient simulation boundary condition to be used to study proteins’ adsorption (of different sizes, and flexibility) on an adsorbing surface covering only one face of the simulation box. The work
described here resolves the definition of this boundary condition, so that simulations of complex systems of proteins or nanoparticles adsorbing on surfaces can correctly be simulated without the typical but unnecessary computational expense of a redundant second adsorbing surface. Results of these simulations are compared to results of systems employing a second adsorbing surface, to validate the behavior of the boundary condition applied. This methodology can then be used to improve the efficiency of any DPD simulation seeking to make use of symmetry in system definition. Improving simulation efficiency by means such as elimination of redundancy is essential to simulate large and complex systems for long times to unlock the full potential of DPD.
Chapter 2

Methodology

2.1 A brief summary of the DPD methodology

Computer simulations using DPD are well-suited to represent protein surface adsorption. To understand the application, the contents and methodology of a typical DPD simulation are described below. To illustrate the work, an intermediate step of a DPD simulation of protein surface adsorption is shown in Figure 2-1. In this example, protein molecules and a large adsorbing surface are represented by spheres referred to as “beads.” The DPD method provides a means to model this behavior using efficient formulae to describe interactions between beads, with simple variable parameters to represent different properties of molecules being simulated. This method, while straightforward, allows for complex simulations featuring large molecules for sufficiently long times to represent protein surface adsorption.
Figure 2-1. Visualization of a representative DPD simulation. A single simulation box is repeated in the X- and Y-directions to illustrate a system described by periodic boundary conditions. Image shows an intermediate time of a simulation of long, thick protein chains (red) adsorbing onto a surface (green). Not shown here are the beads explicitly representing water in this system.

The DPD method as developed by Groot and Warren is summarized as follows 43. Beads interact as particles following Newton’s equations of motion:

\[
\frac{dr_i}{dt} = v_i, \quad \frac{dv_i}{dt} = f_i
\]  

(3)

The forces acting on each particle are defined by three terms: conservative (repulsion), dissipative (drag), and a random force. Figure 2-2 illustrates the summation of these forces on an individual bead.

\[
f_i = \sum_{j \neq i} (F^c_{ij} + F^D_{ij} + F^R_{ij})
\]  

(4)
Figure 2-2. Individual forces acting on a typical DPD bead. Blue circle represents soft sphere defined by cutoff radius. Repulsive force (purple arrow) resulting from close proximity to another soft sphere shown in a). Dissipative force (green arrow) resulting from drag on bead from motion in previous step shown in b). Random force (red arrow) from Brownian motion shown in c). Resultant sum of all forces (black arrow) shown in d).

All three forces are summed for each particle over a defined cutoff radius, \( r_c \), which serves as the normalized length scale used for the simulation. The conservative force is thus defined over two regions:

\[
P_{ij}^c = \begin{cases} a_{ij}(1-r_{ij})F_{ij} & \text{when } (r_{ij} < 1) \\ 0 & \text{when } (r_{ij} \geq 1) \end{cases}
\]

(5)

The term \( a_{ij} \) represents the maximum repulsion between particles \( i \) and \( j \). Interaction between DPD beads is described by a repulsive force of magnitude \( a_{ij} \) when two bead centers are coincident. The force gradually decreases to zero when two bead centers are separated by the cutoff radius, \( r_c \), or greater distance. Note that this definition of the conservative force indicates that DPD beads are considered “soft spheres”, with the cutoff radius defining a sphere about the bead center. Beads are allowed to overlap and experience a gradually increasing repulsion. This interaction can be compared to the typical Lennard-Jones Potential which describes atomic interactions:

\[
u_{LJ} = 4\varepsilon \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6
\]

(6)
Where $\varepsilon$ is the depth of the energy well corresponding to bond strength between atoms, and $\sigma$ is the separation distance corresponding to zero potential (balanced repulsion and attraction).

Figure 2-3 compares these two potentials as they describe repulsion of beads/atoms.

![Figure 2-3](image)

Figure 2-3. Comparison of Lennard-Jones potential to DPD conservative force. The separation distance corresponding to zero interaction is defined as $\sigma$ in the LJ potential, and $r_c$ for DPD. $\varepsilon$ is the depth of the energy well describing atomic bonding with the LJ potential. Reproduced from 83.

Two additional forces are required for DPD, as introduced earlier. The dissipative force is defined as:

$$F^D_{ij} = -\gamma \omega^D (r_{ij}) (\hat{r}_{ij} \cdot \mathbf{v}_{ij}) \hat{r}_{ij}$$  \hspace{1cm} (7)

The friction between two beads is represented as $\gamma$. A distance-dependent weight function, $\omega^D$, is defined to go to zero at $r > r_c$. 

"42"
The random force is defined as:
\[
F_{ij}^R = \sigma \omega^R(r_{ij}) \bar{\theta}_{ij} \bar{F}_{ij}
\]  
(8)

Again, a distance-dependent weight function, \( \omega^R \), is defined to go to zero at \( r > r_c \). The noise amplitude between particles \( i \) and \( j \) is represented by \( \sigma \). Additionally, \( \theta_{ij}(t) \) is a randomly fluctuating variable with Gaussian distribution. Español and Warren determined that the weight functions, \( \omega^D \) and \( \omega^R \), and the friction and noise coefficients for each bead pair, \( \gamma \) and \( \sigma \), must be related by the following equations for the system to sample the canonical probability distribution and to obey the fluctuation-dissipation theorem:

\[
\sigma_{ij}^2 = 2\gamma_{ij} kT
\]  
(9)

\[
\omega^D(r) = \left[ \omega^R(r) \right]^2
\]  
(10)

where \( k \) is the Boltzmann constant and \( T \) is the temperature. Typical choices for the related weight functions are as described by Groot and Warren:

\[
\omega^D(r) = \begin{cases} 
[\omega^R(r)]^2 = (1 - r/r_c)^2 & \text{when } (r < r_c) \\
0 & \text{when } (r \geq r_c)
\end{cases}
\]  
(11)

All molecules are represented as soft-sphere beads, with a repulsive potential as validated by Groot and Warren:

\[
U_{ij}^{GG,r} = \begin{cases} 
\frac{a_{ij}}{2} r_c (1 - r/r_c)^2 & \text{when } (r < r_c) \\
0 & \text{when } (r \geq r_c)
\end{cases}
\]  
(12)

As discussed in Chapter 1, the DPD technique has seen rapid increase in application. This is due in part to modifications to the technique to account for behavior necessary in some systems. Electrostatic interactions have been incorporated into DPD through the definition of a grid upon which the electrostatic field is locally solved and applied to DPD beads. Scaling of DPD models through the entire mesoscale range to capture linear- and non-linear properties of polymer melts is achievable through a tuned coarse-graining which can prevent beads from unrealistically crossing chain backbone bonds. It is important to note that these modifications...
maintain the fundamental method of DPD, such that findings from the current work are applicable to specialized variants of the DPD technique.

2.1.1 Bead types and interaction parameters

This work simulates three bead types: Protein, Surface and Water beads. The interaction between these beads is governed by the $a_{ij}$ parameter in Equation 3, the DPD conservative force calculation. This parameter depends on each bead type in any interacting pair. No explicit attraction parameter values are allowed for in this DPD method; each bead experiences varying degrees of repulsion to other beads. An “effective attraction” results from two beads displaying less repulsion than neighboring bead types. Thus a minimum repulsion of zero represents the maximum “attraction” in these simulations. Table 2-1 details the interaction parameters used in these simulations. These values are based on the Groot and Warren method, wherein the interaction between water beads, which is considered neutral, is defined as 25 \(^{43}\).

Table 2-1. Bead interaction parameters

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Surface</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>$a_{pp} = 25$</td>
<td>$a_{ps} = 15$</td>
<td>$a_{pw} = 25$</td>
</tr>
<tr>
<td>Surface</td>
<td>$a_{ps} = 15$</td>
<td>$a_{ss} = 0$</td>
<td>$a_{sw} = 25, 50, 225$</td>
</tr>
<tr>
<td>Water</td>
<td>$a_{pw} = 25$</td>
<td>$a_{sw} = 25, 50, 225$</td>
<td>$a_{ww} = 25$</td>
</tr>
</tbody>
</table>

In every simulation, the water-water, protein-protein, and water-protein interaction is considered neutral ($a_{ww} = a_{pw} = a_{pp} = 25$). Therefore, there is no preference for protein beads to aggregate in solution, and no chain aggregation is observed in these simulations. The protein-surface interaction is slightly ‘attractive’ (less repulsive) than these neutral interactions, driving protein adsorption. The surface-surface interaction is defined as zero, eliminating large repulsive forces that would arise from the close arrangement of surface beads required to prevent other
beads from passing through. The interaction between surface and water beads is a variable used to identify different systems of differing hydrophilicity. The increased repulsion of the $a_{sw}$ parameter over the $a_{ww}$ parameter ($a_{sw} - a_{ww}$) is referred to as $\Delta a$. A system using $\Delta a = 0$ represents a hydrophilic surface with no additional repulsion for water-surface interaction than there is within the bulk water. A system using $\Delta a = 25$ is mildly hydrophobic, as the water beads are more repulsed by the surface than they are within the solution. Using $\Delta a = 200$ represents a very hydrophobic system where water is strongly repelled from the surface. In all cases, proteins are repulsed by the surface less than water beads; however, systems with large $\Delta a$ values will strongly prefer protein adsorption.

2.1.2 Mapping DPD beads to real dimensions

It is important to note that DPD simulations do not represent a specific size and time scale; the simulation itself only relies on dimensionless units. The simulations can be mapped to represent real systems such that the mapped size and time may be represented by this meso-scale approach. In these simulations, the unit of length is one $r_c$. The unit of energy used is $kT$, and the mass of any DPD particle is one unit of mass. This work uses $\sigma_{ij} = 3$, and individual time steps of $\Delta t = 0.05$. To facilitate comparisons to real-world systems, the dimensionless DPD system is mapped to real physical length dimensions. In this work, each water bead is mapped to represent three water molecules. Because one water molecule is known to have a volume of $\sim 30$ Å$^3$, each bead in these simulations represents $\sim 90$ Å$^3$. Using the water bead density of $\rho = 3$ beads/$r_c^3$, we find one unit volume, $r_c^3 = 3 \cdot 90$ Å$^3$. Thus, our unit of length corresponds to $r_c = 6.5$ Å.

In order to map our simulations to a true time, the diffusion of water is mapped onto the water beads in our system. Following the method of Groot and Rabone $^{87}$, the diffusion constant of a DPD bead is $D_{\text{bead}} = 0.1707 \, r_c^2 / \tau$, where $\tau$ is the time scale. The diffusion of our DPD beads
is matched to an experimentally-determined value for the diffusion constant of water at ambient conditions: \( D_w = 2.43 \times 10^{-5} \text{ cm}^2/\text{s} \). For a coarse-graining of three water molecules per DPD bead, the diffusion constant of a DPD bead is assumed to be 1/3 that of water, \( D_{\text{bead}} = \frac{1}{3} D_w \). This defines the relation \( 0.1707 \frac{r_c^2}{\tau} = \frac{1}{3} \cdot (2.43 \times 10^{-5} \text{ cm}^2/\text{s}) \), and we can determine the time scale of our system, \( \tau \), equal to 88 ps. Figure 2-4 illustrates the mapping of water molecules to DPD beads, and the fluid bead density defined for these simulations.

![Figure 2-4. Mapping of water molecules to DPD water beads. Volume of 3 water molecules, represented in a). Representation of 3 water molecules using 1 DPD bead of same volume, shown in b). Bead radius defined as 1 \( r_c \). Fluid bead density, \( \rho = 3 \) DPD water beads per unit volume shown in c).](image)

### 2.2 Representing surfaces in DPD

Additional DPD parameters and setup are required to represent an adsorbing surface. As discussed in Chapter 1, the surface must function as a barrier to beads within the system. Typically, this is achieved by simulating multiple layers of DPD beads in a staggered lattice, such that beads in one lattice are centered at the midpoint between beads in the next lattice. This
staggering creates an effective barrier to beads within the system. In this work, each adsorbing surface is represented by two layers of 10,000 beads (100x100, spaced 0.5\(r_c\) apart). These layers are staggered by 0.25 \(r_c\) in both the X- and Y-directions, to fill the surface volume and prevent penetration by water or protein beads. In addition to typical DPD forces, an anchoring force must be applied to each bead defining the surface, to maintain the overall connection of the surface, while allowing reasonable fluctuations when interacting with beads in the system. Here, each surface bead is tethered to its initial position by a stiff harmonic spring, represented by the potential in Equation 13:

\[ u_{ij}^{CG, t} = \frac{K_t}{2} r_{i0}^2 \]  

where \(r_{i0}\) is the distance of bead \(i\) from its initial position. The stiffness of these harmonic springs used is \(K_t = 100\). Figure 2-5 illustrates the tethering springs and bead locations of the model surface.

Figure 2-5. Beads and springs to represent an adsorbing surface. Each bead is tethered to its initial position by a spring, as represented by triangles in a). Surface represented by two planar layers stacked and offset, such that bead centers in one layer are directly above/below interstices of adjacent layer, as shown in a zoomed partial view b).
2.3 Representing model protein chains in DPD simulation

2.3.1 Additional forces in DPD to represent bonded chains

To represent molecules of larger volume than that of a single bead, beads making up a large molecule must be connected by an additional linking force, referred to here as a ‘bead bond’. Conceptually this is similar to a chemical bond, except that it connects bead centers, which may represent a large number of individual atoms. To create these bonds required to model structure, the protein beads are linked together with harmonic springs to create semi-flexible linear chains. The equilibrium separating distance between bead centers (the ‘bond length’) is 0.7$r_c$. The potential applied by these springs as beads move out of equilibrium is defined by:

$$u^{CG,s}_{ij} = \frac{K_s}{2} (r_{ij} - r_0)^2$$  \hspace{1cm} (14)

where the value of the bond stiffness, $K_s$, is 100. This force is applied to each bead in a protein chain, in addition to any other previously-defined DPD forces acting on that bead.

All model proteins considered in this work are “elongated” with a nominal linear shape. Thus the potential bond-bending is represented, with equilibrium at linear alignment ($\theta_0=180^\circ$), by:

$$u^{CG,b}_{ij} = \frac{K_b}{2} (\theta - \theta_0)^2$$  \hspace{1cm} (15)

where the value of the bending stiffness springs, $K_b$, is 20. Figure 2-6 illustrates the two forces specific to protein chains in these simulations, as well as a full model chain.
Figure 2-6. Beads and spring forces to represent a model protein. Spring representing connection between beads illustrated in a). Spring representing bending of protein chain illustrated in b). Example of a complete long, thick protein chain shown in c).

2.3.2 Model systems considered in this work

Several model systems are considered in this work, as shown below in Figure 2-7. The largest simulated is referred to as “long, thick” (LT). This model consists of 12 layers of 16 beads (arranged in the section plane 4x4). A single LT molecule is made of 192 beads. Each bead is bonded to up to 6 other beads, at the distance of 0.7rc away: up to 4 adjacent beads within the section, and any bead either directly above or below. No bonds are defined along diagonals, where the bead separation is larger than 0.7rc.

A small system, referred to as “short, narrow” (SN) is made of 6 layers of 5 beads (arranged in the section plane as an “X”). A single SN molecule is made of 30 beads. Beads within the section are bonded to the center bead, and each bead is bonded to one bead above or below.

A third model is referred to as “long, narrow” (LN). These are made of 12 layers of 5 beads, using the total height of the LT model, but the cross-section of the SN. Behavior of this
third geometry may be used to gain insight into any differences between the dynamics of the LT and SN systems.

The three models are chosen to perform a systematic study in three key factors. First, the number of beads in each model varies, resulting in different masses. Second, the arrangement of beads per layer varies, creating a range of molecule lengths and accessible surface areas. Lastly, the number of bonds per bead and per layer is different between the thick and thin models. This difference is expected to result in significantly increased stiffness for the thick models. The actual molecule stiffness, as represented by the radius of gyration ($R_g$) can be measured from simulations and compared to this data to confirm the desired increased stiffness of the highly-bonded thick model. The combination of these geometric parameters will allow examination of the effects of mass, surface area, and stiffness on the adsorption kinetics throughout these simulations. These properties are compared for the various model proteins below in Table 2.2.
Table 2-2. Measurements of molecules in each model

<table>
<thead>
<tr>
<th>Molecule length</th>
<th>Beads per layer</th>
<th>Beads per molecule</th>
<th>Surface Area (r_c^2)</th>
<th>‘Bonds’ per bead</th>
<th>‘Bonds’ per layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Side</td>
<td>End</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long, Thick (LT)</td>
<td>12</td>
<td>16</td>
<td>192</td>
<td>16.17</td>
<td>4.41</td>
</tr>
<tr>
<td>Short, narrow (SN)</td>
<td>6</td>
<td>5</td>
<td>30</td>
<td>2.45</td>
<td>0.49</td>
</tr>
<tr>
<td>Long, narrow (LN)</td>
<td>12</td>
<td>5</td>
<td>60</td>
<td>5.39</td>
<td>0.49</td>
</tr>
</tbody>
</table>

2.3.3 Relating model chains to specific molecules of interest

While the primary focus of these simulations is to evaluate multiple ceiling reflection methods of a “Design system” (described below) with respect to the performance of a “Reference system”, it is essential to keep in mind the underlying intent of any molecular simulation: predicting the behavior of actual molecules. The DPD methodology used here may represent typical molecules such as proteins \(^{89, 90}\), diblock copolymers \(^{91, 92}\), or amphiphilic molecules \(^{93, 94}\) in an aqueous solution, where the dimensionless length scale, \(r_c\), maps to 6.5 Å, as discussed above. The same simulations may also be used, for example, to represent polymers in an acetone solution. Because the unit length, \(r_c\), depends on the mapping used, in the case of considering acetone as the solvent, \(r_c\) increases to 11.1 Å, due to the larger solvent molecule size. Figure 2-8 shows a sample comparison between a model protein simulated in this study and an example real protein. Note that the model protein geometry may be constructed to represent any specific protein of interest.
2.4 The simulation box

The proposed simulation setup, referred to as the Design system, is a simulation box of dimensions $50r_c \times 50r_c \times 50r_c$. This box contains an adsorbing surface at the bottom face (-Z), and uses a ceiling reflection method at the top face (+Z). All the side faces use typical periodic boundary conditions ($\pm X$, $\pm Y$). This box contains 10 identical model proteins (described later), initially oriented vertically at a height of $25r_c$ away from the adsorbing surface. The remainder of the box is filled with water beads at a density of $\rho = 3$, as described earlier. This Design system box is shown in Figure 2-9.
Figure 2-9. Design system, including a reflecting ceiling boundary condition. Ten model proteins (red) are within the box, which is then filled with water beads (blue). The box bottom is bounded by an adsorbing surface (green). The box top is bounded by an analytical boundary which reflects beads back into the box. The four vertical box sides use periodic boundary conditions to replace any bead exiting a side with an identical bead entering from the opposite side.

A second simulation system is also simulated, to validate the simulation results of the proposed Design system. This Reference system is essentially comprised of two mirrored Design system boxes: The box dimensions are $50r_c \times 50r_c \times 100r_c$. The box contains adsorbing surfaces at the top and bottom faces ($\pm Z$), and uses typical periodic boundary conditions at the side faces ($\pm X$, $\pm Y$). This box contains 20 identical model proteins initially oriented vertically. 10 of these begin at a height of $25r_c$ away from the bottom adsorbing surface, and 10 others begin at a height of $25r_c$ away from the top adsorbing surface. The remainder of the box is again filled with water beads at a density of $\rho = 3$. The intent of this system is to represent exactly twice the volume as the Design system, at the same protein concentration, to simulate identical conditions at the adsorbing surface(s). This Reference system box is shown in Figure 2-10.
Figure 2-10. Reference system with two surfaces. Twenty model proteins (red) are within the box, which is then filled with water beads (blue). The top and bottom of the box are bounded by adsorbing surfaces (green). No reflecting boundary is necessary for this system. The four vertical box sides use periodic boundary conditions to replace any bead exiting a side with an identical bead entering from the opposite side.

As discussed in Chapter 1, DPD simulations representing surface adsorption with two surfaces, one each on opposite box faces, have been shown to correlate with experimental observations. Thus, while possibly computationally expensive, this method is an accepted standard with which to compare results of the proposed Design system.
Each configuration simulated is described by the system parameters studied in this work. These parameters include: chain geometry, surface hydrophilicity, and ceiling boundary definition. To ensure validity of these simulations, each box configuration (chain geometry, type of surface and ceiling) is simulated 5 times to generate a reasonable statistical sampling. An example of parameter input values for the software is shown in Appendix A. The initial velocity of each bead is randomly assigned between runs. Water bead placement is random within the entire box volume. The initial x- and y- position of each chain is random, while the z-position and vertical orientation is maintained in all runs. Each bead defining the surface is defined in the same initial position for each run, with a randomly-assigned velocity. Reported results are the average of these runs, with standard deviation included as error bars.

2.5 Boundary conditions

In all cases considered (both Design and Reference system), no physical boundaries are represented along the x- and y-axes. At these faces, periodic boundary conditions are defined in a standard manner: when any bead exits one side, it is replaced with a bead entering the box via the opposite side. The new bead has the same velocity and properties (including bonding) as the bead that exited, maintaining continuity of bead momentum across these periodic boundaries. The harmonic springs representing ‘protein bonds’ correctly allow for part of one protein chain to pass through the side boundary and re-enter the opposite side without unintended bonding or bending forces.

For the simulations of the Reference system, no additional boundary conditions are needed, as the box top and bottom are effectively blocked by the adsorbing surfaces. Because the Design system eschews this boundary method, an explicit boundary condition must be defined at the box’s top where beads can contact the box’s top, and periodic boundary conditions cannot be
used (because beads cannot re-renter the box bottom where the surface blocks their passage). Thus, three separate methods of ceiling boundary conditions are studied in these Design system simulations. An initial “Bead-bouncing” ceiling, a modified “Chain-bouncing” ceiling, and a separate modification for “Multiple-ceiling” boundaries are described below. The specific code used to represent all three ceiling boundaries is included in Appendix B.

2.5.1 “Bead-bouncing” ceiling boundary condition

Initial work with the Design system uses a simply-defined specular reflection of any individual bead contacting the ceiling boundary, referred to as the “Bead-bouncing” ceiling. This ceiling boundary represents a typical analytical specular boundary, as studied by Revenga et al. Revenga\textsuperscript{65}. Any bead whose z-position exceeds the box top has its z-velocity inverted to move it back into the box within the next time step. The x- and y- velocity of the affected beads are unchanged. This boundary is applied identically to any beads contacting the ceiling, either water or protein beads. It is noteworthy that all velocities of any other beads attached to a protein bead by harmonic spring forces are unchanged. Figure 2-11 below shows a graphical representation of a vertical protein chain contacting a Bead-bouncing ceiling.
Figure 2-11. Graphic of the Bead-bouncing ceiling reflection method. Z-direction velocity inverted only for individual bead contacting the ceiling. Ceiling effect on top bead may be offset by lower chain beads. After multiple time-steps of ceiling interaction, chain becomes “trapped” at ceiling boundary.

Figure 2-11 highlights a potential issue with the Bead-bouncing ceiling in this ideal case of a vertical chain contacting the ceiling. It is conceivable that in this ideal case, the reflecting force applied by the ceiling is completely cancelled out by one or more beads in the approaching protein chain. Instead of repelling the chain back into the simulation box, this may simply damp the vertical momentum of the chain, slowing the chain but never forcing it back into the box as intended. As mentioned in Chapter 1, while this behavior appears linked to the geometry of the protein represented in DPD, no discussions of this behavior are yet found in the literature. Correspondingly, there are no literature examples found which address this ceiling affinity behavior. The next two sections propose alternate methods of analytical reflecting ceilings, neither of which is found in use within other DPD simulations.
2.5.2 “Chain-Bouncing” ceiling boundary condition

As mentioned above, in systems containing some protein chain geometries, the Bead-bouncing ceiling might fail to effectively reflect the chains back into the system when they contact the ceiling. Instead, the chains can show an unintended “affinity” for the ceiling boundary. Preliminary results in our group hinted towards this, and thus to investigate and address this issue, a newly-defined “Chain-bouncing” ceiling modifies the effect of the boundary condition on protein beads only, applying the specular inversion to every bead in the protein chain contacting the ceiling. Thus, when any one bead of a protein chain contacts the ceiling, the Z-velocity of every bead in that chain is reversed, ensuring the chain moves back into the box. As with the Bead-bouncing ceiling, the X- and Y-velocities are unchanged for each bead in the protein chain. Figure 2-12 shows a graphical representation of a vertical protein chain contacting a Chain-bouncing ceiling.

![Diagram of Chain-bouncing ceiling reflection method](image)

Figure 2-12. Graphic of Chain-bouncing ceiling reflection method. Z-direction velocity inverted for all beads of protein chain contacting ceiling. Chain moves down from ceiling after one time-step.
2.5.3 “Multiple Ceiling” bouncing condition

An additional, separate modification to the Bead-bouncing ceiling is also considered. This ceiling investigates an alternate explanation for the ineffective Bead-bouncing ceiling behavior seen in some protein chain systems. This “multiple ceiling” method focuses on the effect referred to as “depletion attraction.” Because all interactions between DPD beads in these simulations involve only repulsive forces of varying degrees, a region with fewer interactions can be considered ‘attractive’ by virtue of the absence of repulsion. An example of this depletion attraction situation occurs as a DPD bead approaches the ceiling boundary. As the bead approaches within 1 \( r_c \) of the ceiling, there is an absence of DPD beads beyond the ceiling boundary to repel the approaching bead. Because the bead feels this repulsion from all other directions, the bead is effectively attracted to the ceiling, in an unrealistic and unintended fashion.

Conceptually, this behavior may be similar to the depletion stabilization seen in colloids with high molecular-weight polymers \(^97\). However, in this case, the low concentration resulting in depletion stabilization stems from the analytical boundary, and not a true physical phenomenon. This is evidenced by the lack of similar behavior in reference system simulations which represent the same molecules in the same concentrations, but do not employ an analytical simulation boundary.

This attraction may affect individual beads, such as the water beads in these systems, differently than beads attached to chains representing proteins. While an individual bead attracted to the ceiling may be easily perturbed by contacting beads from the sides, it is possible that the additional mass of an entire chain of beads coming to rest against the ceiling may prevent the interactions with individual water beads from moving that chain. To explore this possibility, a third ceiling condition is considered. This “Multiple-ceiling” case applies the original specular reflection to individual water beads contacting the ceiling. However, the height of the ceiling...
applied to chains of DPD beads is $3r_c$ lower than the typical ceiling height applied to individual water beads. This ensures that there are always water beads above a chain of protein beads, repelling the chain back into the system instead of allowing the depletion attraction effect at the overall ceiling. Figure 2-13 shows a graphical representation of a vertical protein chain contacting a Multiple-ceiling boundary, wherein the leading bead of the protein chain experiences a Bead-bouncing repulsion at a height lower than the water beads in the system would.

Figure 2-13. Graphic of Multiple-ceiling reflection method, which includes box ceiling and a lower chain ceiling. Z-direction velocity inverted only for individual bead contacting either ceiling. Height of chain ceiling is $3r_c$ lower than the box ceiling, which only interacts with water beads. Presence of water beads above chain ceiling ensure chain is repelled down into simulation box.
Chapter 3

Ceiling Boundary: Methodology Validation

As explained in Chapter 1, understanding interactions within complex biological systems is essential to study protein function and transport, and to enable design of biocompatible devices. However, many biological systems of interest, such as protein surface adsorption, cannot be effectively studied by molecular simulations at the atomistic level. To simulate these large systems for the time required for the desired behavior to evolve, “coarse-grain” techniques are often used. Dissipative particle dynamics is one mesoscale technique which makes large size- and time-scales accessible. Current DPD simulations that addressed some of these challenges typically represent two surfaces for adsorption \(^{74, 75}\), even when the second surface is only included to bound the opposite end of the simulation box. To eliminate the computational demand of such a redundant system, we propose the use of a specular reflecting boundary condition as an alternative. This boundary inverts each bead Z-velocity at the box ceiling to bounce them back into the simulation. We identified the requirements for a successful reflecting boundary in Chapter 2. Here, the results of using this boundary are validated against a reference system which contains a second surface and no reflective boundary. Simulation results including surface adsorption, fluid bead density and temperature are used to confirm the equivalence of the results with both boundary methods.

The necessary first step when reviewing new methodologies is to check the validity of the results obtained. It is possible to achieve results which appear reasonable, but which actually have underlying errors that invalidate the result. Results for all simulations are therefore first checked for plausibility. For all plausible cases studied in this work, the simulation results are
further compared to additional simulations using the “Reference system” boundary (which includes an adsorbing surface both at the simulation box top and bottom). As discussed in Chapter 2, this Reference system definition has been shown to correlate with various types of experimental observations, and may be considered a current “accepted” method. Therefore, variance from the Reference system results must be explored in detail, as this may indicate erroneous simulation results which will not correlate with experimental findings. Any errors in the Design system simulations relative to the Reference system simulations are expected to stem from the reflective ceiling boundary employed.

Three model systems are studied in this work: a) Long, thick (LT) chains; b) short, narrow (CN) chains; and c) long, narrow (LN) chains. We have found in our group that the Bead-bouncing ceiling, applied to only the first bead of a chain, can yield implausible results for LT molecules. Thus, here we carefully check the results for the Chain-bouncing ceiling (specifically for long, thick chains) to verify the elimination of incorrect results.

Finally, the performance of a Chain-bouncing ceiling and Multiple-ceiling boundaries are also compared for the Design system. Again this comparison is made for the LT model, and are only focused on simulations with a hydrophilic ($\Delta a = 0$) surface as a representative example. Both ceiling boundary methods are compared to one another, and to the accepted Reference system, to identify any errors or advantages.

3.1 Design system: Bead-bouncing vs. Chain-bouncing ceiling

3.1.1 Results for the long, thick model

The behavior of long, thick molecules when using a Bead-bouncing ceiling was the impetus for this research. We have observed in our previous simulations\textsuperscript{96} that these chains can
display an artificial and unintended affinity for the ceiling boundary of the Design system box. Simulations conducted as part of this research confirm this behavior in Bead-bouncing systems using LT chains. Figures 3-1 through 3-3 illustrate the surface adsorption and also ceiling affinity of chains in a Bead-bouncing system including either a hydrophilic (Δa = 0), mildly-hydrophobic (Δa = 25) or strongly-hydrophobic (Δa = 200) surface. As discussed in Chapter 2, five different samples (with same initial positions but different initial velocities selected at random) are simulated, to obtain the standard deviation. After 4.5μs, half of the LT molecules are adsorbed to the surface, but the other half are “trapped” near the box ceiling, and will not re-enter the box volume to be adsorbed to the surface later. Thus, the simulation is effectively at equilibrium, but half of the adsorption data can never be gathered. By comparison, the behavior of chains in the Chain-bouncing system appears as intended. Initially, chains are distributed throughout the box, including some which are counted in the range near the box ceiling. As the simulation progresses, the number of chains adsorbed to the surface increases, first at a high rate, and later at a lower rate as the concentration of both available chains in solution and available sites on the surface decrease. Accordingly, as the chain concentration in the box volume decreases, the number of chains near the box ceiling approaches zero. Therefore, the Chain-bouncing ceiling appears reasonable and corrects the problem identified in the Bead-bouncing ceiling.

Note that in simulations where ceiling affinity is observed, chains never move back into solution once they display affinity for the ceiling. For this reason, simulations are stopped once all chains are either adsorbed to the surface or are trapped near the ceiling. The chains have achieved an unrealistic equilibrium, and longer simulations will not yield different results.
Figure 3-1. Adsorption results of long, thick chains with $\Delta a = 0$. Note the number of chains near the ceiling approaches zero at long times for the Chain-bouncing ceiling (gray line), but approaches 50% for the Bead-bouncing ceiling (red line). Error bars indicate standard deviation within 5 simulations of each configuration.

Figure 3-2. Adsorption results of long, thick chains with $\Delta a = 25$. Note the number of chains near the ceiling approaches zero at long times for the Chain-bouncing ceiling (gray line), but approaches 50% for the Bead-bouncing ceiling (red line).
Figure 3-3. Adsorption results of long, thick chains with $\Delta a = 200$. Note the number of chains near the ceiling approaches zero at long times for the Chain-bouncing ceiling (gray line), but exceeds 50% for the Bead-bouncing ceiling (red line).

3.1.2 Results for short, narrow protein chains

The primary finding for all simulations using SN chains is that the performance of the Bead-bouncing ceiling appears correct and matches that of the Chain-bouncing ceiling. Figures 3-4 through 3-6 show adsorption characteristics of short, narrow chains onto surfaces of varying hydrophilicity. In each case, the result for the Bead-bouncing ceiling matches that of the Chain-bouncing ceiling. Importantly, the number of chains near the box ceiling in all cases remains low. However, this number does not approach zero for the SN chains in the simulation with a hydrophilic surface, shown in Figure 3-4. This illustrates a statistical sample of the fluid concentration, and does not indicate ceiling affinity of the chains.
Figure 3-4. Adsorption results of short, narrow chains with $\Delta a = 0$. Note the number of chains near the ceiling remains near zero for both the Chain-bouncing (gray) and Bead-bouncing (red) ceilings.

Figure 3-5. Adsorption results of short, narrow chains with $\Delta a = 25$. Note the number of chains near the ceiling approaches zero at long times for both the Chain-bouncing (gray) and Bead-bouncing (red) ceilings.
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3.1.3 Results for long, narrow protein chains

Given the difference in Bead-bouncing ceiling performance with LT proteins versus SN chains, an additional set of simulations examines this dependence of ceiling performance on chain geometry. LN chains, consisting of the same cross-section as the SN chains, and the same length as the LT chains, are studied. Figures 3-7 through 3-9 illustrate the result for both ceiling types when using LN chains. In all cases, the Bead-bouncing ceiling appears to perform as intended. Results match that of the Chain-bouncing ceiling, and the number of chains near the ceiling is low in all cases.
Figure 3-7. Adsorption results of long, narrow chains with $\Delta a = 0$. Note the number of chains near the ceiling approaches zero at long times for both the Chain-bouncing (gray) and Bead-bouncing (red) ceilings.

Figure 3-8. Adsorption results of long, narrow chains with $\Delta a = 25$. Note the number of chains near the ceiling approaches zero at long times for both the Chain-bouncing (gray) and Bead-bouncing (red) ceilings.
Figure 3-9. Adsorption results of long, narrow chains with $\Delta a = 200$. Note the number of chains near the ceiling approaches zero at long times for both the Chain-bouncing (gray) and Bead-bouncing (red) ceilings.

To understand the behavior reported in these simulations, results for both ceiling conditions are compared for one sample, where the starting position and velocities are identical for each case. Figure 3-10a depicts the positions of long, thick chains in a simulation box utilizing the bead-bounce ceiling condition. Figure 3-10b depicts the positions of chains which started at identical configurations within a simulation box utilizing the chain-bounce boundary.
Figure 3-10. Adsorption results of long, narrow chains with $\Delta a = 200$. In a), the bead-bounce ceiling is used. One chain, colored pink, contacts the ceiling at $t=0.092\mu s$. This chain remains at the ceiling shortly after, at $t=0.093\mu s$, and remains at the ceiling at the end of the simulation, $t=4.482\mu s$. In b), the chain-bounce ceiling is used, and the same chain (colored light blue) contacts the ceiling boundary at $t=0.092\mu s$. In this case, the chain moves away from the ceiling boundary by $t=0.093\mu s$, and at the same later time, $t=4.482\mu s$, the chain has eventually adsorbed to the surface.

In Figure 3-10a, at $t=0.092\mu s$, a particular chain (shown as pink) in the bead-bounce condition contacts the ceiling boundary. At $t=0.093\mu s$, the same pink chain in the bead-bounce condition remains at the ceiling boundary. Jumping to a much later time step (4.482$\mu s$), this pink chain remains at the ceiling in the bead-bounce condition (and has never moved away from the ceiling since it first approached). Additionally, 4 other chains have also become trapped by the ceiling boundary and will not re-enter the bulk of the simulation box. This simulation is effectively ended, with half of the chains unintentionally stuck at the ceiling boundary, preventing evaluation of the adsorption characteristics of the intended surface at the box bottom. Reviewing Figure 3-10b shows the exact same configuration in the chain-bounce ceiling simulation. Here, the chain of interest (colored light blue) again encounters the ceiling boundary at $t=0.092\mu s$. 
However, reviewing the result for the chain-bounce ceiling condition at \( t=0.093\mu s \), it is clear that the light blue chain has begin to re-enter the bulk of the simulation box. At the same later time step (\( t=4.482\mu s \)), the light blue chain, and many others, have adsorbed to the surface over time. The simulation may continue until the two remaining chains in solution eventually adsorb to the surface, providing valid data for every chain in the simulation.

Clearly there are situations wherein the traditional Bead-bounce ceiling yields acceptable results, and other situations wherein such simulations are unacceptable. To our knowledge this discrepancy has not been reported in the literature. Since the bead-bouncing technique is an accepted boundary for single beads at ceilings, it is usually extrapolated for more complex systems representing larger molecules by connected beads. However, there might be cases where this choice of boundary might be affecting the results. To explore this phenomenon, the differences between the geometry of chains simulated are further examined. As described in Table 2-2 earlier, the LT chains are significantly less flexible than either narrow chain geometry. The measure of flexibility used at the outset, comparing the number of ‘bonds’ defined, can now be replaced by a robust measurement from simulation results. The radius of gyration (\( R_g \)) is a measure of the molecule size. The radius of gyration is calculated as:

\[
R_g = \sqrt{\frac{1}{N} \sum_{k=1}^{N} (r_k - r_{mean})^2}
\]

where \( N \) is the number of beads in each chain, and \( r \) is the \((x,y,z)\) position of a bead. The position of each bead, \( k \), is compared to the mean position of all beads comprising that chain. Deviation of this value corresponds to the chain flexibility and mobility. The larger the deviation of \( R_g \) for a specific geometry, the more flexible the molecule is. In Figures 3-11 through 3-13, the radius of gyration for each model is plotted. The radius of gyration is measured from the simulations, over 100 individual chain conformations. To compare only chains that are freely moving, care is taken to exclude chains which are adsorbed to the surface.
Figure 3-11. Radius of gyration and standard deviation for long, thick chains.

Figure 3-12. Radius of gyration ($R_g$), and standard deviation for short, narrow chains. Standard deviation is larger than that of long, thick chains, despite the shorter chain length. Short narrow chains therefore demonstrate greater flexibility than long thick chains.
Figure 3-13. Radius of gyration ($R_g$), and standard deviation for long, narrow chains. Mean value is comparable to long, thick chains due to equal chain length. Significantly larger standard deviation than long, thick chains again indicates increased chain flexibility.

Figure 3-14 illustrates the significantly greater flexibility of both narrow chains, as measured by the greater standard deviation of the radius of gyration. This flexibility increases chain mobility in solution, and appears to play a key role in the success of the Bead-bouncing ceiling when that boundary condition does yield reasonable results.
Figure 3-14. Radius of gyration ($R_g$), and standard deviations, for chains simulated. Long, thick chains were the least flexible, with the smallest standard deviation. Both narrow chains exhibit much larger standard deviation, corresponding to greater chain flexibility and therefore mobility.

Because the radius of gyration value reflects chain size, direct comparison between long and short chains doesn’t capture the magnitude of difference in flexibility. The ratio of standard deviation to chain length normalizes the observed variation in chain size by chain length, allowing a more direct comparison between varied chain lengths, as seen in Table 3.1.

Table 3-1. Radius of gyration ($R_g$) of simulated proteins

<table>
<thead>
<tr>
<th>Chain length (# layers)</th>
<th>Radius of Gyration</th>
<th>Standard Deviation</th>
<th>S.D. normalized by chain length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long, thick</td>
<td>12</td>
<td>2.703</td>
<td>0.026</td>
</tr>
<tr>
<td>Short, narrow</td>
<td>6</td>
<td>1.408</td>
<td>0.064</td>
</tr>
<tr>
<td>Long, narrow</td>
<td>12</td>
<td>2.573</td>
<td>0.099</td>
</tr>
</tbody>
</table>
3.2 Design system vs. Reference system

The above results indicate that the Chain-bouncing ceiling produces reasonable results for all chain geometries studied. However, those results do not confirm the accuracy of the ceiling boundary itself. A critical step in showing that simulations using a reflecting ceiling yield accurate results is to validate that those results match the result for a similarly-configured system which does not use a reflecting ceiling. The Reference system is twice the size of the Design system, featuring twice the protein chains and two adsorbing surfaces but no ceiling boundary. The Reference system represents a typical setup of simulations examining surface adsorption. While the Design system is more efficient, requiring fewer beads (and therefore calculations) per simulation, the Design system cannot be shown as more desirable if it cannot reproduce the results of the more computationally-intensive Reference system.

Performance of these two systems is compared in two ways. The adsorption profile of LT chains onto surfaces of varying hydrophilicity is an essential global measure of system performance. Typically, adsorption isotherms are the primary focus of adsorption studies, both experimentally and computationally. Any discrepancies between the Design and Reference system in this measure will indicate a significant issue with the Design system.

A second means of comparing the two systems focuses on local behavior at the Design system ceiling, which correlates to the mid-height of the Reference system box. Local measures tracked include the fluid bead density and temperature. Tracking the fluid bead density at various heights within each box will provide a local measure of effects of the Design system ceiling throughout the box. Provided that the ceiling of the Design system is sufficiently far from the area of interest near the adsorbing surface, minor inaccuracies near the ceiling may not significantly affect simulation results. The magnitude and affected area of any inaccuracies is
determined by comparing the bead temperature near the ceiling boundary with that of beads far from the ceiling. Comparing the fluid bead density and temperature profiles can provide insight into the magnitude of any inaccuracy at the ceiling, as well as the range of the box volume affected by any ceiling-controlled behaviors.

3.2.1 Global measure of system behavior: Surface Adsorption

The adsorption of LT protein chains is compared between the Design and Reference systems in Figures 3-15 through 3-17. In all cases, the results of the Design system (blue lines) closely match those of the Reference system (purple lines). This global measure of performance is critical because this typically is the most important outcome studied in such simulations. Because the Reference system method has been shown to correlate well with experiments (as discussed in Chapter 1), equally good correlation is expected using a Design system that matches adsorption results from the Reference system. The good agreement between Design and Reference systems thus allow further inspection of the efficient Design system.
Figure 3-15. Adsorption results of long, thick chains with $\Delta a = 0$: Design system vs. Reference system. Design system simulation result tracks that of the Reference system.

Figure 3-16. Adsorption results of long, thick chains with $\Delta a = 25$: Design system vs. Reference system. Design system simulation result tracks that of the Reference system.
3.2.2 Local measure of system behavior: Fluid Bead Density

While correlation of surface adsorption profiles between the Design and Reference systems is considered paramount, smaller effects of the ceiling boundary can be monitored via local effects near the ceiling boundary. Therefore, in addition to comparing overall system performance by monitoring adsorption characteristics, the effect of the Chain-bouncing ceiling is evaluated locally through the measure of fluid bead density. Figure 3-18 shows the fluid bead density profile for the Design system with Chain-bouncing ceiling as compared to the reference system with no ceiling. This plot evaluates fluid bead density in 1 $r_c$ height increments along the
entire box height. The fluid bead densities for both the Reference and Design systems fluctuate about 3.0, matching the system definition as described in Chapter 2. The density of fluid beads is low in the area of the surface (where the tethered surface beads prevent water beads from occupying that volume). For this work, the surface definition is unchanged between systems, and the behavior near the box ceiling is the primary focus. Therefore, the fluid density in the bulk (excluding the surface regions) is of particular interest. In this region, the fluid bead density of the Reference system is 3.023, with standard deviation of 0.019. This calculation is an aggregate of 5 simulation runs for each system, based on simulation results at \( t = 0.11 \mu \text{s} \) (250,000 cycles). This density is slightly higher than 3.0 because the excluded volume of the surface and chains reduces the available area for water beads. The fluid bead density in the same region of the Design system is 3.014, with standard deviation of 0.017. Notably, a spike in fluid bead density occurs near the Design system ceiling.

Figure 3-18. Fluid bead density profile along box Z-axis: Design system vs. Reference system. Profile measured every 1.0\( r_c \). Design system data for \( Z > 0 \) is mirrored above box ceiling (dashed line) to represent full box height. Low values at box top/bottom correspond to adsorbing surfaces. Standard deviation is smaller than marker size for both series (S.D. <0.02).
A separate plot compares the fluid bead density of all systems in higher resolution near the ceiling of the Design system. Figure 3-19 shows the fluid bead density in finer increments of 0.1$r_c$. This refinement better captures the fluctuation of the fluid bead density near the ceiling of the Design system, as compared to the equivalent location, the box mid-height, of the Reference system. This refined plot shows fluctuation within 1.5$r_c$ of the Design system ceiling. At distances greater than 1.5$r_c$, the fluid bead density is indistinguishable between the Design and Reference systems. However, this fluctuation may be cause for concern if it can be shown to affect overall system behavior. This prospect can be explored by monitoring the temperature of the simulation as it progresses, as discussed in the following section.

Figure 3-19. Fluid bead density profile along box Z-axis: Design system vs. Reference system. Profile measured every 0.1$r_c$. Design system data for $Z > 0$ is mirrored above box ceiling (dashed line) to represent full box height. Design system profile fluctuates within 1$r_c$ of ceiling, as a result of the reflecting ceiling.
3.2.3 Local measure of system behavior: Temperature and Kinetic Energy

Given the finding of non-uniform fluid bead density at the reflecting ceiling, it is important to evaluate the effect of this distribution on the overall system behavior. A concern is that the ceiling boundary may impart significant energy onto an interacting bead, which would increase the system temperature over time. The system temperature can be calculated as a sum of the individual bead temperatures, where temperature is related to the bead kinetic energy by the relationship:

\[ \frac{1}{2}mv^2 = \frac{3}{2}kT \]  \hspace{1cm} (14)

Where \( m \) is the mass of each bead, \( v \) is that bead’s velocity, \( k \) is the Boltzmann constant and \( T \) is the temperature associated with that bead.

Using this relation we can observe changes on overall system temperature by monitoring the kinetic energy of each bead. Because the Design system contains hundreds of thousands of individual beads, a reasonable approach employed is to calculate the temperature of beads in height increments of \( 1r_c \). Initially, only beads near the top of the box will have their energy modified by the reflecting ceiling. If this ceiling boundary affects the temperature of the entire system, the average temperature of the system will increase over time. Thus, the temperature of beads near the ceiling can be compared to beads throughout the system to ensure the elevated bead temperature is a localized phenomenon. This comparison can also be made as the simulation progresses, to ensure the overall energy of the system is not changing over time. Figure 3-20 shows the temperature of beads at the box ceiling, as well as \( 2r_c \) below the ceiling, and the average for the system, as a function of time. This result shows that the temperature at the Design system’s ceiling is less than 7% higher than within the bulk. However, the
temperature of beads only 2 \( r_c \) below the ceiling (height = 48 \( r_c \)) are not affected by this temperature increase. Instead, beads of height 48\( r_c \) and lower track the desired system temperature of 1kT. Importantly, this finding indicates that the discontinuous energy added to the system as a result of the ceiling boundary is well within the ability of the DPD thermostat to overcome. Because the temperature of beads throughout the system is shown to fluctuate around the intended average temperature, the system temperature is unchanged by the reflecting ceiling of the Design system and matches the Reference system result.

Figure 3-20. Temperature of beads at the ceiling is increased due to ceiling interactions, versus time (log scale). Temperature at the ceiling boundary is approximately 7% higher than the system average. Within 2\( r_c \) from the ceiling (height = 48\( r_c \)), the temperature matches the overall system average. This system average tracks that of the Reference system. Note that equilibrium is reached at approximately 0.4 ns (100 cycles).

A closer examination of bead temperature versus height can illustrate both the localization of elevated temperatures to the box ceiling, and the equilibration of the system.

Figure 3-21 illustrates the bead temperature for an example simulation, which uses the Design
system with Chain-bouncing ceiling, and Long, thick chains. After an initial 10 cycles (4.45E-05 µs) the temperature throughout the system is well above the target temperature of 1kT (Figure 3-21a). At this early stage, the randomly-placed water beads are forced out of unrealistic overlapping positions with the placed surface and chains. As the simulation progresses through 100 cycles (4.45E-04 µs), the temperature throughout the system begins to stabilize near 1kT (Figure 3-21b). The elevated temperature at the surface is resolved, as water beads which had been placed in or below the surface made their way into the system. The temperature increase at the ceiling boundary is now clearly confined to within 2 r_c of the ceiling. Moving forward to 10,000 cycles (4.45E-02 µs), overall system temperature continues to be 1kT as the chains move throughout the solution (Figure 3-21c). There is very little change in average temperature as compared with the 100 cycle image. Jumping ahead to 1,000,000 cycles (4.45µs), the system temperature remains stable as most chains have moved throughout the system and found their way to the adsorbing surface (Figure 3-21d). While slightly elevated temperature can still be observed within 2r_c of the ceiling, the system temperature has remained the desired 1kT temperature throughout the simulation. Therefore, the increased density and temperature near the ceiling is seen to have no effect on the overall simulation result.
Figure 3-21. Temperature of beads versus height for a sample simulation run (Design system with Chain-bouncing ceiling, using Long, thick chains). a) After 4.45E-5 µs (10 cycles), the average temperature throughout the system is well about the target of 1kT. Temperature spikes at both the surface and ceiling, as beads seek equilibrium. b) After 4.45E-4 µs (100 cycles), the average temperature throughout the system is nearly converged at the target of 1kT. Elevated temperatures at the surface and box ceiling are resolved. c) After 4.45E-2 µs (10,000 cycles), the temperature is changed little from 100 cycles. Distribution of chains within solvent can be observed. d) After 4.45 µs (1,000,000 cycles), the temperature has remained stable at 1kT throughout the simulation. Most chains have adsorbed to the surface.

3.3 Design system: Chain-bouncing vs. Multiple-ceiling boundary

The Chain-bouncing ceiling was implemented in an effort to address the ceiling affinity displayed by LT molecules. The intent of that boundary modification was for the ceiling to give the protein chain a stronger “push” back into the system when contacting the ceiling, to overcome
the net forces holding the chain at the ceiling boundary. An alternative method was also
simulated. Using a Multiple-ceiling boundary, the protein chains are confined by a lower
effective ceiling. This ceiling uses the original Bead-bouncing technique, applied only to chain
beads (not water) to prevent further upward motion. This system allows water beads to travel
upward an additional 3 $r_c$ before encountering an identical Bead-bouncing boundary at the full
box height of 50$r_c$. The result ensures there are always water beads above the protein chains,
eliminating the effect of depletion attraction caused by the box ceiling on the chains. To compare
the effect of this Multiple-ceiling boundary to the Chain-bouncing ceiling, five simulations were
run and compared to the Chain-bouncing ceiling result for the same initial configurations. These
simulations used the Design system, with LT molecules. The surface was hydrophilic ($\Delta a = 0$),
to minimize preferential surface adsorption of the chains, so that chains interacted with the ceiling
boundary as much as possible. Simulations were run for 2 million cycles, representing a time of
over 8.5 $\mu$s. At this time nearly every simulation resulted in 100% surface adsorption of protein
chains. A comparison of the results for the Chain-bouncing ceiling and Multiple-ceiling
boundary follows.

3.3.1 Global measure of system behavior: Surface Adsorption

Just as for previous sections, the fundamental measure of ceiling performance is the
determination of the adsorption isotherms of the proteins throughout the simulation. A ceiling
boundary having no significant effect on the simulation will result in unchanged adsorption rates,
when compared to the validated Reference ceiling simulation method. Figure 3-22 compares the
adsorption profile for LT protein chains onto the hydrophilic surface within the smaller Design
The adsorption rate is seen to be statistically the same between both ceiling boundary types. The differences are very small, with error bars overlapping between the two data sets. Additional simulations may reduce the error to increase confidence in the statistical significance of this difference between systems.

Figure 3-22. Equivalent adsorption results of long, thick chains with $\Delta a = 0$ in Design system: Chain-bouncing ceiling vs. Multiple-ceiling boundary.

### 3.3.2 Local measure of system behavior: Fluid Bead Density

In addition to comparing overall system performance by monitoring adsorption isotherms, both Design system methods are compared with the Reference system via the local measure of fluid bead density. Figure 3-23 identifies the fluid bead density for the Multiple-ceiling boundary as nearly identical to that of the Chain-bouncing ceiling, and thus the reference
system. This plot evaluates fluid bead density in 1 \( r_c \) height increments along the entire box height. The bulk fluid bead density is 3.013 with standard deviation of 0.018, which is comparable to both the Chain-bouncing ceiling and the Reference system result. This calculation is again performed using simulation data at 0.11 \( \mu \text{s} \) (250,000 cycles). At this time in the Chain-bouncing Design system there are chains within 3\( r_c \) of the box ceiling. By definition, no chains are this close to the box ceiling in the Multiple-ceiling Design system simulations. As seen earlier for the Chain-bouncing ceiling, a spike in fluid bead density occurs near the Multiple-ceiling boundary. The close agreement in fluid bead density between the two Design systems indicates that the potential number of chain beads near the ceiling is too small to differentiate the local fluid bead density of either reflecting ceiling method.

Figure 3-23. Fluid bead density profile along box Z-axis. Profile measured every 1.0\( r_c \). Design system data for \( Z > 0 \) is mirrored above box ceiling (dashed line) to represent full box height. Negligible difference between Chain-bounce vs. Multiple-ceiling. Low values at box top/bottom correspond to adsorbing surfaces. Design system data for \( Z > 0 \) is mirrored to represent full box height. Standard deviation of all three series is smaller than series markers (S.D. < 0.02).
A separate plot compares the fluid bead density of all systems in higher resolution near the ceiling of the Design system. Figure 3-24 shows the fluid bead density in increments of 0.1\(r_c\). This refinement better captures the fluctuation of the fluid bead density near the ceiling of the Design system, as compared equivalent location, the box mid-height of the Reference system. As seen with the Chain-bouncing ceiling, the Multiple-ceiling boundary results in fluctuation within 1.5\(r_c\) of the Design system ceiling. Again, it is important to evaluate this effect by examining temperature of the system.

![Fluid bead density profile along box Z-axis: Chain-bounce vs. Multiple-ceiling. Profile measured every 0.1\(r_c\). Design system data for Z > 0 is mirrored above box ceiling (dashed line) to represent full box height. Both reflecting methods demonstrate fluctuation within 1\(r_c\) of ceiling, as a result of the reflecting ceiling. Chains are present at the ceiling for some of the Chain-bouncing simulation results shown here. No such chains can be present for the Multiple-ceiling boundary; close agreement between these two boundary methods indicates the presence of chain beads does not have a significant effect on measured fluid bead density.](image)
3.3.3 Local measure of system behavior: Temperature and Kinetic Energy

As with the chain bounce ceiling result, the temperature per bead is examined for the Multiple-ceiling simulation. This result, in Figure 3-25, shows the temperature of beads at the box ceiling, as well as 2r_c below the ceiling, and the average for the Multiple-ceiling Design system. This result parallels that of the Chain-bounce ceiling. Bead temperature is again elevated at the ceiling. However, the temperature of beads only 2 r_c below the ceiling (height = 48 r_c) are not affected by this temperature increase. The average system temperature remains the desired 1kT. Note that in this system the chains never move within 3r_c of the overall ceiling, and therefore do not experience this elevated temperature at all. As was the case with the Chain-bounce ceiling, the Multiple-ceiling boundary yields temperatures that match the Reference system in all regions excepting the ceiling vicinity.
Figure 3-25. The temperature of the Multiple-ceiling simulation is similar to that of Chain-bounce ceiling. Temperature of beads at the ceiling is increased due to ceiling interactions, approximately 6% higher than the system average. Within $2r_c$ from the ceiling (height = 48$r_c$), the temperature matches the overall system average. Note that this is above the height of the ceiling applied to chains, so that the chains completely avoid this high-temperature region. Again, system average tracks that of the Reference system, and equilibrium is reached at approximately 0.4 ns (100 cycles).

### 3.4 Evaluating the performance of ceiling boundaries in the Design system

#### 3.4.1 Acceptability of using a reflecting ceiling boundary

Results for simulations using the Bead-bouncing ceiling are reasonable only when using individual beads and narrow protein chains. It appears that chains which are not flexible behave improperly at the Bead-bouncing ceiling. While this method is appropriate for some cases, the
range of systems in which it is inappropriate is not currently well-defined. When also considering the negligible added computational expense of either a Chain-bouncing ceiling or a Multiple-ceiling boundary, use of the Bead-bouncing ceiling is not recommended.

The Chain-bouncing ceiling method described here produced reasonable simulation results in all cases. This method directly addresses the error found in some Bead-bouncing simulation results. The results when using a Chain-bouncing ceiling in a Design system compare well with those for a Reference system utilizing no ceiling boundary, both for transient chain behavior and steady-state system behavior. Generally speaking, use of the Design system with either a Chain-bouncing ceiling or Multiple-ceiling boundary can be recommended.

There are cases wherein the use of any Design system is strongly recommended over the Reference system. Systems which have a large height of bulk fluid between the upper and lower adsorbing surfaces are not well-suited to analysis via the Reference system, as the majority of simulated particles would represent the bulk. This adds significant computational expense without adding value to the simulation result.

The simulations employing a Multiple-ceiling boundary in a Design system also achieve reasonable results for long, thick chains. It appears likely that elimination of the depletion attraction of chains at the ceiling boundary also resolves the error resulting from the Bead-bouncing ceiling. In other words, maintaining water beads above the chains at all times applies more force on the chain than the Bead-bounce ceiling applied. Conceptually, this method bears resemblance to vicinal water\(^9\). (However, it is important to note that the water beads represented in this work, which simulate three water atoms per bead, cannot capture the atomic-level property differences of water near interfaces versus those of bulk water.) Additionally, the
simulation result for the Design system using a Multiple-ceiling boundary compares well with both the Design system using a Chain-bouncing ceiling, as well as the Reference system itself.

At present, the Multiple-ceiling boundary appears as good a recommendation as the Chain-bouncing ceiling for use in Design system simulations. A strong factor in deciding which of these ceiling boundaries to use is simply the ease of implementation in a given analysis code.

3.4.2 Efficiency of using a reflecting ceiling boundary

The primary driver for exploring the use of the Design system over the typical Reference system is the minimization of computational expenses. As scientists strive to understand more and more complex systems, the combination of hardware and software used must both be efficient. This work studies simulations only using one central processing unit (CPU). It is important to understand how scaling of software versus hardware efficiencies compare. While using multiple CPUs (or multiple graphics processing units, GPUs), is a common practice to reduce the total time required for a simulation, this hardware benefit cannot overcome all hurdles. Because there is additional computational expense (“overhead”) required to assimilate the calculations from individual processors working in parallel throughout a simulation, the reduction in total computation time scales less than linearly with increasing processors available. This is particularly true of “weak-scaling” applications, where the calculations assigned to each processor are dependent on calculations made by other processors. The additional overhead arising from these simulations, including DPD simulations, results in a marked drop-off in per-processor efficiency as the number of processors is increased. As an example, Figure 3-26 compares the efficiency of increasing processors for a simulation of a polymer chain melt using
the LAMMPS software discussed in Chapter 1. This 32,000 atom molecular dynamics simulation is comparable to the computational cost of a DPD simulation of similar number of beads. Potential improvement in computation time is seen when using up to 8 processors, but a sharp decline in per-processor efficiency occurs when using more than 8 processors.

![Graph showing the impact of increasing processors on parallel efficiency.](image)

Figure 3-26. Solving the same LAMMPS polymer chain melt simulation with increasing numbers of processors available. Scaling beyond 8 processors results in significantly decreased per-processor efficiency.  

Scalability plays a similar role when using multiple processors to simulate systems of increasing size. Figure 3-27 plots the per-processor efficiency when increasing the simulation size in proportion to the increase in processors. The decreasing slope of per-processor efficiency illustrates that increasing the available cores cannot maintain the efficiency of simulations when considering larger systems. Thus it becomes critical to efficiently define simulations to allow study of ever-larger and more complex systems.
Figure 3-27. Increasing the size of a LAMMPS polymer chain melt simulation in proportion to increasing the number of CPUs available results in decreased per-processor performance. Very large systems can be studied this way, but at a significant penalty in performance.  

The current work compares a typically-defined Reference system to a Design system using half the DPD beads and employing a reflecting ceiling to bound the simulation box opposite the adsorbing surface. As expected, simulations using the Design system require half the computational resources as the double-sized Reference system. Each Design system simulation of 50,000 cycles requires approximately 24 hours on a single CPU. The same 50,000 cycle simulation of a Reference system model requires approximately 48 hours. The simulation result relative to each surface is nearly equal between the Design and Reference systems, meaning the number of cycles for equilibration, transition from high- to low-rate adsorption, and final steady-state behavior are obtained using either system. Thus, simulating a single sample of 8.8µs of surface adsorption behavior requires 1920 hours using the Reference system, and only 960 hours using the Design system on a single processor. As discussed above, using additional
hardware to account for the extra computational expense of a larger system results in decreased efficiency. Thus, it will always be beneficial to define a smaller simulation which achieves comparable results.

3.4.3 Statistical benefit of using a reflecting ceiling boundary

In addition to tailoring software efficiency, adsorption simulations which define a single adsorbing surface can provide a specific benefit when studying low concentration systems as in the current work. As mentioned in Chapter 1, researchers typically identify the use of two surfaces as an effective measure of two samples when using the Reference system definition. However, this assertion does not address the potential issue of varying chain concentration. In the Reference system, while two surfaces are represented, the concentration of chains is not maintained as equal for each surface. It is logically possible for proteins to migrate toward the opposite end of the box in the Reference system simulations, resulting in unbalanced chain concentration near the supposedly-identical adsorbing surfaces. For low initial concentration systems such as the ones studied in this work, relocation of only one chain creates a 20% net difference in concentration between the two box halves. An example of this behavior in the Reference system is shown in Figure 3-28.
Figure 3-28. Example of unbalanced surface adsorption in Reference system. Initially, 10 chains are in each half of the simulation box. As the simulation progresses to 1.10µs, chain concentration is unequal within the box, with more chains near the lower surface. By 2.67µs, 4 times more chains have adsorbed to the lower surface than the upper surface.

To understand the prevalence of this unbalanced surface adsorption using the Reference system, the adsorption averaged between two surfaces is compared to actual adsorption per surface. Figure 3-29 shows the chains adsorbed in simulation using the Reference system with a strongly-hydrophobic surface ($\Delta a = 200$). This figure shows the average adsorption for each surface, by dividing the total adsorption by the number of surfaces. This figure matches the intent of the simulation, with chain adsorption increasing over time and approaching 50% of chains on each surface. However, the actual number of chains adsorbing on each surface typically varies much more, as shown in Figure 3-30. Here it is seen that 58% of all chains are adsorbed onto one surface, while 39% are adsorbed onto the other surface (with 3% of chains remaining in solution).
In all simulations, each pair of surfaces are defined identically, such that interactions do not bias adsorption to either surface. The particular surface with higher adsorption (top or bottom) varies by simulation. But, every simulation results in one surface with a larger amount of adsorbed chains. This trend is seen for both hydrophobic and hydrophilic surfaces, as shown in Figures 3-31 and 3-32.

Figure 3-29. Surface adsorption in Reference system simulation of strongly-hydrophobic surface ($\Delta a = 200$), plotting average of adsorption onto two surfaces. Expected overall adsorption behavior observed, if one assumes equal adsorption on each surface.
Figure 3-30. Surface adsorption in Reference system simulation of strongly-hydrophobic surface ($\Delta a = 200$), with both the average on two surfaces (purple) and explicitly-tracked concentration per surface (light and dark blue). In all cases, adsorption is not balanced between both surfaces.

Figure 3-31. Surface adsorption in Reference system simulation of mildly-hydrophobic surface ($\Delta a = 25$), showing both average (purple) explicitly tracking concentration on each surface (light and dark blue). Again, adsorption is never balanced between both surfaces.
Figure 3-32. Surface adsorption in Reference system simulation of hydrophilic surface ($\Delta a = 0$), showing both average (purple) explicitly tracking concentration on each surface (light and dark blue). In all cases, adsorption is not balanced between both surfaces.

While it can be argued that this difference is acceptable in physical experiments with greater limits on control of system parameters, there is less ground to make such a point with computer simulations. Here it is shown that two separate simulations yield better statistical results of the target system than one simulation utilizing two surfaces. This improvement comes at no additional computational cost. Thus it is recommended that two parallel simulations using the Design system yields better results at the same cost as one simulation of the Reference system.
Chapter 4

Surface Adsorption: Results and Discussion

4.1 Rate of surface adsorption

Once the veracity of the Design system simulations has been assured, the content of those simulations can be explored. The simulations performed here provide a wealth of data regarding behavior of the model protein chains themselves. For example, the simulations of LT proteins used for comparison between systems also give insight into the actual adsorption behavior of these model proteins on surfaces. In this section, simulation results using the Design system with Chain-bouncing ceiling are explored. Chain adsorption onto surfaces is examined, according to the criteria defined in Chapter 2, based on proximity to the surface: If 30% of the beads of a chain are within $2r_c$ of the adsorbing surface, the chain is considered adsorbed. As discussed in Chapter 2, no chain aggregation is seen in any of these simulations, because there is no difference in the protein-protein, protein-water or water-water interaction parameters. The adsorption behavior is further explored by varying both the geometry of the chains, and the hydrophilicity of the adsorbing surfaces. These findings expand upon the work of Patterson, et al. \(^{41}\), which studied the same LT chains, and SN chains studied in this work. Hydrophilicity and initial chain distance from the adsorbing surface was varied in that work. While starting height was not varied in this work, the effect of hydrophilicity on adsorption rate seen in that work will be compared to similar findings of the simulations discussed here.
4.1.1 Adsorption of long, thick chains

Figure 4-1 shows adsorption behavior of these chains on a hydrophilic surface ($\Delta a = 0$). There is a clear initial phase of high-rate adsorption, followed by a marked transition to a phase of slow adsorption. This slower adsorption rate continues until nearly all chains are adsorbed to the surface after 8.8 µs of simulation (2 million cycles). This adsorption is expected, with the characteristic behavior representing both a decrease in available adsorption sites on the surface, and a decrease in the total number of chains in solution. Figures 4-2 and 4-3 show similar adsorption behavior for both a mildly-hydrophobic ($\Delta a = 25$) and strongly-hydrophobic ($\Delta a = 200$) surface. This result matches the findings of Patterson, et al. 41, which found that LT chains starting near the surface had a similar initial adsorption rate for hydrophobic and hydrophilic surfaces. Increasing the initial separation between the chains and surface in that work correlated with a much larger difference in adsorption rate. This can be attributed to the low mobility of these stiff chains (as indicated by the standard deviation of the radius of gyration, as discussed in Chapter 3). For these chains, mobility plays a much greater role in adsorption kinetics than the hydrophilicity of the surface. This is consistent with experimental findings, which indicate that diffusion of a macromolecule to the surface is generally the rate determining step of the adsorption process 100. In all cases, adsorption of LT chains begins at a higher rate, then decreases until all chains in the simulation are adsorbed. In simulations representing a hydrophilic surface ($\Delta a = 0$), the rate of adsorption is much lower once approximately 75% of chains are adsorbed. In simulations including either the mildly- or strongly-hydrophobic surface ($\Delta a = 25$ or $\Delta a = 200$ respectively), the decrease in rate of adsorption occurs when approximately 50% of the chains are adsorbed. The rate change occurs earlier than for the hydrophilic surface, and the decrease in rate is correspondingly smaller. In these simulations of LT chains, the results are nearly identical for either hydrophobic surface. Differences between the hydrophilic and
hydrophobic surface results are comparatively small as well, indicating that for this chain geometry, the kinetics of chain movement in solution plays a much larger role in adsorption rate than the hydrophilicity of the adsorbing surface. Once the LT chains find the surface, they adsorb and do not desorb (although they do move on the surface). It is also uncommon for LT chains which adhere ‘end-on’ to change conformation to be adsorbed on their side. Again, the stiffness of the chain impacts this behavior, as the chain side, despite the larger surface area, is kept out of range of the surface by the adsorbed end and lack of chain bending.

![Adsorption of Long, Thick Proteins (Δa = 0)](image)

**Figure 4-1.** Surface adsorption of long thick proteins on hydrophilic surface (Δa = 0). Adsorption occurs at a high rate initially. After about 75% of the chains are adsorbed, the rate is much lower for the duration of adsorption.
Figure 4-2. Surface adsorption of long thick proteins on mildly hydrophobic surface ($\Delta a = 25$). Adsorption occurs at a high rate initially. After nearly 50% of the chains are adsorbed, the rate is lower for the duration of adsorption. The rate change occurs earlier than for the hydrophilic surface, and the change in rate is correspondingly smaller.

Figure 4-3. Surface adsorption of long thick proteins on strongly hydrophobic surface ($\Delta a = 200$). Adsorption occurs at a high rate initially. After about 50% of the chains are adsorbed, the rate is lower for the duration of adsorption. Behavior is very similar to that of the mildly hydrophobic surface.
4.1.2 Adsorption of short, narrow chains

The adsorption behavior of SN chains is markedly different than that of the LT chains. The findings reported here again agree with those of Patterson, et al. \(^4\), which found that SN chains are strongly sensitive to surface hydrophilicity. Adsorption occurs more rapidly than with the LT chains. Desorption from hydrophilic surfaces is common, while chains desorb from hydrophobic surfaces much less often. Figure 4-4 shows adsorption behavior of these short chains on a hydrophilic surface \((\Delta a = 0)\). An initial phase of rapid adsorption quickly gives way to a steady-state adsorption value of approximately 16%. At this point, the SN chains desorb readily from the surface, such that most chains remain in solution at any given time.

![Adsorption of Short, Narrow Proteins (\(\Delta a = 0\))]  

Figure 4-4. Surface adsorption of short narrow proteins on hydrophilic surface \((\Delta a = 0)\). Adsorption occurs at a high rate initially, but a low steady state value of 16% is maintained. Chain desorption from the surface is very common in this case.

The ease of desorption of SN chains is illustrated in Figure 4-5. At this stage, one chain adsorbed to the surface is highlighted (in orange). In subsequent time steps, the chain bends and lifts from the surface, as water beads moving randomly provide enough energy to dislodge the
small chains from the surface. This behavior continues throughout simulations of SN chains on the hydrophobic surface, as is expected when simulating a surface which adsorbs water without a significant energy penalty.

![Figure 4-5](image)

Figure 4-5. Example of desorption of short narrow chains on hydrophilic surface. A chain (orange) is observed adsorbed to the surface at 2.627 µs. At time 2.628 µs, this chain begins to lift off the surface, due to interactions with water beads (not shown). By 2.629 µs, the orange chain has desorbed because the energy of randomly interacting water beads was sufficient to overcome the reduced energy of the adsorbed chain. At 2.630 µs, the chain contacts the surface again, and is fully re-adsorbed at 2.631 µs.

When changing the property of the surface simulated, the short narrow chains display different behavior. Figure 4-6 shows simulation results for adsorption of these chains on a mildly-hydrophobic (Δa = 25) surface. Here an initial phase of rapid adsorption occurs and continues to over 0.5 µs before leveling off at a steady-state value of approximately 78% of chains adsorbed. As before, chains are still able to desorb from the surface, preventing 100% adsorption results. However, because the surface prefers the chains over the water beads, much greater energy is required to dislodge an adsorbed chain, limiting the frequency of desorption.
Figure 4-6. Surface adsorption of short narrow proteins on mildly hydrophobic surface ($\Delta a = 25$). Adsorption occurs at a high rate initially and is maintained until a steady state adsorption of 78% is achieved. At this point, less frequent desorption of the chains from the surface ensure some chains remain in solution.

When simulating a strongly-hydrophobic surface ($\Delta a = 200$), short narrow chains are seen to adsorb more rapidly than either earlier case, and desorption is rare. This results in nearly 100% adsorption. Additionally, a decrease in the adsorption rate is seen as the available sites for adsorption on the surface decrease. Results for this simulation are shown in Figure 4-7.
Figure 4-7. Surface adsorption of short narrow proteins on strongly hydrophobic surface ($\Delta a = 200$). The initial adsorption rate is higher than with the mildly hydrophobic surface. Instead of reaching a steady state adsorption value immediately, the rate slows and nearly all chains are eventually adsorbed. However, even with the strongly hydrophobic surface, desorption continues to occur uncommonly.

4.1.3 Adsorption of long, narrow chains

Simulations of LN chains yield similar results to those of SN chains. These chains have twice the side surface area, which increases the strength of adsorption. However, this also covers more area of the surface, reducing the sites available for adsorption of additional chains. The implications of these differences, with faster initial adsorption which slows as the number of adsorbed chains increases, are seen in Figure 4-8. This figure shows results for surface adsorption of long narrow chains on a hydrophilic surface ($\Delta a = 0$). Here the initial rate of adsorption is greater than that of the short narrow chains, indicating that these chains are sufficiently mobile and more strongly attracted to the adsorbing surface. Correspondingly, the steady-state adsorption value is also much higher (55%), with adsorption and desorption nearly balanced.
Figure 4-8. Surface adsorption of long narrow proteins on hydrophilic surface ($\Delta a = 0$). Adsorption occurs at a high rate initially, but slows and eventually reaches a steady state value of 55%. This value is significantly higher than that of the short narrow chains on the same surface, as greater energy is required to induce desorption.

The difference between short and long narrow chains is more pronounced on hydrophobic surfaces, where the change in surface energy from adsorption is more significant. Figure 4-9 shows results for adsorption of long narrow chains on mildly-hydrophobic ($\Delta a = 25$) surface. The initial period of adsorption is described by a higher rate than for the short narrow chains. Additionally, these chains do not stop at a steady-state value when rapid adsorption ends. Instead, adsorption continues at a slower rate to 100%, with desorption uncommonly observed.
Figure 4-9. Surface adsorption of long narrow proteins on mildly hydrophobic surface ($\Delta a = 25$). Adsorption occurs at a high rate initially and then slows to a lower rate until all chains are adsorbed. Desorption is much less common than with the short narrow chains.

During the later phase of slow adsorption, chain desorption is very unlikely. Figure 4-10 illustrates an example of desorption in this later phase. Here, a highlighted chain (in orange) moves across the mildly-hydrophobic surface while all chains are adsorbed. The selected chain encounters another adsorbed chain, and is forced to pass over the chain, eliminating the interactions with the surface. As the selected chain continues moving overtop the other adsorbed chain, the selected chain bends up into solution and desorbs from the surface. This temporary separation is quickly reversed as the surface repels water beads rapidly, resulting in 100% chain adsorption in this case.
Figure 4-10. Example of required conditions for desorption of Long, narrow chains from the mildly-hydrophobic ($\Delta a = 25$) surface. At time $t = 1.817 \mu s$, an adsorbed chain (orange) traverses the surface. At time 1.818 $\mu s$, this chain encounters another adsorbed chain and is forced overtop, beginning to separate from the surface. At 1.819 $\mu s$, the orange chain is only contacting the surface at one end. By 1.820 $\mu s$, the water beads (not shown) have pushed the orange chain sufficiently hard to overcome the remaining surface adsorption. At 1.821 $\mu s$, the orange chain is fully desorbed. All observed desorption events of these chains from this surface are caused by similar chain-to-chain interactions.

This adsorption behavior of LN chains on the strongly-hydrophobic ($\Delta a = 200$) surface is comparable to that of the mildly-hydrophobic surface. Figure 4-11 shows the resulting adsorption isotherm obtained for these simulations. The rapid adsorption phase is very similar between the two surfaces, but slightly more chains adsorb in this regime. This effectively reduces the rate in the slow regime, as there are fewer chains present in solution. Unlike with the mildly-hydrophobic surface, no desorption is observed for Long, thick chains on the strongly-hydrophobic surface.
Figure 4-11. Surface adsorption of long narrow proteins on strongly hydrophobic surface ($\Delta a = 200$). The initial fast adsorption rate is maintained until over 90% of chains are adsorbed. Eventually, all chains are adsorbed and desorption is rare.

The availability of all data within the simulation allows for precise measurement of the modeled behavior at the surface. The energy of the surface may be calculated by summing the repulsive forces between the surface and nearby interacting water or chain beads. Because adsorption in these simulations is driven by a favorable (smaller) repulsive force between the surface and chain beads than the surface and water bead interaction, the energy of the adsorbing surface may be calculated throughout the simulation. An example of this result is shown below in Figure 4-12. Here, the repulsion of the surface against adsorbed chains is calculated (for a single simulation), and presented in parallel to the adsorption rate (for an average of 5 simulations). The surface energy related to surface-chain interactions tracks the adsorption of the chains. Because the surface area of the 10 chains included in these simulations remains much smaller than the total area of the adsorbing surface, the energy related to surface-water interactions dominates the overall surface energy. However, this method would be well suited to
calculating the surface energy of higher concentration simulations, where an adsorbed monolayer would significantly reduce the total energy of the surface.

Figure 4-12. Surface adsorption of long narrow proteins on mildly-hydrophobic surface ($\Delta a = 25$). The surface energy contribution of adsorbed beads, as measured by the repulsion of chain beads to the surface (dark blue), tracks the overall chain adsorption rate (light blue).

### 4.2 Effect of chain geometry on adsorption

In addition to studying individual adsorption results, the array of chain geometries and surface hydrophilicities allows for investigation of trends between variables. Figure 4-13 compares adsorption results for all chains on a hydrophilic surface. The results are compared for 3.6 $\mu$s, which defines the full behavior of the narrow chains (the thick chains require nearly 9 $\mu$s for the same adsorption). While the adsorption of the LT chains was seen to result in 100% adsorption in all cases, it is noteworthy to see the difference in adsorption rate when compared to narrow chains. While more of the LT chains adsorb eventually, the rate is much slower. The mobility of both narrow chains allows them to reach steady-state near 0.5 $\mu$s. The LT chains, on the other hand, continue to adsorb at their initial rate for over 3 $\mu$s. While this initial rate is faster
than the final rate for LT chains, it is an order of magnitude slower than the more mobile narrow chains. However, once adsorption occurs for LT chains, desorption is unlikely even on the hydrophilic surface, as the larger surface area, mass and stiffness of the long thick chains prevent individual water beads from dislodging the chains. A comparison between the short and long narrow chains illustrates what was discussed earlier: the steady state adsorption value for LN chains is much higher than SN chains, as the reduction in surface energy via adsorption is larger.

Figure 4-13. Surface adsorption of different chains on hydrophilic surface ($\Delta a = 0$). Short narrow chains quickly reach a low steady state value (under 0.25µs). Long narrow chains reach a higher steady state value in about twice the time (just over 0.5µs). Long thick chains adsorb as a much slower rate than the other chains. This rate decreases after 3µs, but adsorption continues to approach 100% throughout the simulation.

Comparing the long thick chains to either narrow chain is more straightforward on hydrophobic surfaces, as seen in Figures 4-14 and 4-15. In both cases, the short narrow and long narrow chains rapidly adsorb and reach either steady state or complete adsorption well under 3 µs, while the LT chains don’t reach 100% adsorption until close to 9 µs. The differences between SN and LN chains are much smaller in these simulations. While the SN chains don’t reach 100%
adsorption on the mildly-hydrophobic surface, the initial adsorption rate tracks closely between short and long chains. As with the hydrophilic surface, the flexibility of the narrow backbone increases chain mobility, resulting in rapid adsorption. Even while the surface energy is reduced much more with the adsorption of LT chains, the time required is approximately three times longer.

Figure 4-14. Surface adsorption of different chains on mildly hydrophobic surface ($\Delta a = 25$). Both narrow chains adsorb much faster than seen on the hydrophilic surface. The short narrow chains reach steady state after 0.5µs, while the long narrow chains approach 100% adsorption. However, the long thick chains continue to adsorb much more slowly, and initially adsorb slower than even on the hydrophilic surface over the time scale shown here. At longer times the result is very similar for both hydrophilic and mildly hydrophobic surface.
Both narrow chains behave very similar, rapidly adsorbing for 0.5µs before slowing as they approach 100%. Again, the long thick chains continue to adsorb much more slowly, and initially adsorb slower than even on the hydrophilic surface over the time scale shown here. At longer times the result is very similar for both hydrophilic and strongly hydrophobic surface.

4.3 Effect of surface hydrophilicity on adsorption

4.3.1 Long thick chains on surfaces of varying hydrophilicity

As discussed in the previous section, the LT chain mobility, dictated by its mass and flexibility, controls its adsorption rate. This result is illustrated in Figure 4-16, comparing adsorption of LT chains on all surfaces. While the transition between fast and slow adsorption rates varies between surfaces, the initial rate and final adsorption is nearly identical for all surfaces.
Figure 4-16. Surface adsorption of long thick chains on all surfaces. Initial and final adsorption rates are very similar on all surfaces. At intermediate times (2-6µs), faster adsorption is seen on the hydrophilic surface. Overall, the adsorption seems controlled more by the kinetics of these chains than by the surface hydrophilicity.

4.3.2 Short narrow chains on surfaces of varying hydrophilicity

Short narrow chains show a significant sensitivity to surface hydrophilicity. Figure 4-17 compares results of these chains across all surfaces. While the high mobility of these chains allows for rapid early adsorption (with a comparable rate for all surfaces), the low surface area and mass allows for chain desorption in all cases. Predictably, the surface hydrophilicity controls the rate of desorption for these chains.
Figure 4-17. Surface adsorption of short narrow chains on all surfaces. Surface hydrophilicity controls adsorption of these mobile chains. Hydrophilic surfaces find the chains only slightly energetically favorable to water beads themselves. Hydrophobic surfaces result in much greater adsorption, though desorption continues to occur.

4.3.3 Long narrow chains on surfaces of varying hydrophilicity

The behavior of LN chains remains similar to the SN chains. The larger surface area and mass results in higher adsorption, but the overall adsorption remains dependent on surface hydrophilicity. Figure 4-18 shows minor differences between adsorption onto either hydrophobic surface, which both adsorb LN chains more strongly than the hydrophilic surface. As with the SN chains, the initial rate of adsorption is equivalent for all three surfaces considered.
Figure 4-18. Surface adsorption of long narrow chains on all surfaces. Adsorption again correlates strongly with surface hydrophilicity. Hydrophilic surfaces prefer the chains over water beads, and the larger surface area makes desorption less frequent than with the short narrow chains. Hydrophobic surfaces quickly adsorb virtually all chains available.

4.4 Summary of simulation findings for protein surface adsorption.

The simulations discussed in this work provide insight into the behavior of model protein chains with varying flexibility (measured via deviation of radius of gyration), and correspondingly varied mobility. The kinetics of each chain within solution is found to control the rate of surface adsorption, consistent with expectations\(^{100}\). Additionally, the effect of surface hydrophilicity is studied. While this property plays a minor role in the adsorption rate of the slowly-moving LT chains, surface hydrophilicity plays a key role in the adsorption rate and occurrence of desorption for the more mobile SN chains and the LN chains. Hydrophilic surfaces display the greatest chain desorption, as evidenced by the steady-state adsorption/desorption observed. Again, this finding is consistent with literature, though the model chains simulated here are too simple to represent denaturation of a globular protein, which accounts for even
stronger adsorption to hydrophobic surfaces\textsuperscript{101}. Table 4-1 summarizes the adsorption behavior observed in these simulations.

Table 4-1. Adsorption isotherm data from simulation

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<tr>
<th>Surface $\Delta a$</th>
<th>Hydrophilicity</th>
<th>Initial Slope</th>
<th>Secondary Slope</th>
<th>Steady-state adsorption amount (%)</th>
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<tr>
<td></td>
<td>Hydrophilic</td>
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<td></td>
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<td>100%</td>
</tr>
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<td></td>
<td>0.18</td>
<td>0.08</td>
<td>100%</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>0.19</td>
<td>0.07</td>
<td>100%</td>
</tr>
<tr>
<td>Short, narrow</td>
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<td>16%</td>
</tr>
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</tr>
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<td>0.47</td>
<td>100%</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>2.35</td>
<td>0.47</td>
<td>100%</td>
</tr>
<tr>
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<td>2.16</td>
<td>0.03</td>
<td>100%</td>
</tr>
<tr>
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<td></td>
<td>2.16</td>
<td>0.03</td>
<td>100%</td>
</tr>
</tbody>
</table>

The results presented in Table 4-1 summarize the key comparative findings of these DPD simulations of protein surface adsorption. Studies of basic properties, such as protein flexibility and surface hydrophilicity, yield results consistent with expectations derived from basic experimental studies reported in the literature. While these models are simple to facilitate understanding of the ceiling boundary methods explored, the methods employed here can readily be expanded to studies of greater scientific inquiry. Examples of complex behavior which may be explored with the methods used in this work include: representing specific complex protein chains, observing denaturation-related conformation changes after adsorption, and adsorption onto tailored surfaces including specific binding sites for a variety of specific potential ligands.
Chapter 5

Future Work

The results described in Section 3.1 illustrate a fundamental difference in the performance of a Bead-bouncing ceiling depending on the geometry of protein chains simulated. While the Bead-bouncing ceiling yields acceptable results when using SN protein chains, the results when using LT chains are unacceptable for surface adsorption studies. A preliminary attempt to explain this geometry dependence of ceiling performance was made by simulating LN proteins. These chains behave similar to SN chains, and the simulation results using the Bead-bouncing ceiling appear reasonable. Future studies could investigate other iterations on these chain geometries, to define precisely when it is critical to avoid use of an ordinary Bead-bouncing ceiling when simulating DPD chains of any type, and why this is the case.

For all ceiling boundary types, the ceiling is enforced by inverting the Z-velocity of targeted beads. In the case of the Chain-bouncing ceiling, this affects all beads of any chain contacting the ceiling, significantly affecting motion of the chain. It is possible that a more refined method of targeting the entire chain when using the Chain-bouncing ceiling could improve accuracy of chain movement in the region near the box ceiling. For example, instead of inverting the Z-velocity of each bead in the chain, the ceiling could instead invert only the Z-velocity of the entire chain, distributing the changes to Z-velocity throughout the beads in the chain. This would still result in chain movement that is affected by the ceiling, but the resulting motion could be a better representation of chain response to contacting the ceiling. Alternatively, simulations could more directly consider any chain contacting the ceiling as one leaving the box.
Instead of reflecting that chain back into the box, the simulation could insert a chain randomly entering the box with random and unrelated velocity at any location at the top of the box.

Sections 3.2.2 and 3.3.2 identify unwanted fluctuations of the fluid bead density near the box ceiling for both the Chain-bouncing and Multiple-ceiling boundaries. While this behavior is minor and localized at the box ceiling, revisions to the box ceiling could incorporate changes to smooth out these fluctuations. Depletion attraction is expected to play a role in this behavior, even for individual fluid beads. A proposed method to eliminate this effect is to augment the ceiling bouncing mechanism with one or more layers of fluid beads frozen in place above the box boundary. These beads would not serve to prevent beads or chains from moving beyond the box ceiling, and therefore would be defined at the intended fluid bead density (3.0 in these simulations), instead of an artificially high density required to prevent passage of other beads. These frozen beads would only serve to provide comparable repulsion to fluid beads as seen elsewhere in the system. While adding computational expense, it is possible that one or more of these layers could eliminate the fluctuations of fluid bead density when using a simulation box with a reflecting ceiling of any type.

Finally, an important limitation of all Design system simulations studied here is the effect of changing concentration throughout the simulation. A significant computational benefit of this system is that it allows for setup of a simulation representing only the portion of the fluid near an adsorbing surface. This enables simulation of systems where the actual height of the fluid is much greater than the dimension included in the simulation. However, the current setup is not equipped to maintain chain concentration within the simulation box realistically. If the box ceiling is intended to represent an area of bulk properties of the fluid, it is unrealistic for simulations to adsorb every chain present in the simulation box. In actuality, if the concentration of chains within the box were to significantly drop due to surface adsorption, additional chains from the fluid bulk would diffuse into the volume represented by the Design system simulation.
box. It would be worthwhile to modify the current simulation method to manage chain concentration within the box. If the number of chains moving freely within the fluid volume of the box were to drop below the initial concentration for some period of time due to chains adsorbing to the surface, additional identical chains could be introduced into the simulation box, to maintain the chain concentration from the bulk fluid. This modification would be relatively complex, but would greatly enhance the range of systems at constant concentration conditions which can be accurately simulated using the Design system.
Chapter 6

Conclusions

Understanding of protein surface adsorption isotherms is essential to the design of biomaterials. Mesoscale computer simulations are capable of representing the necessary complexity of systems to adequately characterize this behavior. While existing techniques, such as dissipative particle dynamics (DPD) can be readily applied in standard ways, it is important for the advancement of this field to emphasize efficient simulation design to use computational power to directly solve the most complex problems. Therefore this work explores the use of a proposed “Design system” in DPD simulations, which includes one adsorbing surface and an analytical reflecting boundary opposite to that surface. The use of a conventionally defined “Bead-bouncing” boundary which applies specular reflection to any bead at the simulation box ceiling is tested. This bead-bouncing ceiling yields unsatisfactory results, with simulations of long, thick protein chains ending in unrealistic chain behavior in the ceiling vicinity. Two methods to modify the analytical ceiling boundary, use of a chain-bouncing ceiling which reflects an entire chain at once, or a multiple-ceiling boundary which enforces a lower ceiling height for protein chain beads, are both considered as remedies for the bead-bouncing ceiling deficiency. Each of these methods proves adequate to overcome the drawback of the bead-bouncing ceiling, which is found to be related to the geometry of the protein chains studied. While a bead-bouncing ceiling appears adequate only for smaller, flexible chains, the use of either the chain-bouncing ceiling or multiple-ceiling boundary are found to be accurate for all protein chain geometries studied.
The results from DPD simulations of this Design system, using both the chain-bouncing and multiple-ceiling boundaries, are compared to results from a conventionally defined “Reference system” simulation using two adsorbing surfaces to bound the top and bottom faces of the simulation box. These comparisons show the Design system capable of predicting adsorption isotherms for model proteins on surfaces of varying hydrophilicities with no discernable difference from the adsorption isotherm results of Reference system simulations. Localized fluctuations in fluid bead density and temperature are observed at the Design system ceiling, but these local effects do not compromise the overall simulation integrity, because the DPD technique is shown to be sufficiently robust in maintaining desired overall system properties. Specifically, the DPD thermostat is shown to maintain the system temperature as desired throughout the duration of the simulation.

Simulations using the Design system are seen to be computationally efficient due to their simpler definition while yielding the same targeted results. In addition, it is shown that Reference system simulations, by representing two continuous simulation boxes at once, lose the ability to maintain precise control of protein chain concentration within the system. The Reference method of using two adsorbing surfaces with twice the adsorbate concentration consistently results in biased chain concentration near one of the surfaces, which can significantly alter results in systems with low protein chain concentrations.

The simulations performed all provide insight into the adsorption behavior of three types of protein chains. Protein chain diffusion within solution is seen to dominate adsorption rates, consistent with reported experimental findings. Therefore, the Long, thick chains with their lower observed flexibility and mobility, adsorb much slower than either long or short flexible chains. This effect of diffusion rate on adsorption is found to play a larger role in adsorption than surface hydrophilicity does. When considering flexible chains, the effect of surface hydrophilicity becomes visible in both adsorption rate and steady state adsorption values. Chains
with similar flexibility but substantially larger surface area are found to adsorb more strongly than smaller chains onto identical surfaces. All flexible chains adsorb less strongly to hydrophilic surfaces than hydrophobic ones, as measured by desorption rates. Both of these findings are also consistent with expectations from literature.

The simulations performed in this work highlight the applicability of mesoscale DPD simulations to the study of protein surface adsorption. The validated Design system allows a more efficient approach to DPD simulation of these systems, setting up future simulations of much more complex systems.
Appendix A

Sample Simulation Input

LT_25_a200_100KBB_run1 <- Identifier for current run files
1392 <- Seed value for random number generator
3 <- Number of bead types in system
50 <- Box Width, X
50 <- Box Length, Y
50 <- Box Height, Z
0.05 <- Simulation time step
50000 <- Number of cycles for current run
200 <- Frequency for trajectory output
Yes <- Does system contain protein chains?
elongated <- Shape of chains generated
25 <- Initial height of chains from surface
0 <- Surface-Surface interaction
15 <- Protein-Surface interaction
225 <- Water-Surface interaction
25 <- Protein-Protein interaction
25 <- Protein-Water interaction
25 <- Water-Water interaction
Yes <- Is this run a restart?
LT_25_a200_50KBB_run1_final_config.rstrt <- Previous run restart file
Yes <- Assign letters for bead types
Appendix B

DPD Simulation Code

The DPD simulation code (developed by K. Patterson and M. Lisal), is available upon request. Detailed here is the original code section for the Bead-bouncing ceiling, and modifications implemented in this work for the Chain-bouncing ceiling and Multiple-ceiling boundaries. These sections are applied at the end of every time-step, when calculating the final velocity of each bead based on the assumed intermediate velocity.

Bead-bouncing ceiling

```
DO i=1,N
   itype = part_type(i)
   mass_i = mArray(itype)
   vx(i) = vx(i) + dt* (fx(i) + fx_old(i)) / (2*mass_i)
   vy(i) = vy(i) + dt* (fy(i) + fy_old(i)) / (2*mass_i)
   IF (rz(i) >= Lz/2.0) THEN
       vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
       vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
   ELSE IF (rz(i) <= (-1.*Lz/2.0)) THEN
       vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
       vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
   ELSE
       vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
       vz(i) = vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i)
   END IF
   itype = part_type(i)
   mass_i = mArray(itype)
   KE = KE + (mass_i/2.) * (vx(i)**2 + vy(i)**2 + vz(i)**2)
   Px = Px + mass_i * vx(i)  ! Count for total momentum
   Py = Py + mass_i * vy(i)
   Pz = Pz + mass_i * vz(i)
END DO
```
Chain-bouncing ceiling

!-----Loop to check each chain for ceiling-bounce condition-----
DO i=20001,21920
   ichain = (1 + ((i-20001)/192))
   IF (rz(i) >= Lz/2.0) THEN
      bounceflag(ichain) = bounceflag(ichain) + 1
   END IF
END DO

!-------Final velocity of all beads, inverting Z-vel of any bead
whose chain hits ceiling
DO i=1,N
   itype = part_type(i)
   mass_i = mArray(itype)
   vx(i) = vx(i) + dt* (fx(i) + fx_old(i)) / (2*mass_i)
   vy(i) = vy(i) + dt* (fy(i) + fy_old(i)) / (2*mass_i)
   IF (itype .eq. 2) THEN
      ichain = (1 + ((i-20001)/192))
      IF (bounceflag(ichain) .gt. 0) THEN
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      ELSE
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i)
      END IF
   ELSE
      vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
   END IF
   KE = KE + (mass_i/2.) * (vx(i)**2 + vy(i)**2 + vz(i)**2)
   Px = Px + mass_i * vx(i)
   Py = Py + mass_i * vy(i)
   Pz = Pz + mass_i * vz(i)
END DO
Multiple-ceiling boundary

!-----------Loop to check each chain for ceiling-bounce condition----------
DO i=20001,21920
   ichain = (1 + ((i-20001)/192))     ! integer value of ichain is
truncated (plus one) to identify chain number
   IF (rz(i) >= (Lz/2.0)-3) THEN
      bounceflag(ichain) = bounceflag(ichain)+1   ! increment chain
      bounce flag if bead hit ceiling
   ELSE IF (rz(i) <= (-1.*Lz/2.0)) THEN
      bounceflag(ichain) = bounceflag(ichain)+1   ! increment chain
      bounce flag if bead hit floor (unlikely)
   ELSE
   END IF
END DO

DO i=1,N
   itype = part_type(i)
   mass_i = mArray(itype)
   vx(i) = vx(i) + dt* (fx(i) + fx_old(i)) / (2*mass_i)
   vy(i) = vy(i) + dt* (fy(i) + fy_old(i)) / (2*mass_i)
   IF (itype == 2) THEN                            ! apply ceiling 3 units
      IF (rz(i) >= (Lz/2.0)-3) THEN
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      ELSE IF (rz(i) <= (-1.*Lz/2.0)) THEN
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      ELSE
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i)
      END IF
   ELSE
      IF (rz(i) >= Lz/2.0) THEN
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      ELSE IF (rz(i) <= (-1.*Lz/2.0)) THEN
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
      ELSE
         vzi(i) = -1.0*(vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i))
         vz(i) = vz(i) + dt* (fz(i) + fz_old(i)) / (2*mass_i)
      END IF
   END IF
   KE = KE + (mass_i/2.) * (vx(i)**2 + vy(i)**2 + vz(i)**2)
   Px = Px + mass_i * vx(i)                     ! Count for total momentum
   Py = Py + mass_i * vy(i)
   Pz = Pz + mass_i * vz(i)
END DO
References


7. Vaddiraju, S.; Tomazos, I.; Burgess, D.J.; Jain, F.C.; Papadimitrakopoulous, F. Emerging synergy between nanotechnology and implantable biosensors: A review, Biosensors and Bioelectronics 25, 2010


74. Suttipong, M.; Grady, B.P.; Striolo, A. Self-assembled surfactants on patterned surfaces: confinement and cooperative effects on aggregate morphology, *Physical Chemistry Chemical Physics* **16** (31), 2014


96. Smith, B. Toward Biomaterials Design by Computer Simulations, B.S., The Pennsylvania State University, 2011


