

Dynamics of Bengal Rose Encapsulated in the Meijer Dendrimer Box

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Abstract: We used molecular dynamics (MD) techniques to examine the encapsulation of Bengal Rose (BR) molecules in the Meijer dendrimer box (DBox) formed by the addition of *tert*-butyloxycarbonyl-L-Phe (tBOC-L-Phe) cap molecules to the 64 terminal primary amines of a fifth generation poly(propyleneimine) (PPI-5) dendrimer. Using a large periodic box (including DBox, four to six BR, and CH₂Cl₂ solvent, totaling ~25 000 atoms), we examined the MD of these systems for ~0.5 ns. Without the cap we find that BR molecules establish a concentration dependent equilibrium between the interior and surface regions of PPI-5 and the solvent region outside the dendrimer. The number of BR molecules calculated to associate with the interior of the PPI-5 dendrimer agrees exactly with experiment (at the same BR/PPI concentration). MD simulations on the DBox in CH₂Cl₂ show that the tBOC-L-Phe surface is completely impermeable to encapsulated BR molecules, even when an excess is forced inside the box. The close correspondence of the theory with experiment suggests that these methods can be used to design such systems in advance of experiment.

I. Introduction

Dendrimers and hyperbranched polymers represent a novel class of structurally controlled macromolecules derived from a branches-upon-branches structural motif.¹ Dendrimers are well defined, highly branched macromolecules that radiate from a central core and are synthesized through a stepwise, repetitive reaction sequence that guarantees complete shells for each generation, leading to polymers that are monodisperse.² The synthetic procedures developed for dendrimer preparation permit nearly complete control over the critical molecular design parameters, such as size, shape, surface/interior chemistry, flexibility, and topology.^{1,2} Synthetic techniques proved effective include the Starburst divergent strategy (Tomalia and co-workers^{1,2}), the convergent growth strategy (Fréchet and co-workers³), and the self-assembly strategy (Zimmerman and co-workers⁴). These methods have proved effective in generating macromolecules with a unique combination of properties.^{5,6}

The geometric characterization of dendrimer structure has lagged this rapid progress in synthesis and design.¹ The problem is that these molecules possess an enormous number of energetically permissible conformations, and in solution there is rapid interchange between them. Thus diffraction techniques yield little structural information. Also each generation involves the same monomers, making it difficult to extract precise

information about the local structure from infrared or NMR experiments. Thus the most precise experimental data about overall structure comes from size exclusion chromatography (SEC).^{1,2} The main experimental data about the geometric character of particular sites has come from NMR relaxation times for molecules able to partially penetrate into the dendrimer.⁷

Consequently, we have used atomistic simulations to characterize structure and other properties of these systems.^{1,8} The previous results on how the structure and character of the PAMAM and PE dendrimers change with generation^{1,10} were confirmed experimentally.^{1,7}

An intriguing potential application of dendrimers is selective encapsulation of molecules for later separation and release into another environment. Indeed Meijer and co-workers⁶ have demonstrated the capabilities of the dendrimer box (DBox) for encapsulation and selective liberation of numerous guest molecules.¹ They demonstrated that the fifth generation poly(propylene imine) (PPI-5) dendrimer capped with *tert*-butyloxycarbonyl-L-Phe (tBOC-L-Phe) will encapsulate, for example, up to four Bengal Rose (BR) dye molecules from CH₂Cl₂ solvent.

In order to determine how well theory can serve in predicting encapsulation properties of dendrimers, we have studied the Meijer DBox system. Thus we carried out molecular dynamics (MD) theoretical studies of the DBox in the same solvent and find the same encapsulation properties. The close correspondence with experiment indicates that such theoretical studies could be carried out prior to experiment in order to design new molecular systems that might, for example, contain a central core suitable for recognizing particular molecules but might involve an outer generation that controls encapsulation and

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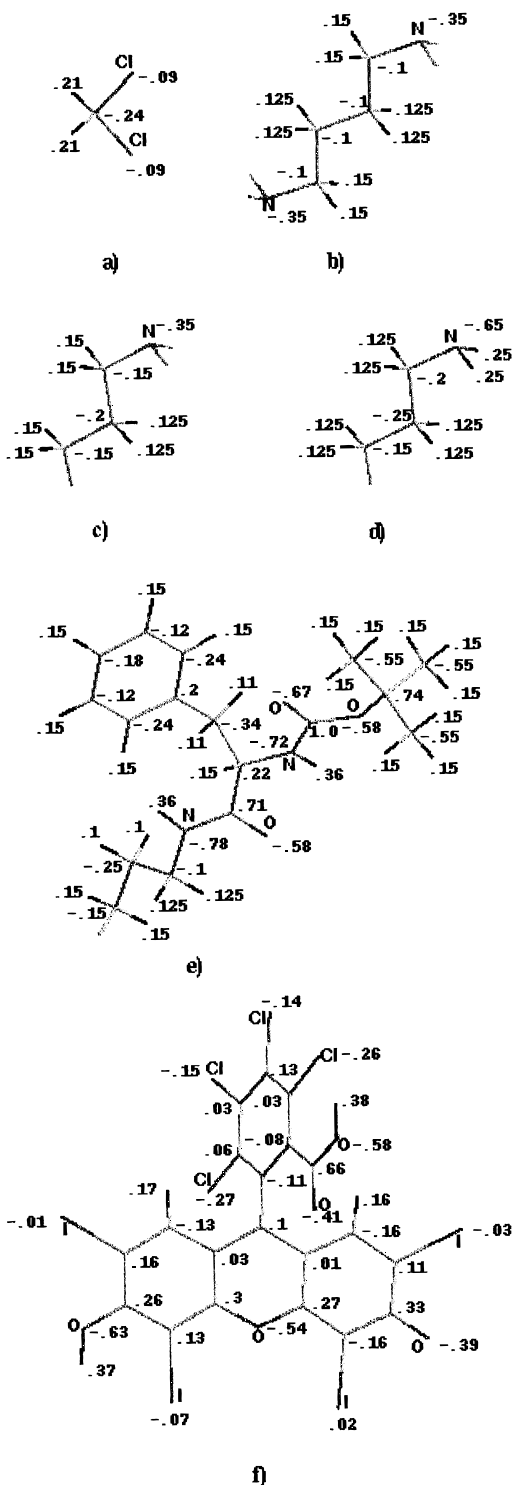


Figure 1. Charges: (a) CH_2Cl_2 ; (b) butylamine (BA) core; (c) polypropylamine (PA) interior; (d) exterior PA; (e) PA plus tBOC-L-Phe cap; (f) BR. All atoms except H and C are labeled explicitly.

which targets particular recognition sites. This might have applications for drug delivery, for protection of dyes from photoinduced oxidation, and for incorporation of long-lived additives to protect polymers.

Section II summarizes the theoretical methods, while section III applies them to the dendrimer box. The significance of the calculations and relevance to experiment are discussed in section IV.

II. Computational Details

II.A. Force Field, Charges, MD. We used the DREIDING force field (FF) which has been found to be accurate for organic

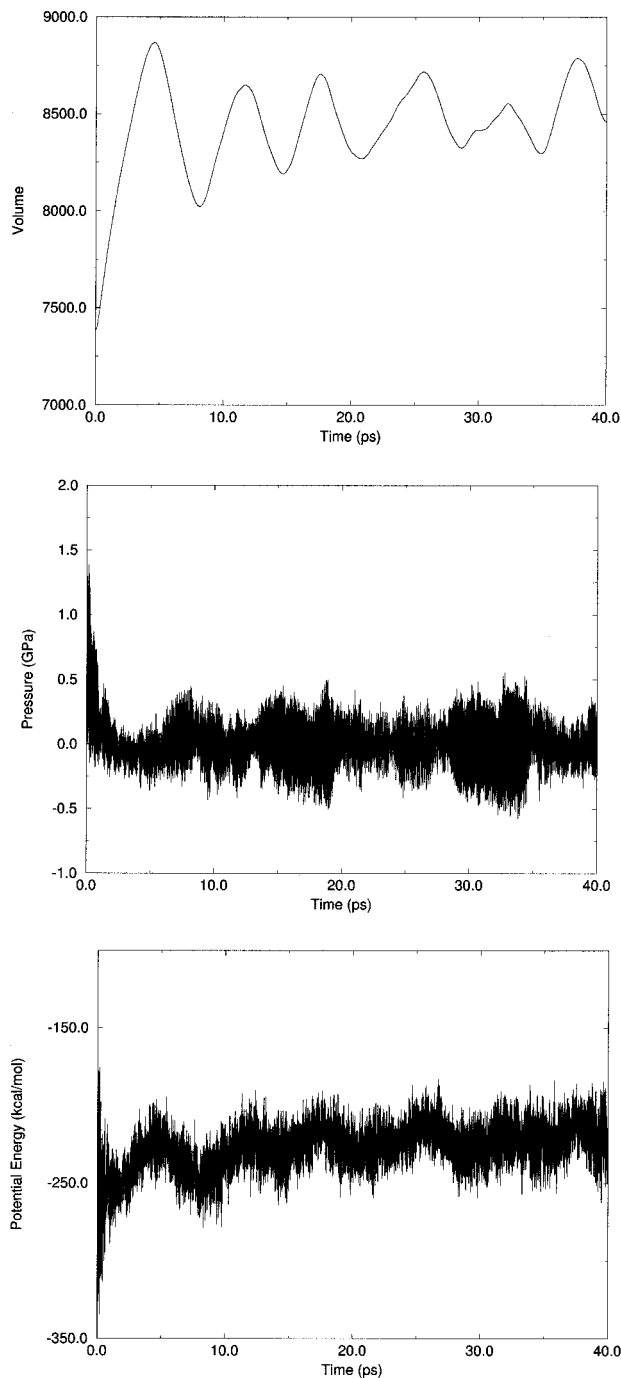


Figure 2. NPT equilibration of CH_2Cl_2 box (74 molecules per cell): (a) volume, (b) pressure, and (c) potential energy.

systems.⁹ Charges for the PPI-5 dendrimer were obtained using charge equilibration (QEq).¹⁰ Charges for various molecules (BR, CH_2Cl_2 , and the tBOC-L-Phe cap with propylamine) were determined from the electrostatic potential (potential derived charges) calculated from quantum chemistry^{11,12} (Hartree-Fock with the 6-311G** basis set). These charges are summarized in Figure 1.

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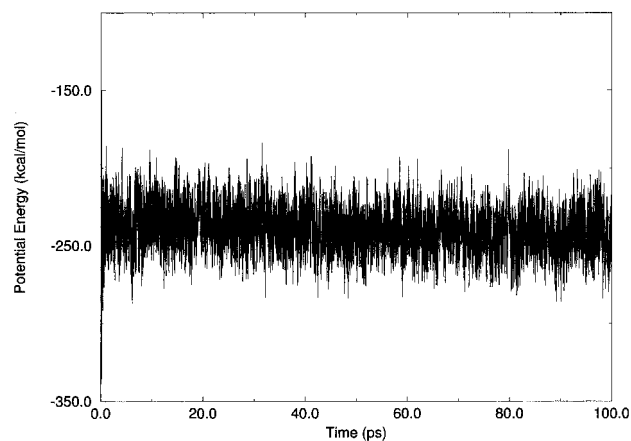


Figure 3. NVT equilibration of CH₂Cl₂ box (74 molecules per cell) with $a = 20.5 \text{ \AA}$.

Table 1. Total Number of Molecules and Atoms in the MD for Various Cases

system	dendrimer (atoms)	BR (atoms)	CH ₂ Cl ₂ (molecules)	total (atoms)
solvent box	0	0	4736	23 680
4BR/DBox	3622	4 × 37	4269	25 115
6BR/DBox	3622	6 × 37	4258	25 134
4BR/PPI-5	1382	4 × 37	4570	24 380
6BR/PPI-5	1382	6 × 37	4550	24 350

The molecular dynamics (MD) calculations used periodic boundary conditions (PBC) with a cubic box of dimension 82 \AA . We used the MPSim program¹³ developed for very large scale MD on the Kendall Square Research (KSR) computer. We used the Reduced Cell Multiple Method (RCMM)^{14,15} with far-field updating every five time steps. Unless indicated otherwise, we carried out NVT (constant volume and temperature) MD at a temperature of 300 K, using the Nosé temperature thermostat with a relaxation time of 0.02 ps and a time step of 0.001 ps.

II.B. Solvent Box. To obtain an accurate description of the solvent, we first performed NPT dynamics (constant pressure and temperature) at 300 K with 74 CH₂Cl₂ molecules¹⁶ per unit cell using Ewald summations¹⁷ for nonbond interactions (Coulomb and vdW). Averaging over the last 30 ps of a 40 ps equilibration (see Figure 2) leads to a density of 1.211 g/ml, corresponding to a cube of length 20.5 \AA . Using the cubic unit cell of dimension 20.5 \AA , we then equilibrated the 74 CH₂Cl₂ molecules for 100 ps with NVT dynamics (see Figure 3). The 20.5 \AA unit cell after the 100 ps of equilibration was replicated 63 times to create the 82 \AA unit cell (4736 CH₂Cl₂ or 23 680 atoms) used in simulations of the dendrimers. See Table 1 for a summary.

II.C. Building Procedure. The fifth generation PPI dendrimer and DBox were built by optimizing the geometries (in vacuum) to minimize the energy after adding each generation

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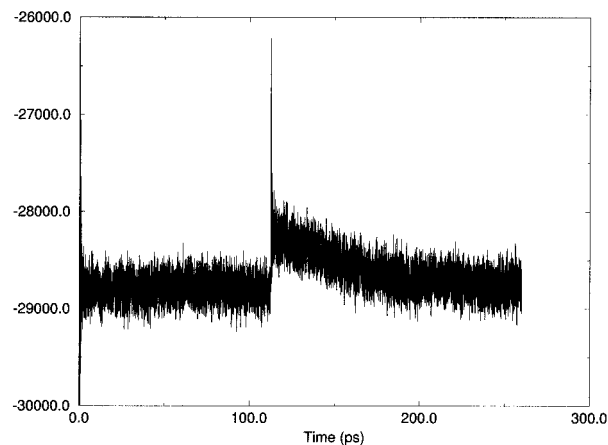


Figure 4. NVT equilibration of 4BR/DBox. Shown is the potential energy. For the first 112.5 ps (75 000 steps) we used a time step of 0.0015 ps to accelerate equilibration, followed by dynamics using a time step of 0.001 ps for the remaining 147.5 ps (147 000 steps). The perturbation in the trajectory at 112.5 ps arises because we reinitialized the velocities when the time step was reduced to 0.001 ps. The potential energy (vertical axis) is in kcal/mol.

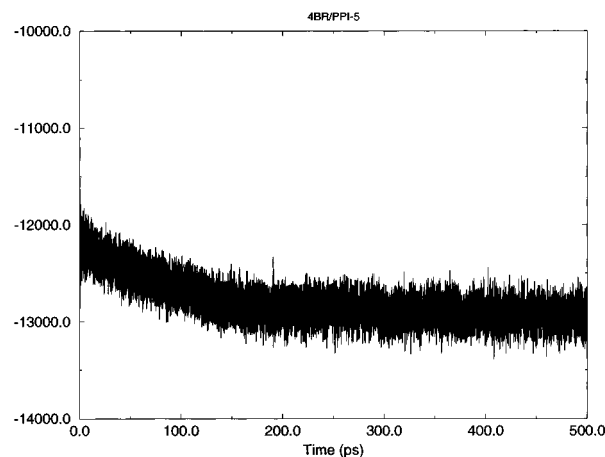


Figure 5. NVT equilibration of four BR/PPI-5. Shown is the potential energy (kcal/mol).

of monomers. The BR molecules were optimized in the vacuum and then inserted into the dendrimer at regions containing the largest cavities and then the composite structure was optimized. The starting structure for each dendrimer system (including BR) was then placed in the solvent box, and all solvent molecules within 1.4 \AA of any of the dendrimer or BR atoms were excluded. This leads to ~ 25 000 atoms per periodic box. Then 1 ps of dynamics at 0 K were performed for each solvated system to remove bad contacts. Then we proceeded with NVT dynamics at 300 K. The potential energies in Figures 4 and 5 show that convergence was reached in ~ 200 ps.

II.D. Regions of the PPI-5 Dendrimer. There are three regions for the PPI-5 dendrimer.

i. The *core region* is defined as the region within the radius of gyration

$$R_g^2 = \frac{\sum_i m_i (R_i - R_{cm})^2}{M_T}$$

where R_{cm} is the center of mass, m_i is the mass of atom i , M_T is the total mass, and the sum is over only the dendrimer atoms. For a sphere of radius R and uniform density $R_g = \sqrt{3/5}R$.

ii. The *surface region* is defined as the region from R_g to R_t where the tip radius R_t is defined as

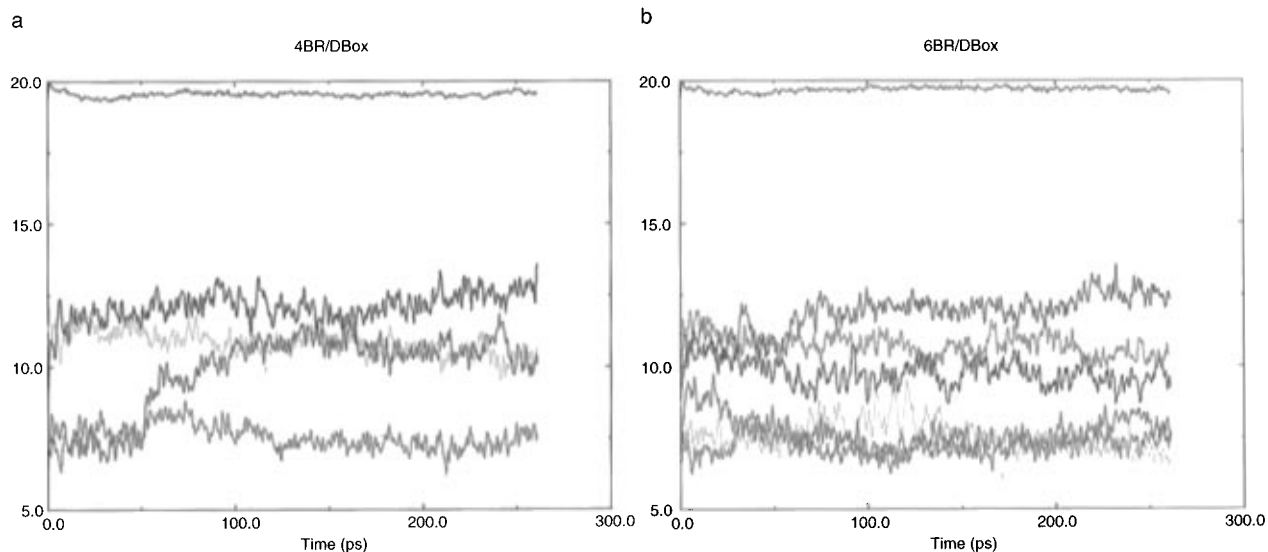


Figure 6. Distances of the BR molecules from the CM of the DBox (from NVT MD). The $R_g \sim 19 \text{ \AA}$ and the BR range from ~ 5 to 12 \AA : (a) 4BR/DBox and (b) 6BR/DBox.

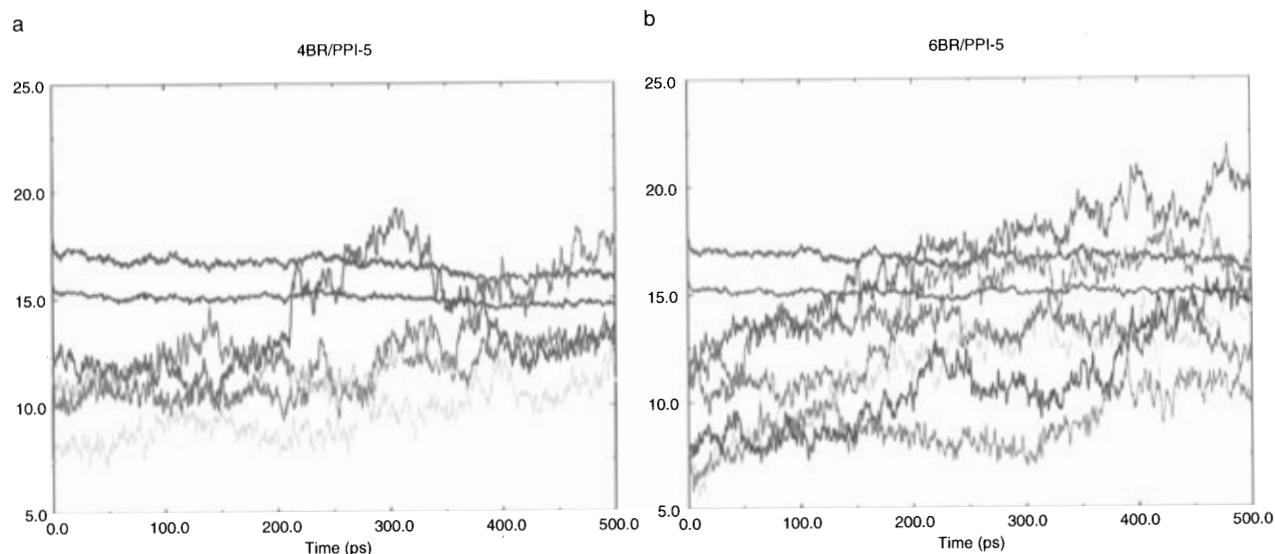


Figure 7. Distances of the BR molecules from the CM of the PPI-5 dendrimers (from NVT MD). Here $R_g \sim 15 \text{ \AA}$ in black, $R_t \sim 16 \text{ \AA}$ in red. (a) 4BR/PPI-5 (uncapped box). Here three interior BR are at $10\text{--}15 \text{ \AA}$, while one surface BR is at $16\text{--}18 \text{ \AA}$. (b) 6BR/PPI-5 (uncapped box). Here four interior BR are at $10\text{--}15 \text{ \AA}$, one surface BR is at $16\text{--}18 \text{ \AA}$, and one exterior BR is at $18\text{--}22 \text{ \AA}$.

$$R_t = \sum_i (R_i - R_{cm})^2 / N_t$$

Here the sum is over only the last nitrogen atom of the leaf monomer of each dendrimer branch and $N_t = 64$ is the number of such tip atoms. For a uniform sphere of radius $R_t = R$.

iii. The *solvent region* outside the dendrimer is defined as outside R_t .

III. Results

III.A. BR in Capped Dendrimer Box. We first determined the structure and properties for four or six BR molecules encapsulated inside DBox. For 6BR/DBox, Figure 6 shows the distance of the center of mass (CM) of each BR molecule from the CM of the dendrimer over 200 ps of NVT MD. In both cases all BR molecules remain well within the interior of the box.

III.B. BR in Uncapped PPI-5. We started with the structures of four or six BR in the capped DBox obtained after 162.5 ps of MD, deleted the caps, resolvated, and then carried

out NVT MD for 500 ps. For 6BR/PPI-5 Figure 7 shows the distances between the CM of each BR molecule and the CM of the dendrimer (along with R_g and R_t). In both cases, an equilibrium distribution is established for the BR positions over the regions of the dendrimer. For 4BR/PPI-5 three BR are in the interior, while one resides in the surface region. For equilibrated 6BR/PPI-5 four BR are in the interior, while one is in the surface and one stays outside the surface.

These results agree well with experiment, which finds that the number of encapsulated BR molecules depends on the BR/PPI-5 molar ratio (in CH_2Cl_2) prior to addition of the tBOC-L-Phe capping molecules.⁶ Thus $[\text{BR}/\text{PPI-5}] = 4$ leads to encapsulation of 3BR/DBox, while $[\text{BR}/\text{PPI-5}] = 6$ and higher leads to encapsulation of 4BR/DBox. The MD calculations find exactly these results, namely for $\text{BR}/\text{PPI-5} = 4$, we find three molecules in the interior region, and for $\text{BR}/\text{PPI-5} = 6$, we find four molecules in the interior. This indicates that during the encapsulation reactions, those BR molecules located within the interior of the dendrimer are encapsulated while those located on or outside the surface are not.

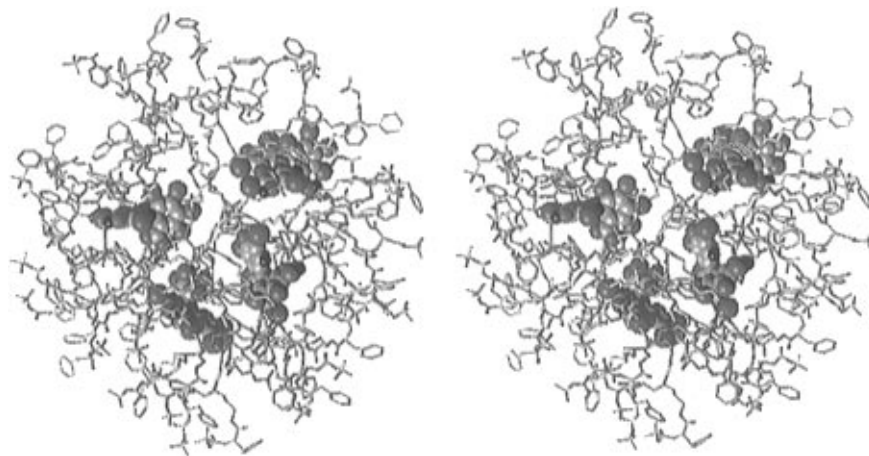


Figure 8. Stereoview of 4 BR/DBox after 200 ps. Here the BR are shown as CPK balls while the DBox is shown as sticks.

IV. Discussion

The MD shows that the BR molecules migrate around and in and out of the uncapped dendrimer box. Thus Figure 7a shows that the green BR moves to a distance beyond the average distance of the tip atoms at $t = 250\text{--}330$ ps, and then returns while the blue BR gets to this tip radius at $t = 400$ ps. This reflects the ability of the PPI-5 to bind a maximum of four BR molecules. The BR exhibit Brownian motion and are not strongly coupled. Figure 7b for 6BR/PPI-5 shows that two of the BR leave the interior of the dendrimer. The green BR leaves in about 200 ps, while the blue one leaves in about 400 ps. One BR remains in the surface region while the other goes outside.

Figure 7 shows that over the 500 ps the R_g decreases by 0.4% while R_t drops by 9%. This reflects the initial condition in which these systems were first equilibrated with the caps. The caps do not change the overall size (R_g) of the dendrimer; however, they do cause the last PPI generation to be more extended.

Figure 6b shows that even with six BR in the interior, the tBOC-L-Phe cap retains the BR in the interior. This shows that the DBox is indeed effective at encapsulation.

To see the spatial relationship between these BR molecules, we show in Figure 8 a stereoview of the molecules after 200 ps. Here the BR molecules are shown as CPK balls while the DBox is shown as sticks.

Figure 9 shows the environment around one of the interior BR molecules in the 4-Br/PPI-5 system after 500 ps. Here we see good hydrogen bonds with several of the amide sites.

V. Conclusion

We show that molecular dynamics (MD) calculations of the Meijer dendrimer box leads to encapsulation properties in close agreement with experiment. Thus the number of BR in the interior region of the PPI-5 dendrimer in CH_2Cl_2 corresponds to the number encapsulated upon adding caps. BR in the surface or exterior regions are not encapsulated. We also find that *tert*-butyloxycarbonyl-L-Phe (tBOC-L-Phe) is a quite effective cap, which prevents even six BR from leaving the dendrimer box until the caps are removed.

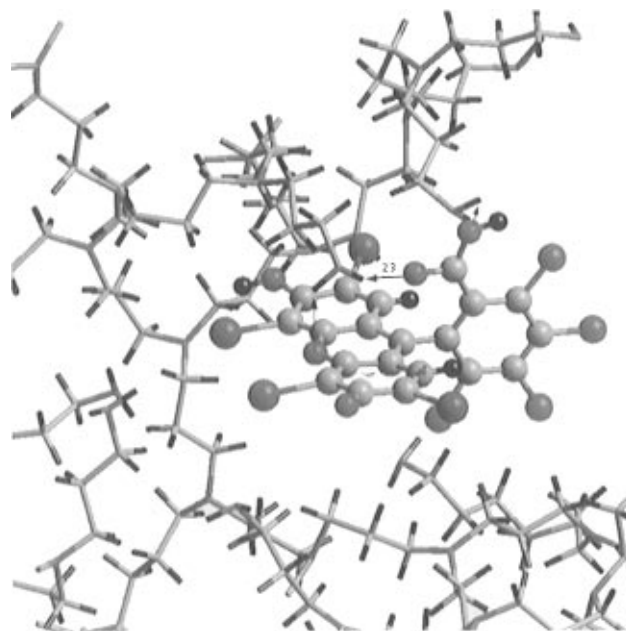


Figure 9. Structure around an interior BR for 4BR/PPI-5.

This close correspondence of the theory with experiment suggests that such atomistic theoretical methods can be used to design and test dendrimer encapsulation systems in advance of experiment.

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