Multiscale modeling for complex macromolecular systems: Methodologies and applications

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Multiscale modeling for complex macromolecular systems: Methodologies and applications

by

Seung Ha Kim

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

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CHAPTER 1. Introduction

1.1 Background and Motivation

Computer simulations are an increasingly important way to gain new insight about molecular-scale phenomena that occur in complex macromolecular systems. However, many important processes, such as host-guest binding, involve events that have characteristic length and time scales that span orders of magnitude. Multiscale modeling approaches are needed to overcome this challenge. The subject of this thesis concerns the development and application of multiscale molecular models and simulations capable of describing macromolecular systems from length and time scales commensurate with electronic structure theory to length and time scales in the nanoscale regime.

An overview of multiscale molecular modeling strategies for complex formation between a polyamidoamine (PAMAM) dendrimer and a polyaromatic hydrocarbon in aqueous solution is illustrated in Figure 1.1. As is shown in Figure 1.1, the molecular modeling strategy should be changed according to the temporal and spatial length scales of interest.

Atomistic molecular dynamics (MD) simulations have been used to examine the physical behavior of macromolecules[1, 2, 3] to measure the thermal and the physical properties by the molecular configurations. However, there are two limitations to describe the macromolecules by atomistic simulations. One is that many important biological phenomena in the complex system, such as host-guest binding mechanism for drug delivery, take place over characteristic length and time scales that are well beyond the current capacities of classical molecular dynamics simulation. The other is that atomistic simulations are difficult to explain the phenomena related to the electronic structures of the system such as the interactions which are related to the hydrogen bonding and the $\pi$ stacking by the aromatic rings, which are occurred between
Figure 1.1: Illustration of the temporal and spatial scales of interest for complex formation in a system of polyamidoamine dendrimers (PAMAM) and the polyaromatic hydrocarbon phenanthrene (Phe). There are snapshots from four different modeling approaches included in this figure: a PAMAM branch interacting with a Phe molecule (ab-initio), fragments of a G5 PAMAM dendrimer with 4 Phe molecules (fragment molecular orbital method), G5 PAMAM dendrimer (all-atom simulation), and a coarse-grained G5 PAMAM and a solution of 216 coarse-grained G5 PAMAM dendrimers with 216 Phe molecules (solvent-free coarse-grained model).
proteins and ligands, or residues of the proteins.

Mesoscale methodology is one of the solutions to describe the mesoscopic behaviors of the biological systems, which connect between the atomistic and the macroscopic resolution to overcome the limitation of atomistic simulations. The mesoscale simulations can be classified by two methods: coarse-grained (CG) molecular dynamics simulations and statistical field theories.

Coarse-grained (CG) simulations have effectively provided large-scale structural information because coarse-graining reduces the degree of freedom of the systems to enter into macroscopic properties at length and time scales much larger than is possible with all-atom molecular models. To connect between atomistic and coarse-grained (CG) resolutions, there are a number of CG approaches reported in the literature; most can be classified into one of two categories. In the first category, termed indirect parameterization, are methods in which the potential parameters of a pre-selected analytical form are optimized by calibration against thermodynamic or structural properties. An example is the MARTINI force field for biological molecules[4], whose parameters are based on oil/water partitioning coefficients. In the second category, called direct parameterization, the CG potentials are determined from an explicit atom MD simulation. One example is the multiscale coarse-graining (MS-CG) method, which derives CG parameters from force matching[5]. In the former category, a force field such as MARTINI can be easily applied whenever the target system is changed with little to no reparameterization required. In the latter category, the MS-CG method has the advantage of being systematic; the CG force field is evaluated from data collected along the trajectories of atomistic MD simulations. An alternative approach, called the solvent-free MS-CG model, derives effective CG potentials between sites on the solute molecules while integrating out the explicit representation of the solvent molecules[6].

Statistical field theories, an analytical method, self-consistent field theory (SCFT), and a nonlocal density functional theory, are computational methods where the degrees of freedom of the systems are fluctuating fields, which are field-based models rather than particle-based models such as atomistic and coarse-grained (CG) simulations[7]. In particular, self-consistent
field theory (SCFT) has been widely used for the prediction of equilibrium mesophases in the polymeric systems[8, 9]. Moreover, field approaches readily provide the decomposition of the free energy into entropic and enthalpic terms, so the field theories can be used to understand the binding mechanism of dendrimers with guest molecules using the balance between entropic and enthalpic terms[9].

The calculations using the ab-initio level are used for determining the ground state potential energy and electronic structures, which can be able to see more smaller length scale compared to atomistic simulations. By the computational studies using this approach, many interesting features such as the hydrogen bonding, the electron delocalization from the aromatic rings, and the hydrophobic interactions between molecules have been explained. However, most of the biological systems have too many atoms to calculate the electron density because the computational cost increase drastically with the system size. To reduce the computational cost, the several techniques are used: density functional theory, semiempirical methods, and quantum mechanics/molecular mechanics. Recently, the fragment molecular orbital (FMO) method were introduced by Kitaura and coworkers to describe a large system at ab-initio level[10, 11]. There are many advantages using FMO: Pair interaction energy decomposition analysis is available to see the partial interactions between fragments, and the polarizable continuum model[12] (PCM) can be used as the implicit solvation method with FMO[13].

The main material of the research topic is a dendrimer. Dendrimers are highly branched synthetic macromolecules, and have received increasing attention over the past several decades as attractive candidate materials for a variety of applications. The size and shape of a dendrimer can be designed by uniform stepwise reactions for generational growth. The synthesized methods for dendrimers can be classified into two major approaches: divergent and convergent synthesis (Figure 1.2). In the divergent approach, the construction of the dendrimer takes place in a stepwise manner staring from the core and building up the molecule towards the periphery. In contrast to the divergent method, the synthesis using the convergent approach begins with the peripheral groups forming dendrons as they converge upon the core where it becomes a dendrimer. This method was first invented by the Fréchet group to create polyether
dendrimers[14]. There are many merits for the convergent method compared with the divergent approach. One advantage is that the number of reactions per molecule per growth remains constant whereas the number of reaction site per molecule grows exponentially so that it is hard to generate a higher generation of dendrimers. The other advantage is that two different dendrons can be combined at any step to create an asymmetric core or branching points[15]. The first dendritic molecule was synthesized by the Vogtle and co-workers in the end of 1970s[16]. After that, polyamidoamine dendrimers (PAMAM) are commonly used since the first report by Tomalia and co-workers in the mid-1980s[17].

The controlled features of their synthesis give benefits in terms of a low polydispersity index and their highly branched architecture provides a well defined structure. Figure 1.3 shows the general structure of a dendrimer including their functionality. These advantages brings the modification of the functional groups easy, so that physical properties of dendrimers such as pH sensitivity, interior density, targeted binding affinity, and degradability can be controlled by engineering these functional groups [18]. As a common dendrimer, the structures and the physical properties of PAMAM dendrimers are widely studied by various experimental tools related to the interactions of PAMAM dendrimers with guest molecules. SAXS and SANS experiments are used to study the size and the shape of the microstructures[19] and UV-vis absorption and fluorescence spectroscopies have been carried out to investigate the complex of PAMAM dendrimers with metal ions like gold particles[20, 21]. NMR and FRET (Fluorescence resonance energy transfer) spectroscopies have been also applied in other to study the dynamics for the binding mechanism between dendrimers and DNA, medicines, or metal ions[22, 3]

Because of the binding interactions with guests, such as DNA and drugs, PAMAM dendrimers can serve as a host for therapeutic and imaging agents[23, 24, 25], and a high performance chelating agent for the removal organic pollutants and toxic metals from water and soil[26, 27].
Figure 1.2: Scheme of a dendrimer synthesis using a divergent and a convergent methods. (Reprinted from Ref. [15].)

Figure 1.3: A sketch of a general structure of dendrimer. The functionality of the structure is divided into four regions: core, surface groups, interior branching, and void spaces.
1.2 Research Objectives

The objective of this dissertation is to understand the binding mechanism between flexible macromolecules and guest species in solution using multiscale molecular modeling strategies, including: *ab initio* electronic structure theory, all-atom classical molecular dynamics simulations, coarse-grained molecular dynamics simulations, and statistical field theory. A brief summary of the subsequent chapters in this thesis is provided. Chapters 2 - 7 and Appendix A are self-contained units complete with literature review and bibliography. Chapters 2, 3, 5, 6, and Appendix A have been adapted from the following publications:

**Chapter 2:** S. H. Kim and M. H. Lamm, "Multiscale modeling for host-guest chemistry of dendrimers in solution", *Polymers* (invited review), submitted.


**Chapter 5:** S. H. Kim and M. H. Lamm, "Reintroducing explicit solvent to a solvent-free coarse-grained model", *Physical Review E* 84 (2011) 025701(R).


**Appendix A:** S. H. Kim and E. W. Cochran, "Localization of spherical nanoparticles within lamellar AB diblock copolymer melts through Self-Consistent Field Theory", *Polymer* 52 (2011) 2328-2339.

Chapter 2 reviews recent computational studies aimed at providing a better understanding of the relevant physicochemical parameters at play in the binding and release mechanisms between dendrimers and guest species. We highlight recent contributions that model supramolecular dendrimer-guest complexes over the temporal and spatial scales spanned by simulation methods ranging from all-atom molecular dynamics to statistical field theory. The role of solvent effects on dendrimer-guest interactions and the importance of relating model parameters across multiple scales is discussed.
Chapters 3, 4, and 5 investigate the binding mechanism between G5 polyamidoamine (PAMAM) dendrimers and phenanthrene molecules in solution. In Chapter 3, the binding phenomena between G5 PAMAM dendrimers and phenanthrene molecules in solution is studied by fluorescence resonance energy transfer (FRET) and all-atom simulations. Different solvent pH conditions are considered in order to understand the encapsulation and release mechanisms for guest molecules as a function of pH. Since the total number of atoms in one G5 PAMAM dendrimer is over 4000, it is difficult to thoroughly investigate systems containing more than one dendrimer in the simulation box. In Chapter 4, a coarse-grained modeling approach is applied to understand the binding interactions of guest molecules (phenanthrenes) and host molecules (G5 PAMAM dendrimers) in solution. For the coarse-grained approach, the solvent-free condition model is applied to the system based on the multiscale coarse-graining (MS-CG) methods to enable the calculation of a complex at high concentrations to compare with the experimental results. From this approach, we study the possible binding sites of phenanthrene molecules to the PAMAM dendrimer. The MARTINI CG model is also used to explain the binding behavior of the PAMAM-Phe complex, but in this model, the CG Phe molecules are highly bound to PAMAM dendrimers compared with the atomistic simulations. And the binding energies of PAMAM with Phe molecule are calculated at the ab-initio level to study the binding sites of PAMAM dendrimer. Also, pair interaction energy decomposition analysis (PIEDA) is carried out to explain the binding interactions according to the binding sites of PAMAM dendrimer.

In Chapter 5, a new coarse-grained modeling scheme is introduced. This approach combines a systematic, solvent-free force-matched coarse-graining algorithm for a complex macromolecule with an existing coarse-grained solvent model. Using the specific example of dendrimers binding phenanthrene in water, it is shown that this procedure efficiently and reliably describes the relevant interactions for flexible macromolecules in solution.

Chapter 6 investigates the self assembly of poly(amidoamine) (PAMAM) dendrimer and fullereneol to explore the stoichiometric ratio, thermodynamics, and molecular dynamics of the binding. Using isothermal titration calorimetry, spectrofluorometry, and computer simulations it is shown that both generations 1 (G1) and 4 (G4) PAMAM dendrimers can host one fullereneol
per two primary amines. Energetically, it was found that the interactions between G4 and multiple fullerenols ($\Delta G_{\text{bind}} = -5.44 \text{ kcal/mol}$) were more spontaneous than that between G1 and multiple fullerenols ($\Delta G_{\text{bind}} = -2.16 \sim -4.5 \text{ kcal/mol}$), due to the higher surface charge density and more internal voids of the G4 dendrimer. In addition to hydrogen bonding between the hydrogens of the dendrimer primary amines and the oxygens of the fullerenols, hydrophobic interaction, electrostatic interaction, and complex formation mediated via ionic bonding and Lewis acid-base reaction were the mechanisms assigned for the dynamics and conformation of the assembly. The dendrimer-fullerenol assemblies studied here have applications in drug delivery and may have implications for mitigating the environmental impact of discharged nanomaterials.

Chapter 7 studies the formation of a supramolecular complex between a PAMAM dendrimer and a protein. Coarse-grained molecular dynamics simulations are used to model the interactions between poly(amidoamine) (PAMAM) dendrimers and human serum albumin (HSA). These simulations model the conformational changes of both the dendrimer and the protein as the complex is formed. Based on isothermal titration calorimetry data reported in the literature [28], six possible binding sites on the protein were evaluated and it is found that HSA prefers to interact with the amide and tertiary amines of the PAMAM dendrimer. The results from this study contribute new knowledge to the general discussion of the so-called protein corona effect [29, 30], that is hypothesized to play an important role in determining the whether a synthetic species, such as a dendrimer or nanoparticle, is biocompatible or has some degree of toxicity.

Chapter 8 summarizes the complete of the research and discusses future research directions for modeling the cellular uptake of engineered nanomaterials based on the multiscale methods developed and implemented in this thesis.

For completeness, an earlier study on the behaviors of macromolecules using the statistical field theory is included in Appendix A. This work presents results for a three dimensional hybrid self-consistent field theoretic (HSCFT) model describing the equilibrium particle distribution of spherical nanoparticles within symmetric AB diblock copolymer melts. Holding
the polymer composition and morphology fixed, we consider a comprehensive parameter space comprised of the Flory interaction parameter describing interactions between B segments and the particle surface compared to the segment-segment interaction parameter ($\chi_{BP}$), the particle volume fraction ($\phi_P$), and the ratio of the particle diameter to block copolymer domain spacing ($\frac{d_P}{\sigma_{AB}}$). Analysis of the free energy over this parameter space yields phase diagrams showing the conditions under which particles segregate to the intermaterial diving surface (IMDS) or the center of the domain. Interestingly, we predict a particle concentration dependent “reentrant” phase transition in which particles move from the domain interior, to the IMDS, and back as $\phi_P$ increases. This prediction is consistent with recent experiments on block copolymer nanocomposites and the results are interpreted as a subtle consequence of the competition between enthalpic polymer-particle interactions and the chain packing frustration imposed by the particulate inclusion.
References


CHAPTER 2. Multiscale modeling for host-guest chemistry of dendrimers in solution

A paper submitted to the journal *Polymers* (Invited Review)

Seung Ha Kim and Monica H. Lamm

2.1 Introduction

Dendrimers have received increasing attention over the past several decades as attractive candidate materials for a variety of applications such as therapeutic delivery systems[1, 2, 3, 4, 5], imaging agents[6, 7, 4], templates for catalytic metal nanoparticles[8, 9, 10], and extraction agents for the removal organic pollutants and toxic metals from water and soil[11, 12, 13, 14]. The breadth across applications is attributed to dendrimers being well-defined, monodisperse nanostructures for critical nanoscale design parameters[15, 16], such as size, shape, rigidity, surface functionality, and solvent affinity, can be tuned. For this reason, a number of guest molecules have been studied with dendrimer hosts[2, 17]: drugs[1, 2, 3, 4], gene[18, 19, 20], contrast agents[7, 21, 22], metal ions[11, 12, 13, 14, 9, 10], polymers[23], and organic pollutants[24, 25].

A number of experimental approaches have been conducted to elucidate the interaction of dendrimers with guest molecules. The binding strength of the complex has been determined by isothermal titration calorimetry (ITC)[26, 27, 28, 29], high performance liquid chromatography (HPLC)[30] and fluorescence spectroscopy[31, 32, 33]. The detailed orientations and formation of the dendrimers with guest molecules have been studied by nuclear magnetic resonance (NMR)[34, 35, 36] and the size of the aggregates has been measured by dynamic light scattering (DLS)[31, 23]. For instance, supramolecular assemblies of phenanthrene, an organic pollutant, and a polyamidoamine (PAMAM) dendrimer have been studied using fluores-
cence resonance energy transfer (FRET) to understand the interaction between dendrimers and guest molecules in aqueous solution [25]. Using FRET experiments, the most stable state for PAMAM-phenanthrene binding was for a pH of 8 and 1:2 molar ratio (phenanthrene:PAMAM). However, the fundamental binding mechanism between PAMAM dendrimers and phenanthrene molecules, such as preferred binding sites and why the binding was optimum for specific conditions, could not be explained by the experimental techniques alone. In another case, fullerol-PAMAM assemblies have been studied using isothermal titration calorimetry, dynamic light scattering, and fluorescence spectroscopy to understand the binding mechanism [29]. These experiments measured the binding constant and the stoichiometry between PAMAM dendrimers and fullerenols, but lacked a detailed molecular description of binding mechanism. Theoretical and computational approaches can complement fundamental experimental studies like these to facilitate rational materials design of dendrimer carriers.

Various theoretical and computational approaches have been used to examine molecular-scale phenomena that occur in complex dendrimer systems with guest molecules [37, 38, 39]. However, modeling methodologies have been determined by the temporal and length scale of the features of the dendrimer complex systems because many macromolecular processes involve events that couple multiple characteristic length and time scales that span orders of magnitude. For this reason, multiscale modeling approaches are necessary to understand the phenomena of dendrimer complex systems because multiscale methodologies are capable of describing macromolecular systems from length and time scales commensurate with electronic structure theory to length and time scales in the nanoscale regime [40].

Figure 2.1 represents the overall multiscale strategies for the dendrimer complex in solution as the temporal and spatial length scales are changed to show different computational approaches. From the bottom to the top of Figure 2.1, four computational approaches are described: (a) ab-initio, (b) fragment molecular orbital (FMO) method, (c) all-atom simulation, and (d) mesoscale simulation. At an intrinsic level, the nuclear coordinates and electronic structures in dendrimer complex systems have been described using ab-initio quantum mechanics and density functional theory (DFT) (Figure 2.1(a)). The configurational bar-
Figure 2.1: Illustration of the temporal and spatial scales in the complex system of polyamidoamine dendrimers (PAMAM) and phenanthrenes (Phe). There are four systems included in this figure: (a) PAMAM branch and Phe molecule (ab-initio) (b) fragments of G5 PAMAM dendrimer with 4 Phe molecules by a rainbow color notation (FMO) (c) G5 PAMAM dendrimer (All-atom simulation) (d) coarse-graining G5 PAMAM and 216 CG G5 PAMAM dendrimers with 216 Phe molecules (Mesoscale simulation).
riers of the dendrimer[41], the binding energies and the binding sites of the dendrimer complex with guest molecules, especially metal ions[42, 43], have been studied using these fundamental methodologies. However, this approach can be used for only the lowest generation of the dendrimer because it is computationally intractable to carry out these types of calculations for systems with more than 1000 atoms. To reduce the computational cost at this time and length scale, several techniques are used: semi-empirical methods, and quantum mechanics/molecular mechanics. Recently, the fragment molecular orbital (FMO) method were introduced by Kitauro and coworkers to describe a large system at ab-initio level (Figure 2.1(b))[44, 45]. There are many advantages using FMO: Pair interaction energy decomposition analysis is available to see the partial interactions between fragments, and the polarizable continuum model[46] (PCM) can be used as the implicit solvation method with FMO[47]. Even though the complex of dendrimers with guest molecules can be studied at the quantum level using the fragment molecular orbital (FMO) method, there are limitations such as the optimization of the structures at the same level and the convergence of the energy. For this reason, the FMO approach needs to be coupled with methods such as docking or classical molecular dynamics to model the interaction of dendrimers with guest molecules.

As the next higher scale of calculation, all-atom and mesoscale simulations have been widely applied to study the binding mechanism of dendrimers with guest molecules[48, 38, 39, 49]. There are two main approaches for mesoscale simulations: coarse-grained simulations and statistical field theories. This review will cover those computational methodologies that have been used to model dendrimers and dendrimer-guest complexes in solution: all-atom (Section 2), coarse-grained methodologies (Section 3) and statistical field theories (Section 4).

### 2.2 All-atom simulations

All-atom molecular dynamics simulations are suited to modeling phenomena involving up to $10^6$ atoms over time scales on the order of tens of nanoseconds (Figure 2.1(c)). Thus, these simulations are routinely used to investigate conformational changes of a single dendrimer in explicit solvent with respect to dendrimer generation (size) and process conditions, such
as temperature and solvent pH [50, 51, 52, 53]. All-atom simulations may also be used to investigate the dynamics of binding between of a single dendrimer and one or more ligands in explicit solvent [54, 25, 38, 39].

### 2.2.1 All-atom force fields for modeling dendrimers

Various force fields have been used to model dendrimers in solution with all-atom simulation. In the literature reviewed below, four force fields are commonly used: AMBER, CHARMM, CVFF, and Dreiding. The AMBER force field and generalized AMBER force field (GAFF) for organic molecules give good predictions for the binding between small organic ligands, nucleic acids, and proteins [55, 56]. The CHARMM force field is specially developed for modeling the interactions between biological molecules such as proteins, nucleic acids, carbohydrates, and lipid membranes [57, 58]. CVFF is a generalized valence force field optimized for organic molecules [59]. The Dreiding force field is a generalized force field for use with organic and biological systems [60]. As with all applications of molecular simulation, selecting an appropriate force field for modeling dendrimers and dendrimer-ligand complexes is critical to obtaining the correct fundamental behavior. In the case of PAMAM dendrimers, it has been frequently shown that predictions from molecular simulation for the swelling behavior of dendrimers as a function of solvent pH and salt concentration are very sensitive to the force field used in those simulations [50, 52, 53]. The hydrogen bonding parameters in the Dreiding III force field were recently optimized to prevent the swelling of PAMAM dendrimers at low pH and to predict radii of gyration that are consistent with small-angle neutron scattering experiments [61].

### 2.2.2 The physical properties of dendrimers in solution

Using all-atom molecular dynamics simulation, the effect of solvent pH on the PAMAM dendrimer were first considered by Baker and co-workers [62]. In this study, the primary and tertiary amines in the PAMAM dendrimers were protonated to model low, neutral, and high pH effects of PAMAM dendrimer based on pH titration data, and the CVFF force field was used. The solvent was modeled implicitly and this caused the estimations of the radii of gyration for the dendrimers to be higher than those obtained from experiments.
Maiti et al. used atomistic molecular dynamics simulation to study the pH response of generation 4-6 PAMAM dendrimers [50] and a generation 8 PAMAM dendrimer [51] with explicit water molecules. From these calculations, the structure of the PAMAM dendrimer was shown to swell, with the branches extending as the solvent pH decreased. The open structures occur due to the electrostatic repulsions among the protonated tertiary (low pH) and primary amines (low and neutral pH) of the PAMAM dendrimers and due to the counterions and solvent molecules penetrating into the more open interior of the dendrimer at lower solvent pH. This pH responsive phenomena of PAMAM dendrimers in solution makes them attractive candidates for applications requiring controlled encapsulation and release, such drug delivery and environmental remediation.

Maiti and coworkers also carried out long (10-20 ns) atomistic molecular dynamics to see the counterion distribution and observe the \( \zeta \) potential as a function of generation (3 to 7) for the PAMAM dendrimers at neutral pH and understand the electrostatic binding between PAMAM dendrimer and nucleic acids[48]. In the counterion distribution, they found that the concentration of counterion density is increased in the interior regime of the dendrimer at higher generation dendrimer. The reason is that the electrostatic repulsion occurs inside of the dendrimer as the amount of backfolding increases in the higher generations. To screen the electrostatic penalty, the counterions penetrated inside the dendrimer, so the concentration of the counterions increases. They predicted the \( \zeta \) potential of the dendrimer using the counterion density profile, and found that the \( \zeta \) potential slowly increases with an increase of dendrimer generation, even though the surface potential exponentially increases. Thus, these simulations predict that the increased concentration of counterions does not affect the \( \zeta \) potential of the dendrimer. The observation of \( \zeta \) potential using molecular dynamics correspond to the results obtained for colloidal particles using Monte Carlo simulations and the Poission-Boltzmann theory. Because the diffusion properties of carriers are highly correlated to drug delivery performance, Maiti and coworkers studied the self-diffusion of dendrimers up to generation 8 [63]. The self-diffusion of the dendrimer with explicit solvent and different solvent pH condition were predicted by the long atomistic simulations. In this work, they found that
PAMAM dendrimers did not follow the scaling laws of the Stokes-Einstein relation for diffusion. Because PAMAM dendrimers are flexible macromolecules with lots of interior cavities, water and ions penetrate to the interior of the dendrimers. Based on the different diffusion behavior observed for dendrimers compared to linear polymers, it appears necessary to treat water and counterions explicitly when modeling dendrimers in solution.

Recently, Goddard and coworkers modified the Dreiding III force field to more accurately model the pH responsive structural changes observed in small-angle neutron scattering experiments on PAMAM dendrimers. In the experiments, the observed radius of the gyration ($R_g$) for a generation 4 PAMAM dendrimer was independent of solvent pH[61]. Using new hydrogen bonding parameters obtained from quantum mechanics, the molecular dynamics simulations confirmed that there was no change in radius of gyration and predicted that internal distribution of atoms in the dendrimer varied with solvent pH, from a 'dense core' (high pH) to a 'dense shell' (low pH).

2.2.3 The physical properties of complex systems of dendrimers with guest molecules in solution

All-atom molecular dynamics simulations have frequently been used to model the direct interactions of dendrimers with guest molecules or ligands. The types of guest molecules considered has included small organic drug molecules and pollutants [64, 25, 65, 29], and nucleic acids [66, 67, 38, 68, 69, 70].

Dendrimer-drug complexes have generated a lot of interest due to the need for understanding the fundamental mechanisms for encapsulation and release of drug payloads inside a dendrimer carrier. Tanis et al. studied ibuprofen, a nonsteroidal anti-inflammatory drug, as a guest molecule bound to a generation 3 PAMAM dendrimer at different solvent pH conditions using atomistic molecular dynamics simulations with GAFF[64]. They found that the hydrogen bonding between G3 PAMAM dendrimer and ibuprofen are mainly occurred at neutral pH, which is the hydrogen of the amide group and oxygen of the carbonyl group of ibuprofen. At low pH, the more open structure of PAMAM dendrimer is caused by the electrostatic repulsion between protonated amines of dendrimers, but the hydrogen bond formation between a den-
Figure 2.2: Representative images of G5-PAMAM dendrimer with 25 of phenanthrene molecules after 1 ns as an atomistic molecular dynamics: (a) low pH (b) neutral pH (c) high pH. The dark red arrows indicate the stacked Phe molecules. Permission request from Ref. [25]

Figure 2.3: Interaction of PAMAM dendrimers with fullerenols at neutral pH: (a) Two fullerenols in proximity to one G1 dendrimer within 1.5 nm of the center of mass of the dendrimer (b) 21 fullerenols in proximity to one G4 PAMAM dendrimer within 3.5 nm of the center of mass of the dendrimer. Permission request from Ref. [29]
the hydrophobicity of ibuprofen. At high pH, the drugs were mostly located on the surface of the dendrimer due to the electrostatic interactions between the drugs and the primary amine groups. Therefore, the hydrogen bond and the electrostatic interaction between drugs and PAMAM dendrimer have played an important role for the dendrimer-drug complex systems in solution. Similar behaviors were predicted by our group in a study of the phenanthrene-G5 PAMAM dendrimer complex using Drieding force field[25]. In this study, the phenanthrene molecules were highly bound to the G5 PAMAM dendrimer at neutral pH even though the dendrimer structure is more open at low pH, so there are more cavities in the interior of the dendrimer(Figure 2.2). The main reason of the high efficiency of the binding at neutral pH is that the hydrophobic interactions were the driving force for hydrophobic phenanthrene molecules to penetrate into the interior of the PAMAM dendrimer compared to a low pH case. Baker and coworkers studied 2-methoxyestradiol (2-ME), a potential anticancer agent, with modified PAMAM dendrimers at low pH condition, which the terminal group was converted from primary amines to acetyl, hydroxyl, and carboxyl group[65]. To understand the binding mechanism, they carried out atomistic molecular dynamics with CVFF force field. From the simulations, they confirmed the binding position of 2-ME molecules in the dendrimer. They found that the structure of amine, acetyl, and hydroxyl surface groups were more open that that of carboxyl group, so it is easier to release the 2-ME molecules under acidic conditions. Recently, our group has investigated complex formation between fullerenol, a fullerene derivative, and PAMAM dendrimers at neutral pH using GAFF [29]. The binding capacity of G1 and G4 PAMAM dendrimers for fullerenol was determined by molecular dynamics simulations. Using umbrella sampling simulations, the binding constant between PAMAM dendrimer and fullerenol was predicted to be independent of dendrimer size. However, the binding capacity of the G4 PAMAM dendrimer was 20-fold that of the G1 PAMAM dendrimer (Figure 2.3). The main reason is that the surface area of G4 PAMAM dendrimers is larger than that of G1 PAMAM dendrimers, thereby increasing the probability for binding fullerenol in G4 dendrimers compared to G1 dendrimers. After analyzing the types of hydrogen bond pairs formed during binding, the greatest fraction of hydrogen
bond events occurred between the primary amines on the dendrimer and the oxygen on the  
hydroxyl group of fullerenols, which supports the increased surface area hypothesis.  

Dendrimers have been explored as a non-viral vector for gene therapy and that has moti-  
vated modeling studies of the binding mechanism between dendrimers and DNA [2]. Dendrimers  
and proteins have similar physical properties and hence, complexes of DNA and dendrimers  
have also been investigated as model systems for gaining insight about the fundamental bind-  
ing interactions between proteins and genes. Maiti and Bagchi carried out atomistic molecular  
dynamics simulations using the Dreiding force field for dendrimers and AMBER95 force field  
for DNA to understand complexes formed between a 38 base single-stranded DNA (ssDNA)  
and PAMAM dendrimers of generations G2-G4 [66]. They included explicit water and ions  
with the ssDNA-PAMAM complex, whereas the Debye-Hückel approximation had been ap-  
plicated to model the electrostatic interactions between genes and dendrimers in the previous  
studies [54, 71]. In this study, the binding interaction between PAMAM dendrimers and ss-  
DNA was divided into three contributions, which are the bending energy (entropic effect), the  
electrostatic energy and the base pairing and stacking energy (enthalpic effect). For low gen-  
eration (G2, G3) PAMAM dendrimers, the surface charges of a dendrimer were not enough  
to neutralize ssDNA, so the electrostatic interaction between dendrimers and ssDNA was not  
stronger than the bending interaction. Therefore the dendrimers did not cause ssDNA to adopt  
a coiled confirmation. However, in the higher generation (G4) PAMAM dendrimer, ssDNA was  
neutralized by the protonated amines of the dendrimer, so the enthalpic gain from the electro-  
static energy overcame the entropic loss in the bending energy and ssDNA coiling was observed.  
They also calculated the free energy surface as a function of the distance between a dendrimer  
and ssDNA, and as a function of the local bending parameter defined as the summation of the  
distance between each phosphate site and the center of mass of the dendrimer. Based on the  
free energy calculation, they explained the stability of the coiled ssDNA-dendrimer complex in  
the following way. During the binding process, there is a metastable state, which occurs due to  
unfavored pairings in the coiled ssDNA, but in this state a large entropic loss was encountered  
from the elastic energy preventing the coiling of ssDNA. They also found the global minimum
state for the dendrimer-ssDNA complex, which had a lower local bending energy and a shorter
distance between the dendrimer and ssDNA. Maiti and coworkers have also simulated complex
formation between a 38 base pair double-stranded DNA (dsDNA) and generation (G3-G5)
PAMAM dendrimers[38]. In this study, they proposed that the critical variable for wrapping
nucleic acids around dendrimers is the charge ratio, which is defined as the number of positive
amines in a dendrimer divided by the number of negative phosphates in a nucleic acid. The
simulations showed that dsDNA completely wrapped around a G5 PAMAM dendrimer when
the charge ratio is over 1 (1.64) even though the a 38 base pair dsDNA has length short enough
(13 nm) that it is considered to be a rigid rod (Figure 2.4). In the case of G3 and G4 PAMAM
dendrimers (charge ratio <1), the dendrimer did not neutralize the dsDNA, so the dsDNA
did not totally wrap the dendrimers like what was observed with the G5 PAMAM dendrimer.
In addition, the lower generation (G3 and G4) dendrimers were deformed during the interaction
with dsDNA, presumably due to the smaller surface area of the dendrimers. They also
investigated the stability of the dsDNA-dendrimer complex using helicoidal parameters (rise,
roll, twist, tilt, shift, slide) to characterize the overall backbone structure of the dsDNA in the
complex and it was found that the G4 dendrimer-dsDNA complex was more stable than the
G5 dendrimer-dsDNA complex. Free energies of the complex were calculated using the molecu-
lar mechanics Poisson Boltzmann surface area (MM-PBSA) method with two thermodynamic
models for the entropy calculation. The free energies of the complex showed that the binding
strength increases with higher generation of dendrimer and that the binding energy per pro-
tonated primary amine (dendrimer) was maximized for the G4 dendrimer, suggesting that the
G4 dendrimer could be the optimum candidate for gene therapy applications. Mills et al. per-
formed molecular dynamics simulations with the CHARMM 27 force field to study the binding
between a dendrimer and dsDNA [67]. In this work, the local environment effects, such as de-
formation and the electrostatic interactions, were characterized and related to single molecule
pulling experiments. The potential of mean force for a G3 PAMAM dendrimer-dsDNA (24 bp)
complex was calculated by umbrella sampling and used to derive a mesoscopic stochastic model
based on a Monte Carlo method. From the model they calculated the mesoscale force-extension
Several all-atom molecular dynamics simulations studies have investigated dendrimer-RNA complexes. Pavan et al. investigated the G4-G6 PAMAM dendrimer-Firefly Luciferase (GL3) siRNA complex in solution [72]. The AMBER force field was used and the parameters for the PAMAM dendrimer atom types were derived using \textit{ab initio} calculations. The force field parameterization was validated against measurements of the hydrodynamic radius of PAMAM dendrimers using dynamic light scattering. The flexibility of the binding behavior, as a function of dendrimer generation generation and solvent pH, was characterized using an energetic flexibility (EF) index, defined as the ratio of the enthalpic contribution ($\Delta H$) to the entropic contribution ($T\Delta S$) of the binding energies calculated by the MM-PBSA method with the normal mode analysis for the entropy. Defined in this way, a higher EF index indicates stronger binding behavior, which results from strong intermolecular attractions, such as electrostatic interactions, and a lower entropic cost. Using the flexibility (EF index), it was found that the binding efficiency for G4 PAMAM dendrimers does not change with variation in solvent pH. Vasumathi and Maiti studied G3 and G4 PAMAM dendrimer-siRNA (21 bp) complexes using the Dreiding force field for PAMAM dendrimers and the AMBER03 force field for siRNA[69]. In this study, they considered the effects of counterion distribution, salt concentration, and the number of the dendrimer molecules involved in the complex. In these simulations, it was observed that a G4 PAMAM dendrimer gained more entropy from releasing Na\textsuperscript{+} ions to bind with siRNA than the G3 PAMAM dendrimer. The simulations predicted that as salt concentration increases, the binding affinity decreases because the salt ions screen the favorable electrostatic interactions between PAMAM dendrimers and siRNA. In addition, they calculated the binding energy for the complex using the MM-PBSA method. As with the computational studies about dendrimer-dsDNA complexes discussed above, they found that the binding affinity is correlated to the charge ratio between the dendrimer and siRNA. To understand the stability of the binding, the radial distribution function was analyzed between the primary and tertiary amines in the dendrimer, sorted by the subgeneration of dendrimer and the phosphates in backbone of siRNA. From this analysis, it was found that the first peak of the curve is related
Figure 2.4: (a) Structure of the DNA-G5 dendrimer complex during various stages of complex formation at the interval of a few nanoseconds (b) Time evolution of the radius of gyration ($R_G$) of DNA, the dendrimer, and the complex for the complexation with G3, G4, and G5 dendrimers at neutral pH. Permission request from Ref. [38]
to the protonated primary amines, and the second peak is related to the wrapping of siRNA and hence, the stability. Concerning the basis of the radial distribution function analysis, the two G4 dendrimer-siRNA complex was not stable even though the charge ratio indicates the binding affinity would be stronger than any other case considered.

2.3 Coarse-grained simulations

Coarse-grained molecular dynamics simulations are an effective strategy for obtaining large-scale structural information for dendrimer-guest assemblies, because the reduction in degrees of freedom permits computational investigations of length and time scales much larger than is possible with all-atom molecular models. The freely jointed chain model is one of the coarse-grained methodologies used to describe the behaviors of dendrimers in solution \cite{73, 74, 75}. The advantage of this approach is that the key physical details governing the observed macroscopic properties can be easily extracted from the simulation predictions. However, the intermolecular interactions for this class of model are phenomenological and do not directly originate from the all-atom force fields or the thermodynamic properties of a specific dendrimer chemistry. To maintain chemical fidelity in coarse-grained molecular simulations, alternative multiscale coarse-graining strategies have been applied. In these multiscale strategies, most coarse-grained approaches can be classified into one of two categories. In the first category, termed indirect parameterization, are methods in which the potential parameters of a pre-selected analytical form are optimized by calibration against thermodynamic or structural properties. An example is the MARTINI force field for biological molecules \cite{76, 77}, whose parameters are based on oil/water partitioning coefficients. In the second category, called direct parameterization, the coarse-grained potentials are determined from all-atom molecular dynamics simulations. One example is the force matching method \cite{78, 79, 80, 49}.

2.3.1 The physical properties of dendrimers in solution

Tian \textit{et al.} studied the structure and the size of a charged dendrimer as a function of the counterion valency and different salt concentrations using a freely jointed bead-spring model for G4 cationic dendrimer\cite{75}. In this study, they found that the strong electrostatic interaction
from high valence ions neutralized the negatively charged surface group of the dendrimer and reduced the electrostatic repulsion. Thus, the high valency counterions effectively cause an osmotic pressure drop in the interior region of the dendrimer. In addition, the conformations of the dendrimer changed from extended to collapsed to a weak swollen state with increasing concentration of multivalent salt ions. These intriguing results warrant further investigation of dendrimer-polyelectrolyte complexes, by both experimentation and by all-atom molecular simulation.

Maiti et al. developed a generic coarse-grained model for PAMAM dendrimers to describe higher generations of dendrimers [81]. The mapping of coarse-grained sites to atoms is similar to the model developed by Lee and Larson [82] (Figure 2.5). Coarse-grained parameters for non-bonded and bonded potentials were derived using an all-atom molecular dynamics simulation of a G6 PAMAM dendrimer in the gas phase. The results obtained with this potential compare well with previous all-atom simulations [83] of PAMAM in the gas phase (poor solvent) but the potential is incapable of providing insight about behavior in water (good solvent).

2.3.2 The physical properties of complex systems of dendrimers with guest molecules in solution

The binding chemistry between dendrimers and guest molecules has been studied using coarse-grained simulations to understand the behaviors of complex systems beyond all-atom simulations. As guest molecules, polyelectrolyte polymers (nucleic acids) interacting with dendrimers have been studied using coarse-grained simulations [73, 74]. Even though all-atom simulations have been carried out to understand the detailed binding mechanisms between nucleic acids and dendrimers at full atomistic resolution [66, 38, 72, 69], there is a high computational cost for all-atom simulations to properly describe these complex systems in solution. Lyulin et al. systematically studied the electrostatic interactions, which varied by the multivalency of the counterions and the linear chain in the explicit solvent condition [73]. The freely jointed bead-spring model was used to model a system containing a G4 dendrimer with 48 positive charges on its surface and a linear polyelectrolyte chain with 10 negatively charged beads. Using this model, the simulations predicted that the structure of the dendrimer shrinks
Figure 2.5: (a) Mapping of dendrimer PAMAM segments into coarse-grained beads. Each monomer is represented by two beads N1 or N2. N1 is represented by O or S, N2 is represented by N or P. Note that N1-N2 need not be equal to N2-N1. In order to keep track of this difference we used O,3 and S,3 atom types for N1 and N,3 and P,3 atom types for N2. Other than the definition of bond distances the force field attributes of N,3 and P,3 (O,3 and S,3) are identical. The same segment in PAMAM has different bond lengths and we apply special protocol to label each segment. The distance between the center of beads O and N is different from the distance between the center of beads N and S, although beads O and S represent exactly the same segment (-CH2-CO-NH-CH2). The reason is that the segment is not symmetrical in two directions. Beads O and S are labeled as N1 and bead N is labeled as N2. So the N1-N2 (O-N) distance (within the same generation) is different from the N2-N1(N-S) distance (between the two successive generation). Permission request from Ref. [81]
due to the strength of the electrostatic interactions whereas the structure of the polyelectrolyte chain was unchanged. In the complex, the electrostatic interaction between the chain and its counterions was screened by the dendrimer-chain complex, and the dehydration of the chain was occurred by the electrostatic interaction. Therefore they effectively explained the effect of electrostatic interactions by the multivalency of the counterions and the polyelectrolytes in drug and gene delivery. However, there is a discrepancy of the binding position of the polyelectrolyte to the dendrimer compared to the all-atom simulations[66]. In the coarse-grained simulations, the chain beads were located to the center of the dendrimer, but in the all-atom simulations, a ssDNA was located far away from the center of the dendrimer. The main reasons of the discrepancy of the binding position is related to the steric hinderance of the dendrimer and the chain, which can be provided by the introduction of angle and torsional potentials. Tian et al. investigated the effect of the chain rigidity using the freely jointed bead-spring model[74]. In this study, the stiffness of the polyelectrolyte was varied by the strength of bond angle constants. The increase of the linear chain rigidity provided the interesting conformational transformations from coil to U or V then to rod shape due to the increase of the bending energy occurred by the chain stiffness. Also they found that the size and the shape of the dendrimer are changed by the stiffness of the charged linear chain and the Bjerrum length, which represents the strength of electrostatic interactions in the system.

To see the interaction of dendrimers, Tian et al. investigated the energy barriers depending on solvent pH, counterions, and modification of terminal groups of dendrimers using the MARTINI coarse-grained model, which is a generalized coarse-grained force field for proteins and cell membranes[84]. For the solvent pH effect, they found that the decrease of the solvent pH leads the repulsive interactions between dendrimers stronger due to the electrostatic interactions. In the higher valency of the salt ions, the release of the free energy between dendrimers is getting higher, so the dendrimers are easily aggregated and make more stable clusters. For the modification of the terminal groups of the dendrimers, the aggregation of the dendrimers were not changed even though the stability of the clusters was lower in the charged functional groups.
Figure 2.6: Two-dimensional probability distribution function, $P(r)$, of Phe molecules from the core of each dendrimer. The white circles indicate the branch points of the dendrimers. The numbers in the circles represent the generation of the dendrimer, and Q is the abbreviation for $Q_d$. The color bars express the intensity of $P(r)$. The unit of X and Y axis is nm. (a) Atomistic simulation, (b) solvent free CG, (c) explicit solvent CG (27 dendrimers) (d) explicit solvent CG (216 dendrimers) Permission request from Ref. [49]
Our group has studied the interactions between G5 PAMAM dendrimers and phenanthrene molecules using a new coarse-grained modeling scheme that combines a systematic, solvent-free multiscale coarse-graining algorithm for a complex macromolecule with an existing coarse-grained solvent model[49]. The solvent-free coarse-graining approach[79] does not work well for flexible macromolecules in solution because of the significant configurational entropy loss from the absence of explicit solvent molecules. To overcome this problem, the configurational entropy was restored by reintroducing coarse-grained solvent molecules to the system. The new coarse-grained modeling approach predicted the experimentally measured binding capacity and reproduced the distribution of phenanthrene molecules obtained with all-atom molecular dynamics simulations(Figure 2.6). The advantages of this coarse-grained methodology is to obtain the coarse-grained potentials derived from the all-atom simulations for the flexible macromolecules in solution such as drug delivery systems, so the coarse-grained potentials provide better descriptions of the binding mechanism at a specific system even though this method could not provide generic coarse-grained potentials.

2.4 Statistical field theories

Statistical field theories, such as self-consistent field theory (SCFT) or nonlocal density functional theory where the degrees of freedom are fluctuating fields, are an alternative to particle-based methods such as all-atom or coarse-grained molecular dynamics [85]. In particular, SCFT has been widely used for the prediction of equilibrium mesophases in polymeric systems[86, 87, 88, 89, 90]. Field-based simulation approaches readily provide the decomposition of free energy into entropic and enthalpic contributions. This is advantageous for modeling the binding mechanism between dendrimers and guest molecules when the balance between entropic and enthalpic terms is desired [88]. However, no kinetic data regarding binding can be obtained from statistical field theories.

Boris and Rubinstein proposed the dense-core model for the equilibrium structure of the starburst dendrimers using a modified SCFT approach [86]. Since that time, the mean field theory has been performed to understand the relationship between enthalpic and entropic driv-
Giupponi et al. studied the conformational change of polyelectrolyte dendrimers as a function of dendrimer generation using molecular dynamics simulations and mean field theory [87]. In this study, the explicit free ions and the implicit solvent models were applied to more accurately describe the electrostatic interaction of the dendrimer rather than the Debye-Hückel approximation, since there are important nonlinear phenomena present in the counterion distribution. Using this model, they found that the variation of the dendrimer structure was weaker with increasing concentration of salt ions than the structure prediction given by the Debye-Hückel approximation. Giupponi et al. explained that the weaker dependence of the dendrimer structure was due to the osmotic pressure of the trapped counterions because the electrostatic interactions were strongly screened by the local charge neutrality. They performed mean field theory calculations to determine the osmotic pressure of the dendrimer based on the free energy calculation. From these calculations, the osmotic brush regime for the dendrimer, in which the electrostatic interactions are strongly screened, dominated to cause swelling of the dendrimer structure. Ting et al. studied hole formation and rupture of a membrane caused by the insertion of a fully charged dendrimer "nanoparticle" using SCFT to rationally design vectors for gene delivery systems [89].
They simplified the system using the discrete Gaussian chain model, considering electrostatic interactions with constraints of one reaction coordinate and a fixed position for the charged dendrimer. Using this simplified model, they considered two different cases for membrane rupture, a tensionless membrane and a membrane under tension, with the latter case being motivated by the proton sponge hypothesis for cell membranes. In the tensionless membrane, the membrane deformed and partially wrapped the dendrimer (Figure 2.7); no stable pore was formed in the membrane. For the membrane under tension, pore formation of the membrane was stable if the tension exceeded a critical value. They also found that higher generations of the dendrimer provided more stable pore structure in the membrane; this result is consistent with that using coarse-grained simulation based on the MARTINI model [91]. Recently, Ting et al. proposed more sophisticated model using SCFT combined with the string method [92] to calculate the minimum energy path to membrane pore formation and rupture. [90] The string method automatically determines the reaction coordinate of the minimum energy path and thus, provides a means by which one may consider the actual nucleation events for the pore formation induced by the dendrimer.

2.5 Conclusions

Dendrimers have been widely studied to understand the structure and the properties at a molecular level using the computational methodologies from ab-initio to mesoscale simulations depending on what kinds of properties of the dendrimer or the dendrimer complex are investigated. All-atom simulations have been carried out to investigate the change of the properties depending on the size and solvent pH, which is related to the balance between entropic and enthalpic interactions of the system based on the the descriptor derived from the atomistic force field. With the improved computational facilities, recent studies have been considered the explicit solvent condition, and shown that the explicit solvent effect is necessary to be consistent with the experimental data. Whereas the dendrimer structure is shrunk since the absence of the interaction between the dendrimer monomers and the solvent molecules in the vacuum state[62, 83], the explicit solvent condition successfully describes the swelling effect of
the dendrimer structure in solution[50, 51]. Using the explicit solvent condition, many studies have been well predicted the diffusion[63], \( \zeta \) potentials[48], and the radius of the gyration of the structures[50] of the dendrimers. In addition, several studies have been found that the behaviors of the dendrimers such as the response of the solvent pH in solution is sensitive to the atomistic force field[61]. After the consideration of the explicit solvent and the proper force field, the host-guest interactions can be understood using all-atom simulations, which are the encapsulation and the release of the guest molecules such as drug[64, 65, 29], gene[66, 67, 38], and pollutant[25] with dendrimers. However, for questions involving the interaction of many high generation dendrimers with explicitly modeled solvent molecules, the system size and time scale requirements are much larger than what is routinely feasible for atomistic simulations. For these situations, coarse-grained simulations and statistical field theories are commonly used to provide a view of mesoscale structures in place of atomistic force fields (Figure 2.1(d)).

Coarse-grained simulations have been performed to understand the behaviors of the higher generation of dendrimers and systematically study the generic features of the dendrimer complex with the variation of several parameters such as multivalent counterions, concentration of salt ions, generation of the dendrimer, and functional group on the surface of the dendrimer. In general, freely jointed bead-spring model, one of the simplified coarse-grained models, has been widely used to understand the interactions between a dendrimer and salt ions or guest molecules considering a comprehensive parameter space. Using this coarse-grained model, the transition of the conformational state of the dendrimer was explained by the electrostatic interaction between salt ions and the charged surface group of the dendrimer and the osmotic pressure drop inside the dendrimer[75]. Also, the binding chemistry between a dendrimer and a polyelectrolyte chain have been explained by the balance between electrostatic interaction and bending interaction[73, 74]. However, there is the difference of the detailed molecular behavior of the binding mechanism between dendrimers and guest molecules such as binding sites compared to the all-atom simulations. For this reason, more accurate coarse-grained potentials of the dendrimer system are necessary to explain the binding behaviors. MARTINI coarse-grained force field has been widely used to more accurately describe the binding phenomena
of the dendrimer system compared to the simplified model\cite{91, 37, 84}, but this force field is generalized for studying the interaction between a lipid membrane and a protein, so the MARTINI coarse-grained model does not well explain the binding behaviors of dendrimers in some cases. To overcome this limitation, the force matching method has been applied to derive the coarse-grained potential at a specific system. However, to consider the system including higher generation of the dendrimers, the solvent-free coarse-grained model based on the force matching method should be considered because of the memory requirement\cite{49}. However, the absence of the interaction of the solvent molecules provides the significant configurational entropy loss inside the dendrimer like the dendrimer in gas phase. To improve this, a new coarse-grained modeling scheme was developed, which combines a systematic, solvent-free multiscale coarse-graining algorithm for a flexible dendrimer with an existing coarse-grained solvent model\cite{49}.

Of the mesoscale simulations, statistical field theory has been applied to understand the equilibrium structures of the complex of lipid membrane and dendrimers (charged nanoparticle) based on the free energy calculation\cite{89, 90}. The stable pore formation of the membrane was explained by the electrostatic interactions between a charged particle and the membrane, and the membrane tension.

Overall, multiscale strategies for the complex systems of dendrimer with guest molecules in solution should be determined based on the characteristic temporal and spatial length scales.
References


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CHAPTER 3. Fluorescence resonance energy transfer between phenanthrene and PAMAM dendrimers


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3.1 Abstract

We describe herein an adsorption-induced energy transfer between phenanthrene, a major environmental pollutant, and a fluorescently labeled dendrimer acting as a host molecule. We find experimentally that such energy transfer is the most efficient at a solvent pH of 8 and for a phenanthrene:dendrimer molar ratio of 1:2. Using molecular dynamics simulations we show that the strongest binding interactions occur between phenanthrene and the primary amines of the dendrimer. The simulations provide evidence that at low pH, phenanthrene-phenanthrene interactions are favorable and compete with phenanthrene-dendrimer binding. This study offers a new scheme for detecting dendrimer molecular assembly and a physical basis for exploiting dendrimer nanotechnologies for water purification and environmental remediation.

3.2 Introduction

Dendrimers are synthetic polymers which are constructed using repetitive chemistry[1]. The structure of a dendrimer consists of a central core and layers of symmetric branches, or "generations", emanating out of the central core. Dendrimers can be synthesized with the structural precision of a small molecule, yet provide the functional advantages of a macromolecule. Since dendrimers such as poly(amidoamine) (PAMAM) have been shown as largely bio-benign, they have been used to modify carbon nanotubes with specific cancer cell targeting
folic acids[2], and as hosts for the encapsulation of DNA, drugs, prodrugs, and contrast agents for drug delivery and MRI imaging[1, 3]. Owing to their unique physical properties (low viscosity, nanosize, and hydrophobicity)[1, 4], dendrimers, along with their structurally relevant but defective counterparts-hyperbranched polymers-have been used as coating additives, viscosity mediators, and inhibitors for the growth of gas hydrates in oil pipelines[1].

It has been shown from both experimental[5, 6, 7, 8, 9] and simulation studies[10, 11] that the structure (size, flexibility) and surface charge of dendrimers, especially that of PAMAM, can be readily controlled by altering solvent pH and electrolyte strength. As a result, dendrimers have recently been proposed, and demonstrated, as effective loading and releasing agents for the chelation of heavy metal ions such as Cu(II), for the removal of perchlorates, pesticides, volative organic compounds, and polychlorinated biphenyls in drinking water, and for the recovery of uranyl from wastewater streams[5, 6, 7, 8, 9, 12].

In contrast to the vast promise of biological, environmental, and industrial applications for dendrimers, the physical assembly of dendrimer hosts and their guest molecules remains poorly understood[13]. Here we present a spectrofluorometry study of the interaction between phenanthrene (Phe) - a polycyclic aromatic hydrocarbon and a major environmental pollutant[14, 15, 16], and a PAMAM dendrimer (Scheme 3.1). By detecting the energy transfer between the autofluorescent Phe and the fluorescently labeled PAMAM dendrimer, we obtain the molar ratio and solvent pH dependence to determine their optimal binding conditions. In complement to the experimental measurements, which aim at offering a new detection scheme for dendrimer-based nanotechnologies, we also conduct molecular dynamics simulations to probe the microscopic details of binding between Phe and PAMAM, from acidic to basic conditions.

3.3 Methods

The initial structure of the G5-PAMAM dendrimer was obtained from Maiti et al[17]. All molecular dynamics simulations were carried out using LAMMPS[18], which is an open source code for large-scale molecular dynamics simulation. The Dreiding force field[19] was used to
Figure 3.1: **Experimental scheme.** (Left panel) Free Phe molecules are excited by UV light. Free PAMAM dendrimer is non-fluorescent. (Right panel) FRET induced fluorescence quenching of Phe and emission of dye-labeled dendrimer upon their binding.

Figure 3.2: a) Fluorescence spectra indicate the occurrence of FRET between Phe (donor, in purple) and PAMAM dendrimers (acceptor, in blue) upon mixing (green). Molar ratio of Phe:PAMAM is 1:2, and solvent pH = 8. b) Spectra of a mixture of Phe and PAMAM dendrimers. An addition of 1 mL of Phe at time 9.2 min caused an anti-correlation between the donor and acceptor spectra, followed by an equilibration in approximately 2 min. Sample original pH: 7.

<table>
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</table>
Figure 3.3: a) Molar ratio dependence of FRET efficiency for Phe and labeled G5-PAMAM dendrimers, at pH 8. b) pH dependence of FRET efficiency for Phe and labeled G5-PAMAM dendrimers. Incubation time 24 h. The molar ratio of Phe:PAMAM is 1:2.

describe the molecular interactions of the PAMAM and Phe molecules. Water is explicitly modeled using the TIP3P force field[20]. In this simulation, we fixed the cell size to a cubic box length of 125 Å. The PAMAM dendrimer was placed in the center of a cubic box with periodic boundary conditions. The primary and tertiary amines in the dendrimer were protonated using DS Visualizer 2.0 to model low, neutral, and high pH effects as follows[10]: i) high pH (> 10), no protonation; ii) medium or neutral pH (∼ 7), all primary amines protonated; iii) low pH (< 4), all primary and tertiary amines protonated. To make each system charge neutral, we added Cl- counterions near the protonated amine groups of the dendrimer. The characteristic simulation parameters about protonation and explicit water molecules are given in Table 3.1. To get the equilibrium structure of PAMAM with explicit water molecules at each pH case, the relaxed structures were subjected to one temperature cycle (from 300 to 600 K and back) using NPT Nose/Hoover temperature thermostat[21] and Nose/Hoover pressure barostat[22]. The time constants for the Nose-Hoover temperature thermostat and pressure barostat were 0.1 ps and 1 ps, respectively. This generated a system trajectory consistent with the isothermal-isobaric ensemble; the structures were equilibrated at 300 K and 1 atm for 500 ps. After this initial equilibration, the water molecules were removed, and 25 Phe were added to the equilibrated dendrimer structure. Water molecules were then reintroduced
to the system. Molecular dynamics simulations of dendrimer and Phe with explicit water molecules were carried out for 2 - 4 ns at 300 K and 1 atm. To put the Phe molecules near the surface of the PAMAM dendrimer, a spring force acting between the center of masses of the Phe and the dendrimer was used for 10 ps, and then turned off. Equilibration was determined by calculating the all-atom pair distribution function (not shown) at different time points during the simulation and noting the time beyond which no significant variations in the function were present. We note that the structures of the PAMAM-Phe complexes reached equilibrium an order of magnitude faster than would be expected based on previous molecular dynamics simulations of PAMAM-DNA involving explicit solvent and counterions[23]. However in the latter case, the complexation was driven mostly by electrostatic interactions between the positively charged PAMAM and the negatively charged DNA, while the present complexation driving force is due to hydrophobic interactions. In all simulations the velocity-Verlet time stepping scheme was used and the integration time step was 1 fs. PPPM (particle-particle particle-mesh) was used for electrostatic interactions and a cutoff of 10 Å was used for van der Waals interactions.

We characterized the interaction between Phe and the surface of the PAMAM dendrimer using the normalized distribution of Phe molecules, the pair distribution function (PDF) and the second-order Legendre polynomial, P2. The normalized distribution function of Phe molecules is computed by the distance between the centers of mass of the Phe molecules and the center of mass of the dendrimer. The PDF was calculated by the distance between nitrogens in primary amine group (near surface of dendrimer) and the centers of mass of Phe. To study the alignment of the Phe with the nitrogen atoms in the primary amine group of PAMAM, P2 was calculated from the expression[24]:

$$P_2 = \frac{1}{2}(\langle \cos^2 \theta \rangle - 1)$$  \hspace{1cm} (3.1)

where $\theta$ is the angle between the normal vector of the Phe plane and the vector from the nitrogen in the primary amine group of PAMAM to the center of mass of Phe molecule. The brackets indicate that the value is averaged over the production simulation time (i.e., after
removing the spring force between PAMAM and Phe) and all the Phe molecules. Each data point is calculated based on block averaging method, with block sizes of 250 ps.

### 3.4 Results and Discussion

#### 3.4.1 Fluorescence Resonance Energy Transfer

Fluorescence resonance energy transfer (FRET) denotes the dipolar interaction between a fluorescence donor and a fluorescence acceptor, which are separated by a spatial distance of 10 nm or less\[25\]. Over the past two decades the FRET technique has proven effective for detecting inter- and intramolecular fluctuations and interactions, at both ensemble and single-molecule levels\[26, 27, 28, 29, 30\]. Experimentally, FRET is manifested by an anticorrelation between the emission spectrum of the donor and that of the acceptor. As exemplified in Figures 3.2a and 3.2b, upon mixing Phe (fluorescence donor, guest) with Alexa Fluor 350-labeled PAMAM dendrimers (fluorescence acceptor, host) and when excited at 250 nm, the fluorescence peaks of Phe at 350 nm, 365 nm, and 380 nm were drastically reduced (purple to green), while the fluorescence peak of the labeled dendrimers at 443 nm was enhanced (blue to green) (Fig. 3.2a).

In addition to displaying an anticorrelation between the fluorescence spectra for the Phe and the labeled PAMAM dendrimer, Fig. 3.2b further presents time traces of these spectra when Phe was added to a Phe-dendrimer mixture. It took approximately 2 min for the fluorescence signals to recover and stabilize. Our ratio and pH dependence data, therefore, were collected from samples incubated overnight to ensure stable binding between the Phe and the PAMAM dendrimers.

**3.4.1.1 FRET Dependence on Molar Ratio**

Figure 3.3a shows the molar ratio dependence of FRET between Phe and Alexa Fluor 350-labeled PAMAM dendrimers. An increased FRET efficiency was found when the molar ratio of Phe:PAMAM was decreased from 25:1 to 1:2, probably due to the enhanced dispersion (or reduced aggregation/stacking) of the Phe molecules at the lower molar ratios. An optimal molar ratio of Phe:PAMAM, shown here at 1:1 to 1:2, ensured an efficient energy transfer between the fluorescence donor and the acceptor. A further reduction in the molar ratio (1:4)
yielded a slightly decreased FRET efficiency, probably resulting from the less efficient energy transfer between the donor and its multiple competing acceptors. The error bars in Figure 3.3 were mainly caused by the nonuniform labeling of the dendrimers, aggregation of the Phe molecules, and handling (pipetting and mixing) of the Phe-PAMAM samples.

3.4.1.2 FRET Dependence on Solvent pH

The pH dependence of the FRET between the Phe and the labeled dendrimers is shown in Figure 3.3b, using the optimal Phe:PAMAM molar ratio of 1:2. A maximal FRET efficiency was obtained for the mixture at pH 8, while both acidic and basic conditions caused a reduction in FRET efficiency. At low pH (4 and 6), the primary and the tertiary amines of the dendrimers are protonated[10], which would swell the dendrimer branches to encourage the entry of water; such hydrophilic conditions in the interiors of the dendrimers would be less accessible for the hydrophobic Phe and limit binding. At neutral pH (7 and 8), only the primary amines of the dendrimers are protonated[10], and the Phe is favored to bind to the interiors of the dendrimers through hydrophobic interactions. At high pH (10), the dendrimers are fully neutralized[10] and become less water soluble. The aggregation of the dendrimers, as indicated by a 20% reduction in absorbance at pH 10 vs. pH 8 (data not shown), provided fewer interior sites for the Phe molecules to bind. Furthermore, aggregation of the dendrimers at high pH would shield their functionalized Alexa Fluor 350 dyes from being accessed by the Phe molecules, thus hindering the FRET process from occurring.

3.4.2 Molecular Dynamics Simulations of the Phe-PAMAM complex

To probe the molecular details of the binding between Phe and PAMAM, atomistic molecular dynamics simulations were conducted for one G5-PAMAM dendrimer and 25 Phe molecules in explicit water at low (< 4), neutral (~ 7), and high pH (> 10). Representative snapshots of the G5-PAMAM dendrimer complexed with Phe are shown in Figure 3.4. These images show the solvent pH effects on dendrimer conformation. The dendrimer adopts an open conformation at low pH and increasingly compact as the pH is raised. This is consistent with previous experimental[31, 32, 33] and theoretical[10, 34, 23, 35, 36, 37, 38] studies on PAMAM
Figure 3.4: Representative images of G5-PAMAM dendrimer with 25 of phenanthrene molecules after 1 ns as an atomistic molecular dynamics: a) low pH, b) neutral pH and c) high pH. The dark red arrows indicate the stacked Phe molecules.

Figure 3.5: Normalized distribution of Phe relative to the center of mass of the dendrimer a) low pH, b) neutral pH, and c) high pH. RN indicates the radius of gyration for the nitrogen atoms in the primary amines.

dendrimer conformation in solution.

3.4.2.1 Distribution of Phe in the Phe-PAMAM Complex

Characterizing the distribution of Phe among the branches of the PAMAM dendrimer gives a good indication of how effectively the dendrimer encapsulates the guest species. Figure 3.5 shows the normalized distribution of Phe molecules relative to the center of mass of the dendrimer for the low, neutral, and high pH cases. For reference, the radius of gyration for the nitrogen atoms in the primary amines, $R_N$, is shown to indicate the approximate surface of the PAMAM dendrimer. Notably, the value of $R_N$ decreases from 29 Å to 22 Å when the solvent pH is increased from low to high (Fig. 3.5a to 3.5c), indicating a more compact conformation of the dendrimer at high pH due to its neutralized primary and tertiary amines[10]. At low
Figure 3.6: a) Pair distribution function (PDF) $g(r)$ versus the distance between nitrogens in primary amine group of G5-PAMAM and the center of masses of Phe at 1 ns for low pH (blue line), neutral pH (purple line), and high pH (green line). b) Phe order parameter $P_2$ versus the distance between nitrogens in primary amine group and the center of masses of 30 Phe for low pH (blue line), neutral pH (purple line), and high pH (green line).

and neutral pH, Phe distributes throughout the interior of the dendrimer (defined as distances $< R_N$), while at high pH the interior distribution of Phe is limited to locations that are close to the dendrimer surface. More Phe molecules penetrate into the interior of the dendrimer at neutral pH than low pH; this agrees with the experiment measurements which showed a higher FRET efficiency at neutral pH than low pH (Fig. 3.3b). In the case of high pH, Fig. 3.5c shows that Phe molecules distribute preferentially at two locations (20 Å and 25 Å near the surface area of the dendrimer. The more ordered arrangement of Phe is a direct result of the dendrimer structure being more compact at high pH.

### 3.4.2.2 Interaction between Phe and PAMAM Dendrimer Surface

To characterize the interaction between the surface of the dendrimer and Phe molecules as a function of solvent pH, we calculated the pair distribution function $g(r)$ for distances between nitrogens in the primary amines of the dendrimer and the center of mass of the Phe molecule. These results are shown in Figure 3.6a. At low pH, the first peak of $g(r)$ appears at 3 Å, followed by a second peak at 9.3 Å. At neutral pH, the first peak also appeared at 3 Å, but with a higher intensity than at low pH. This is another indication that there is a stronger interaction between G5-PAMAM and Phe, through Phe binding with the primary amines, at
neutral pH than at low pH, which is consistent with the FRET measurement (Fig. 3.3b). At high pH, the first peak in g(r) appears at 6.8 Å, where once again, the compact structure of the dendrimer branches at high pH prevent close interactions with the primary amines.

The propensity for Phe to interact with the primary amines of the dendrimer suggests that the Phe molecules adopt preferred orientations when binding interactions are observed to be strong. To assess the orientation order between the nitrogens in the primary amine groups and the Phe molecules, the second-order Legendre polynomial, $P_2$, was calculated. Values of $P_2$ equal to one indicate that the Phe molecule is aligned with the normal vector from the aromatic ring plane pointing toward the primary amine groups. At $P_2$ equal to zero, the Phe molecule assumes a completely random orientation. Negative values of $P_2$ mean that the aromatic ring plane of the Phe molecule is perpendicular to the amine group of the PAMAM dendrimer.

Figure 3.6b shows $P_2$ values as a function of the distance between nitrogens in the primary amine groups of the dendrimer and the center of mass of the Phe molecule, at the three different pH cases. In cases of low and neutral pH, the $P_2$ value reached 1 at 2.5 Å, with a lower degree of orientational order ($P_2 \sim 0.5$) at 5 - 5.5 Å. In the high pH case, the highest value of $P_2$ attained was 0.7 at 3.5 Å, followed by random orientations ($P_2 < 0$) between 6 - 8 Å.

Also, the snapshots (Fig. 3.4) illustrate how the structure of the dendrimer-Phe complex changes from open to compact with increasing pH. At lower pH the dendrimer has a more open structure which permits the Phe molecules to align parallel to the primary amine groups of the dendrimer and, in some cases, to stack parallel to other Phe molecules (Fig. 3.4a). These ordered arrangements of the Phe molecules are expected to interfere with the energy transfer from the Phe to the PAMAM, and result in low FRET efficiencies at low solvent pH (Fig. 3.3b).

### 3.5 Conclusions

In summary, we have devised a novel FRET scheme for detecting dendrimer-based supramolecular assembly. In this scheme a PAMAM dendrimer acted as a host molecule for the adsorption and encapsulation of Phe guest molecules. Optimal FRET efficiencies for Phe-PAMAM bind-
ing were determined experimentally at an intermediate solvent pH 8 and a Phe:PAMAM molar ratio of 1:2 (Fig. 3.3). Both the pH and molar ratio dependence can be understood as a consequence of the physiochemical state of the Phe coupled with that of the PAMAM. At high pH the primary and tertiary amines of the PAMAM dendrimer were neutralized, which subsequently contracted its radius of gyration and shielded its interior from being accessed by the Phe molecules (Figs. 3.4 and 3.6). Such neutralization further promoted mutual aggregation of the dendrimers, discouraged functionalized dyes on the dendrimers to be accessed by the Phe, and consequently reduced FRET efficiency (Fig. 3.3b). At low pH, stacking of the Phe molecules (Fig. 3.4a) hindered their interaction with the cationic PAMAM dendrimers, which inevitably impaired the energy exchange between the Phe and the PAMAM (Fig. 3.3b).

In addition to the physiochemical states of the Phe and the PAMAM, the efficiency of dye labeling (2:1 to 4:1 for dye to dendrimer, see the Experimental Section) could also have played a role in defining the optimal molar ratio of the Phe:PAMAM for FRET (Fig. 3.3a). It is conceivable that a lower labeling efficiency would reduce the FRET efficiency for all pH values, and a higher labeling efficiency would undesirably alter the properties (amphiphilicity and polarity) of the PAMAM dendrimer. The direct binding between Phe (hydrophobic) and Alexa Fluor 350 dye (hydrophilic) is energetically unfavorable. On the other hand, the sensitivity and temporal resolution of our experiment may be significantly improved by using single molecule devices, an effort which will be pursued by the authors in the near future. It is our belief that the methodologies and understanding from this study will have implications and applications for the design and detection of dendrimer supramolecular assembly, water treatment, and environmental protection, and may prove beneficial for the development of dendrimer nanomedicine where drugs and prodrugs—much like Phe—are prevalently hydrophobic and aromatic.

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References


CHAPTER 4. Multi-scale modeling for binding PAMAM dendrimers with organic molecules

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4.1 Abstract

The multiscale methods are used to understand the dendrimers binding with organic molecules, and one of the specific examples is G5 polyamidoamine (PAMAM) dendrimers with phenanthrenes (Phe). In the coarse-grained method, the solvent-free condition model is applied to the system based on the multiscale coarse-graining (MS-CG) methods to enable the calculation of a complex at high concentrations to compare with the experimental results. From this approach, we study the possible binding sites of phenanthrene molecules to the PAMAM dendrimer. The MARTINI CG model is also used to explain the binding behavior of the PAMAM-Phe complex, but in this model, the CG Phe molecules are highly bound to PAMAM dendrimers compared with the atomistic simulations. For the comparison with the molar ratio dependency from the experimental results, the configuration of the simulation increases from 1:8 to 1:1 (PAMAM:Phe), and the binding capacity of the CG simulations is qualitatively consistent with the experiments. And the binding energies of PAMAM with Phe molecule are calculated at the ab-initio level to study the binding sites of PAMAM dendrimer. Also, pair interaction energy decomposition analysis (PIEDA) is carried out to explain the binding interactions according to the binding sites of PAMAM dendrimer.
4.2 Introduction

Dendrimers are highly branched synthetic macromolecules, and the size of a dendrimer can be designed by the uniform stepwise reactions for generational growth. Among a variety of dendrimers, polyamidoamine dendrimers (PAMAM) are commonly used since the first report by Tomalia and co-workers in the mid-1980s[1]. Physical properties of PAMAM dendrimers such as pH sensitivity, interior density, targeted binding affinity, and degradability can be controlled by engineering these amine functional groups because of the tertiary amines for the interior structure and the primary amines for the surface groups[2]. PAMAM dendrimers can serve as a host for therapeutic and imaging agents[3, 4, 5], and a high performance chelating agent for the removal organic pollutants and toxic metals from water and soil[6, 7]. As a consequence, a clear description of the host-guest interactions with PAMAM dendrimers is an essential factor for many applications[3, 4, 2, 8].

The interactions of PAMAM dendrimers with guest molecules have been studied by numerous experimental techniques. SAXS and SANS experiments are used to study the size and the shape of the microstructures[9] and UV-vis absorption and fluorescence spectroscopies have been carried out to investigate the complex of PAMAM dendrimers with metal ions like gold particles[10, 11]. NMR and FRET (Fluorescence resonance energy transfer) spectroscopies have been also applied in other to study the dynamics for the binding mechanism between dendrimers and DNA, medicines, or metal ions[12, 13].

In parallel, various theoretical and computational approaches have been used to examine the physical behavior of dendrimers[14, 15]. Atomistic molecular dynamics (MD) simulations are suited to investigate the solution behavior and binding phenomena of single dendrimers in explicit solvent directly compared with experimental data, providing microscopic structures, solvation, and electrostatic information of dendrimers[16, 17, 13]. However, for questions involving the interaction of many high generation dendrimers with explicitly modeled solvent molecules, the system size and time scale requirements are much larger than what is routinely feasible for atomistic MD. For these situations, coarse-grained (CG) molecular models are commonly used in place of atomistic force fields.
There are a number of CG approaches reported in the literature; most can be classified into one of two categories. In the first category, termed indirect parameterization, are methods in which the potential parameters of a pre-selected analytical form are optimized by calibration against thermodynamic or structural properties. An example is the MARTINI force field for biological molecules[18], whose parameters are based on oil/water partitioning coefficients. In the second category, called direct parameterization, the CG potentials are determined from an explicit atom MD simulation. One example is the multiscale coarse-graining (MS-CG) method, which derives CG parameters from force matching[19]. In the former category, a force field such as MARTINI can be easily applied whenever the target system is changed with little to no reparameterization required. In the latter category, the MS-CG method has the advantage of being systematic; the CG force field is evaluated from data collected along the trajectories of atomistic MD simulations. However, although the MS-CG method has been implemented for lipids in an efficient way[20], storage and memory requirements make it computationally difficult to derive CG potentials for complex biological systems with greater than ten defined CG site interaction types[19]. An alternative approach, called the solvent-free MS-CG model, derives effective CG potentials between sites on the solute molecules while integrating out the explicit representation of the solvent molecules[21]. This approach was recently implemented for a lipid bilayer[21].

By applying these current CG MD methodologies, the interactions between PAMAM dendrimers and a lipid bilayer were studied[22, 23]. However, these coarse grained approaches are not much applied for explaining the interactions on the complex of PAMAM dendrimers with organic molecules such as DNA and drugs. In this article, we demonstrate the multi-scale modeling for studying the binding mechanism of 5 poly(amidoamine) (G5-PAMAM) dendrimers and phenanthrene (Phe), one of specific organic molecules, in water to study the host-guest interactions with PAMAM dendrimers using the full atomistic molecular dynamics and the above coarse-grained methods. To accurately study the molecular binding interactions between PAMAM dendrimers and Phes, we also perform the full electron calculation on the complex of one Phe with a branch of PAMAM dendrimer using the ab-initio level calculations.
4.3 Experimental Methods

4.3.1 All-Atom Simulation

The initial structure of the G5-PAMAM dendrimer was obtained from Maiti et al[24]. The general AMBER force field (GAFF)[25] was used to describe the molecular interactions of the PAMAM dendrimers and Phe molecules, and the TIP3PBOX water model[26] were applied to the water molecules. All simulations including the coarse-grained methods were performed using GROMACS[27]. The primary amines in the dendrimer were protonated to model neutral pH. To make the system charge neutral, we added 128 Cl\textsuperscript{−} counterions near the protonated amine groups for one dendrimer. To prevent the energy entrapment, the simulated annealing was carried out under 1 atm with heating and cooling rate of 50K per 10000 steps from 300K to 500K (4 repeated cycles). After the optimized structure of G5 PAMAM dendrimer was obtained, we randomly added 27 G5-PAMAM dendrimers and 216 Phe to a box which the cell size is fixed to a cubic box length of 35 nm, and solvated the system with 1389280 explicit water molecules and 3456 Cl\textsuperscript{−} counterions. The whole system equilibrated in the \textit{NPT} ensemble. The atomistic configurations and force data for the MS-CG method were collected from a 5 ns run in the \textit{NVT} ensemble; a total of 5000 configurations were sampled for generating the solvent-free CG potentials. The system temperature was maintained at 300 K using the Nosé-Hoover thermostat with a relaxation time of 0.1 ps. In all the atomistic simulations, the time step was 2 fs.

4.3.2 Coarse-Graining Methods

We performed coarse-grained(CG) molecular dynamics(MD) simulations of a complex of G5 PAMAM dendrimers and Phes using MS-CG method and MARTINI model in order to interpret the observed mesoscopic behavior from experimental data. For the CG models, dendrimers and phenanthrenes have been grouped into four CG sites as illustrated in Figure 5.1. The coarse-grain mapping scheme used by Ref. [23] was applied; this scheme is based on the MARTINI CG force field[18].

For the MS-CG method, the solvent-free CG condition is necessary for this large reference
Figure 4.1: Atomistic (left) and coarse-grain (right) representation. In the atomistic structure, gray spheres represent carbon; blue, nitrogen; red, oxygen; and white, hydrogen. In the coarse-graining structure, blue spheres represent $N_0$; red, $N_{da}$; and green, $Q_d$. (a) PAMAM (for clarity, only the core is shown). For coarse-graining, blue circles of the atoms map into $N_0$; red, $N_{da}$; and green, $Q_d$. (b) Phenanthrene. One benzene molecule is represented by single CG site, $SC_4$. (c) A snapshot of G5 PAMAM dendrimer for CG-mapping.
system (4292364 atoms). If a CG water site was explicitly included in the MS-CG procedure, there would be 361,630 total CG sites and a memory requirement of > 11 GB per configuration. Solvent-free CG potentials for PAMAM and Phe in water at neutral pH were obtained by the force matching procedure described elsewhere\cite{28, 29, 21} based on the previous sampling of the atomistic simulations. A brief summary follows. The first step of the MS-CG procedure is to obtain the atomic positions and forces sampled from an equilibrated, atomistic MD simulation. The degrees of freedom in the atomic configurations are reduced by mapping groups of atoms to defined CG sites and computing the net forces acting on the coarse-grained sites. If $f_{ij}(r_i, r_j)$ is the non-bonded CG force acting on the $i^{th}$ CG site due to the $j^{th}$ CG site, and it is assumed to depend linearly on $m$ unknown parameters $p_1, p_2, \cdots, p_m$, the CG pair force is expressed by $f_{ij}(r_i, r_j, p_1, p_2, \cdots, p_m)$. To obtain the CG potential in a systematic way, cubic spline or B-spline functions are fitted to the CG forces to enable a smooth curvature across mesh points. Specifically, B-spline functions improve the force matching performance because they can reduce the memory requirement and increase the accuracy\cite{19}. Then, the $m$ unknown parameters are optimized based on a least-squares method, which minimizes the difference between the net forces and the fitted pair forces and is expressed as

$$\chi^2 = \sum_{i=1}^{N_i} \left| F_{i}^{\text{atomic}} - F_{i}^{\text{predicted}}(r_i, p_1, p_2, \cdots, p_m) \right|^2, \quad (4.1)$$

where $F_{i}^{\text{atomic}}$ and $F_{i}^{\text{predicted}}$ are the reference atomistic force field and the calculated force field, respectively. The parameters obtained in this way from each configuration are averaged over the total number of atomic configurations sampled\cite{19}. In this work, the solvent-free potentials were calculated under the solvent-free condition of the force matching method, in which the solute molecules only are considered during the reference atomistic MD simulation to get the CG pair forces, and thus, the solvent effect is implicitly included in the CG potentials of the solute molecules\cite{21}.

For the solvent-free CG potentials of bonded interactions, a harmonic potential was applied:

$$V(r) = \frac{1}{2} k_r (r - r_0)^2 \quad (4.2)$$

$$V(\theta) = \frac{1}{2} k_\theta (\theta - \theta_0)^2 \quad (4.3)$$
where \( V(r) \) and \( V(\theta) \) are bond and angle potentials, and \( k_r \) and \( k_\theta \) are the bond force constant and the angle force constant, respectively. The parameters for bonded potentials were determined by inverse Boltzmann fitting of the bond distributions from the atomistic simulation[30]. For the bond angle potentials, the angle force constants, \( k_\theta \), are chosen in an iterative way because of the flexibility of the dendrimer; the equilibrium angles are obtained from the angle distributions.

We also used the CG MARTINI force field to model the PAMAM dendrimers and Phes. For G5 PAMAM dendrimer, the force constants were reparameterized by Ref. [23], and for Phes, SC4, CG MARTINI atom type, is used because of the benzene rings. The whole complex consists of 27 CG PAMAM dendrimers, 216 Phe molecules, and 346456 CG water with 3456 Cl\(^-\) counterions.

The CG MD simulations were carried out for 100 ns in the \(NVT\) ensemble with the same system size as the atomistic simulations. The temperature was maintained at 300 K using the Berendsen thermostat with a relaxation time of 1 ps. A time step of 10 fs was used in the MS-CG method, and a time step of 32 fs was used in the MARTINI model for the CG MD simulations.

4.3.3 Quantum Mechanics

The full electron calculations were carried out to study the binding interactions between PAMAM dendrimers and phenanthrenes. In these ab-initio calculations, the structure of PA-
MAM dendrimers is divided into three binding candidates: the tertiary amine, the secondary amide group, and the protonated primary amine at the end of the branch. The only one branch of PAMAM dendrimer in the tertiary amine is considered for the calculation, and the other branches are removing and substituted by ethane molecules like Figure 4.2.

The geometry optimization runs of the branch of PAMAM dendrimer and Phe including the complex of the branch and Phe molecule, and the hessian calculations of them are implemented at the ROHF/PCM/6-31G(d) level of theory, and the energies are determined through MP2//ROHF/PCM/6-31G(d) calculations on the ROHF fully optimized geometries. The solvent effects are implicitly taken care of by the polarized continuum model (PCM)[31]. After the optimized structures of the branch of PAMAM dendrimer and Phe molecule are obtained, Phe molecule are inserted near one of the binding sites of the branch. The binding energies are calculated by this formula:

\[
\Delta E_{PAMAM-PHE} = (E_{PAMAM-PHE} + ZPE_{PAMAM-PHE}) - (E_{PAMAM} + ZPE_{PAMAM}) - (E_{PHE} + ZPE_{PHE})
\]

(4.4)

where \( E_{PAMAM-PHE} \) is the binding energy of the branch of PAMAM dendrimer and Phe molecules, and \( ZPE_{PAMAM-PHE} \) is the zero point energy (ZPE) of the complex, which is calculated by the second derivatives of the energy[32]. It is used for correction factors to calculate the binding energies[32, 33].

To study the pair interaction between Phe molecule and the binding sites of PAMAM branch, we also performed the pair interaction energy decomposition analysis (PIEDA) at the FMO2/MP2/6-31G(d) level[34, 35]. The interaction energy in PIEDA represents the strength of the affinities between two different fragments, which is based on the fragment molecular orbital (FMO) method introduced by Kitaura and co-workers[36, 35]. The main advantage of PIEDA is that the pair interaction generated by PIEDA is divided into the electrostatic (ES), the exchange-repulsion (EX), charge transfer and higher order mixed terms(CT+mix), and dispersions (DI) like this equation:
\[ \Delta E_{i,j}^{\text{int}} = \Delta E_{i,j}^{ES} + \Delta E_{i,j}^{EX} + \Delta E_{i,j}^{CT+\text{mix}} + \Delta E_{i,j}^{DI} \] (4.5)

Here, the GAMESS quantum chemistry package\cite{37} was used for all of the quantum mechanics calculations.
4.4 Results and Discussion

4.4.1 All-Atom and Coarse-graining simulations

The CG molecular simulations using two different CG approaches were carried out to study the molecular details of the binding between PAMAM dendrimers and Phe molecules. Before studying the binding capacity, the potentials and the structures from the CG simulations were compared with the atomistic results to validate the CG potentials.

Figure 5.S3 compares non-bonded CG potentials for the MARTINI model and the solvent-free CG model. In the pair interaction of the same CG atom types defined by Figure 5.1, the CG potentials of the solvent-free CG model become sharply repulsive at shorter distances rather than those of the MARTINI model. Interestingly, the interactions between $N_0$-SC$_4$ and $N_{da}$-SC$_4$ are related to the binding behaviors with Phe molecules, which consist of SC$_4$ CG atoms. As a consequence of the different CG potentials between two different approaches, the binding capacities between PAMAM dendrimers and Phe molecules calculated by these two CG method should be different.

In Figure 5.2, the site-site RDFs of between CG types of G5-PAMAM and Phe by the MARTINI model (the top row) and the solvent-free condition (the bottom row) are compared with those from the atomistic MD simulations to investigate the local structures. For all site-site RDFs shown, the intensities of the RDFs peaks are higher for the solvent-free condition (the bottom row) than for the atomistic simulations, indicating that dendrimer structures have aggregated for the solvent-free case because the solvent degrees of freedom are integrated out after applying the solvent-free condition to the MS-CG method, which the solvent effect is included implicitly to the system in the solvent-free CG model. For the MARTINI model, whereas the intensities of the RDFs for $N_0$-$N_0$ and $N_0$-$Q_d$ atom types are lower than those of the atomistic configurations, which are involved in the PAMAM dendrimer itself, the intensities of the $N_0$-SC$_4$ and $N_{da}$-SC$_4$ atom types are higher than for the atomistic simulations and even for the solvent-free condition, which are related to the interactions between PAMAM dendrimers and Phe molecules. These RDFs indicate that Phe molecules highly interact with the PAMAM dendrimers, so Phe molecules are too close to the dendrimers compared to the structures of
the atomistic simulations. In MARTINI model, the potentials are fitted to the pre-selected function, which is the Lennard-Jones potential, so there are limitations to describe the host-guest mechanism between PAMAM dendrimers and organic molecules. Recently, we develop the new methodology, the explicit solvent CG model, to overcome these unusual behaviors from the solvent-free CG and the MARTINI model compared to the all-atom simulations\[38\]. This new method is made by the combination of the solvent-free CG and the MARTINI model, and the RDFs of the method are shown in Figure 5.2 to show the improvement.

Figure 4.5 shows partial snapshots of the simulation box for atomistic simulation, MARTINI model, and the solvent-free CG model to investigate the binding of Phe molecule to PAMAM dendrimer, which are obtained by the radius of the range between 1.5 and 2.0 nm from the molecular center of mass of one of Phe molecules to see the detail configuration of the complex for the PAMAM and Phe molecule. In the configurations of the atomistic simulation and the solvent-free condition CG simulation, the Phe molecules are most likely placed near the amide groups in the atomistic configurations, and the \(N_{da}\) atom type is related to the amide groups of the dendrimers. However, in the MARTINI model, unlike the atomistic and the solvent-free CG simulations, the Phe molecules are located near the center of the PAMAM dendrimers. Additionally, the partial snapshot of the explicit solvent CG model are provided to compare with other CG approaches in Figure 4.5.

To compare the binding interactions between G5-PAMAM and Phe with the fluorescence resonance energy transfer (FRET) experimental data\[13\], the two-dimensional probability distributions functions, \(P(r)\), for the distance from Phe to dendrimer core are calculated in Figure 4.6, and the average location of each dendrimer branch point are shown to recognize the binding site of Phe molecules in the PAMAM dendrimers. Comparing the solvent-free case to the reference atomistic simulation shows that the peak intensity of the solvent-free model is nearly four times higher, but the most preferred binding site of Phe molecules in the PAMAM dendrimer using the solvent-free CG simulations are in reasonable agreement, whereas the most preferred position of Phe molecule using the MARTINI model is near the core region of the dendrimer. These results are consistent with the previous data, which are the CG potentials and the RDF
analysis. Both results indicate that the binding interactions between the PAMAM dendrimers and Phe molecules in MARTINI model are different from those in the solvent-free CG model. In contrast to the CG potentials by solvent-free CG model, which are obtained by the specific procedure for the system, the CG potentials by the MARTINI model are parameterized based on the CG MARTINI atom types, so it is hard to describe the binding like this system. To investigate the effect of molar ratio on binding capacity in the model system, we increased the number of G5-PAMAM dendrimers from 27 (∼1:10 molar ratio) to 216 (∼1:1 molar ratio), while keeping the number of Phe fixed at 216 for directly comparing the interaction between PAMAM dendrimers and the Phe molecules with the FRET experiments, which contain that FRET efficiency increased as the molar ratio of PAMAM:Phe incread from 1:10 to 1:1[13]. In the simulation with 216 dendrimers, which represents 1:1 molar ratio of PAMAM:Phe, peak intensity has increased compared with the intensity by the 27 dendrimers, which represents 1:10 molar ratio, consistent with the molar ratio dependency effect on binding capacity observed by experiment[13]. This is a large system compared to 27 PAMAM dendrimer, and this scale (∼10^7 atoms)is not feasible with atomistic MD simulations.

4.4.2 Quantum Mechanics calculations for the binding interactions

Studies of the electronic structures and the binding energies of the complexes were carried out at the ROHF/PCM/6-31G(d) and MP2//ROHF/PCM/6-31G(d) levels to investigate the PAMAM branch interacting with Phe molecule. To enable the optimizations of the structures and the calculations of the second derivatives of the energies at the ROHF/PCM/6-31G(d) level, the G5 PAMAM dendrimer is simplified to the branch of PAMAM dendrimer, which two of the three branches from a tertiary amine are substituting by ethane to keep the geometrical effect of three branches for the tertiary amine (Figure 4.2). For the binding of Phe molecule, we select five different cases based on the initial position of Phe molecule: Amide-||, Amide-⊥, Tertiary amine-I, Tertiary amine-II, and (protonated) Primary amine. These regions are slightly different from the previous study for the branch of PAMAM dendrimer[32] because we found that Phe molecules are not easily binding with the core region of the G5 PAMAM dendrimer by the steric effects using the atomistic and the coarse-grained approaches, so the
Figure 4.4: Radial distribution functions (RDF) between CG types of PAMAM dendrimers and phenanthrenes from atomistic (solid line) and coarse-grained (dashed line) molecular dynamics simulations. The dashed lines in the upper figures represent the MARTINI model, and in the bottom figures the solvent-free CG model. For reference, the site-site RDF of the explicit solvent CG model (dotted line) is provided [38]. (a) the site-site RDF for $N_0-N_0$, (b) the site-site RDF for $N_0-Q_d$, (c) the site-site RDF for $N_0-SC_4$, and (d) the site-site RDF for $N_{da}-SC_4$. 
core of the G5 PAMAM dendrimer is neglected for the binding site. Since we considered the neutral pH of G5 PAMAM dendrimers, the solvation effect is necessary for the studies of the binding of PAMAM dendrimers and Phe molecules. Here, to deal with the water solvation effect, the polarized continuum model (PCM) method has been applied for all of the ab-initio calculations except the FMO calculation for PIEDA.

The calculation of the binding energies was performed at the MP2//ROHF/PCM/6-31G(d) using eq (4.4) after optimizing the structure of each binding case at the ROHF/PCM/6-31G(d). The zero point energies (ZPE) for the correction of the binding energies were obtained by the second derivatives of the energies, which were calculated at the ROHF/PCM/6-31G(d). The results of the binding energies are summarized in Table 4.1 for each case, and the interaction strength decreases in the order: Amide-⊥ > Amide-∥ > Tertiary amine-I > Primary amine > Tertiary amine-II. The optimized structures of the complex for PAMAM branch and Phe molecule are illustrated in Figure 4.7. In the optimized geometries, the possible hydrogen bonds between the hydrogen atoms of Phe and the oxygen and the nitrogen atoms of the branch are counted as one of the binding effects. At the case of amide-∥, which Phe molecule is initially place parallel to the amide of PAMAM branch, three possible candidate hydrogen atoms of
Figure 4.6: Two-dimensional probability distribution function, \( P(r) \), of phenanthrenes from the core of each dendrimer. The white circles indicate the branch points of the dendrimers. The numbers in the circles represent the generation of the dendrimer, and \( Q \) is the abbreviation of \( Q_d \). The color bars express the intensity of \( P(r) \). (a) All-atom model, (b) MARTINI model, (c) Solvent-free CG model (27 dendrimers), and (d) Solvent-free CG model (216 dendrimers). The \( P(r) \) data for the explicit solvent CG model were published in Ref. [38].
Phe molecules are closer to oxygen and nitrogen atoms of two amide groups. At the case of amide-⊥, which Phe molecule is perpendicularly located at the amide of PAMAM branch at the beginning, five possible candidate hydrogen atoms of Phe molecules are closer to oxygen and nitrogen atoms of two amide groups. For tertiary amine-I, which initially Phe molecule insert into the region of tertiary amine, three possible candidate hydrogen atoms of Phe interact with the tertiary amine and the amide groups. In the case of tertiary amine-II, which initially Phe molecule insert into the region of tertiary amine at the end of the branch, one possible candidate hydrogen atoms of Phe interact with the tertiary amine. Finally, for the primary amine, which initially Phe molecule insert into the region of protonated primary amine, two possible candidate hydrogen atoms of Phe interact with the primary amine. For the hydrogen bond, the water would be considered as one of the binding sources, but it is not available in this case because the solvent effect is calculated implicitly using PCM due to the high computational load.

To calculate the pair interaction energy between binding sites of the PAMAM branch and Phe molecule, PIEDA was carried out at the FMO2/MP2/6-31G(d) at the gas phase using the optimized structures. The PIEDA results and the fragmentation of the complex of the PAMAM branch and Phe molecule are shown in Figure 4.8. The PAMAM branch is divided into five fragments which are based on the binding sites and consistent with the CG mapping for PAMAM dendrimers. The Phe molecule has three aromatic rings, so we keep the Phe molecule as one fragment due to the electron delocalization by the aromatic rings. In Figure 4.8, the pair interaction energies are divided into four components: electrostatic, exchange-repulsion, charge transfer, and dispersion contributions. Based on the total pair interaction energies, the amide-⊥ and the primary amine cases are more stable compared to other cases. The pair interactions of the cases have a strong electrostatic interactions because the primary amines, which Phe molecules are close in both cases, are protonated, so the strong electrostatic interactions are shown. Also, Phe molecules have three aromatic rings, so the dispersion interactions, which are related to the hydrophobic interactions, are attractive. Here, the pair interaction strength decreases in the order: Primary amine > Amide-⊥ > Amide-∥ > Tertiary amine-I ≃ Tertiary
Table 4.1: Binding energies (in kcal/mol) of complexes of phenanthrene to the branch of PAMAM dendrimer. $E_{\text{PAMAM}} = -65958.84$ and $E_{\text{PHE}} = -337464.94$ (kcal/mol) in MP2//ROHF/6-31G(d)(PCM)

<table>
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<tr>
<th>Unit(Kcal/mol)</th>
<th>Amide-∥</th>
<th>Amide-⊥</th>
<th>Tertiary amine-I</th>
<th>Primary amine</th>
<th>Tertiary amine-II</th>
</tr>
</thead>
<tbody>
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<td>MP2//ROHF/6-31G(d)(gas)</td>
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<td>-997441.08</td>
<td>-997423.22</td>
<td>-997430.22</td>
<td>-997371.66</td>
</tr>
<tr>
<td>MP2//ROHF/PCM/6-31G(d)</td>
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<td>-997514.15</td>
<td>-997502.21</td>
<td>-997498.04</td>
<td>-997441.17</td>
</tr>
<tr>
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<td>-91.40</td>
<td>-78.96</td>
<td>-75.47</td>
<td>-18.68</td>
</tr>
</tbody>
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amine-II. This is different compared to the energies calculated by the MP2//ROHF/PCM/6-31G(d). The differences between the two CG methods are caused by the solvent effect. In contrast to the implicit solvent effect by MP2//ROHF/PCM/6-31G(d), PIEDA has a limitation not to apply the implicit solvent effect (PCM) to the system. For the alternatives, the explicit solvent molecules can be considered during the PIEDA, but it is difficult to deal with the solvent molecules because the computational load is drastically increased because of the degrees of freedom of the solvent molecules.

### 4.5 Conclusions

In the present work, we have explained the binding of PAMAM dendrimer with Phe molecule, which represents the organic guest compound. For the methodology of this study, the atomistic and the coarse-grained simulations were carried out to explain the binding mechanism at the mesoscopic resolution. For the CG simulation, the CG potentials should be validated by the comparison with the atomistic data. For RDF analysis for the structure matching, both the solvent-free CG model and the MARTINI model have some discrepancy about the structures compared to atomistic MD, which, in the solvent-free method, the structures of G5 PAMAM dendrimers, flexible macromolecules, are easily aggregated inside because the explicit solvent effect is integrated out, so the total degrees of freedom decrease. In the MARTINI model, the RDF intensity for one of CG atom types of PAMAM dendrimers and the CG atom type for Phe molecule is very strong compared to the atomistic simulation. For this reason, the MARTINI model does not reproduce the binding structures between PAMAM dendrimers and Phe molecules like the atomistic simulations. For the solvent-free condition, even though
Figure 4.7: Optimized structures of the complex of PAMAM branch and phenanthrene (Phe) by the ROHF/6-31G(d) level. (a) Branch of PAMAM dendrimer. There are four binding candidates for Phe molecule. (b) Amide-||. Phe molecule is placed parallel to the amide of PAMAM branch. (c) Amide-⊥. Phe molecule is perpendicularly located at the amide of PAMAM branch. (d) Tertiary amine-I. (e) Primary amine. (f) Tertiary amine-II.
Figure 4.8: (a) Fragmentation of the PAMAM branch with phenanthrene and (b) total pair interactions (in kcal/mol) for Phe molecule with five fragments of PAMAM branch by the MP2/6-31G(d) level. The pair interaction energy is divided into the electrostatic (ES), exchange-repulsion (EX), charge-transfer+mixed terms (CT+mix), and dispersion (DI) contributions.
the intensities of the binding capacity is higher than those in the atomistic results, the highest peak position for the binding capacity is qualitatively consistent with the atomistic data. To achieve the better CG potentials using the solvent-free condition, we are developing the combined method, which is add the CG water molecules to the solvent-free condition[38].

From the configurational analysis using the partial snapshots, the Phe molecules are likely placed to the amide group of the PAMAM dendrimer in the atomistic and the solvent-free condition simulations, so we performed the MP2//ROHF/PCM/6-31G(d) to study the interaction energy between the Phe molecule and the binding sites, which are consistent with the CG atom types for the PAMAM dendrimer. From the binding energies and PIEDA, the amide groups are strong candidates for binding site of the Phe molecules.

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References


CHAPTER 5. Reintroducing explicit solvent to a solvent-free coarse-grained model

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Seung Ha Kim and Monica H. Lamm

5.1 Abstract

A unique coarse-grained modeling scheme that combines a systematic, solvent-free multi-scale coarse-graining algorithm for a complex macromolecule with an existing coarse-grained solvent model is proposed. We show that this procedure efficiently and reliably describes the interactions for complex macromolecules, using the specific example of dendrimers binding phenanthrenes in water. The experimentally measured binding capacity is predicted by the new coarse-grain modeling approach; the conditions for this simulation are beyond what could be reasonably simulated with an all-atom molecular dynamics simulation.

5.2 Letters

Coarse-grained (CG) molecular models have broad appeal for simulation-based investigations of complex macromolecules, such as polymers, surfactants, proteins, and lipid membranes, because they permit interrogation of these systems at length and time scales much larger than is possible with all-atom molecular models. Most CG approaches can be classified into one of two categories. In the first category, termed indirect parameterization, are methods in which the potential parameters of a pre-selected analytical form are optimized by calibration against thermodynamic or structural properties. An example is the MARTINI force field for biological molecules[1], whose parameters are based on oil/water partitioning coefficients. In the second
category, called direct parameterization, the CG potentials are determined from an explicit atom molecular dynamics (MD) simulation. One example is the multiscale coarse-graining (MS-CG) method, which derives CG parameters from force matching[2]. In the former category, a force field such as MARTINI can be easily applied whenever the target system is changed with little to no reparameterization required. Unfortunately, because the CG parameters are not directly based on the underlying atomistic forces, it is difficult to reproduce accurate local (< 10Å) structural details for a specific system. For example, the MARTINI force field is incapable of predicting the preferred positions for non-covalent binding between a flexible macromolecule and a small organic guest molecule[3]. In the latter category, the MS-CG method has the advantage of being systematic; the CG force field is evaluated from data collected along the trajectories of reference atomistic MD simulations.

The MS-CG method has been implemented for a solvated lipid bilayer with up to 12 CG sites[4]. However, storage and memory requirements make it computationally difficult to derive CG potentials for complex biological systems with greater than 15 defined CG site interaction types[2]. An alternative approach, called the solvent-free MS-CG model, derives effective CG potentials between sites on the solute molecules while integrating out the explicit representation of the solvent molecules[5]. This approach was recently implemented for a lipid bilayer[5] and for polyglutamine peptides[6]. For relatively rigid molecules, such as lipids, the local and long-range structure calculated from the solvent-free CG MD and the reference atomistic MD simulations are nearly identical [5]. For flexible molecules the absence of explicit solvent can cause the solvent-free MS-CG MD simulations to produce an increased tendency toward intramolecular and intermolecular aggregation. This has been observed in the solvent-free CG model for polyglutamine and further evidence for this solvent-free CG effect is provided in the supplementary material[7]. Specifically, the radial distribution functions obtained for the flexible molecules in the solvent-free CG model indicate structures that are highly ordered compared to the explicit solvent CG model. The drastic reduction in degrees of freedom for the solvent-free CG model leads to configurational entropy loss. The loss of configurational entropy upon coarse-graining has been quantified for hydrocarbon chains and is shown to increase as the flexibility of the
chain increases\cite{8}. Thus, while solvent-free CG models may work well for lipids, an explicit solvent CG model is required for problems concerning flexible macromolecules in solution.

In this communication, we present a new method that combines the computational efficiency of deriving solvent-free MS-CG potentials from force matching with the improved reliability of retaining the solvent degrees of freedom (i.e. reducing configurational entropy loss) in the CG MD simulation by using independently derived CG solvent potentials. We illustrate the accuracy and convenience of this new approach by modeling a mixture of generation 5 poly(amidoamine) (G5-PAMAM) dendrimers and phenanthrene (Phe) in water to calculate the binding properties of PAMAM.

Solvent-free CG potentials for PAMAM and Phe in water at neutral pH were obtained by the MS-CG approach described elsewhere\cite{9, 10, 5}. A brief summary follows. The first step of the MS-CG procedure is to obtain the atomic positions and forces sampled from an equilibrated, atomistic MD simulation. The degrees of freedom in the atomic configurations are reduced by mapping groups of atoms to defined CG sites and computing the net forces acting on the coarse-grained sites. If $f_{ij}(r_i, r_j)$ is the non-bonded CG force acting on the $i^{th}$ CG site due to the $j^{th}$ CG site, and it is assumed to depend linearly on $m$ unknown parameters $p_1, p_2, \ldots, p_m$, the CG pair force is expressed by $f_{ij}(r_i, r_j, p_1, p_2, \ldots, p_m)$. To obtain the CG potential in a systematic way, cubic spline or B-spline functions are fitted to the CG forces to enable a smooth curvature across mesh points. Specifically, B-spline functions improve the force matching performance because they can reduce the memory requirement and increase the accuracy\cite{2}. Then, the $m$ unknown parameters are optimized based on a least-squares method, which minimizes the difference between the net forces and the fitted pair forces and is expressed as

$$\chi^2 = \sum_{i=1}^{N_i} \left| F_{\text{atomic}}^i - F_{\text{predicted}}^i(r_i, p_1, p_2, \ldots, p_m) \right|^2,$$

where $F_{\text{atomic}}^i$ and $F_{\text{predicted}}^i$ are the reference atomistic force field and the calculated force field, respectively. The parameters obtained in this way from each configuration are averaged over the total number of atomic configurations sampled\cite{2}. In this work, the solvent-free potentials were calculated under the solvent-free condition of the force matching method, in which
Figure 5.1: Atomistic (left) and coarse-grain (right) representation. In the atomistic structure, gray spheres represent carbon; blue, nitrogen; red, oxygen; and white, hydrogen. (a) PAMAM (for clarity, only the core is shown). For coarse-graining, blue circles of the atoms map into $N_0$; red, $N_{da}$; and green, $Q_d$. (b) phenanthrene. Each aromatic ring is represented by a single CG site, $SC_4$.

the solute molecules only are considered during the reference atomistic MD simulation to get the CG pair forces, and thus, the solvent effect is implicitly included in the CG potentials of the solute molecules[5]. The reference atomistic simulation contained 27 G5-PAMAM dendrimers and 216 Phe with 1389280 explicit water molecules and 3456 $Cl^-$ counterions at neutral pH. A detailed description of the all-atom simulation methodology is provided in the supplementary material.[7]

To determine the bonded interactions in the CG system, a harmonic potential was applied: $V(r) = \frac{1}{2}k_r(r - r_0)^2$ and $V(\theta) = \frac{1}{2}k_\theta(\theta - \theta_0)^2$, where $V(r)$ and $V(\theta)$ are bond and angle potentials, and $k_r$ and $k_\theta$ are the bond force constant and the angle force constant, respectively. The parameters for the bonded potentials were determined by inverse Boltzmann fitting of the bond distributions[11]. The bonded interactions are calculated by the simple Boltzmann probability: $V(r) = -k_BTln[\frac{1}{2} \frac{P(r)}{P(0)}]$, where $P(r)$ is calculated by the bonded interaction distribution of the all-atom simulations. For the bond angle potentials, the initial guess of the angle
Figure 5.2: Radial distribution functions (RDF) between CG types of PAMAM dendrimers and Phe from atomistic (solid line) and coarse-grained (dashed line) molecular dynamics simulations. The dashed lines in the upper figures represent the solvent-free CG model, and in the bottom figures the explicit solvent CG model. (a) the site-site RDF for N$_0$-N$_0$, and (b) the site-site RDF for N$_{da}$-SC$_4$. For clarity in comparison, the distributions are only shown up to 2.5 nm. All distributions shown approach unity at large $r$ (not shown).

force constants, $k_\theta$, are selected by inverse Boltzmann fitting method, and determined in an iterative way because of the flexibility of the dendrimer; the equilibrium angles are obtained from the angle distributions. The nonbonded and bonded force parameters for the G5 PAMAM dendrimers and Phes are given in the supplementary material.[7]

In order to validate the solvent-free CG potentials, the site-site RDFs between solvent-free CG sites from G5-PAMAM and Phe are compared with those from the atomistic MD simulations (the top row of Figure 5.2). N$_0$ and N$_{da}$ are the CG atom types of G5 PAMAM dendrimers, and SC$_4$ is the CG atom type for Phe. The RDF of N$_0$-N$_0$ shows the structures of the den-
drimers and the RDF of $N_{da}$-SC$_4$ indicates the strength of the interactions between PAMAM dendrimers and phenanthrene molecules. The intensities of the RDFs from the solvent-free simulation are higher than in the atomistic simulation, indicating that the dendrimers have aggregated and that the interaction of the dendrimers and Phes in the CG simulations are much stronger than in the all-atom simulations. This tendency of the CG model to overpredict aggregation occurs because of the absence of explicit water molecules and because the dendrimer is a flexible macromolecule. Data provided in the supplementary material shows that this enhanced aggregation occurs because of the absence of explicit CG water molecules in the system, and not due to the coarse-graining scheme itself[7].

To overcome this limitation, we propose a new methodology (explicit solvent CG model), which introduces the CG water molecules to the formerly solvent-free system of G5-PAMAM and Phe. The MARTINI water model was selected because it is compatible with the CG mapping scheme applied to the solutes. Even though the degrees of freedom of the system increase after introducing the CG solvent molecules, the explicit solvent CG model (109944 atoms) is still computationally efficient relative to the atomistic simulation (4292364).

The CG pair potentials for interactions involving water have the Lennard-Jones form, $U_{LJ}(r) = \varepsilon_{ij}[\left(\frac{\sigma_{ij}}{r}\right)^{12} - 100 \times \left(\frac{\sigma_{ij}}{r}\right)^6]$, where $\varepsilon_{ij}$ is the strength of the interaction, and $\sigma_{ij}$ represents the closest distance between $i^{th}$ and $j^{th}$ CG sites. Because the solvent-free CG potentials already include implicit water effects, the strength of the solute-water interaction parameters had to be rationalized based on the physical properties of CG types, such as polarity. This was accomplished as follows. The CG solute sites were divided into two types: hydrophilic ($N_0$, $N_{da}$, $Q_d$) and hydrophobic ($SC_4$). The strengths of the interaction parameters were modified such that $\varepsilon_{\text{hydrophobic}} (8 \text{ kJ mol}^{-1}) < \varepsilon_{\text{hydrophilic}} (15 \text{ kJ mol}^{-1}) < \varepsilon_{\text{water}} (20 \text{ kJ mol}^{-1})$, where $\varepsilon_{\text{water}}$ is same value used in the MARTINI CG water model. CG MD simulations in the NVT ensemble were run after introducing explicit CG waters modeled with the modified MARTINI potential. The RDFs between dendrimer and Phe sites are shown in the bottom row of Figure 5.2. The CG system with explicit water more closely matches the RDFs observed from the reference atomistic MD simulations.
Figure 5.3: Fraction of Phe bound to the dendrimer. The number of Phe molecules is fixed at 216. Atomistic MD is shown by closed circles, solvent-free CG model by open circles, and the explicit solvent CG model by closed triangles.

To test the concentration dependence of the CG potentials derived with the explicit solvent CG approach, the number of PAMAM dendrimers was varied from 4 to 27 while keeping a fixed number (216) of Phe molecules. Figure 5.3 shows the fraction of Phe bound to PAMAM in 216 Phe molecules, computed as the accumulated probability density function of Phe from the core to the surface of the dendrimer. As a consequence, Figure 5.3 indicates the capacity of the dendrimer to encapsulate Phe molecules. As mentioned above, the CG potentials for all of the CG simulations in Figure 5.3 were derived from the atomistic simulation with 27 PAMAM dendrimers and 216 Phe in explicit water. Since the intensity of fraction of bound Phe molecules in the solvent-free CG model is higher compared to the atomistic and the explicit solvent CG model, the strength of the binding interaction of Phe molecules are overestimated. Therefore the solvent-free CG model does not reproduce the interactions of PAMAM dendrimers with Phe, the explicit solvent CG model transfers well to different relative concentrations.

Earlier experimental measurements on the fluorescence resonance energy transfer (FRET) between G5-PAMAM and Phe showed that FRET efficiency increased as the molar ratio of PAMAM:Phe increased from 1:10 to 1:1[12]. To investigate the effect of molar ratio on binding capacity in the model system, we increased the number of G5-PAMAM dendrimers from 27 to 216, while keeping the number of Phe fixed at 216. Figure 5.4 shows the two-dimensional probability distributions functions, \( P(r) \), for the distance from Phe to dendrimer core, overlayed
Figure 5.4: Two-dimensional probability distribution function, \( P(r) \), of Phe molecules from the core of each dendrimer. The white circles indicate the branch points of the dendrimers. The numbers in the circles represent the generation of the dendrimer, and \( Q \) is the abbreviation for \( Q_d \). The color bars express the intensity of \( P(r) \). The unit of X and Y axis is nm. (a) Atomistic simulation, (b) solvent free CG, (c) explicit solvent CG (27 dendrimers) (d) explicit solvent CG (216 dendrimers)
with the average location of each dendrimer branch point. Comparing the solvent-free case to the reference atomistic simulation shows that the peak intensity of the solvent-free model is nearly four times higher. The peak intensity of the CG model with explicit water is much closer to the reference atomistic MD system. In the simulation with 216 dendrimers, peak intensity has increased, consistent with the molar ratio dependency effect on binding capacity observed by experiment[12]. We note that a simulation of this scale is not feasible with atomistic MD.

In this communication we have introduced an approach for restoring the configurational entropy lost when the solvent degrees of freedom are removed from a system that contains flexible macromolecules in solution. In this procedure, the effective solvent-free solute-solute potentials are derived by MS-CG (i.e. force matching) and then the solvent potential is reintroduced by using an independently derived CG solvent model. This approach yields equilibrium structures that are in better agreement with those produced by the reference atomistic MD simulation than the equilibrium structures generated by the solvent-free CG model. We anticipate that this new procedure can be extended to other flexible macromolecules in solution where a MARTINI-like coarse-grained mapping scheme is chemically sensible. Using such an approach may lead to new physical insight for systems where interactions among macromolecules in solution are important driving forces. Application of this method to investigate how flexible macromolecular conjugates used in drug delivery bind to proteins in solution is currently underway. We caution that this CG approach is not designed to recover dynamic properties, such as transport coefficients. For CG models where accurate time correlations for solutes are desired, the friction and noise forces must be included to the equations of motion, via Langevin dynamics or dissipative particle dynamics. Ref. 13 illustrates such a procedure for a CG simulation of a star polymer melt.

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oratory, which is supported by the Office of Science of the U.S. Department of Energy under contract DE-AC02-06CH11357. The authors thank Professor Gregory Voth for providing us with the MS-CG code and Dr. Lanyuan Lu for assistance with technical issues concerning its implementation. We also thank Professor Dennis Vigil for useful discussions.

5.3 Supplementary material

5.3.1 Comparison of solvent effects from the solvent-free and the explicit solvent CG model

In this communication, we show that molecular dynamics (MD) simulations of flexible molecules using solvent-free coarse-grained (CG) potentials predict structures that are more aggregated and compact compared to the reference atomistic MD simulations. To determine that this effect is due to the solvent-free condition, and not due to the coarse-graining scheme itself, one should make a direct comparison between the CG potential with explicit solvent and the CG model with implicit solvent (i.e., solvent-free CG model). However, for the system under consideration in the communication, the explicit solvent CG model cannot be obtained directly from the reference atomistic MD simulation by force matching due to the excessive memory requirement. For this reason, we show here how going from a CG model with explicit solvent to a solvent-free CG model also leads to predicted structures that display more aggregation. The reference system is 27 generation 5 poly(amidoamine) (G5-PAMAM) dendrimers in 1385824 waters. The MARTINI force field[1], with the CG mapping scheme use in Ref [13] is applied. The reference CG MD simulations are carried out for 100 ns in the $NVT$ ensemble. A total of 1000 configurations were sampled. Force matching was applied to determine the solvent-free CG potential. MD simulations using the solvent-free CG potential were performed for 100 ns in the $NVT$ ensemble at the same conditions as the reference CG MD simulations.

Figure 5.S1 compares nonbonded interactions for the reference CG potential with explicit solvent and the solvent-free CG potential. For all sites shown, the solvent-free CG potential is weaker than the CG potential with explicit solvent. The radial distribution functions (RDF) for different pairs of CG sites are shown in Figure 5.S2. The intensities of the RDFs peaks are higher for the solvent-free potential than for the reference CG potential with explicit solvent,
indicating that dendrimer structure has strongly aggregated for the solvent-free case. For this reason, the contributions by explicit solvent molecules are more important than the implicit solvent effects into the CG solute potentials to accurately reproduce the atomistic structures. This finding is in contrast with Ref. [5], which evaluated solvent-free potentials for lipid bilayers. In that study, the lipid structures for the solvent-free and reference atomistic MD simulations were compared and found to be nearly identical[5]. Lipid molecules are relatively rigid objects compared to the more flexible G5-PAMAM dendrimers. Thus, the calculations shown here demonstrate that explicit CG solvent molecules are necessary for reliable CG MD simulations of G5-PAMAM dendrimers in solution. This conclusion is likely to be applicable for all flexible macromolecules.

5.3.2 Details of the all-atom and CG simulations and solvent-free CG methodology

All atomistic and CG MD simulations were performed using GROMACS[14]. The reference atomistic simulation contained 27 G5-PAMAM dendrimers and 216 Phe with 1389280 explicit water molecules and 3456 Cl\(^{-}\) counterions at neutral pH. In this atomistic simulation, we fixed the cell size to a cubic box length of 35 nm. The general AMBER force field (GAFF)[? ] and the TIP3PBOX water model[15] were used. The system was equilibrated in the NPT ensemble. The atomistic configurations and force data for the MS-CG method were collected from a 5 ns run in the NVT ensemble; a total of 5000 configurations were sampled. The system temperature was maintained at 300 K using the Nosé-Hoover thermostat with a relaxation time of 0.1 ps. In all the atomistic simulations, the time step was 2 fs. Using the CG potentials from the MS-CG method, the CG MD simulations were carried out for 100 ns in the NVT ensemble with the same system size as the atomistic simulations. The temperature was maintained at 300 K using the Berendsen thermostat with a relaxation time of 1 ps. A time step of 10 fs was used for the CG MD simulations. The nonbonded CG potentials of the solvent-free CG model and the explicit solvent CG model for G5 PAMAM dendrimers and phenanthrene molecules are provided in Figure 5.S3. For the bonded interactions of CG model, the detail bonded parameters are given in Table A.2.
Figure 5.S1: Exact MARTINI (solid line) and Solvent-free (dash line) pair potentials for the PAMAM dendrimers (a) CG potentials for $N_0$ and $N_{da}$ site, (b) CG potentials for $N_0$ and $Q_d$ site, (c) CG potentials for $N_{da}$ and $N_{da}$ site, (d) CG potentials for $N_{da}$ and $Q_d$ site

Table 5.S1: Simulation parameters

<table>
<thead>
<tr>
<th>pH</th>
<th>No. of atoms</th>
<th>No. of protonated amines</th>
<th>No. of counterions (Cl$^-$)</th>
<th>No. of waters (no Phes)</th>
<th>No. of waters (with Phes)</th>
</tr>
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<tr>
<td>Low</td>
<td>4082</td>
<td>254</td>
<td>254</td>
<td>61065</td>
<td>60133</td>
</tr>
<tr>
<td>Neutral</td>
<td>4676</td>
<td>128</td>
<td>128</td>
<td>61646</td>
<td>60721</td>
</tr>
<tr>
<td>High</td>
<td>4548</td>
<td>0</td>
<td>0</td>
<td>61921</td>
<td>61325</td>
</tr>
</tbody>
</table>
Figure 5.S2: Radial distribution functions (RDF) between CG types of PAMAM dendrimers from MARTINI force field (solid line) and solvent-free condition of the MARTINI force field (dashed line) in the CG simulation. (a) the site-site RDF for N0-Nda, and (b) the site-site RDF for N0-Qd, (c) the site-site RDF for Nda-Nda, (d) the site-site RDF for Nda-Qd
Figure 5.S3: Effective nonbonded potential for the PAMAM dendrimers and the phenanthrenes. 
(a) CG potentials for \( N_0 \) site and (b) CG potentials for \( N_{da} \) site
References


[7] See supplementary material for a direct test of the solvent-free effect and the detail simulation procedure.


CHAPTER 6. Understanding the Self Assembly of Dendrimer-Fullerenol for Nanomedicine and Environmental Remediation

A paper submitted to the journal ACS Nano

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The first two authors contributed equally to this work.

6.1 Introduction

Nanoscale assembly is an area of active research that has great implications for molecular design, biological sensing, environmental remediation, nanofabrication, supramolecular chemistry, energy, and catalysis\[1, 2\]. Dendrimers are a class of polymeric nanomaterials that possess high degree branching and order, low viscosity, monodispersity, pH-responsive surface charge and radius of gyration, and ample interior voids\[3, 4\]. Major classes of dendritic polymers such as poly(amidoamine) (PAMAM), poly(propylene imine) (PPI), and PAMAM-tris(hydroxymethyl)amidomethane have been shown robust in encapsulating guest species of metal cations and anions, polycyclic aromatic hydrocarbons, and inorganic solutes in contaminated waters and soils\[5, 6, 7, 8, 9, 10\]. Specifically, within the pH range of 7-10 PAMAM dendrimers bind to transition metals through multiple mechanisms, including Lewis acid-base complexation with their primary and tertiary amines serving as donors, ion-pairing with charged terminal groups, and non-specific interactions that result from the physical encapsulation of ions in interior cavities which may involve interactions with trapped counterions or water molecules\[6, 7, 10, 11, 12\]. Generally, lower-generation dendrimers bind to guest molecules or ions more effectively due to their more accessible interior which offers decreased mass transfer resistance and facilitates more guest-host collisions than their higher generation counterparts\[11\]. Importantly,
dendrimers can also reversibly release contaminant loads through changes in the solvent pH and electrolyte strength, or via a UV trigger. For example, using PAMAM and PPI dendrimers Diallo et al[6, 7]. selectively removed Cu(II) and perchlorate (ClO₄⁻) from water. Once dendrimer-Cu(II) or dendrimer-ClO₄⁻ complexes were formed, they were eliminated from aqueous solutions by ultrafiltration. Regeneration of the dendrimers, at 90% or above, was realized when the solution pH was lowered to 4 to release Cu(II) and raised to 9 to unload ClO₄⁻. Furthermore, dendrimers can be integrated into existing, commercial ultrafiltration membrane separation processes that permit operation at lower pressure (and thus lower cost) than that normally applied to reverse osmosis membranes for water purification[6].

In addition to environmental and industrial applications, dendrimers can bind either covalently or noncovalently with small and macro-biomolecules as well as metal ions, and act as transporters for the delivery of genes, drugs, prodrugs, MRI contrast agents, and viral inhibitors[4, 13, 14]. The feasibility of such applications is established upon the understanding that PAMAM dendrimers interact readily with phospholipids and show high permeability through cell membranes[15, 16, 17], thereby rendering them non-viral transporters with high efficacy[18]. The biocompatibility of dendrimers has been a topic of concern, but toxicities were reported for dendrimers of generations seven and larger, and only minimally[19, 20].

In contrast to the "soft" polymeric dendrimers, fullerenes and their derivatives are carbon-based, single-molecular particulates that possess appealing mechanical, thermal, electrical, physicochemical, and redox properties; the last two aspects endowed them a name of nanopharmaceuticals[21, 22, 23, 24]. Consequently, fullerenes and their derivatives are building blocks for designing nanoscaled assemblies for promising physical, biological, and medicinal applications. For example, photovoltaic devices made of polymer-fullerene derivatives – where the polymer acts as the electron donor and the fullerene as the electron acceptor - have been studied and commercialized[25]. Conjugation of murine anti-gp240 melanoma antibody to fullerene C₆₀ with cross-linker N-succinimidyl-3-(2-pyridyl)dithio)propionate (SPDP), has been shown to preserve the drug potency and facilitate the development of fullerene immunotherapy[26].

Hydrophobic fullerenes C₆₀ and C₇₀ show a propensity for the amphiphilic lipid bilayer
and can potentially impact cellular processes including electron transport in the photosystems of plant species. Water-soluble fullerene derivatives $C_{60}(OH)_x$ – or fullerenols – have been found effective in suppressing reactive oxygen species and the toxicity of copper, and have been employed as glutamate receptor antagonists and antiproliferative, neuroprotective or anticancer agents[24, 27, 28, 29]. Along with these biological and medicinal applications, the fate of fullerenes and their derivatives in living systems has become a topic of much research effort, especially over the past decade[30, 31, 32, 33, 34, 35]. Using in vitro and in silico studies Sayes et al.[32] and Qiao et al.[33] delineated the differential cytotoxicities of pristine and functionalized fullerenes, and attributed such contrasting cell responses to lipid peroxidation, hydrophobicity, and distribution of potential of mean force associated with the nanoparticles in a lipid bilayer. Others[36] and our group[37, 29] showed that fullerenol could inhibit polymerase chain reaction (PCR) and microtubule polymerization in vitro. Specifically, the surface hydroxyls of fullerenol $C_{60}(OH)_{20}$ complexed with the triphosphate oxygens of nucleotides and nucleic acids and with the alpha helices and the junctions of tubulin dimers through hydrogen bonding (H-bonding), as well as hydrophobic and electrostatic interactions. In addition, water-soluble $C_{60}(OH)_{20}$ compromised plasma membranes to induce necrosis in Allium cepa cells, driven by concentration gradient of the nanoparticles across the hydrophobic plant cell wall[38].

In view of the promises of fullerenes and dendrimers for nanomedicine, and in view of the crucial need for developing new strategies for mitigating the potential adverse effects of environmental discharge of nanomaterials, here we show a novel self assembly of PAMAM dendrimers and fullerenols and elucidate the underlying physical chemistry and thermodynamics for such assembly. Both generations 1 and 4 (i.e., G1 and G4) dendrimers have been employed to take advantage of their versatile morphology, charge density (8 and 64 primary amines per G1 and G4 dendrimer, respectively), and radius of gyration. It is noted that the syntheses of fullerene-terminated dendrimer nanoconjugates have been reported before[39, 40], where $C_{60}$ fullerenes reacted readily with PAMAM dendrimers or dendritic nanoscaffolds in organic solvent to yield their covalent architectures. Such nanoconjugates were used to generate singlet oxygen ($^1O_2$) in oxidation reactions[39], or effectively convert photons to photocurrents in con-
Table 6.1: Characterizations of Fullerenols, PAMAM Dendrimers and Their Assemblies

<table>
<thead>
<tr>
<th>Particle</th>
<th>Hydrodynamic size (nm)</th>
<th>Polydispersity index (PDI)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{60}(OH)_{20}(10 \mu M)</td>
<td>4.4\pm3.8</td>
<td>1.00\pm0.00</td>
<td>-21.8\pm10.9</td>
</tr>
<tr>
<td>C_{60}(OH)_{20}(100 \mu M)</td>
<td>5.1\pm5.1</td>
<td>0.685\pm0.22</td>
<td>-53.8\pm10.5</td>
</tr>
<tr>
<td>G1-NH_{2}(200 \mu M)</td>
<td>2.5\pm1.6</td>
<td>0.837\pm0.12</td>
<td>24.7\pm3.0</td>
</tr>
<tr>
<td>G4-NH_{2}(50 \mu M)</td>
<td>5.3\pm1.3</td>
<td>0.74\pm0.06</td>
<td>23.6\pm4.8</td>
</tr>
<tr>
<td>[G1]/{[C_{60}(OH)_{20}]} = 0.05</td>
<td>79.18</td>
<td>0.25\pm0.03</td>
<td>-25.0\pm6.5</td>
</tr>
<tr>
<td>[G1]/{[C_{60}(OH)_{20}]} = 1.37</td>
<td>1071</td>
<td>0.24\pm0.04</td>
<td>9.7\pm6.4</td>
</tr>
<tr>
<td>[G4]/{[C_{60}(OH)_{20}]} = 0.001</td>
<td>196.7</td>
<td>0.18\pm0.02</td>
<td>-52.1\pm4.9</td>
</tr>
<tr>
<td>[G4]/{[C_{60}(OH)_{20}]} = 0.04</td>
<td>403.2</td>
<td>0.2\pm0.03</td>
<td>-12.7\pm7.8</td>
</tr>
</tbody>
</table>

trolled 3-dimensional assemblies[40]. In contrast, our current study concerns the self assembly of water-soluble fullerene-derivative C_{60}(OH)_{20} with PAMAM dendrimer, and the purpose of this study is to exploit the use of nanoscaled assemblies for medicinal and environmental applications. Furthermore, this study demonstrates a new concept that (fullerenol) nanomaterial discharge – an emerging environmental concern – maybe remedied by a beneficial dendritic nanotechnology.

6.2 Results and Discussion

We have characterized the process of PAMAM dendrimer-fullerenol self assembly using the techniques of dynamic light scattering (DLS), isothermal titration calorimetry (ITC), spectrofluorometry, and molecular dynamics (MD) simulation. Specifically, the use of DLS yielded information on the hydrodynamic diameter and stoichiometric ratio of dendrimer-fullerenol assembly, and the analytical technique of ITC further confirmed the stoichiometric ratio and determined the thermodynamic parameters of the interaction. The fluorescence study showed stronger complex formation with higher generation dendrimers. The MD simulations further revealed how the distribution of hydrogen bonds in the assembly and the dendrimer surface area contributed to the experimental observations.

6.2.1 An Empirically Determined Ratio of Dendrimer-Fullerenol Assembly

As shown in Table 6.1, the hydrodynamic diameter of fullerenol at a concentration of 10 \mu M (used for G1/fullerenol interaction) and 100 \mu M (used for G4/fullerenol interaction)
Figure 6.1: DLS measurements of (A) G1/fullerenol and (B) G4/fullerenol assemblies. An abrupt increase in the hydrodynamic size of the complexes was observed for both G1/fullerenol and G4/fullerenol mixtures at a ratio of number of primary amines of dendrimer/fullerenol $\approx 2$.

ranged between 1.5～8.7 nm, with an average size of 4.4 ± 3.8 nm at 10 $\mu$M and 5.1 ± 5.1 nm at 100 $\mu$M, indicating the association of fullerenol molecules as a result of hydrophobic and hydrophilic partitioning. The zeta potential of the fullerenol aqueous suspension was found to be concentration dependent, in agreement with that reported by Letenko et al[41]. The dendrimers, in contrast, showed a low extent of entanglement due to the high degree of surface functionalities and crowding[42]. Specifically, the hydrodynamic diameter of G1 dendrimer at a concentration of 200 $\mu$M was measured at 2.5 ± 1.6 nm while that of G4 dendrimer at a concentration of 50 $\mu$M was 5.3 ± 1.3 nm. The zeta potentials of both G1 and G4 dendrimers were positive due to their protonated primary amines (Table 6.1).

Upon addition of G1 and G4 dendrimers to the fullerenol suspensions, however, the average hydrodynamic diameter of the dendrimer-fullerenol assembly increased immediately by an order of magnitude (Figure 6.1). Also, the stoichiometric ratio of greater than one fullerenol per primary amine in the case of G4 dendrimer suggested that their binding was more complex than ionic bonding, likely also involving H-bonding and hydrophobic interaction. For G1 dendrimer, saturation in the aggregate size was observed ranging between 710-955 nm, for a G1/fullerenol
Figure 6.2: Representative configurations of G4/fullerenol at neutral pH (20 ns). Ions and water molecules have been omitted for clarity: (A) 1:80 molar ratio and (B) 3:80 molar ratio of G4:fullerenol.

molar ratio of 0.27 (corresponding to 2.19 primary amines per fullerenol) or higher (Figure 6.1A). As shown in Figure S1, the size distribution of dendrimer-fullerenol complexes in the suspensions was fairly narrow and monomodal. For G4 dendrimer, uniform sized aggregates were formed until the ratio of dendrimer/fullerenol reached ~0.03 (corresponding to 2.14 primary amines/fullerenol, Figure 6.1B). As more dendrimers were added to the suspensions, the fullerenols associated with one dendrimer started to interact with those bound to neighboring dendrimers (Figure 6.2B) to trigger the formation of large-scale dendrimer-fullerenol supramolecular complexes, likely mediated by H-bonding. Such inter-cluster interactions also occurred in the case of G1/fullerenol system (data not shown). Interestingly, the number of primary amines/fullerenol at which inter-cluster aggregation occurred was ~2 for both G1 and G4 dendrimers. Coincidentally, a similar observation was made by Jensen et al.[39] for the preparation of fullerene-dendrimer nanoconjugates, where one fullerene bound to two primary amines of a G4-PAMAM dendrimer. Whereas the sizes of the G1/fullerenol and G4/fullerenol complexes were comparable (1,000 - 1,300 nm) at a primary amine/fullerenol ratio of 2, precip-
Figure 6.3: Interaction of PAMAM dendrimers with fullerenols at neutral pH: (A) Two fullerenols in proximity to one G1 dendrimer within 1.5 nm of the center of mass of the dendrimer and (B) 21 fullerenols in proximity to one G4 PAMAM dendrimer within 3.5 nm of the center of mass of the dendrimer.

Neutralization occurred more rapidly for G4 dendrimers (Figures 6.1 and 6.52) indicating a stronger G4/fullerenol association. Moreover, the zeta potential at a G1/fullerenol ratio of 0.05 was $-25.0 \pm 6.5$ mV while at a ratio of 1.37 it became slightly positive, at $9.7 \pm 6.4$ mV (Table 6.1). This change of sign for zeta potential indicates near fullerenol neutralization. For G4 dendrimers, in comparison, the zeta potentials were $-52.1 \pm 4.9$ mV and $-12.7 \pm 7.8$ mV at low (0.001) and high (0.04) G4/fullerenol ratios, respectively (Table 6.1). The lesser negative zeta potential at the high ratio suggests that most of the positively charged G4-dendrimers were neutralized by the negatively charged fullerenols and those remaining in the suspension were dendrimer-fullerenol assemblies.

Molecular dynamics simulations provided additional molecular insight into the assembly of PAMAM dendrimers with fullerenols. The following molar ratios were examined: 1:8, 2:8, and 4:8 for G1:fullerenol, and 1:80, 2:80, and 3:80 for G4:fullerenol. Figures 6.2 and 6.3B illustrate representative snapshots from the simulations for G4-fullerenol complexes, and representative configurations for G1-fullerenol complexes are provided in Figures 6.3A and 6.3S3. Figure 6.2A shows the presence of free fullerenol molecules at low G4 dendrimer concentration (1:80). However, at high G4 dendrimer concentration (3:80), all of the fullerenols were
bound to the dendrimers (Figure 6.2B). To observe the transient binding state at high concentrations, we monitored the configurations of the dendrimers and fullerenols as a function of time; configurations for G4-fullerenol complexes at 0, 10, and 20 ns are provided in Figures 6.4A, 6.4B, and 6.2B, respectively. Initially, free fullerenol molecules were in close proximity to the dendrimers (Figure 6.4A). At 10 ns the dendrimer-bound fullerenols began to interact with other fullerenols in the simulation box (Figure 6.4B). By 20 ns, all of the fullerenols attached to the dendrimers (Figure 6.2B). In contrast, Figure 6.3 shows that in the case of G1-fullerenol complexes, each G1 dendrimer bound to a maximum of 1-2 fullerenols, even at high dendrimer concentration (4:8). Figure 6.3A demonstrates that each fullerenol bound to two primary amines of the G1 dendrimer, in agreement with our DLS measurements. For G4 dendrimer, the fullerenols bound with the peripheral primary amines of the dendrimer, and fullerenol-fullerenol interactions were prominent (Figure 6.3B).

**6.2.2 Thermodynamics of Dendrimer-Fullerenol Assembly**

The enthalpic change ($\Delta H$) of dendrimer-fullerenol binding was found to be negative, indicating a net exothermic reaction (Figure 6.4). As fullerenols in suspension were being consumed by dendrimers, the heat released upon each dendrimer-fullerenol binding decreased until near saturation was reached. The reactions were spontaneous as indicated by the negative values of Gibbs free energy $\Delta G$. The much lower entropy $\Delta S$ of G1 dendrimers in contrast to that of G4 dendrimers upon binding to fullerenols indicates a higher degree of ordering in the G1/fullerenol system. For G1 dendrimer, whose size is comparable to that of fullerenol, the binding stoichiometric ratio $n$ of fullerenol to dendrimer was nearly 1. In consistency with the DLS data and MD simulations, which showed formation of dendrimer-fullerenol supramolecular complexes of nearly uniform sizes above a G1/fullerenol molar ratio of 0.27 (corresponding to 2.19 primary amines of G1/fullerenol), a gradually decreasing heat release above this ratio was observed; this implies that interactions still existed between the dendrimer-fullerenol aggregates. For G4 dendrimer, by contrast, the binding stoichiometric ratio was nearly proportional to the number of primary amines (64) on the dendrimer, at 44.1. The binding curves also suggest that saturation was reached faster in the case of G4 dendrimer at a G4/fullerenol ratio of 0.04.
Figure 6.4: ITC raw data and analysis plots of (A) G1/fullerenol and (B) G4/fullerenol complexes. The interactions between dendrimers and fullerenols resulted in significant heat release ($\Delta H = -21.8$ kcal/mol for G1/fullerenol and $-19.5$ kcal/mol for G4/fullerenols). The fullerenol:dendrimer stoichiometric ratios obtained from data analysis were $1.34 \pm 0.04$ for G1/fullerenol and $44.1 \pm 0.43$ for G4/fullerenol. The $\Delta G$ values for both G1/fullerenol (-7.69 kcal/mol) and G4/fullerenol (-7.24 kcal/mol) indicate the reactions are similarly spontaneous. G1/fullerenol complexes are also more ordered ($\Delta S = -47.4 \times 10^{-3}$ kcal/mol) than G4/fullerenol complexes ($\Delta S = -15.8 \times 10^{-3}$ kcal/mol).
(corresponding to 2.14 primary amines of G1/fullerenol), implying completion of the binding. This is in agreement with the DLS data, where precipitation of the suspension occurred above a G4/fullerenol ratio of 0.04 (Figure 6.1). Such saturation was not reached for G1 dendrimers, suggesting the presence of free fullerenols in the reaction chamber.

The highly negative enthalpic values indicated that the binding of fullerenol with dendrimer was enthalpy driven (Figure 6.4). Whereas the spontaneity of the interactions between G1 and G4 dendrimers with fullerenols was similar, the entropy as well as enthalpy of the interaction between G4 dendrimer and fullerenol were slightly less than that between G1 dendrimer and fullerenol. This could be attributed to the more open and hydrophilic structure of the G1 dendrimer, which afforded more sites for the fullerenols to bind, compared to the more compact and hydrophobic structure of the G4 dendrimer. At neutral pH, the interiors of G1 and G4 PA-MAM dendrimers remained non-charged and hydrophobic, while their exterior primary amines were protonated. Fullerenols, on the other hand, were partially negatively charged at neutral pH, as a result of the high electronegativity of the surface oxygens and deprotonation of the surface hydroxyl groups[43]. The self assembly of dendrimer-fullerenol was therefore possibly attributed by ionic bonding – via interactions between the protonated amines of the dendrimer and the negatively charged oxygens on the fullerenol, and by H-bonding between the fullerenol surface hydrogens and the amine and amide groups of the dendrimers. Apparently, contributions from such interactions could not be discerned by ITC, whose thermodynamic parameters reflected a combined result of ensemble-level nanoparticle and dendrimer self aggregation, as well as dendrimer-fullerenol complexation.

MD simulations were carried out to gain more insight into the binding mechanisms. First, the cluster sizes of fullerenols bound to PAMAM dendrimers were studied to examine the capacity of the dendrimer to bind fullerenols. To measure the cluster size per dendrimer, the distances between the centers of mass (COM) of fullerenols and the COM of a specific dendrimer were measured, and we counted the fullerenols as "bound" if they were located within twice the radius of gyration of the equilibrated dendrimer (=3.5 nm for G4). It was found that for G1 dendrimers, 1-2 fullerenols were bound per dendrimer, and increasing the concentration of
Figure 6.5: Number of fullerenols bound to G4 dendrimers at neutral pH after equilibration (>15 ns) within 3.5 nm from the dendrimer center of mass, which is twice as large as the radius of gyration for the dendrimer. The number of fullerenol molecules is fixed to 80 in the simulation box. The blue line represents the number of fullerenols bound to an individual dendrimer at the highest dendrimer concentration of 3:80 G4:fullerenol. The red line shows the total number of fullerenols bound to all three dendrimers in the simulation box.

dendrimers did not alter the binding stoichiometry (Figure S5), in agreement with our DLS and ITC results. Figure 5 indicates the capacity of G4 dendrimers to encapsulate fullerenols at neutral pH after equilibration (>15 ns). In the case of one G4-fullerenol cluster (blue line in Figure 6.5) the binding capacity of G4 dendrimer at low dendrimer concentration (1:80) is less than that at high dendrimer concentrations (2:80 and 3:80). Thus, as also seen from the DLS measurements (Figure 6.1), the sizes of dendrimer-fullerenol aggregates increased with the concentration of dendrimers and the interactions were instantaneous and remained stable over time (See the transient binding capacity of the dendrimers in Figure 6.S6). The red line in Figure 6.5 shows the binding capacities of all three dendrimers towards the 80 fullerenols in the simulation box. In the case of high concentration of dendrimers (3:80), the total maximum number of fullerenols in proximity to the three dendrimers was found to be ~65. This translates to ~22 fullerenols bound to each dendrimer (See the number of fullerenols bound to dendrimers as a function of time in Figure 6.S6). The discrepancy in binding stoichiometry between the simulations and those found in our ITC study (44 fullerenols per G4 dendrimer) is due to the fact that, because of their hydrophobic moieties fullerenols often exist as agglomerates in water, whereas single fullerenols were considered at the beginning of the simulations.
Figure 6.6: H-bonding between G1 dendrimers and fullerenol molecules at 4:8 G1:fullerenol molar ratio and neutral pH: (A) Relative number of H-bonds for O(amide)-H(hydroxyl group in fullerenol) (blue), H(amide)-O(hydroxyl group in fullerenol) (red), and H(primary amine)-O(hydroxyl group in fullerenol) (green) and (B) radial distribution functions of the above H-bonding pairs.

Figure 6.7: H-bonding between G4 dendrimers and fullerenol molecules at 3:80 G4:fullerenol molar ratio and neutral pH. The bars with the dashed lines represent the number of H-bonds with the interior G3 of the G4 dendrimer: (A) Relative number of H-bonds for O(amide)-H(hydroxyl group in fullerenol) (blue), H(amide)-O(hydroxyl group in fullerenol) (red), and H(primary amine)-O(hydroxyl group in fullerenol) (green) (B) radial distribution functions of the above H-bonding pairs.
The simulation data was further analyzed to characterize the prevalence of the H-bonds between fullerenols and dendrimers. To simplify the system we protonated the primary amines of the dendrimers but considered the fullerenols neutral. Hence, electrostatic forces between dendrimers and fullerenols contributing to ionic bonding were not accounted for in the simulations. However, the H-bonding between the hydroxyl groups of fullerenols and the amine groups of the dendrimers was analyzed to understand the strength of H-bonding between the PAMAM dendrimers and the fullerenol molecules. The possible H-bonds between G1 dendrimers and fullerenols were considered in the case of 4:8 molar ratio (G1:fullerenol) at neutral pH after the binding of dendrimers with fullerenols was saturated (>15 ns) (Figure 6.6A). The possible H-bonding pairs in this case involved the oxygens on the amide group of G1 and the hydrogens on the fullerenols, the hydrogens on the amide group of G1 and the oxygens on the fullerenols, and the hydrogens on the primary amines of G1 and the oxygens on the fullerenols. The peak of Figure 6.6A indicates the relative number of the H-bonds based on the total number of the H-bonds between G1 and fullerenols. Among the three possible pairs for the H-bonds, most of the H-bonds were formed between the hydrogens on the primary amines (H(n4)) and the oxygens on the fullerenols (OF). Figure 6.6B shows the radial distribution functions of the H-bonding capable pairs involving G1 dendrimers and fullerenols. The first peak of H(n4) on the primary amines and OF on the fullerenol appeared at 2 Å, corresponding to a typical H⋯O H-bond length. Compared to the intensity of the first peak of H⋯O from the primary amines, the H-bond types between the oxygens of the amide groups of the dendrimer and hydrogens on the hydroxyl groups of the fullerenol (O(n)-HF), and hydrogens of the amide groups of the dendrimer and oxygens of the hydroxyl group of the fullerenol (H(n)-OF) were less dominant. A similar case can be seen for G4/fullerenol in Figure 6.7. The H-bond formation between the hydrogens on the primary amines and the oxygens on the fullerenol were the most frequent pairing in the complex of G4 dendrimers with fullerenols (Figure 6.7A). This indicates that most H-bonds were formed at the surfaces of the G4 dendrimers. Like the H-bonds in the case of G1, the first peak for the hydrogens on the primary amines of the G4 and the oxygens on the fullerenol appeared at 2 Å in Figure 6.7B. In addition, some hydrogen bonds were also observed...
Table 6.2: Binding Energies ($\Delta G$) as a Function of the Number of Fullerenols Attached to Dendrimers

<table>
<thead>
<tr>
<th>G1 PAMAM</th>
<th>G4 PAMAM</th>
<th>Fullerenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of fullerenols</td>
<td>$\Delta G$[kcal/mol]</td>
<td>No. of fullerenols</td>
</tr>
<tr>
<td>3</td>
<td>-4.5012</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>-2.16</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>-8.3756</td>
<td>1</td>
</tr>
</tbody>
</table>

in the interior region of G4 and fullerenols.

Umbrella sampling simulations were used to calculate binding energies ($\Delta G$) between dendrimers and fullerenols as well as between fullerenols themselves [45, 46]. The $\Delta G$ values were calculated from the potentials of mean force (PMFs) (Figure 6.87). The umbrella sampling simulations were done with respect to the number of bound fullerenols to the dendrimer. The binding energies between G1 and G4 dendrimers and fullerenols were summarized in Table 6.2. For both G1 and G4 dendrimers, the binding energies between the dendrimer and one fullerenol molecule were consistent with the ITC experiments (Figure 6.4), which increased 8.18% for G1/fullerenol and 2% for G4/fullerenol complex compared with the experimental results. For G1 dendrimer, the binding energies varied based on the number of fullerenols bound. It can be seen from Table 6.2 that the case of three fullerenols per G1 dendrimer was more stable than the case of two fullerenols per G1 dendrimer. In the former case, the third pulling fullerenol was far from the other two fullerenols, so the pulling fullerenol was close to the dendrimer by four primary amine branches, whereas in the latter case the fullerenols were adjacent to each other and only two primary amine branches interacted with the fullerenols. For G4 dendrimer, it was also seen that the interaction between one G4 dendrimer and one fullerenol was more spontaneous than with multiple fullerenols based on the binding energies in Table 6.2. However, the strengths of interactions between the G4 dendrimer and multiple fullerenols did not change by the number of fullerenols bound to the G4 dendrimer except for the case of one G4 dendrimer with one fullerenol, and the interactions between G4 and multiple fullerenols ($\Delta G_{\text{bind}} = -5.44$ kcal/mol) were more spontaneous than that between G1 and multiple fullerenols ($\Delta G_{\text{bind}} = -2.16 \sim -4.5$ kcal/mol) as shown in Table 6.2. We considered the
interaction between two fullerenols, and found its binding energy ($\Delta G_{\text{bind}} = -4.15$ kcal/mol) was similar or even more spontaneous than with G1 and multiple fullerenols ($\Delta G_{\text{bind}} = -2.16 \sim -4.5$ kcal/mol), further substantiating the favored stoichiometric ratio of one fullerenol per G1 dendrimer as shown in Figure 6.4.

6.2.3 Relevant Intermolecular Interactions in Dendrimer-Fullerenol Assembly

Fluorescence spectroscopy was used to characterize the predominant intermolecular interactions involved in dendrimer-fullerenol complex formation. Fullerenols have been shown to emit fluorescence, the source being the decay from singlet to ground state in the parent $C_{60}$ upon excitation at an appropriate wavelength[47, 48]. The emission spectra of fullerenols are typically broad due to variance in the number of surface hydroxyl groups attached to the nanoparticles. As shown in Figure 6.8, increased dendrimer concentrations resulted in a quenching of fullerenol fluorescence, which was accompanied by a shift in the fluorescence maximum. This is indicative of complex formation between dendrimers and fullerenols and an inter-complex energy and/or electron transfer. The plateau regions of the spectra imply complete dendrimer-fullerenol assembly. High optical density and turbidity inhibited further measurements at
higher concentrations. In the case of G1 dendrimer, above a ratio of 1.9 for the number of primary amines/fullerenol, scattering from a cloudy solution (large aggregates) resulted in a slight increase in fluorescence, whereas precipitation of the large aggregates above that ratio in the case of G4/fullerenol prohibited us from further measurements. Note this is the same ratio where both DLS and ITC measurements showed interesting phenomena (i.e. gradual increase in the heat released and stability in the size of G1/fullerenol, and saturation in heat released and precipitation of earlier stable colloidal solution in the case of G4/fullerenol). As shown in Figure S8, the fluorescence intensity of fullerenols alone was linearly dependent on its concentration. Dendrimers, in comparison, displayed weak concentration dependence for their autofluorescence (Figure 6.89). However, the quenching and peak shift upon fullerenol binding with dendrimers was notable. Specifically, a blue shift of 21 nm, averages for both G1 and G4-fullerenol, was observed for increased concentrations of dendrimers bound with fullerenols. Although peak shifts in fluorescence are generally observed for energy transfer, Förster resonance energy transfer (FRET) can be ruled out since it usually does not occur with a blue shift for increased concentrations. Ionic bonding between the two species of dendrimer and fullerenol in solution formed stable complexes in the non-excited state. Upon excitation the formation of charge-transfer complexes was indicated by fluorescence peak shifts and quenching. Static
quenching occurs primarily due to complex formation via ionic bonding and Lewis acid-base
reactions, whereas dynamic or collisional quenching is a result of charge-transfer, change in
solvent polarity and/or viscosity, and other weak interactions like H-bonding\cite{49}. Although
the probability of charge-transfer complex formation with electron rich amines was low since
the double bonds present in the parent C_{60} have lost their electron deficient character on hy-
droxylation with the OH groups\cite{50}, charge-transfer from the hydroxyl groups to the protons
on the amines was probable. When both dynamic and static quenching coexists, the decrease
in fluorescence intensity can be described by the Stern-Volmer equation as:

\[
\frac{F_0}{F} = (1 + K_{SV}[Q]) (e^{x p V[Q]})
\]  

(6.1)

For low \([Q]\), Eq. 6.1 yields

\[
\frac{F_0}{F} = (1 + K_{SV}[Q]) (1 + V[Q])
\]  

(6.2)

where \(F_0\) and \(F\) are the fluorescence intensities of the fluorophore (fullerenol) in the absence and
presence of a quencher (dendrimer), respectively, \(K_{SV}\) and \(V\) are the Stern-Volmer and sphere-
of-action quenching constants – indicative of the sensitivity of a fluorophore towards a quencher,
and \([Q]\) is the concentration of the quencher (dendrimer). The higher the value of \(K_{SV}\) or \(V\),
the more effective is the respective quenching. As seen in Figure 6.9, the Stern-Volmer plot is
nonlinear with a positive deviation for G1/fullerenol complexes, and linear for G4/fullerenol
complexes. A positive deviation from linearity indicates simultaneous occurrence of both dy-
namic and static quenching. For low dendrimer concentrations, sphere-of-action quenching or
static quenching via complex formations dominated, giving \(K_{SV} \ll V\), and the value of \(V\)
calculated from the data fitted with Eq. 6.2 yielded 0.028 \times 10^5 \text{ M}^{-1}. For higher dendrimer
concentrations, dynamic quenching through charge-transfer, H-bonding and electrostatic inter-
actions between the already formed complexes and newly added dendrimers dominated, yielding
\(K_{SV} = 0.96 \times 10^5 \text{ M}^{-1}\). A smaller value of \(V\) indicates that the fullerenol fluorescence was
quenched primarily by dynamic quenching between the dendrimer and the fullerenol. In the
case of G4/fullerenol, the Stern-Volmer plots are linear throughout, indicating the quenching
was primarily dynamic. The linear Stern-Volmer constant \(K_{SV}\) obtained from the fitted data
is $3.3 \times 10^5 \text{ M}^{-1}$. The three-fold higher value of KSV for G4/fullerenol complexes is consistent with the MD simulation, which showed that the higher surface area of the G4 dendrimers facilitated many more intermolecular contacts with fullerenol aggregates than those observed for G1 dendrimers. Therefore, the higher surface area of G4 dendrimers increases their efficiency for quenching fullerenols. A modified Stern-Volmer Eq. 6.3 offers new insight into the binding affinity of the static quenching process\cite{51}: 

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a [Q]} + \frac{1}{f_a}$$  \hspace{1cm} (6.3) 

where $\Delta F$ is the difference of the fluorescence intensities in the absence and presence of a quencher, $K_a$ is the effective quenching constant for accessible fluorophores and is directly related to the binding constant for the quencher-acceptor system (assuming the decrease in fluorescence stems from static collision due to complex formation), and $f_a$ is the fraction of the fluorophore that is initially accessible to the quencher. The value of $K_a$ calculated from a plot of $F_0/\Delta F$ vs $[Q]^{-1}$ for G1/fullerenol is $1.0 \times 10^5 \text{ M}^{-1}$ and G4/fullerenol is $2.64 \times 10^5 \text{ M}^{-1}$ (data not shown). The value of binding constant obtained in case of G4/fullerenol is in very close agreement with our ITC results ($2.69 \times 10^5 \text{ M}^{-1}$). In contrast, the value is much lower in the case of G1/fullerenol from our ITC measurements ($4.35 \times 10^5 \text{ M}^{-1}$). Note that ITC measures the binding constant as a result of combined electrostatic interactions, complex formations, H-bonding, as well as hydrophobic interactions. The binding constant values obtained from fluorescence measurements primarily resulted from complex formation via ionic bonding and Lewis acid-base reaction. This reiterates our hypothesis that G4 formed stronger complexes with fullerenols than G1 throughout the concentration range used due to a higher density of protonated primary amines, however, complex formation between G1 and fullerenols was the strongest in the lower concentration region, after which, large agglomerates were formed (as shown from the DLS data) due to other interactions through hydrogen bonding and hydrophobic interactions. The fraction of accessible fullerenols calculated from the modified Stern-Volmer equation is $f_a = 1.09$ for G1/fullerenol and 1.23 for G4/fullerenol. This implies that, initially, there might be more than one binding site of fullerenol for dendrimers and the molecular environment of fullerenol was easily accessible to the dendrimer. In addition, the
spectral shift observed could be attributed to an induced dipole - dipole interaction between the hydroxyl groups of the fullerenols and the primary amines of the dendrimers\[52\] or, due to selective quenching of exposed vs. buried fluorophore sites of the fullereno[49].

6.2.4 Conclusion

The thermodynamics, stoichiometric ratio and binding mechanisms of dendrimer-fullerene assembly have been revealed by the experimental techniques of DLS, ITC, and spectrophotometry, and by all-atom molecular dynamics simulations. The formation of dendrimer-fullerene assemblies, at a maximum loading capacity of \(~\text{2 or 44 (experiment)}\) fullerene per G1 or G4 dendrimer (corresponding to \(~\text{2 primary amines per fullerene in both cases}\) was found to be energetically favorable. In addition, inter-cluster interactions were evident, as a result of electrostatic forces, H-bonding, ionic bonding, as well as Lewis acid-base reaction. Apparently, such inter-cluster formation can be controlled by adjusting both the concentrations of the fullerene and the dendrimer, and by tuning the molar ratio of dendrimer to fullerene. While inter-cluster interaction should be minimized for the delivery of fullerene derivatives by a dendrimer – in light of their diffusion in the bloodstream and eventual cell uptake, inter-cluster interaction is deemed desirable for mitigating the accidental release of nanomaterials in the environment. Based on our study, we recommend a G1/fullerene loading ratio of 0.2-1.6, and G4/fullerene loading ratio of 0.005-0.02 for drug delivery (the range below precipitation), and a G1/fullerene loading ratio of above 1.6, and a G4/fullerene loading ratio of above 0.02 for environmental remediation. Furthermore, for both nanomedicinal and environmental applications, the assembly of dendrimer and fullerene – as exemplified in the current study – may be extended to that of branched/hyperbranched polymers and nanoparticles of opposite charge.

6.3 EXPERIMENTAL AND COMPUTATIONAL METHODS

6.3.1 Materials

Amine terminated PAMAM dendrimers with ethylenediamine cores of generations 1 (MW 1430, 9.98 wt % in H₂O) and 4 (MW 14,215, 14.04 wt % in H₂O) (G1 and G4, respectively) were purchased as aqueous solutions from Dendritech, Inc. Polyhydroxy-C₆₀ (C₆₀(OH)ₙ,
fullerenol hereafter, n~18-22) was purchased from BuckyUSA. An average of 20 OH groups per fullerenol molecule was assumed for all measurements. All materials were used as received. Stock fullerenol suspension of 1 mM was prepared in deionized water by bath sonication for 30 min.

6.3.2 Dynamic Light Scattering and Zeta Potential Measurements

The average hydrodynamic diameter, particle size distribution and polydispersity indices (PDI) of the dendrimer-fullerenol assemblies were measured using a Nanosizer (S90, Malvern Instruments). The pristine dendrimer and fullerenol aqueous solutions were filtered with Anotop filters (Whatman) of 20 nm pore size prior to the measurements. 19 injections of dendrimer solutions of 8 µL each were added to 1.46 mL of fullerenol suspension in a standard plastic macro-cuvette of path length 1 cm. The dendrimer-fullerenol mixtures were allowed to incubate for 5 min after each successive injection and 30 sec mixing prior to the measurements. The pH of the final mixture was 6.5. Three repeats were performed for statistical error analysis. Surface charges of the pristine dendrimer and fullerenol suspensions, and that of the dendrimer-fullerenol mixtures at different stoichiometric ratios were measured using a Zetasizer (NanoZS, Malvern Instruments).

6.3.3 Isothermal Titration Calorimetry

ITC was performed with a VP-ITC Isothermal Titration Microcalorimeter (MicroCal, Inc.) with dendrimers in the injection syringe and fullerenol in the experimental cell, while the reference cell contained deionized water. Concentration of fullerenol in the experimental cell was 10 µM (0.0106 g/L) for reactions with G1 dendrimer and 100 µM (0.106 g/L) for G4 dendrimer. The concentrations of G1 and G4 dendrimers in the injection syringe were 200 µM and 50 µM, respectively. The initial volume of fullerenol in the reaction cell was 1.46 mL. Each experimental run consisted of 31 to 35 injections of 8 L each at an interval of 3 min between successive injections. The sample cell was maintained at 25°C and stirred at 200 rpm. Heats of dilution of dendrimers were subtracted from the final ITC results. Due to the negligible dilution of fullerenol, heats of dilution of fullerenol were minimal. Apparatus cleaning
was performed according to the manufacturer’s recommendations prior to the experiments. Baseline corrections and data fitting were performed using automated routines in Origin v. 7.0 data analysis and acquisition software (OriginLab Corp.). Minor corrections were done at user’s discretion. Figure 6.4 shows the raw ITC data of power vs. time and the resulting peak integrations are plotted as energy per mole of injectant ($\Delta H$) vs. the molar ratio of dendrimers per fullerenol (n) in the sample cell after each injection. Analysis of the ITC data was done using the One set of sites model. Due to the larger size of dendrimers compared to fullerenols, in our experiments, dendrimers were considered as macromolecules and fullerenols as ligands. Hence, during data analysis, a selection of ‘Ligand in Cell’ was made. A much larger raw heat is observed in the case of G4 dendrimers than in the case of G1 dendrimers since, in the case of G4 dendrimers, 44 fullerenols bind to each dendrimer instantaneously releasing a larger amount of heat, whereas only one fullerenol binds to G1. It is noted that with fullerenols in the injection syringe and dendrimers in the experimental cell, we would have observed a lesser raw heat release due to the presence of excess dendrimers and fewer fullerenols. In such case, it would have taken a longer time to reach saturation in heat release.

6.3.4 Fluorescence Spectroscopy

A Cary Eclipse fluorescence spectrophotometer (Varian, Inc.) was used to measure the fluorescence of the dendrimer-fullerenol assemblies. 1 $\mu$L of aqueous dendrimer solution was added in gradient concentrations to 500 $\mu$L of fullerenol in a 1 mm path length quartz cuvette and allowed to incubate for 5 min after a 30 sec mixing. Spectrum scans between 400-600 nm of the fluorescence emitted by the control samples and the mixture upon excitation at 340 nm were conducted after 5 min incubation each time. Fluorescence intensities were recorded until complete quenching was observed. Measurements were repeated with three samples for statistical error analysis. Recorded fluorescence spectra were corrected for their respective blanks (i.e., fullerenols and dendrimers only).
6.3.5 Molecular Dynamics Simulations

The initial structures of the G1 and G4 PAMAM dendrimers were obtained from Maiti et al.[53]. The general AMBER force field (GAFF)[54] was used to describe the molecular interactions of the PAMAM dendrimers and fullerene (C\textsubscript{60}(OH)\textsubscript{20}) molecules, and the TIP3PBOX water model[55] were applied to the water molecules. The partial charges on the dendrimers and fullerene molecules were assigned by the Gastiger method[56]. The primary amines in the PAMAM dendrimer were protonated to model neutral pH effects. Cl- counterions were added to make each system charge neutral near the protonated amine groups of the dendrimer. The characteristic simulation parameters about protonation are given in Table 3. To obtain the equilibrium structure of PAMAM dendrimers with explicit water molecules and prevent energy entrapment of the dendrimers, simulated annealing was carried out at 1 atm with heating and cooling rate of 50 K per 10,000 steps from 300 K to 500 K (4 repeated cycles) in the NPT ensemble, and the structures of the dendrimers were equilibrated at 298 K and 1 atm using Nose-Hoover[57, 58] and Parrinello-Rahman[59] schemes, with constant couplings of 0.1 and 1.0 ps, respectively for 5 ns. All of the dendrimers were solvated in the cell size to a cubic box length of 10 nm. Equilibration of the dendrimers was determined by the stabilities of the radii of gyration of the dendrimers. After equilibration, the water molecules and counter ions were removed, and the equilibrated structures of dendrimers were obtained. Multiple copies of the equilibrated structures of dendrimers and fullerene molecules were added, solvated, and the system was made charge neutral using Cl- counter ions. All of the simulations were carried out in the NVT and NPT ensemble for 200 ps where the solute molecules, dendrimers and fullerene molecules, were fixed to their initial positions by a harmonic potential with a force constant of 1000 kJ mol\textsuperscript{-1}nm\textsuperscript{-2} to relax the water molecules at 298 K and 1 atm using Nose-Hoover thermostat[57, 58] and Parrinello-Rahman barostat[59] with constant couplings of 0.1 and 1.0 ps, respectively. After the water molecules were relaxed, the harmonic potentials were released to relax the solute molecules (the dendrimers and the fullerenols) under NPT ensemble for 1 ns. After equilibration, simulations for data collection were performed for 20 ns under 298 K and 1 atm using Nose-Hoover thermostat[57, 58] and Parrinello-Rahman barostat[59] with
constant couplings of 0.5 and 2.0 ps, respectively. The binding energies of fullerenol molecules with the dendrimers were measured by calculating the potential of mean force (PMF) using the umbrella sampling method[60]. A harmonic biasing potential was added between the COMs of fullerenol molecules and dendrimers, and a force constant of 1000 kJ mol$^{-1}$nm$^{-2}$ was applied in increments of 0.2 nm. After fixing the position for the solute molecules, the system was relaxed for 1 ns. After equilibration, data was collected for 10 ns to obtain the PMF profile using the weighted histogram analysis (WHAM) method[61].

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Figure 6.S2: Image of G4/fullerenol complexes at a ratio of number of primary amines of G4/fullerenol = 3 (left) and 2.2 (right).

Figure 6.S3: Representative configurations of G1 PAMAM dendrimers at neutral pH (20 ns): (A) 1:8 molar ratio (B) 4:8 molar ratio. The system reached equilibrium and free fullerenols were observed. Ions and water molecules were omitted for clarity.
Figure 6.S4: Molecular configurations of G4-PAMAM dendrimer-fullerenol assembly at neutral pH. Ions and water molecules have been omitted for clarity: (A) 3:80 molar ratio at 0 ns and (B) 3:80 molar ratio at 10 ns.

Figure 6.S5: Number of fullerenols bound to each G1 dendrimer at neutral pH and 4:8 molar ratio within 1.5 nm, which is twice the radius of gyration of the dendrimer. The fourth dendrimer does not capture fullerenols.
Figure 6.S6: Number of fullerenols bound to an individual G4 dendrimer at neutral pH within 3.5 nm, which is twice the radius of gyration of the dendrimer. In each molar ratio case (1:80, 2:80, and 3:80), one dendrimer was taken to count the attached fullerenols near the dendrimer.

Figure 6.S7: The potential of mean forces (PMF) between PAMAM dendrimer with fullerenols and fullerenol in solution as a function of the center-of-mass separation distance: (A) the PMFs between G1 PAMAM dendrimer and fullerenol molecules at neutral pH and between two fullerenol molecules (B) the PMFs between G4 PAMAM dendrimer and fullerenol molecules at neutral pH.
Figure 6.S8: (A) Fluorescence emission of C60(OH)20 at different concentrations. Ex.: 340 nm. Arrow indicates the direction of increasing concentration. (B) Plot of intensity of fluorescence emission of C60(OH)20 vs. concentration at Ex/Em = 340/520 nm.

Figure 6.S9: Fluorescence emission of (A) G1 and (B) G4 at different concentrations. Ex.: 340 nm. Arrow indicates the direction of increasing concentrations (on the right) in μM.
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CHAPTER 7. Computer Simulations of Binding Between a PAMAM Dendrimer and Human Serum Albumin

A manuscript in preparation for the journal *ACS Macro*

Seung Ha Kim and Monica H. Lamm

7.1 Letter

Polyamidoamine (PAMAM) dendritic polymers are widely used as attractive carriers because dendrimers are globular, well-defined, and highly branched nanomaterials with functional groups structures[1, 2]. For this reason, PAMAM dendrimers are applied to the biological applications which are related to the delivery of genes and drugs. However, the responses of biological systems to PAMAM dendrimers is still unclear. Of the numerous studies on the biological responses to dendrimers, the toxicity to the biological materials are one of the interesting topics since the toxicity of the dendrimers may induce unpredictable and irreversible effects which are unfavorable folding, aggregation, and denature of the proteins[3]. Related to the concerns about the nanotoxicity of PAMAM dendrimers, the biocompatibility and bioavailability of a dendrimer-based drug injected into the blood stream is an important factor in the success of the application. Human serum albumin (HSA), which is the most abundant protein in blood plasma, binds anionic and cationic ligands, such as PAMAM dendrimers, and the formation of such a conjugate may alter the binding affinity of the PAMAM dendrimer to its gene or drug target[4]. Recently, a number of experimental approaches have been conducted to elucidate the interaction of HSA proteins to PAMAM dendrimers and understand the toxicological effects of PAMAM dendrimers during the drug or gene delivery[5, 6]. The binding constants of PAMAM dendrimers to HSA have been determined by experimental techniques including isothermal titration calorimetry, and fluorescence spectroscopy and are dependent on
the generation and the chemical structures of functional groups in PAMAM dendrimers\cite{7, 4, 8}. In addition, experimental studies have shown that binding interactions between PAMAM dendrimers and human serum albumin alter protein conformation and suggest that the protein may even partially unfold. The alteration of the protein’s secondary structure may impact its ability to effectively regulate intercellular fluxes\cite{5, 7, 4}. However, there is a need for a fundamental understanding of how PAMAM dendrimers interact with HSA proteins. For this reason, the theoretical approaches are necessary for understanding the interaction of HSA to PAMAM dendrimers.

Various theoretical and computational approaches can be considered to examine the interaction of HSA to PAMAM dendrimers. Atomistic molecular dynamics (MD) simulations are suited to investigate the solution behavior and binding phenomena of single dendrimers in explicit solvent\cite{9, 10, 11}. However, for questions involving the interaction of many high generation dendrimers (PAMAM) and proteins (HSA) with explicitly modeled solvent molecules, the system size and time scale requirements are much larger than what is routinely feasible for atomistic MD. To overcome these limitations, coarse-grained (CG) molecular models can be applied in place of atomistic force fields to understand the physical behaviors of the HSA-PAMAM complex in solution.

In this letter, we performed coarse-grained (CG) molecular dynamics (MD) simulations of the mixture of human serum albumin (HSA) and polyamidoamine (PAMAM) dendrimers in solution in order to elucidate the interactions of the protein-dendrimer complex and predict the conformational changes of HSA. The CG method is recently developed which combines the computational efficiency of deriving solvent-free MS-CG potentials from force matching\cite{12} with the improved reliability of retaining the solvent degrees of freedom by using independently derived CG solvent potentials\cite{13}.

The solvent-free potentials were calculated under the solvent-free condition of the force matching method, in which the solute molecules only are considered during the reference atomistic MD simulation to get the CG pair forces, and thus, the solvent effect is implicitly included in the CG potentials of the solute molecules. The reference atomistic simulation contained 4
Figure 7.1: Coarse-graining human serum albumin.

Figure 7.2: Coarse-grained representation for human serum albumin.
human serum albumins and 10 G5-PAMAM dendrimers with 246810 explicit water molecules and 580 Cl- counterions at neutral pH. We made coarse-grained configurations for human serum albumin (Figure 7.1) based on the mapping rule in Figure 7.2. The nonbonded CG potentials of the explicit solvent CG model for HSA and G5 PAMAM dendrimers are provided in Figure 7.3.

In Ref. [7], using isothermal titration calorimetry and capillary electrophoresis, the binding capacity of HSA is 4-6:1, but the binding site locations are not clear. Based on ion formation, steric effect, and vicinity, 6 possible binding sites were considered. We measured the binding energies between the possible binding site and a dendrimer using an umbrella sampling technique. Based on the binding energies, site 3 and 4 have strong interactions with a dendrimer whereas site 1, 5, 6 have weak interactions with a dendrimer. To study the detailed binding structures, we compared the radial distribution function according to the binding sites. In Figure 7.4, site 3 and 4 have strong interactions with dendrimers, and this is consistent with the binding energies. Interestingly, the first peak position in the end group, Qd, of PAMAM dendrimer is longer than in N0 and N0. Even though Qd is a positive charged group of PAMAM dendrimer (primary amine), HSA prefers to interact with the amide and the tertiary

Figure 7.3: Effective nonbonded potential for HSA and PAMAM dendrimers. (A) CG potentials for N0 site with NP0 and P5 from HSA (B) CG potentials for Qd site with NP0 and P5 from HSA (right).
Figure 7.4: (A) possible binding sites of HSA with G4 PAMAM dendrimer (B) binding energies between the binding site candidates of HSA and G4 PAMAM dendrimer (right).

amine group of PAMAM dendrimer.

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References


[7] Dzmitry Shcharbin, Magdalena Janicka, Michal Wasiak, Bartlomiej Palecz, Magdalena Przybyszewska, Marian Zaborski, and Maria Bryszewska. Serum albumins have five sites


Multiscale modeling approaches are necessary to understand the phenomena of complex macromolecular systems because multiscale methodologies are capable of describing materials from length and time scales commensurate with electronic structure theory to length and time scales in the nanoscale regime. Here, we interpret macromolecular phenomena using multiscale modeling approaches. Dendrimers have been studied as model system because dendritic polymers have received increasing attention over the past several decades as attractive candidate materials for a variety of applications such as therapeutic delivery systems, templates for catalytic metal nanoparticles, and extraction agents for the removal organic pollutants and toxic metals from water and soil. To understand the binding mechanisms relevant to these applications, a clear description between dendrimers and guest molecules such as DNA, medicines, and metal ions is an essential factor. The major contributions of this research which have been shown in earlier chapters of this thesis are as follows.

First, atomistic molecular dynamics simulations were conducted for one G5 polyamidoamine (PAMAM) dendrimer and 25 phenanthrene (Phe) molecules in explicit water at different solvent pH cases to understand the molecular details of the binding between Phe and PAMAM. We found that the binding efficiency between a dendrimer and phenanthrene molecules is high at neutral pH, and this is consistent with the experimental results. We explained the binding behaviors the configuration changes of PAMAM dendrimers at each pH.

Second, the coarse-grained simulations were carried out to explain the binding mechanism between PAMAM and Phe molecules at the mesoscopic resolution. To obtain the CG potentials for the system, the MARTINI CG model and the solvent-free model based on the force matching method were applied. Both the solvent-free and MARTINI CG models show some discrepancy about the structures compared to atomistic MD in the structural analysis.
Third, a new CG method, which is explicit solvent CG model, was developed to reproduce more accurate structures compared to the atomistic configurations, and test this method using PAMAM-Phenanthrene complex system.

Finally, the explicit solvent CG model was applied to describe more effectively the flexible macromolecular complex systems which are fullerenols and human serum albumins (HSA) with PAMAM dendrimers. In these studies, the explicit solvent CG model are used to search the binding sites of proteins (HSA) with dendrimers and explain the experimental observations for binding interactions (fullerenol).

Future work, based on the current research contributions to multiscale modeling, may address systems with more complexity such as the system described in the following section.

8.1 Cellular uptake of nanomaterial complex systems

Dendrimers have been widely used as a nanomaterial carrier for the delivery of drugs, genes, and imaging sensors. If dendrimers are used for the delivery of the guest molecules into living systems, the interaction with the cell membrane is very important to investigate the biocompatibility. Although experiments have investigated the interactions of the membrane with the dendrimers[1, 2, 3], theoretical approaches are necessary to understand the molecular level of the interactions of the membrane with the nanomaterials.

All-atom and coarse-grained simulations have been carried out to understand the interaction of a membrane bilayer with dendrimers and to explain phenomena such as a hole formation in the bilayer[4]. However, to our knowledge, there is no calculation to understand the membrane interaction with the complex system of dendrimers and guest molecules in solution because the system including the dendrimers, guest molecules, and membranes is beyond the all-atom simulations. In addition, the binding chemistry between dendrimers and guest molecules are important to describe the accurate interactions of the membrane and the nanomaterial system as well as that between dendrimers and cell membrane.

Recently, we developed a new coarse-grained (CG) methodology to accurately connect between atomistic and mesoscale resolution for the complex systems of dendrimers and guest
molecules in solution, and confirmed the methodologies to the systems of the dendrimers with
different guest molecules, which are phenanthrenes, fullerenols, and human serum albumin.
This methodology can provide the essential clues of the interaction of the cell membrane and
the nanomaterial complex systems to understand the nanotoxicity.
References


APPENDIX A. Localization of spherical nanoparticles within lamellar AB diblock copolymer melts through Self-Consistent Field Theory

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consider a comprehensive parameter space comprised of the Flory interaction parameter describing interactions between B segments and the particle surface compared to the segment-segment interaction parameter ($\chi_{BP}/\chi_{AB}$), the particle volume fraction ($\phi_P$), and the ratio of the particle diameter to block copolymer domain spacing ($d_P/d_{AB}$). Analysis of the free energy over this parameter space yields phase diagrams showing the conditions under which particles segregate to the intermaterial diving surface (IMDS) or the center of the domain. Interestingly, we predict a particle concentration dependent “reentrant” phase transition in which particles move from the domain interior, to the IMDS, and back as $\phi_P$ increases. These results are interpreted as a subtle consequence of the competition between enthalpic polymer-particle interactions and the chain packing frustration imposed by the particulate inclusion. These results are consistent with recent experiments on block copolymer nanocomposites.

A.1 Introduction

Polymer nanocomposites (NCPs), mixtures of nanoscale filler particles and polymers, have been a topic of increasing importance over the past decade. As a consequence of the enormous surface area of the particle/matrix interface, synergistic interactions arise with potential applications exploiting the unique electrical,[1] optical,[2] or mechanical properties[3, 4] characteristic of these materials. Fundamental to the development of NCP technology is the ability to control the particle distribution;[4] for this reason researchers have turned to block copolymer (BCP)
matrices due to the ability of microphase separation to selectively direct the localization of the
nanoparticles, allowing the properties of the composites to be tailored by molecular design.[5]
Block copolymers tend to organize themselves into exquisitely ordered periodic mesophases,[6]
and the positioning of particles within mesodomains can be dictated, in principle, by controlling
the preference of the particle for one domain over the others.

In the simplest case, the matrix is an AB diblock copolymer, which in the absence of
particulate inclusions typically assumes one of essentially four periodic equilibrium phases,
ddictated by the fraction of A segments \( f_A \) and the Flory segment-segment interaction param-
eter \( \chi_{AB} \).[7, 8, 9, 10, 11] The introduction of nanoparticles is accompanied by considerable
complexity. The least complicated possibility has to date received the most attention, i.e.
monodisperse spherical nanoparticles, introducing at the minimum 5 additional parameters:
(1) the particle volume fraction \( \phi_P \), (2) the ratio of particle size \( d_P \) to a characteristic di-
mension of the matrix, e.g. the BCP domain spacing \( d_{AB} \), (3) particle-particle interactions,
(4) particle-A interactions, and (5) particle B-interactions. In practice a reduced set of only 3
parameters should dictate phase behavior: native particle-particle interactions are so strong as
to preclude dispersion in favor of aggregation, and in experiments particles are generally pas-
sivated through the attachment of ligands chemically similar to A (or B) segments;[5, 12] this
effectively eliminates particle-particle interactions and renders the particle surface neutral to
A segments, yielding a single domain selectivity parameter \( \chi_{BP} \) which characterizes enthalpic
interactions between the particle surface and B segments. Even when \( \chi_{AP} \neq 0 \), so long as
macrophase separation is not an issue one can define \( \chi_{AP}^* = \chi_{AP} - \chi_{AP} \) as a reference state
such that \( \chi_{AP}^* = 0 \) and \( \chi_{BP}^* = \chi_{BP} - \chi_{AP} > 0 \).

As a consequence the particle distribution becomes a function of the canonical parameter
set \( f_A, \chi_{AB}, \chi_{BP}, \phi_P, \) and \( \frac{d_P}{d_{AB}} \). Formulated in this manner, nanospheres will tend to distribute
to the A domain interior (DOM) or the intermaterial dividing surface (IMDS) subject to these
parameters; a number of experimental and theoretical approaches have been conducted over
the years to elucidate the conditions favoring these two extreme states.

Of the numerous experimental studies, Lauter-Pasyuk et al. performed the neutron specular
reflection to study the morphology of iron oxide nanoparticles in the lamellar phase of a symmetric polystyrene-polybutylmethacrylate (PS-PBMA) diblock copolymer. They found that the preference of the particle position in the diblock copolymer matrix is dependent on the size of nanoparticle, such that small particles exhibited IMDS placement while larger particles are concentrated in the center of the PS domain.\[13, 14\] Bockstaller et al. reproduced this behavior using thin films of poly(styrene-b-ethylene propylene) embedded with mixtures of aliphatic-coated gold (small particles, IMDS) and silica nanoparticles (large particles, DOM).\[15\] This behavior was rationalized based on entropic effects according to the particle size ($\frac{dP}{dAB}$), but the enthalpic interactions (i.e., by changing the nature of the ligands, $\chi_{BP}$) were not investigated.

To explain the enthalpic interaction between nanoparticles and block copolymers, Chiu et al. synthesized particles coated with controlled mixtures of $A$ and $B$ oligomers at fixed particle size and concentration, demonstrating directly how IMDS vs. DOM placement can be tuned through $\chi_{BP}$.\[12\] Kim et al. showed similar behavior by controlling the PS ligand graft density on gold nanospheres in a lamellar poly(styrene-b-vinyl pyridine) (PS-PVP) block copolymer; here the Au particle surface prefers the PVP domains, and thus the PS graft density directly influences $\chi_{BP}$.\[16, 17, 18, 19\] In summary, experimental studies show that entropy-dominated NCPs, i.e. with small particle size, favor IDMS positioning whereas enthalpically dominated systems favor DOM positioning.

In parallel, various theoretical approaches have been conducted to understand the morphology of nanocomposites according to changing the physical and chemical properties of nanoparticles, e.g., Monte Carlo,\[20\] molecular dynamics\[21, 22\], and dissipative particle dynamics.\[23\] Huh et al. study a phase diagram of nanocomposites with particles using Monte Carlo simulation.\[24\] These methods are able to illustrate the kinetics of the evolution of the nanoparticle distribution within the block copolymer, however it is difficult to discern the equilibrium structure of the system. On the other hand, statistical field theories provide no kinetic data but can provide an effective way of studying the ordered phases of nanocomposites at equilibrium.\[25\] There are many methods for statistical field theory, like an analytical method, self-consistent field theory (SCFT), and a nonlocal density functional theory (DFT). For an analytical approach,
Pryamitsyn and Ganesan have performed the density distribution of particle calculation using the strong-stretching theory (SST) with several approximations[26]. And using DFT, Cao and Wu predict the preferred position of particles with nanocomposites like experiments[27]. In particular, self-consistent field theory (SCFT) has been remarkably successful in the prediction of equilibrium mesophases in various heterogeneous polymer systems.[28, 8, 29, 25, 10] Moreover, field theoretic approaches readily allow the decomposition of the free energy into entropic and enthalpic terms; thus applied to BCP nanocomposites, SCFT can be used to quantify how entropic and enthalpic terms control the phase behavior of the nanoparticles in the matrix.[4]

However, the strong excluded volume interaction associated with solid particles in a polymer matrix complicates the application of SCFT to these systems. Two approaches to this problem have predominated the literature. In 2000 Thompson et al. described the system through implementing a density functional theory (DFT) to describe the nanoparticles in conjunction with the use of SCFT for the BCP matrix.[30] Although ordered phases of the nanocomposites are well explained by the theory, the interaction between the polymers and the particles is described by the mean field approach, in which the polymers are effectively excluded from the average particle positions.[31]

To improve the excluded volume interaction of nanoparticles and polymers, Matsen introduced the use of “cavity” functions to explicitly exclude the polymer segments from areas prescribed to be occupied by nanoparticles; this approach was first used to model block copolymer thin films confined between two planar “walls”, i.e., the polymer/substrate and polymer/air interfaces.[32] In this study, the confined surface density profile at the interfaces was described by a continuous function $\rho_P(r)$, which assumed a value of 1 in the “wall” regions and 0 in the polymer film. Polymer-interface enthalpic interactions are encapsulated at non-limiting values of $\rho_P(r)$ through the introduction of a Flory-like binary interaction term of the form $\chi_{BPPBPP}$. Application of the incompressibility constraint enforces $\rho_A(r) + \rho_B(r) + \rho_P(r) = \rho_0$ throughout the simulation volume. Since the specification of $\rho_P$ is arbitrary, this method allows both polymer thin film calculations as well as polymer systems with particulate inclusions of arbitrary shape and size. Numerous case studies involving both BCP thin films and BCP
nanocomposites have appeared over the past decade based upon this approach.[33, 34, 35, 36]

A limitation of the “cavity” method is that once prescribed, $\rho_P$ is a static function and thus particle coordinates are fixed — i.e., there is no direct method to simultaneously equilibrate both polymer and particle degrees of freedom. As a first attempt at circumventing this restriction, Sides et al. introduced a hybrid SCFT (HSCFT) method which retains the nanoparticle positions as explicit degrees of freedom to calculate a coupling between the nanoparticles and the polymers.[37] Here a two-dimensional simulation was employed in which an ensemble of cylindrical nanoparticles were positioned randomly within the simulation area, and SCFT was used to calculate the nearest saddle point for this configuration. Equilibrium particle positions were estimated using a steepest descent scheme (i.e., Brownian dynamics with no stochastic term), with additional SCFT saddle-point calculations between particle moves until the polymer morphology stabilizes. Using this method these researchers were able to reproduce experimentally observed phase transformations in pseudo-analogous experimental systems with spherical nanoparticles in lamellar and cylindrical BCP phases. Due to the computational complexity of these simulations, however, calculations were constrained to 2D and more sophisticated algorithms such as force-biased Monte Carlo particle moves were impractical. Nonetheless this study was instrumental in validating the application of the “cavity” method to experimental systems, highlighting that not only does the BCP matrix direct the assembly of the nanoparticles, but also that the nanoparticle excluded volume can also have a strong effect on the BCP morphology.

Recently, Matsen and Thompson calculated the particle distribution including the explicit interaction between spherical nanoparticles and block copolymer in a lamellar phase, to investigate the dependence of the surface affinity and diblock composition using the hybrid SCFT method, which is based on the cavity function like Sides et al. to describe the particle-polymer interface, in the dilute limit for the nanoparticles, so that particle/particle interactions are negligible.[31] They fixed the particle position at a specific point in the nanocomposite and also simplified the system to use an axial symmetry in the $z$ axis with reflecting boundary conditions for reducing the computational time, allowing the expansion of the system from 2D
to 3D as compared to the Sides study. In this study a continuous particle distribution was inferred from the free energy as a function of the particle position with respect to the IMDS. Neutral and partially selective particles were considered for two different particle sizes and 3 nearly symmetric polymer compositions. Qualitatively, neutral particles preferred the IMDS while the selective ones localized within the domain center, irrespective of the other parameters; quantitatively, the sharpness of the particle distribution varied considerably. They compared their approach with the SCFT/DFT and strong segregation methods (SST).

In this article we report a comprehensive 3D study of the preferred particle location in a symmetric lamellar $AB$ diblock copolymer melt at intermediate segregation strength over a range of $\phi_P$, $\chi_{BP}$, and $\frac{d\phi_P}{d\chi_{AB}}$. We present a computationally efficient HSCFT model similar to that of Matsen and Thompson,[31] but with periodic boundary conditions allowing us to consider the effects of the nanoparticle composition. Our approach strikes a balance between the simplicity of the ideal gas limit and the computational complexity of Sides’ treatment of many-particle systems; we achieve this by invoking a “mean-field” approximation with respect to the lateral particle position, i.e. the manner in which particles pack. We employ a periodic unit cell calculation in which each cell contains a single particle; the dimensions of the periodic unit cell are adjusted to control particle volume fraction. This allows us to precisely analyze the energetics of semi-dilute nanocomposites over a comprehensive parameter space, incorporating effects of particle perturbations to polymer conformations. This simplified model is in agreement with and unites the various experimental reports relating particle size and surface affinity to either IMDS or DOM positioning, and furthermore predicts a $\phi_P$-dependent “reentrant” DOM-IMDS-DOM phase transition that, to our knowledge, has not been reported through either experimental or theoretical approaches.

A.2 Experimental

In this work we treat the system as two periods of a symmetric diblock copolymer with a single spherical nanoparticle confined between two planar interfaces (“walls”), with periodic boundary conditions, as illustrated in Fig A.1. Here the “walls” in the $YZ$ plane serve to
Figure A.1: Schematic diagram of the mixture of lamellae-forming diblock copolymer with a nanoparticle in 3 dimensions. The particle was placed at the interface between A and B segments or the domain interior of an A segment. $d_{AB}$ is the period of a diblock copolymer lamellar morphology, and walls were located at the period of the structure and attractive with A domain. The length of Y and Z axes are the same.

immobilize the polymer mesophase with respect to the position of the particle; the use of two periods ensures that within the interior of the simulation volume the confinement effects of the “walls” may be neglected in the vicinity of the nanosphere. This construction mimics the Dirichlet boundary condition in the $x$ direction, serving to prevent the translation of the lamellar mesophases based on the particle location. Previous calculations by Stein[36] and Mishra[38] have indicated that the presence of confining walls on the energetics of the polymer only persist for roughly one half-period; the interior of these confined systems do not feel the presence of the walls directly. To demonstrate that this is indeed the case we have also conducted a smaller set of calculations in an $ABABABA$ cell for comparison with our results from the more computationally efficient $ABABA$ cells.

The simulation volume is either hexagonal or orthorhombic with dimensions $L_x \times L_y \times L_z$, where $x$ is the direction normal to the “walls” and $L_y = L_z$. Hexagonal lattices were compared to orthorhombic to assess any potential qualitative influence of particle packing on our simulation results. To determine the preferred position of the particle in the polymer matrix, we calculate the free energy of the system using SCFT, which considers the explicit interac-
tions between the particle and the matrix in the same manner as Matsen.\[32\] The equilibrium nanosphere placement is determined by the minimal free energy as a function of particle position $r_P$. The Helmholtz free energy ($A$) is calculated as:

$$A(n, V, T) = -k_B T \ln Z_C(n, V, T) \approx H^*$$  \hspace{1cm} (A.1)

where $Z_C$ is the canonical partition function, and $H^*$ is the effective Hamiltonian at the mean-field (saddle-point) configuration \{$w^*, r_P$\}. Including the particle position in the formulation of the saddle-point configuration extends the mean-field approximation to the ensemble of particle coordinates. Due to periodic boundary conditions particles are spaced equidistant from each other in the lamellar plane, i.e. $|r_{P,i} - r_{P,j}| = nL_y$, where $n$ is an integer. This approximation is reasonable for semi-dilute particle concentrations, i.e. where nearest-neighbor particle-particle spacings ($\sim L_y$) are greater than 1–2 $R_g$. In this regime we are able to account for weak particle-particle interactions that arise as a consequence to their displacement of polymeric segments, retaining computational tractability and — since there is only one particle per unit cell — the ability to dissect the free energy into its components to analyze the mechanisms by which particles tend to localize. Vanishing particle concentration ($L_y \rightarrow \infty$) recovers the ideal-gas limit employed by Matsen [31]; at high particle concentration ($L_y \rightarrow 0$), the situation treated by Sides in 2D [37] becomes relevant and a mean-field treatment of particle packing is not applicable. Moreover, in this regime specific particle-particle interactions will significantly influence particle aggregation and ultimately macrophase separation.

The effective Hamiltonian is given by:

$$\frac{A}{nk_BT} = \frac{H^*}{nk_BT} = \frac{1}{V} \int_V \text{d}r \left[ \rho_A \rho_B \chi_{AB} N + \rho_B \rho_P \chi_{BP} N \right.$$  
$$\left. - w_A \rho_A - w_B \rho_B + p(\rho_A + \rho_B + \rho_P - \rho_0) \right]$$

$$- \ln Q[w_A, w_B]$$ \hspace{1cm} (A.2)

where $\chi_{AB}$ is the interaction parameter between A and B segment, $\chi_{BP}$ is the interaction parameter between cavity and B segments, $N$ is the total polymerization index, and $\rho_A$, $\rho_B$, and $\rho_P$ are the average densities of $A(B)$ segments and cavities (spherical particle and “wall”) respectively. The single-chain partition function ($Q[w_A, w_B]$) is evaluated from $Q[w_A, w_B] =$
\[ \frac{1}{V} \int_V \, d\mathbf{r} \, q(\mathbf{r}, N; [w_A, w_B]). \]

By incompressibility, the density profiles of A and B segments, and the cavity are constrained to \( \rho_A(\mathbf{r}) + \rho_B(\mathbf{r}) + \rho_P(\mathbf{r}) = \rho_0, \) where \( \rho_0 \equiv nN/V \) is the average segment density.

The cavity function is calculated as follows:

\[ \rho_C(\mathbf{r}) = \rho_P(\mathbf{r}) + \rho_W(\mathbf{r}) = \rho_0 \left[ h(|\mathbf{r} - \mathbf{r}_P|) + h(x - x_W) \right] \quad (A.3) \]

The choice of \( h(x) \) determines the shape of the polymer/cavity interface, approaching a value of 1 in the particle interior and 0 far from the particle surface. The choice of generating function may be nearly arbitrary;[39] here we use a product of tanh functions allowing \( \rho_C \) be continuous over the entire simulation volume:

\[ h(x) = \frac{1}{2} \left[ 1 + \tanh \left( s \frac{R - x}{t} \right) \tanh \left( s \frac{R + x}{t} \right) \right] \quad (A.4) \]

Such a profile, sketched in Figure A.2, is convenient since both interfaces are contained within a single continuously differentiable function when used with periodic boundary conditions. \( h(x) \) is maximal at \( x = 0 \), reaches a value of 0.5 at \( x = \pm R \), and decays from \( \left( \frac{1}{2} + \frac{1}{2} \tanh(s) \right) \) to \( \left( \frac{1}{2} - \frac{1}{2} \tanh(s) \right) \) over a distance of \( t \), the interfacial thickness. All spatial dimensions are in units of \( R_g \), the unperturbed radius of gyration. To approximate a surface \( t < R_g \) should hold; we have found that all values of \( t \) for \( t < 0.5R_g \) lead to qualitatively identical results. Moreover, exceedingly small values of \( t \) delay convergence and require an expensive degree of spatial resolution, and thus we hold \( t = 0.5R_g \) in all calculations. For spherical particles \( R = \frac{dP}{2} \) is simply the particle radius, whereas for the “walls” \( 2R \) is the wall thickness. This should be the smallest value possible that prevents the overlap of the two interfaces; we use a choice of \( R = t \). The parameter \( s \) is chosen such that the thickness defined by \( t \) corresponds to the distance over which \( h(x) \) decays from a value of 0.995 to 0.005; \( s = \tanh^{-1}(0.99) \). In a simulation box of dimensions \( L_x \times L_y \times L_z \), the effective polymer film thickness is \( d = L_x - 2R \).

\( \rho_A(\mathbf{r}) \) and \( \rho_B(\mathbf{r}) \) are described by:

\[ \rho_A(\mathbf{r}) = -\frac{\delta \ln Q}{\delta w_A} = \frac{\rho_0}{VQ} \int_0^f ds \, q(\mathbf{r}, s) q^\dagger(\mathbf{r}, s) \quad (A.5) \]

\[ \rho_B(\mathbf{r}) = -\frac{\delta \ln Q}{\delta w_B} = \frac{\rho_0}{VQ} \int_f^1 ds \, q(\mathbf{r}, s) q^\dagger(\mathbf{r}, s) \quad (A.6) \]
where here \( s \) denotes the contour variable that describes the location of a segment along the backbone of the chain, and \( q(r, s) \) and \( q^\dagger(r, s) \) are the chain propagator and complementary chain propagator, respectively; these satisfy the modified diffusion equation:

\[
\frac{\partial}{\partial s} q(r, s; [w_A, w_B]) = \frac{b(s)^2}{6} \nabla^2 q(r, N; [w_A, w_B]) - w(r, s)q(r, N; [w_A, w_B])
\] (A.7)

where \( b(s) \) is the statistical segment length and \( w(r, s) \) is the auxiliary field. In these simulations, we set \( b = b_A = b_B \).

The MDE was solved using a fourth-order backward difference formula (BDF): [10]

\[
\frac{25}{12}q_{n+1} - 4q_n + 3q_{n-1} - \frac{4}{3}q_{n-2} + \frac{1}{4}q_{n-3} = \Delta s[\nabla^2 q_{n+1} - w(r)(4q_n - 6q_{n-1} + 4q_{n-2} - q_{n-3})]
\] (A.8)

where \( \Delta s \) is the step size along the chain contour. The Laplacian operator is treated implicitly in \( k \)-space through discrete Fourier transforms. Saddle-point configurations of the pressure field were calculated using a semi-implicit relaxation scheme devised by Ceniceros and Fredrickson,[40] while explicit Euler relaxation was used to calculate the mean-field chemical potential fields. The lamellar domain spacing \( d_{AB} \) was optimized through a golden section search.
to minimize the system free energy as a function of $L_x$ for each simulation. Each calculation used a linear AB diblock copolymer architecture with fixed $f_A = 0.5$ and $\chi_{AB}N = (25,30,35)$ for which in the neat state lamellae is the stable phase. The number of collocation points, $n_x$, $n_y$, and $n_z$, are obtained by $(L_x/n_x, L_y/n_y, L_z/n_z) \approx R_g/20$ and $\Delta s = 10^{-3}$ was chosen such that the relative approximate error in $\frac{F}{nk_BT}$ is $\frac{F_{iter+1}-F_{iter}}{F_{iter+1}} < 10^{-6}$.

**A.3 Results**

A typical calculation begins with preconverged ABABA lamellae oriented parallel to the “walls”. A single nanospherical cavity is then introduced with $r_P = (x,0,0)$, where $x = 0$ is defined as the center of the interior $A$ domain. We take the particle concentration as the particle volume fraction within a single lamellar period, $\phi_P = \frac{\pi d_P^3}{6d_{AB} L_y^2}$. Thus by adjusting $L_y = L_z$, changes in the lateral unit cell dimensions correspond to changes in the particle volume fraction; by fixing the particle position we are prescribing a fixed packing (particles are on either a square or hexagonal lattice). We also performed a limited set of calculations in a cell containing the extended structure ABABABA to determine the extent the proximity of the nanosphere to the confining wall has on the results of our simulations.

For example, Figure A.3 shows the determination of the equilibrium, metastable, and unstable values of $x$ through calculations of $\frac{F}{nk_BT}$ vs. $x$ over the range $0 \leq x \leq 1.5 \frac{d_P}{d_{AB}}$ for $\chi_{AB}N = 25$, $\phi_P = 0.05$, particle size $\frac{d_P}{d_{AB}} = 0.15$, and particle selectivity in the range $0 \leq \chi_{BP}/\chi_{AB} \leq 1$. Evidently $F(x = 0)$, the free energy of particle placement in the $A$ domain center, is essentially invariant to the particle selectivity since $B/P$ contacts are nearly excluded at this position. Placement in the $B$ domain interior, $\frac{x}{d_{AB}} = 1$, is metastable through $\frac{\nu_P}{\chi_{AB}} \approx 0.8$, whereas IMDS placement is the equilibrium positioning for selectivity values $0 \leq \frac{\nu_P}{\chi_{AB}} \leq 0.5$. The equilibrium IMDS position varies slightly as a function of selectivity, $0.4 \leq \frac{x}{d_{AB}} \leq 0.5$ for this particular system. A representative density trace through the center of the three dimensional simulation volume for $\frac{d_P}{d_{AB}} = 0.15$ is shown in Figure A.4, illustrating how the polymer matrix responds to the inclusion of the “walls” and the nanosphere. Figure A.5 shows density cross-sections through the particle center for dilute and concentrated systems with both IMDS and DOM
Figure A.3: Free energy (with respect to reference of $\chi_{BP} = 0, x = 0$) vs. particle position for a 2D simulation of a single nanoparticle in an $ABAB$A unit cell ($A$ blocks adjacent to the walls are not shown). Here $\phi_P = 0.05, \chi_{AB}N = 25, \frac{d\phi_P}{d_{AB}} = 0.15$. The curves (from bottom to top) represent $\chi_{BP}N$ values of $\{0, 5, 10, 15, 20, 25\}$. The color scale (blue/red) represents the polymer segment density of the $A/B$ blocks.

Using similar calculations we have computed the equilibrium particle position over a wide range of particle size, concentration, and selectivity. We restrict our attention to the lamellar phase over the entire parameter space. It is important then to qualify our high-$\phi_P$ results with the caveat that particle-driven morphology transformations, observed in both theory and experiment,[37, 19] are neglected. Nonetheless, we assert that in qualitative terms these results are still applicable since as shown by Matsen,[31] the effects of the copolymer composition on the equilibrium particle placement serves only to broaden the particle distribution. Thus, for example, while we would perhaps expect a ($f_A = 0.5, \phi_P = 0.15$) system to form a cylindrical morphology, a nanocomposite system forming lamellae (e.g., $f_A = 0.35, \phi_P = 0.15$) exhibits the same energetic behavior we report for a ($f_A = 0.5, \phi_P = 0.15$) system constrained to the lamellar phase. In support of this claim, we have repeated a subset of our calculations adjusting $f_A$ such that $f_A + \phi_P = 0.5$ within the central $A$ domain (see Figure A.7) illustrating that the predicted particle placement is a weak function of polymer composition.

Further calculations, summarized in Table A.1 proceed rapidly ($< 5000$ field iterations) using converged solutions from similar values of $\phi_P$, $\frac{d\phi_P}{d_{AB}}$, and $x$; this feature enables fully 3D
Figure A.4: Representative saddle-point density traces of diblock/nanoparticle mixtures (through the particle center) in 3 dimensions. $\phi_A$, $\phi_B$, and $\phi_i$ are the segment densities of $A$ (solid line) and $B$ (dashed line) blocks, and the cavity (red line), which describes the wall and the nanoparticle, respectively. a) The particle is located at the interface between $A$ and $B$ blocks. b) The particle is placed to the domain interior of $A$ block.
Figure A.5: 3D density snapshots at fixed $\chi_{AB}N=25$ and $d_P/d_{AB}=0.2$ with spatial dimensions in units of $d_{AB}$. a) IMDS and b) DOM positioning with $\phi_P=0.05$, c) IMDS and d) DOM phases at $\phi_P=0.2$. 
Table A.1: Summary of the parameter space sampled in this study using our SCFT model. In every case $f_A = 0.5$ and the particle selectivity $\frac{\chi_{BP}}{\chi_{AB}}$ was sampled in the range $[0, 1.2]$ in increments of 0.02.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>$\phi_P$</th>
<th>$\frac{d_P}{d_{AB}}$</th>
<th>$\chi_{AB}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.01 – 0.17</td>
<td>0.05</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>0.02 – 0.18</td>
<td>0.10</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>0.02 – 0.21</td>
<td>0.15</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>0.005 – 0.075</td>
<td>0.075</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>0.005 – 0.13</td>
<td>0.15</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>0.005 – 0.13</td>
<td>0.15</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>0.005 – 0.13</td>
<td>0.15</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>0.005 – 0.20</td>
<td>0.20</td>
<td>25</td>
</tr>
</tbody>
</table>

calculations over the parameter space with only modest computational resources. We include analogous 2D calculations for the purposes of comparison.

The results of our calculations are summarized in the phase diagrams presented in Figures A.8 (2D) and A.9 (3D). The two dimensional calculations, corresponding to nanocylindrical particles (Figure A.8), show a monotonic decrease in the selectivity required to favor DOM placement, with respect to the particle concentration, irrespective of the particle diameter. At dilute particle concentrations, $\phi_P \lesssim 0.05$, smaller particles more strongly favor IMDS placement. In the dilute limit, we find that the particle selectivity ($\frac{\chi_{BP}}{\chi_{AB}}$) must be greater than unity ($\chi_{BP} > \chi_{AB}$) in order for the DOM phase to become stable. As the cylinder diameter increases, the selectivity of the IMDS-DOM transition is reduced to about $\frac{\chi_{BP}}{\chi_{AB}} \approx 0.85$. This is consistent with experimental observations that small particles tend to favor the IMDS phase. As the particle concentration increases, however, the selectivity of the IMDS-DOM transition drops more rapidly for smaller particles than for larger ones, such that at moderate concentrations larger particles are more likely to localize to the IMDS.

Our 3D calculations with nanospheres (Figure A.9) show similar behavior with respect to the influence of particle diameter: at dilute concentrations smaller particles more strongly favor IMDS placement while as the concentration increases they tend towards DOM placement.
compared to larger particles. The most significant distinction between the 2D and 3D calculations emerge, however, as we examine the concentration dependence of DOM vs. IMDS placements: while the selectivity required for DOM placement decreases monotonically for the smallest particle size ($\frac{dp}{d_{AB}} = 0.075$), as the particle concentration increases larger particles more strongly favor IMDS placement until a critical concentration beyond which DOM placement again becomes more preferable.

Figure A.6 investigates the possibility that thin-film effects, i.e. those arising from the proximity of the “walls” to the lamellar period containing the nanoparticle, are dominant in the determination of the particle placement phase diagram. This example compares the 3-period system employed throughout the study with an analogous 7-period system; the latter case requires more computational effort but considerably diminishes the influence of the confining walls. At a representative selectivity and particle size of $\chi_{AB}N = 25/\frac{dp}{d_{AB}} = 0.15$, Figure A.6 shows qualitatively identical particle placement behavior; this result indicates that the DOM-IMDS-DOM transition is not an artifact of the confining walls.

Figure A.7 explores the role of copolymer composition on equilibrium particle placement behavior. Under the constraints of our study, we consider only the lamellar phase. Both experiments\cite{12} and theory\cite{37} demonstrate that an immediate effect of increasing particle concentration is to swell the polymer domain preferred by the particle (here the A domain), eventually inducing an order-to-order transition, e.g. LAM-HEX. At the higher end of the $\phi_P$ range we consider, the A domain is swollen disproportionately with nanoparticles and thus the lamellar phase is not likely the lowest-energy mesophase. In Figure A.7, we compare two systems with $\chi_{AB}N = 25$ and $\frac{dp}{d_{AB}} = 0.15$, chosen as a representative point in parameter space corresponding to typical experimental conditions. In one scenario, corresponding to the bulk of our other calculations, the copolymer composition is constant, $f_A = 0.5$, as the particle concentration increases. In the second scenario depicted in Figure A.7, we adjust the copolymer composition such that $f_A + \phi_P = 0.5$, i.e. the net volume of the swollen A phase remains equal to that of the B phase, where the lamellar morphology should be stable. There is no discernible difference between the two cases, which is not surprising given Matsen’s finding that
Figure A.6: 3D phase diagram indicating the equilibrium placement of nanospheres at $\chi_{AB}N = 25$ and $\frac{dP}{d_{AB}} = 0.15$ as a function of particle concentration ($\phi_P$) and selectivity ($\frac{\chi_{BP}}{\chi_{AB}}$) for the original ABABA morphology (●) and the extended ABABABA morphology (○).

Figure A.7: 3D phase diagram indicating the equilibrium placement of nanospheres at $\chi_{AB}N = 25$ and $\frac{dP}{d_{AB}} = 0.15$ as a function of particle concentration ($\phi_P$) and selectivity ($\frac{\chi_{BP}}{\chi_{AB}}$) at the original morphology (●), and the morphology which the particle volume was extracted from A block (○).

The copolymer composition dependence of particle placement in lamellar mesophases is quite weak. This comparison is important in that it demonstrates that while our restriction to the lamellar morphology may be artificial in that it precludes particle-induced phase transitions, analogous systems with polymer composition adjusted to form lamellae can be expected to exhibit the same behavior.

These examples of “reentrant” behavior are unique to the 3D system and thus imply a shift in the balance of entropic/enthalpic contributions to the free energy. For example, the screening of polymer segment-segment interactions represents an enthalpic mechanism for IMDS placement; mechanisms such as these could be strongly dependent on the degree of polymer segregation strength and qualitative differences in the particle placement phase diagram are intuitively possible. In consideration of this possibility we repeated a subset of our 3D calculations at $\frac{dP}{d_{AB}} = 0.15$ varying $\chi_{AB}$. These results are shown in Figure A.10. The importance of segment-segment screening is evidenced through the shift to higher requisite particle selectivity for DOM placement as $\chi_{AB}$ increases. However, the concentration dependence of the IMDS-DOM transition, and the concentration-dependent reentrant behavior thereof, is qualitatively independent of polymer segregation strength. Interestingly, the strong dependence of
the IMDS-DOM transition on $\chi_{AB}$ indicates that strongly segregated melts clearly favor IMDS placement, and that DOM placement only becomes likely as the weak segregation regime is approached.

### A.4 Discussion

In this work, we have implemented a simplified model that treats three-dimensional mixtures of symmetric diblock copolymer and spherical nanoparticles. Polymer statistical thermodynamics are treated in the mean-field limit, i.e. with SCFT, and the particles are modeled as cavity functions that encapsulate their strong excluded volume. Through the use of confining walls in the $yz$-plane, we constrain the polymer morphology to lamellae which are unable to translate in the $x$-direction. We use periodic boundary conditions, so that placing 1 particle in the simulation volume (a) fixes the lateral packing of the particles and (b) the particle concentration may be adjusted through the $L_y$ and $L_z$ cell dimensions. This amounts to a mean-field
Figure A.10: Phase diagram of the mixture of diblock copolymer and nanoparticle ($\phi_p = 0.15$) in 3 dimensions about the preferred particle position between IMDS and DOM phases determined by the free energies depending on $\chi_{AB,N}$. $\chi_{BP}$ is the interaction parameter between B segment and particle, and $\chi_{AB,N}$ varies 25 (closed circle, solid line), 30 (open circle, solid line), and 35 (closed triangle, solid line).
approximation with respect to the ensemble of particle positions; such an approximation is relevant for semi-dilute nanocomposites.

Within this model, particles interact with other only through their perturbations to the polymer segment density between particles; these interactions are responsible, for example, the depletion effect[41]. Accordingly this study bridges ideal gas limit presented by Matsen[31] and the study by Sides[37] in three important ways: (1) Treatment of particle-particle interactions: Matsen’s work neglects particle-particle interactions entirely while Sides’ more fully accounts for them through steepest-descent sampling of particle positions within a 2D unit cell. The present study partially accounts for particle-particle interactions as described above. (2) Computational cost: Sides’ approach is most costly due to multiple particle move/SCFT convergence cycles, restricting the treatment to 2D on a fairly coarse grid (72 × 80 , or ≈ 0.11R_g per collocation point). In contrast Matsen’s approach is quite inexpensive since it exploits the azimuthal symmetry of the single-particle system and thus models three spatial dimensions with only two computational dimensions. Our method incurs intermediate expense with three dimensional sampling at ≈ 0.05R_g per collocation point yet with only a single SCFT convergence cycle per particle position. Our results illustrate that SCFT predicts significantly different behavior in 2D vs. 3D systems. (3) Amenability to analysis: Matsen’s paper treats only a single particle and thus changes in contributions to the free energy are directly attributable to changes in the particle position; in the present study we retain the same ability to attribute energetic effects directly to particle position since we consider a single particle per unit cell. Sides’ study does not readily allow this type of analysis since there are many particles within the simulation domain.

Macrophase separation is a possibility that we do not consider within this model. While a potential concern for inadequately passivated nanocomposites, numerous experimental studies covering the range of parameter space that we consider do not indicate macrophase separation [15, 13, 14, 12, 17]. As a further comparison, we summarize the results of these studies in Table A.2 and plot them against our 3D particle placement diagram in Figure A.11; these studies find particle placements in agreement with the predictions of our model.
Table A.2: Summary of experimental particle placement data extracted from Refs [15, 13, 14, 12, 17]. These points are plotted in Figure A.11 using the lettered notation.

<table>
<thead>
<tr>
<th>$d_P/d_{AB}$</th>
<th>$\phi_P$</th>
<th>$\chi_{PS-P2VP}$</th>
<th>Domain/IMDS</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>0.02</td>
<td>Unknown (Fe$_2$O$_3$)</td>
<td>IMDS</td>
<td>Lauter 1997 [13]</td>
</tr>
<tr>
<td>0.24</td>
<td>0.01</td>
<td>Unknown (Fe$_2$O$_3$)</td>
<td>PS center</td>
<td>(PS-PBMA) with $\gamma$-Fe$_2$O$_3$</td>
</tr>
<tr>
<td>0.06</td>
<td>0.02</td>
<td>~1 (PEP prefer: Au)</td>
<td>IMDS (a)</td>
<td>Bockstaller 2003 [15]</td>
</tr>
<tr>
<td>0.26</td>
<td>0.02</td>
<td>(~1 (PEP prefer: SiO$_2$)</td>
<td>PEP center (b)</td>
<td>(PS-PEP) with Au and SiO$_2$</td>
</tr>
<tr>
<td>0.154</td>
<td>0.15</td>
<td>~1 (PS prefer: Au)</td>
<td>PS center (c)</td>
<td>Chiu 2005 [12]</td>
</tr>
<tr>
<td>0.154</td>
<td>0.15</td>
<td>~1 (P2VP prefer: Au)</td>
<td>P2VP center (d)</td>
<td>(PS-P2VP) with Au</td>
</tr>
<tr>
<td>0.154</td>
<td>0.25</td>
<td>~0.25 (PS-P2VP mixed: Au)</td>
<td>IMDS (e)</td>
<td></td>
</tr>
<tr>
<td>0.18</td>
<td>0.15</td>
<td>0 (~$(\chi_{PS-P2VP})$)~1</td>
<td>P2VP center (f)</td>
<td>Kim 2007 [17]</td>
</tr>
<tr>
<td>0.18</td>
<td>0.15</td>
<td>0.36 (~$(\chi_{PS-P2VP})$)</td>
<td>P2VP center (g)</td>
<td>(PS-P2VP) with Au</td>
</tr>
<tr>
<td>0.18</td>
<td>0.15</td>
<td>0.25 (~$(\chi_{PS-P2VP})$)</td>
<td>IMDS (h)</td>
<td></td>
</tr>
<tr>
<td>0.18</td>
<td>0.15</td>
<td>0.0036 (~$(\chi_{PS-P2VP})$)~0.88</td>
<td>PS center (i)</td>
<td></td>
</tr>
</tbody>
</table>

Figure A.11: Comparison of the SCFT 3D phase diagram (black, identical to Figure A.9) with experimental data (red and blue) from Refs [15, 13, 14, 12, 17], which are summarized in Table A.2. For the experimental data, each point is labeled (a)–(i) in reference to the corresponding entry in Table A.2; the symbol indicates the preferred placement by its color (red: DOM, blue: IMDS) and the particle size ($d_P/d_{AB}$) by its shape (closed circle: 0.075, open circle: 0.15, and triangle: 0.26).
To facilitate our interpretation of the particle placement phase diagrams presented in the Results section, we decompose the dependence of free energy, Eq A.2, on \((\phi_P, \frac{d_P}{d_{AB}}, \frac{\chi_{BP}}{\chi_{AB}})\) into contributions from four competing terms: 

\[
F_{AB} \equiv \frac{1}{V} \int_V d\mathbf{r} \phi_A(\mathbf{r}) f_B(\mathbf{r}) \chi_{AB},
\]

\[
F_P \equiv \frac{1}{V} \int_V d\mathbf{r} f_B(\mathbf{r}) \rho_P(\mathbf{r}) \chi_{AB},
\]

\[
F_{\ln Q} \equiv -\ln Q,
\]

\[
F_{w\phi} \equiv -\frac{1}{V} \int_V d\mathbf{r} f_A(\mathbf{r}) w_A + f_B(\mathbf{r}) w_B.
\]


\(F_{AB}\) and \(F_P\) represent enthalpic contributions from unfavorable \(A/B\) and \(B/P\) contacts, respectively. \(F_{\ln Q}\) is an entropic term representing the chain stretching energy required to perturb the Gaussian coils into their equilibrium configuration. Finally, \(F_{w\phi}\) represents the energy required to generate a particular composition pattern, including both entropic and enthalpic effects. Comparison of these terms allows us to readily discern the predominant driving forces for IDMS vs. DOM placement throughout the parameter space. For example, Figures A.12 and A.13 shows how these components of the free energy vary versus either \(\phi_P\) or particle selectivity \(\frac{\chi_{BP}}{\chi_{AB}}\) with both DOM and IMDS positions. In all cases this dependence is nearly linear; the slopes of these curves, 

\[
\frac{\partial F_i/nk_BT}{\phi_P} \bigg|_{\chi_{BP}/\chi_{AB}} \quad \text{and} \quad \frac{\partial F_i/nk_BT}{d_{AB}/\chi_{AB}} \bigg|_{\phi_P},
\]

represent the sensitivity of the free energy contributions to changes in particle concentration and selectivity, respectively.

We are thus able to obtain a broader view of these observations by extracting the sensitivity from all calculations by treating the \(\frac{F_i}{nk_BT}\) vs. fixed \(\frac{\chi_{BP}}{\chi_{AB}}\) or \(\phi_P\) data as linear for all \(\phi_P\) or \(\frac{\chi_{BP}}{\chi_{AB}}\). We denote the sensitivity as \(\epsilon_{i,j}\), the average slope of the free energy contribution \(i\) as parameter \(j\) (concentration or selectivity) varies:

\[
\epsilon_{i,j} \equiv \left\langle \frac{\partial \left( \frac{F_i}{nk_BT} \right)}{\partial j} \right\rangle_k \quad \left\{ \begin{array}{l} i = AB, P, \ln Q, w\phi \\ j = \phi_P, \frac{\chi_{BP}}{\chi_{AB}} \\ k = \frac{\chi_{BP}}{\chi_{AB}}, \phi_P \end{array} \right. \quad (A.9)
\]

The introduction of \(\epsilon_{i,j}\) facilitates the analysis of our results since serves to eliminate an entire degree of freedom from the parameter space.

\(\epsilon_{i,\phi_P}\) describes how sensitive free energy component \(i\) is to changes in \(\phi_P\). Figure A.14 plots this quantity versus \(\frac{\chi_{BP}}{\chi_{AB}}\) for two particle sizes, \(\frac{d_P}{d_{AB}} = 0.075\) and \(\frac{d_P}{d_{AB}} = 0.015\), for both DOM and IMDS placement. Each point corresponds to the average slope extracted from \(F_i\) vs. \(\phi_P\) data such as those presented in Figure A.12 at a particular selectivity. The value of \(\epsilon_{i,\phi_P}\) is
Figure A.12: Dependence of the free energy on $\phi_P$ for IMDS (●) and DOM (○) particle placement at fixed $\frac{\chi_{BP}}{\chi_{AB}} = 0.8$ and $\frac{d_P}{d_{AB}} = 0.15$. 
Figure A.13: Dependence of the free energy on $\frac{\chi_{BP}}{\chi_{AB}}$ for IMDS (●) and DOM (○) particle placement at fixed $\phi_p = 0.025$ and $\frac{d_P}{d_{AB}} = 0.15$. 
Figure A.14: Sensitivity with respect to $\phi_P$ over sampled values of $\frac{\chi_{BP}}{\chi_{AB}}$ of the free energy contribution to IMDS (filled) and DOM (open) placement for $\frac{dp}{d_{AB}} = 0.075$ (\triangle) and $\frac{dp}{d_{AB}} = 0.15$ (○).
Figure A.15: Sensitivity with respect to $\frac{\chi BP}{\chi AB}$ of the free energy contribution to IMDS (filled) and DOM (open) placement versus particle concentration for $\frac{dp}{d_{AB}} = 0.075$ ($\triangle$) and $\frac{dp}{d_{AB}} = 0.15$ ($\circ$).
only weakly dependent on $\frac{\chi_{BP}}{\chi_{AB}}$; that is, the manner in which composition effects the energetics is essentially $\frac{\chi_{BP}}{\chi_{AB}}$-dependent.

$\epsilon_{AB,\phi_P} < 0$ for particles situated at the interface represents the “screening” effect; i.e., as the concentration of particles at the interface increases, $A/B$ contacts are eliminated in favor of polymer/particle interactions. This is far more pronounced for smaller particles as a consequence of their higher specific surface area. Interestingly, for DOM placement $\epsilon_{AB,\phi_P} > 0$, tending to destabilize the DOM configuration as the particle concentration increases since polymer expelled from the domain interior results in increased $A/B$ interfacial area. This mechanism further stabilizes IMDS placement for enthalpic reasons, and explains in part why the particle selectivity must be so close to unity for the stability of the DOM configuration as shown by our calculations and also experimental observations.[17] Moreover, this explains why IMDS placement becomes more favorable as $\chi_{AB}N$ increases. As is to be expected, the role of particle selectivity is expressed primarily through $F_{BP}$; $\epsilon_{BP,\phi_P} > 0$ for particles with IMDS placement indicative of the increased $B/P$ contact with $\phi_P$. Since here the particle selectivity $\frac{\chi_{BP}}{\chi_{AB}} < 1$, the enthalpy of the system is reduced as more particles are placed at the IMDS compared to the domain center.

Entropic effects associated with increasing particle concentrations may be understood through $\epsilon_{\ln Q,\phi_P}$ and $\epsilon_{w,\phi_P}$. As particles are added to the system, further perturbations to the Gaussian coil dimensions occur and accordingly the elastic energy associated with chain stretching should increase. $\epsilon_{\ln Q,\phi_P}$ gauges the average elevation of the chain stretching energy (i.e., translational entropy) as $\phi_P$ is raised; it has a positive value for both IMDS and DOM placement and is increases as the particle diameter is reduced. Small particles feature stronger degrees of curvature and thus require more drastic chain rearrangements than do larger ones; this is reflected in Figure A.14 through larger $\epsilon$ values for the small particle case. The introduction of particles to the domain interior evidently causes more chain stretching than at the IMDS; at the IMDS the disruption induced by the particle is nearly balanced between $A$ and $B$ segments, whereas in the domain interior it is essentially the $A$ segments that must rearrange to accommodate the inclusion. The disparity between IMDS and DOM positioning on the chain
stretching sensitivity $\epsilon_{\ln Q, \phi_P}$ is larger for small particles than for larger ones, consistent with the notion that entropic effects tend to favor the placement of small particles at the IMDS.

In contrast to chain stretching contributions, the effect of particle placement on the composition pattern as manifested through $\epsilon_{w\phi, \phi_P}$ is markedly different for the IMDS and DOM states. In SCFT, interchain interactions are decoupled through the introduction of “chemical potential” fields $w_A$ and $w_B$. Within domain interiors, there is little spatial dependence on the value of $w_i$, whereas at domain interfaces these fields change drastically to drive the formation of the resultant density pattern. Thus the energy associated with the formation of a particular $A/B$ interface and the corresponding translational entropy thereof is encapsulated within the $F_{w\phi}$ term of the Hamiltonian. Particulate inclusions at the interface cause significant perturbations to the structure of the $A/B$ interface; the characteristic frequency of this perturbation is related to the particle size. Accordingly, the injection of smaller particles is accompanied with a significantly larger value of $\epsilon_{w\phi, \phi_P}$ compared to larger ones. At the domain interior, the structure of the $A/B$ interface changes little since the particles are “hidden” from the interface; in this situation the $F_{w\phi}$ contribution actually decreases, and the particle size has little influence.

While there is only slight dependence $\frac{\partial F_i / (nk_B T)}{\partial \phi_P} \bigg|_{\chi_{AB}}$ on $\phi_P$, it is very small deviations from linearity that give rise to the “reentrant” behavior we predict in the particle placement phase diagram (Figures A.9, A.10). The origins of this behavior are better understood by considering the sensitivity of the free energy to changes in the particle selectivity, $\epsilon_i, \chi_{BP}$, as a function of particle concentration (Figure A.15). Figure A.15 shows that for particles in the DOM position, the only component of the free energy that is sensitive to the particle selectivity is $F_{AB}$; here $\epsilon_{AB, \chi_{BP}}$ becomes increasingly negative as the particle concentration increases. That is, for particles in the DOM position, systems with highly selective particles tend to sharpen the $A/B$ interface thereby reducing the value of $F_{AB}$. At the IMDS, however, all components of the free energy are highly sensitive to the particle selectivity, and the dependency of this sensitivity on $\phi_P$ is the underlying mechanism for the reentrant DOM-IMDS-DOM behavior that we predict. At dilute particle concentration, only a small fraction of the system is influenced directly by
displacement of $A/B$ contacts in favor of $B/P$ contacts. That is, the $A/B$ interface is only locally perturbed by the presence of the nanoparticles. In view of Figure A.15a, $\epsilon_{AB, x_{AB}}$ for $d_P / d_{AB} = 0.15$ is nearly negligible at the IMDS until the inflection point at $\phi_P = 0.05$ is reached.

At this concentration particles begin to “communicate” indirectly through their influence on the interfacial structure. Likewise, we observe inflection points in $\epsilon_i, x_{AB}$ in Figure A.15b–d for $d_P / d_{AB} = 0.15$ at $\phi_P = 0.05$. Thus at dilute particle concentration the most sensitive contribution to the free energy is $F_{BP}$, the enthalpic contribution from $B/P$ contacts.

As a size-dependent critical particle concentration is reached, particles become close enough to one another that the effects of particle selectivity on the composition pattern become evident. $\epsilon_{BP, x_{AB}}$ vs. $\phi_P$ tapers, but increases in $x_{AB}$ expel more $B$ segments from the particle vicinity, forcing the formation of additional $A/B$ contacts and more strongly stretching polymer chains in avoidance of $B/P$ contacts. Up until this critical concentration, the selectivity range over which IMDS placement is favorable becomes larger primarily due to the disproportionate stabilization from the composition pattern component of the free energy, $F_{w\phi}$, in comparison with the enthalpic and chain stretching components.

Very interestingly, the reentrant behavior indicated by Figures A.9 and A.10 is only observable if fully 3D calculations are conducted. Our 2D results (Figure A.8), corresponding to semi-infinite cylindrical particles, show no reentrant behavior. This is most likely a direct consequence of the greater portion of the particle surface that is directly immersed in the $A/B$ interface in the 2D case. The qualitative difference in these 2D vs. 3D IHSCT results suggests that experimental comparisons with simulations conducted with the former restriction should be compared with caution.

A.5 Conclusions

We have investigated the self-assembly the spherical nanoparticles in $AB$ diblock copolymer melts using a simple model in which one $A$-selective particle per periodic unit cell is placed into the either the $A/B$ intermaterial dividing surface (IMDS) or the $A$ domain interior. This simplified approach has allowed for the first time an expansive study of this system over a
vast range of particle sizes, particle selectivity, and particle concentration. Our results show in general that greater particle selectivity is required to localize smaller particles to the domain interior, in agreement with experimental results.[13, 14, 5] However, this preference for the IMDS is highly dependent on particle concentration and also polymer segregation strength. Moreover, we have reproduced the experimental discovery of Kim et al. that significant particle selectivity is required to realize domain interior particle segregation.[17] Most significantly, we predict that moderately selective systems may show a “reentrant” behavior, in which domain interior placement is favored at very dilute and very concentrated systems, whereas IMDS placement is preferred at intermediate concentrations. This approach, reinforced by its agreement with existing experimental data[15, 13, 14, 12, 17], should be useful for further design of experimental nanocomposite systems.

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