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Coarse-Grained Simulations of Poly(Propylene Imine) Dendrimers in Solution

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The behavior of poly(propylene imine) (PPI) dendrimers in concentrated solutions has been investigated using molecular dynamics simulations containing up to a thousand PPI dendrimers of generation 4 or 5 in explicit water. To deal with large system sizes and time scales required to study the solutions over a wide range of dendrimer concentrations, a previously published coarse-grained model was applied. Simulation results on the radius of gyration, structure factor, intermolecular spacing, dendrimer interpenetration and water penetration are compared with available experimental data, providing a clear concentration dependent molecular picture of PPI dendrimers. It is shown that with increasing concentration the dendrimer volume diminishes accompanied by a reduction of internalized water, ultimately resulting in solvent filled cavities between stacked dendrimers. Concurrently dendrimer interpenetration increases only slightly, leaving each dendrimer a separate entity also at high concentrations. Moreover, we compare apparent structure factors, as calculated in experimental studies relying on the decoupling approximation and the constant atomic form factor assumption, with directly computed structure factors. We demonstrate that these already diverge at rather low concentrations, not because of small changes in form factor, but rather because the decoupling approximation fails as monomer positions of separate dendrimers become correlated at concentrations well below the overlap concentration.

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I. INTRODUCTION

Dendrimers are a group of macromolecules with a treelike architecture. Short spacers are added to a multifunctional core, where each spacer forms a reactive end for a new series of short spacers. Dendrimers are thus synthesized in an iterative fashion where in each generation the number of reactive ends is multiplied. The synthesis is very precise compared to most other polymers leading to well-defined uniform structures.

Because of their wide range of potential applications, dendrimers have been investigated extensively for quite some time, also using computer models. In these computer models the focus commonly is on the average shape or internal structure of a single dendrimer. These simulations can only reproduce a subset of experimental results, for experiments on dendrimers are typically conducted on solutions of various concentrations. For concentrated solutions the interactions between polymers come into play and dendrimers behave remarkably different than linear polymers. Dendrimers are globular, have fewer entanglements, and offer opportunities to create nanoscale materials with a greater level of control. To simulate phenomena involving dendrimer–dendrimer interactions requires large numbers of dendrimers and solvent molecules and demands that substantial time scales are regarded. This is prohibitive to fully atomistic molecular dynamics (MD) simulations yet possible with a coarse-graining (CG) scheme wherein groups of atoms are lumped together to form a single interaction site.

Previous endeavors in computational modeling of multiple dendrimers started with the melt phase, i.e., mobile dendrimers without solvent. The earliest examples are short atomistic MD simulations (0.4 ns) of small numbers of PPI dendrimers, i.e., 14 third generation (14 G3), 8 fourth generation (8 G4) and 4 fifth generation (4 G5) dendrimers. These studies were followed by longer simulations of melt containing larger numbers of dendrimers and using simpler models (125 bead-spring G4, 100 ns 216 CG G4 (45 beads) polyphenylene dendrimers) as well as a longer atomistic simulation of poly(amide amine) dendrimers (15 ns 8 G4). In solutions, the behavior of dendrimers is an interplay of dendrimer–solvent and dendrimer–dendrimer interactions. A starting point for simulations is the study of 2 dendrimers in solution. This has been investigated with bead-spring dendrimers of G4 or G5 in implicit solvent and in explicit water. Solutions of more dendrimers have been simulated a couple of times with bead-spring models in implicit (Monte Carlo 500 G4, 32
FIG. 1. The fifth generation 1,4-diaminobutane poly(propylene imine) dendrimer drawn as the molecular structure representation (left) and the coarse-grained form (right) with the coarse-grained sites indicated in the middle.

$G^3$ and explicit solvent ($30 G^4$).

Compared to those papers, here we perform larger scale simulations (up to 1430 $G^4$ PPI of 62 particles each or 968 $G^5$ PPI of 126 particles each) in explicit water, over a full range of concentrations, each simulation covering 24 ns. For this we use our previously developed CG poly(propylene imine) ($G^5$: DAB–dendr–$(\text{NH}_2)_{64}$) dendrimer model$^5$ shown in Figure 1.

In the next section we describe the coarse-grained PPI model and the simulation arrangements matching experiments available in literature. This is followed by the simulation results providing descriptions of the various dendrimer properties measured. First, the radii of gyration, radial distribution functions and structure factors are calculated to demonstrate the similarity between the simulations and experiments. Next, the simulations are used to answer further questions raised by experiments, i.e., to quantify the amount of overlap of dendrimer branches under different concentrations, to determine the ‘average distance’ between dendrimers, and to observe possible dendrimer shape changes upon increasing concentration.

II. MODEL

In this study, we use the coarse-grained model developed before to examine the behavior of poly(propylene imine) (PPI) and poly(propylene imine)-urea-adamantyl (PPIUA) dendrimers in water$^5$. This coarse-grained model for PPI is illustrated in Figure 1. Its
parameterization was based on a combination of matching thermodynamic data (like for our earlier lipid model\textsuperscript{18-20}) and a Boltzmann inversion scheme on bond and angle distributions of virtual coarse-grained sites accumulated from atomistic simulations of G5 PPIUA. The parameters\textsuperscript{5} are reproduced in Section SI of the Supplemental Material\textsuperscript{21}. This CG model compared well with atomistic simulations as well as experiments\textsuperscript{22} over a range of generations (G1 to G7), providing a complete molecular picture of dilute dendrimers including dendrimer shapes, monomer distributions, spacer expansions and atomic form factors. Those simulations as well as all simulations described in this paper were performed using our in-house developed MD platform PumMa\textsuperscript{23}.

For the validation of the simulations of dendrimer solutions in this paper two available small-angle neutron scattering (SANS) studies of PPI in good solvent are used. First the measurements of G5 PPI in D\textsubscript{2}O by Ramzi \textit{et al.}\textsuperscript{24} (volume percentages: 5\% to 60\%), and second the measurements of G4 and G5 PPI in methanol by Topp \textit{et al.}\textsuperscript{25} (mass percentages: 1\% to 80\%). To allow for comparison with these experiments, the coarse-grained simulations were also performed on PPI dendrimers of generations 4 and 5, with dendrimer mass percentages of approximately 0.1\%, 1\%, 2\%, 6\%, 23\%, 43\%, 64\%, 83\%, and 100\%. Note that the actual volume percentages are rather similar to the mass percentages up to 64\% after which they drop (see Supplemental Material\textsuperscript{21} Section SII).

The initial simulation boxes (Figure 2) consist of duplicates of a dendrimer isolated from the earlier simulations\textsuperscript{5} with the number of water particles reduced to get the desired concentration. To minimize empty space between duplicates, for each generation one dendrimer was selected for its small radius and minimal asphericity. These dendrimers were subsequently desolvated, multiplied, resolvated and minimized. This procedure resulted in 5 G4 PPI dendrimers with 250105 water particles for 0.1\% up to 1430 dendrimers for 43\% and beyond, and 2 G5 PPI dendrimers with 204078 water particles for 0.1\% up to 968 dendrimers for 43\% and beyond. Simulation box volumes range from 12203.1 nm\textsuperscript{3} to 37431.2 nm\textsuperscript{3} (for all configurations see Supplemental Material\textsuperscript{21} Section SII).

For each combination of concentration and generation one simulation was performed with constant pressure (1 bar) and constant temperature (298 K) using Berendsen pressure and temperature coupling. With a time step of 24 fs, each simulation lasts 24 ns, of which the first 6 ns are considered equilibration time, and the final 18 ns production run. Autocorrelation functions for dendrimer $R_g$, asphericity and rotation\textsuperscript{26} indicate that the 6 ns
equilibration time suffices as the relaxation times are well within this time scale (see Supplemental Material\(^{21}\) Section SIII). During the production run every 0.06 ns a configuration was saved for further analysis.

III. RESULTS AND DISCUSSION

The final configurations of simulations at four different concentrations are shown in Figure 2. Overall the dendrimers do not form large aggregates, instead they constantly collide and separate over the course of the simulations. Yet in concentrations as low as 23 % mutual distances are often so small that any clustering algorithm would still lump most dendrimers together.

A. Radius of Gyration

We first investigate the dendrimer size as a function of concentration. This dendrimer size is calculated by the radius of gyration, a measure of the mass distribution of a molecule around its center of mass:

FIG. 2. Snapshots of the simulation states at the end of the G5 PPI simulations for the dendrimer mass percentages indicated.
FIG. 3. Radius of gyration at varying concentrations. The dotted lines represent the $R_g$ calculated for solitary dendrimers\(^5\). The dashed lines are the best fits for $R_g$ as a function of mass fraction $\varphi$, for G4: $R_g(\varphi) = R_{g,0} \left( 1 - \left( \frac{\varphi}{499.3} \right)^{2.11} \right)$ and G5: $R_g(\varphi) = R_{g,0} \left( 1 - \left( \frac{\varphi}{291.7} \right)^{2.58} \right)$. Averages and standard deviations have been calculated over all dendrimers (see Supplemental Material\(^{21}\) Section SV). Diamonds represent experimental values from Ramzi \textit{et al.}\(^{24}\), Scherrenberg \textit{et al.}\(^{22}\) and Topp \textit{et al.}\(^{25}\).

\begin{align}
\langle R_g^2 \rangle &= \frac{1}{\sum_{i=1}^{N} m_i} \left\langle \sum_{i=1}^{N} m_i |\mathbf{r}_i - \mathbf{c}|^2 \right\rangle \\
\mathbf{c} &= \frac{\sum_{i=1}^{N} m_i \mathbf{r}_i}{\sum_{i=1}^{N} m_i}
\end{align}

where $\langle \rangle$ denotes the ensemble average, $N$ the number of CG particles, $m_i$ the mass of the $i$th particle, $\mathbf{r}_i$ its position and $\mathbf{c}$ the center of mass of the molecule.

In Figure 3 these values are compared to the radii of the solitary G4 and G5 dendrimers as calculated in our preceding paper\(^5\). Evidently in low concentrations the radii are the same, but with increased concentration the dendrimers become more and more compacted.

The $R_g$ values at dilute concentrations (1%) were measured experimentally as 11.6 Å for G4 PPI\(^{22}\) and 13.9 Å for G5 PPI in water\(^{22,24}\) and 12.4 Å for G4 PPI\(^{25}\) and 15.6 Å for G5 in methanol\(^{25}\). Our simulation values lie between these reported values. The shape-persistence at low to medium concentrations is in agreement with SANS measurements of G4 PPI functionalized with urea phenyl in dimethylacetamide\(^{27}\), where no difference in shapes was observed between volume percentages 15% and 23%. Under those conditions similarly flexible linear polymers show much more pronounced shape changes. Over a larger
concentration range (0.5 % to 50 % in water) solution densitometry and viscometry measurements of G4 and G5 PPI functionalized with acetyl chloride did show their hydrodynamic volumes decreasing\textsuperscript{28}. The authors postulated that these dendrimers either interpenetrate at high concentrations, or expel solvent. We will examine the extent of both phenomena in Sections III D and III F, but first continue with a direct comparison of the simulations with experimental SANS measurements.

B. Structure Factor

Small-angle neutron scattering is a technique for examining the structure of, e.g., a solution of dendrimers by casting a beam of neutrons at it and measuring the deflections caused by the nuclei of the atoms within. The structure may then be reconstructed from the measured total coherent scattering intensity which is a function of the scattering angle \(I(q)\). Random orientation of the dendrimers in solution results in angular isotropy of the signal such that \(I(q) = I(q)\), where \(q\) denotes the magnitude of the scattering wave vector \(q\) in \(\text{Å}^{-1}\).

In dilute solutions the scattering due to distant dendrimers is extremely weak and may be ignored. The measured scattering is dominated by scatterers within the individual dendrimers, which can be used to assess their shape. This atomic form factor \(F(q)\) thus describes the correlation of scatterers in the dendrimer:

\[
F(q) = \frac{1}{N} \left\langle \sum_{j=1}^{N} \sum_{k=1}^{N} e^{-iq \cdot (r_j - r_k)} \right\rangle \\
= \frac{1}{N} \left(\sum_{j=1}^{N} \sum_{k=1}^{N} \sin(q|r_j - r_k|) / q|r_j - r_k|\right) \tag{3}
\]

The atomic form factors from simulations are compared with experimental ones in a Kratky plot (Figure 4). At small angles the scatter intensity is associated with distant scatterers. The left hand side of the Kratky plot thus matches the overall dendrimer shape, while at the right hand side (large angles) the local internal structure is represented. Experimentally that is increasingly hard to do well while for the coarse-grained simulation the particles’ size limits the smallest possible distances. As a result the difference between the form factor curves becomes more pronounced at larger angles. Still the simulated atomic form factors follow the experimental one quite well. The clear peak means the dendrimer is a
FIG. 4. Kratky plot of $G^4$ and $G^5$ PPI from experiments$^{22}$, current 0.1 % coarse-grained simulations and atomistic simulations of a single dendrimer$^5$. Simulation values are scaled to match the height of the peaks.

fairly dense globular structure$^{29,30}$. The difference between the isolated atomistic dendrimers of the previous paper$^5$ and the current dilute coarse-grained simulations is imperceptible on the upwards slope of the peak.

From simulations $F(q)$ can also be calculated for higher concentrations. The actual form factors for these higher concentrations differ only slightly, as evidenced in Figure 5. An inset is required to illustrate that the form factor broadens with rising concentration, which is expected as the dendrimers become more compressed as observed in Figure 3.

In experiments this $F(q)$ however cannot be measured directly. Measured is the full scattering intensity $I(q)$ which can be calculated similarly as:

$$I(q) = \frac{1}{\nu N} \left\langle \sum_{m=1}^{\nu} \sum_{n=1}^{\nu} \sum_{j=1}^{N} \sum_{k=1}^{N} e^{-i\mathbf{q} \cdot (\mathbf{r}_{m,j} - \mathbf{r}_{n,k})} \right\rangle$$

$$= \frac{1}{\nu N} \left\langle \sum_{m=1}^{\nu} \sum_{n=1}^{\nu} \sum_{j=1}^{N} \sum_{k=1}^{N} \sin(q |\mathbf{r}_{m,j} - \mathbf{r}_{n,k}|) \right\rangle$$

(4)

where $\nu$ is the number of dendrimers in solution and periodic boundary conditions are applied.

The coherent scattering plots shown in Figure 6 are comparable to experimental ones$^{24}$ with the caveat that the latter were only investigated for concentrations up to 60 %. The change in internal dendrimer structure with increasing concentration shows in the divergence from a single curve at high $q$-values.
FIG. 5. The atomic form factors of generations (a) 4 and (b) 5 in various concentrations. The inset is to highlight the increasing differences between the least concentrated and beyond.

FIG. 6. Log-log plot of the scattering intensities $I(q)$ of PPI dendrimers of generations (a) 4 and (b) 5 at various concentrations as indicated by the legends.

As shown in Figure 6 the overall scattering intensity changes dramatically with concentration. This is mostly due to scattering between dendrimers. Two measures for the short range order of the dendrimers in the solution are the structure factor ($S(q)$) and the radial distribution function ($g(r)$) of the dendrimer centers. The calculation for the structure factor is effectively the same as for the atomic form factor, but with the dendrimers’ centers of mass instead of their atoms$^{15}$:
\[ S(q) = \frac{1}{\nu} \left\langle \sum_{m=1}^{\nu} \sum_{n=1}^{\nu} e^{-i \mathbf{q} \cdot (\mathbf{c}_m - \mathbf{c}_n)} \right\rangle \tag{5} \]

The radial distribution function describes the probability of finding a dendrimer at a certain distance of another dendrimer relative to this same probability in a random distribution with equal density:

\[ g(r) = \frac{2 n_{\text{hist}}(r)}{\nu \rho_0 \frac{4}{3} \pi \left( (r + \frac{dr}{2})^3 - (r - \frac{dr}{2})^3 \right)} \tag{6} \]

where \( n_{\text{hist}}(r) \) is a histogram of interdendrimer distances \( r = |\mathbf{c}_m - \mathbf{c}_n|, \ n > m \) with bin size \( dr \), and \( \rho_0 \) is the overall density, i.e., the number of dendrimers divided by the volume of the simulation box \( (\rho_0 = \nu / V) \). Note that \( g(r) \) can only be calculated for \( r \leq R \), half the minimum box length; \( g(r) \) is set to 1 for \( r > R \) as per the definition of noncorrelation at large distances.

The structure factor and the radial distribution function are related via:

\[ S(q) = 1 + 4 \pi \rho_0 \int_0^R dr r^2 \frac{\sin(q r)}{q r} (g(r) - 1) \tag{7} \]

Both have their own interesting features and both are shown for a subset of the simulations in Figure 7.

The spatial correlation between dendrimers is shown in the radial distribution graphs wherein the level of order in a system is represented by the number and steepness of peaks. For example, in 6 % G5 the first peak is hardly discernible, indicating the dendrimer distribution is unordered. For 23 % G5 and 6 % to 23 % G4 one clear peak is present, representing weak correlation between first neighbors only. The higher concentrations show secondary and tertiary peaks corresponding with a liquid-like order. Real crystalline distributions with dendrimers at discrete distances would show as sharp discrete peaks, but no such distribution is observed here.

While less intuitive, the structure factor graphs are better suited for comparison with experiments. The trend exhibited by the curves at low q-values are quite similar to the experimental ones. In these graphs the height of the primary \( S(q) \) peak is said to represent the level of crystallinity of the solution. Crystallization occurs at \( S(q) \) values near 2.85\textsuperscript{31}, while 1.5 suggests liquid-like order\textsuperscript{24}. Following this rule of thumb, it seems unusual that the 64 % and 83 % simulations have a higher peak than the 100 % simulations. For both G4 and G5 dendrimer simulations, even at 100 % the dendrimers exhibit no long range order,
FIG. 7. Interdendrimer distances of generations 4 and 5 in various concentrations. (a, c) Radial distribution functions (bin size 1 Å). (b, d) Corresponding structure factors.

as evidenced by \( g(r) \) and \( S(q) \). In the \( g(r) \) graphs the 100 \% simulations exhibit closer packing, but not increased order. Visual inspection of the simulations shows the dendrimers remain mobile with respect to each other, even if their size and crowdedness leads to a high viscosity. Similar \( S(q) \) peak height progression is apparent in the experimental results of Topp et al.\(^{25}\) where the highest peaks are around 20 \% \( G5 \), while for \( G4 \) the structure factors plateau early. The 5 \% to 60 \% structure factor results of Ramzi et al.\(^{24}\) do not show any peak (see Figures 8c and 8f for these experimental results).

Knowing all atom positions in the simulations, the differentiation between internal scatterers for the atomic form factor and scattering between dendrimers for the structure factor
FIG. 8. The differently calculated structure factors of generations 4 (a, d) and 5 (b, e) in multiple concentrations. The dotted lines are the actual $S(q)$ values directly calculated from the simulations as in Figure 7. The dashed lines are the apparent structure factors calculated by dividing the coherent scattering by the current atomic form factor, while the solid lines are the apparent structure factors ($S_{\text{app}}(q)$) calculated with the atomic form factor of the diluted dendrimers as is customary in experiments (here 0.1%). (c, f) The results of such experiments by Ramzi et al.$^{24}$ and Topp et al.$^{25}$ for similar concentrations of G5 PPI.

is straightforward. For the SANS experiments it is more difficult to make this distinction in order to determine the structure factor. Recall that the total coherent scattering intensity is a combination of internal scattering and scattering due to other dendrimers. Assuming the correlation of interdendrimer distances and the correlation of intradendrimer monomer distances is completely separate, i.e., the positions of individual monomers of one dendrimer do not correlate with the positions of monomers of any other dendrimer, the total coherent scattering intensity can be factorized as the product of the form factor and the structure factor ($I(q) \cong F(q)S(q)$). Thus with this decoupling or factorization approximation the structure factor can be calculated if the atomic form factor is known. At least in infinitely diluted solution $S_0(q) = 1$ and $F_0(q) = I_0(q)$. One must then assume this atomic form
factor is constant over all concentrations for the apparent structure factor to be calculated: 

\[ S_{\text{app}}(q) = \frac{I(q)}{F_0(q)} \]

It is clear that with increasing concentration, dendrimers come in close contact and the validity of both the decoupling approximation and the constant form factor assumption weakens. By comparing the actual structure factor with the apparent structure factor(s), the vulnerability of the experimental procedure for increased concentrations is examined. The results are shown in Figure 8. For comparison the actual structure factors from Figure 7 are repeated. That comparison does not appear favorably for the indirect calculations. Only the lowest concentrations of 6\% are reproduced with minimal disparity. At 23\% the \( S_{\text{app}}(q) \)'s are still quite similar, but it gets progressively worse. At 43\% the peaks are becoming subdued. The same is true for 64\%, but now the required asymptotic value of 1 is not reached anymore. At 83\% the expected peak is only visible for G5, and at 100\% the peaks are practically nonexistent. Utilizing the actual form factors changes the apparent structure factors only minutely—which is expected as they do not differ by much (Figure 5)—but not for the better either considering the peak values. The very assumption that \( I(q) \) can be decoupled into factors \( S(q) \) and \( F(q) \) is what fails, the monomers of separate dendrimers are correlated even at fairly low concentrations. The error in the assumption that \( F(q) \) remains \( F_0(q) \) is of lesser importance.

These results compare well to Monte Carlo simulations of archetypical G4 dendrimers where a dilute state and the overlap concentration state were simulated\(^{15}\). Especially the 43\% state is surprisingly similar to their overlap concentration simulation. That in our simulations the decoupling approximation deteriorates well before the overlap concentration is reached may be because the explicit solvent molecules actively conduct monomer movement thereby enhancing their correlation.

C. Intermolecular Spacing

Ever since the application of small angle scattering techniques to particles dispersed in solvent, researchers have noticed a small peak appearing in the scattering curve at higher concentrations (e.g., Figure 6). A common interpretation ties the position of this peak (\( q_{\text{max}} \)) to the so-called average distance between neighboring particles (\( \bar{d} \)) using a theoretical validation provided by Guinier and Fournet\(^ {32}\)—who simultaneously warned of the fallacy
in defining neighbors in a substance without long-range order. By assuming the system consists of spherically symmetric particles that lie on a distorted face-centered cubic lattice (an fcc-paracrystal) they derived an average nearest neighbor distance function:

\[ d = \sqrt{\frac{3}{2}} \frac{2\pi}{q_{\text{max}}^2} \approx 1.22 \frac{2\pi}{q_{\text{max}}} \]  

(8)

This relation has been used to infer the average dendrimer–dendrimer distance in many experimental sources including PPI–PPI\textsuperscript{24,25}. We applied this technique to our simulations to see whether it yields distances comparable to those that were measured directly. For this distance (\( \overline{d} \)) we take the most common nearest neighbor distance given by the first peak in the radial distribution function \( g(r) \) (Figure 7a, c).

First, using the peak positions of the scattering curve \( I(q) \) for \( q_{\text{max}} \) in Equation 8, just like in the experiments\textsuperscript{24,25}, markedly overestimates the distance values. Fitting the clear peak positions (60 %-100 %) to the known distances would result in a smaller factor, namely 1.122.

The difference between the fitted value and \( \sqrt{3/2} \) may stem from the fact that the Guinier model\textsuperscript{32} originally assumed hard spheres for which the interparticle distances correspond with the structure factor. By taking for \( q_{\text{max}} \) the peak position of \( S(q) \) as calculated from the dendrimer centers in our simulations (Figure 7), the factor \( \sqrt{3/2} \) indeed performs better (Figure 9a), though still slightly overstates the average distance. Fitting the peak positions, which are in these \( S(q) \) curves visible for all concentrations (5 %–100 %), yields a factor of 1.189. Moreover, in a separate test mirroring the Guinier model we placed 108000 particles on a face-centered cubic (fcc) lattice perturbed with random noise. Without noise the \( \sqrt{3/2} \) factor performed very well, yet with increasing randomness applied the smaller factor also did better (see Supplemental Material\textsuperscript{21} Section SIV).

Given that the apparent structure factor as derived in experiments is only an approximation of \( S(q) \), we also considered \( q_{\text{max}} \) from \( S_{\text{app}}(q) \) (Figure 8, G4: 20 %-80 %; G5: 40 %-100 %). This leads to a factor of 1.203 upon fitting. In conclusion, while the common factor of 1.22 seems an overestimate, especially in case \( q_{\text{max}} \) is determined from \( I(q) \), it provides a reasonable estimate for the average distance given experimental error in determining the peaks.

How dispersed are the dendrimers in the simulations? At the 100 % end of the concentration range the dendrimers are packed in a smeared fcc lattice, the densest possible
FIG. 9. Average nearest neighbor distances between dendrimers as derived from the radial distribution function. (a) The distance compared to calculations from the structure factor. The common factor 1.225 overstates the distance, 1.189 provides a better fit. (b) Dendrimer–dendrimer distance with dashed ranges denoting complete aggregate (horizontal line) and maximal dispersion (exponential line, \(d(\phi)\)). (c) The distance compared with experimental results\(^{24,25}\).

packing for spherical particles. Should the dendrimers not dissolve in water, they would form an equally dense aggregate where the nearest neighboring distance does not change upon dilution. Should the dendrimers however be completely dispersed through solvation, conceptually an expanded fcc lattice remains a convenient representation for their positions, as an fcc lattice is merely the most efficient way of packing spheres with the largest possible nearest neighbor distances. The volume of an fcc box \(V_{\text{fcc}}\) is defined as \(2\sqrt{2}d^3\) with \(d\) the nearest neighbor distance. It contains 4 spherical dendrimer volumes \(V_D\), fit at 100 \% with a volume fraction \(\eta\) defined as \(\frac{7}{3\sqrt{2}} \approx 0.74\). Then the maximal minimum distance between dendrimers as a function of the mass fraction \(\phi\) is:

\[
d(\phi) = \sqrt[3]{\sqrt{2} \frac{1}{\phi \eta} V_D} = \sqrt[3]{\frac{6 V_D}{\phi \pi}}
\]

In dilute solvent the dendrimers behave somewhere between complete aggregation and maximal dispersion (Figure 9b). From visual inspection, it is clear they do not aggregate, yet fleeting pair forming is observed. As for concentrations of 64 \% and above the upper bound \(d(\phi)\) converges to the dendrimer diameter, the dendrimers are forced into an aggregate, albeit a porous one at first.

Figure 9c shows the simulation results combined with results from the SANS experiments on PPI. While the simulations go up to 100 \%, at the 64 \% end of the experiments the results are similar (especially for \(G4\)), which is an indication the subsequent trend is plausible. Lower
than 30 % however, the experimental distances rise faster than the simulation equivalents. The simulation dendrimers are often closer together than in the experiments, which would indicate that the dendrimers should be a little more repulsive. This is probably due to the fact that the charges in the dendrimer are made implicit in the CG particles. Thus, while the splaying of dendrimer branches due to interparticle repulsion is correct, the long range repulsion between dendrimers is not made explicit. On the other hand, the most striking outlier (experimental measurement of 76.7 Å at 10 %24) would, according to Equation 9, only be possible in solutions with concentrations up to 5 %.

Recently 30 bead-spring trifunctional core dendrimers at 60 mass percent were studied17. The novelty being that the end groups are charged, there are explicit counterions, and the electrostatic interactions are varied with the dielectric permittivity constant. Noteworthy is that apparently the third generation dendrimers (91 beads) form structures in accordance with the body-centered cubic (bcc) lattice, while the fourth generation builds fcc structures. For both our dendrimer sizes, from 20 mass percent and up, the emerging structures adhere to the smeared fcc pattern.

D. Dendrimer Interpenetration

When the intermolecular spacing is low, the dendrimers come into close contact with each other. What happens at the interface? Do the dendrimers interpenetrate and become part of a larger entity or do they repel each other so each dendrimer retains its independence while being a bit compressed? And what method is most suitable to calculate such interpenetration?

Attempts have been made to examine dendrimer aggregates in vitro. Cryo-TEM of dilute G10 poly(amido amine) (PAMAM) in water shows clustering but no apparent interpenetration in the clusters33. That is, instead of a single large blob, discrete dendrimers can be recognized. However, the fact that these large aggregates emerge in such dilute conditions leads the authors to speculate that this could be a side effect of the preparation of the specimen.

To calculate the level of interpenetration in the simulations of increased densities, we used a convex hull method. This method entails the creation of a convex hull for each dendrimer, i.e., the minimal polygon which encompasses all the positions of the dendrimer’s
FIG. 10. Dendrimer interpenetration in various concentrations, calculated using the cell method and the convex hull method. See the text for the different meaning of penetration percentage for these methods. The percentages calculated per saved time step are so alike that all standard deviation bars fall within the symbols drawn for the averages.

particles. Then the number of particles of other dendrimers that fall within the hull are counted as penetrating the dendrimer. The penetration percentage is that amount divided by the number of original dendrimer particles, times 100. Note that the convex hull does not span the Van der Waals radii of the particles, but rather the particles’ centers.

The results are shown as the two curves near the bottom of Figure 10. It is clear the interpenetration is practically the same for both generations. At low concentrations there is almost no interpenetration, the dendrimers are separate although they may collide. Even at 64% dendrimers, the penetration percentage is lower than 5%. In melt the penetration percentage reaches approximately 15%, that is on average 8.7 invading particles for G4 (62 particles) and 19.6 invading particles for G5 (126 particles).

Previously Zacharopoulos and Economou came to a different view for their PPI melt simulations\(^7\), namely that with larger dendrimers the amount of interpenetration decreases considerably. They calculated an interpenetration percentage with a cell method by dividing the simulation box into cubic cells. From the perspective of an individual dendrimer, it is said to be penetrated if a cell containing its particles also contains other dendrimers’ particles. The penetration percentage of the dendrimer is then defined as the number of cells with a mixed presence divided by the number of occupied cells, times 100. Applying this methodology on our simulation data yields the other curves shown in Figure 10, whereby the
cell size is chosen such that at 100% the interpenetration values match with their results\textsuperscript{7} (i.e., 6.2 Å gives 41.6% vs. the original 40.9% for G4 and 34.0% vs. 33.2% for G5, for a maximum of 6 particles per cell). These results would indeed imply G4 has a higher interpenetration than G5, even across all concentrations. However, note that the cell size highly influences the result in such a set-up. Even in a hypothetical case with no actual interpenetration, at high concentrations all cells at dendrimer interfaces contribute to the penetration score. Furthermore, the smaller the dendrimers considered, the larger the part of the simulation box that contains interfaces. This is the origin of the difference in the results and conclusions.

Yet another method was applied on a melt of general bead-spring trifunctional core dendrimers under increasing temperatures\textsuperscript{34}. There the interpenetration was calculated by looking at the average radial monomer density from the core bead, and where this graph overlaps with the average monomer density of other dendrimers this is counted as interpenetration. Firstly this assumes that dendrimers are perfectly spherical, and secondly as the monomer density slowly approaches zero, more and more monomers are included that merely have the good fortune to be at the same distance from the core bead as one monomer once was. Still the interesting observation that interpenetration increases with decreasing temperature, and this dependence is greater for smaller dendrimers\textsuperscript{34}, deserves further investigation.

Given that even in melt the number of hull penetrating particles is fairly low, what does this mean for the opposing views on dendrimer structure simplified as dense shell or dense core? In the dense shell model all branches are envisioned to extend towards the surface, leading to a vacant core region and crowding at the surface\textsuperscript{35}. In the dense core model the branches occupy any possible region through bending and back-folding, thereby having a dense core and not-so dense shell\textsuperscript{36}. Even the dendrimer with the most particles penetrating the hull at the end of the simulation (48 particles, 38.1%) is still easily recognized as a separate dendrimer (Figure 11). In fact, although the dendrimer hull is highly permeated, one side is not penetrated at all. A high level of penetration does not mean a bunch of intertwined branches, nor overlapping splayed dendrimers with each other’s branches in cavities near their cores. In this sense, the view that dendrimers in melt are dense impenetrable spherical cores with permeable shells may be a tad simplistic, it at least gives the right impression. It is also in agreement with our previous simulations where the radial monomer densities and spacer expansion profiles support the dense core view\textsuperscript{5}. 
E. Overlap Concentration

A commonly used term in classifying the behavior of polymers in solution is the overlap concentration ($C^\ast$). It is loosely defined as the concentration where the distance between macromolecules equals their size. At concentrations below $C^\ast$ the solution is dilute, whilst above $C^\ast$ it becomes concentrated. In concentrated solutions the macromolecules either interpenetrate or deform to accommodate the high concentration. Because dendrimers are pretty dense compared to linear molecules, their overlap concentration is fairly high.

While excellent for formulating ideas, difficulties arise when actually calculating the overlap concentration, as the size of macromolecules is not clearly defined. We use the average convex hull volumes of the single dendrimer simulations to calculate appropriate diameters of unconstrained dendrimers:

$$D = 2\sqrt[3]{\frac{V_{\text{hull}}}{\pi}}$$

resulting in $D_{G4} = 24.8 \, \text{Å}$ and $D_{G5} = 33.2 \, \text{Å}$.

By taking the average distance between dendrimers as $g(r)_{\text{max}}$ as shown in Figure 9b and looking at where the diameter intersects, we obtain the overlap concentration. For G5 it is at 55%, for G4 even at 68%. From the fairly flat character of the distance graph, it is clear that slightly larger calculated sizes would lead to lower overlap concentrations. Nevertheless, such high overlap concentrations are not unheard of with dendrimers (e.g., 56.6 %).

As all experimental graphs shown in Figure 9c are steeper, and their molecular sizes are calculated differently, the reported overlap concentrations are lower. Topp et al. use the relationship $R = \sqrt{\frac{5}{3}R_g}$ for the dendrimer size ($D_{G4} = 32.0 \, \text{Å}$ and $D_{G5} = 40.3 \, \text{Å}$) so the
intersection with the average distance graph puts $C^*$ for both $G4$ and $G5$ at 25 %. Ramzi et al.\textsuperscript{24} calculated $C^*$ by dividing the theoretical bulk volume by the measured hydrodynamic volume, arriving at 36.5 % for $G5$ PPI.

While the calculated overlap concentration of around 60 % is high compared to the SANS experiments, in the simulations (Figure 10) the penetration percentage does indeed shift between practically non-existent before $C^*$ and increasing thereafter. Moreover, Figure 9b shows overlapping after $C^*$ is unavoidable, even if the dendrimers were maximally dispersed.

\section*{F. Water Penetration}

Aside from what the fate of the dendrimer branches is with increasing dendrimer concentration, the destination of the diminishing solvent has its own hypotheses. In one view the solvent enters pockets within the dendrimers while the dendrimers expand to fill the space, which can be imagined like stacked sponges. In another view the solvent fills cavities between stacked dendrimers, comparable with submerged marbles.

The level of water penetration in the dendrimers can be calculated by counting the water particles inside the convex hull of each dendrimer. The water density, the number of water particles divided by the volume of each convex hull, is shown in Figure 12a as a function of the dendrimer concentration. For convenience, the water density is calculated to molecular values (i.e., 1 particle represents 4 water molecules). The dilute dendrimers at 2 % contain on average 217.3 internal water molecules in $G5$ and 88.1 molecules in $G4$. The most concentrated dendrimers, at 83 %, contain 75.9 water molecules in $G5$ and 27.7 molecules in $G4$. Even then the number of water particles that appear in two convex hulls is quite low, for in $G5$ it happens to only 2.4 % of the 27714 water particles. With increasing concentration the convex hull volume average decreases from 8.1 nm$^3$ to 6.8 nm$^3$ for $G4$ dendrimers. For $G5$ dendrimers this decrease is from 19.1 nm$^3$ to 16.0 nm$^3$. By comparing the convex hull volumes for the 0.1 % dendrimer simulations with those in bulk follows that for dilute $G4$ PPI 12.9 % of the dendrimer volume is from solvent and for $G5$ that percentage is 16.1 % (see Supplemental Material\textsuperscript{21} Section SV). Figure 12a suggests that the larger $G5$ dendrimers absorb relatively more water than the $G4$ dendrimers. However, when the total absorbed water percentage is plotted against the percentage of box volume occupied by dendrimers (as calculated with the convex hull method), both generations behave alike (Figure 12b).
FIG. 12. The level of water penetration into the dendrimers at the final state at varying concentrations. (a) The number of water molecules per dendrimer convex hull volume. (b) The percentage of water particles that fall inside the convex hulls plotted against the percentage of box volume occupied by dendrimers. The dotted diagonal indicates the theoretical percentage should the water particles be distributed uniformly over the simulation box. Even at 100% dendrimers, the total volume occupied by convex hulls amounts to 73.2% for G4 and 84.8% for G5 (see Supplemental Material21 Section SII). (c) Radial monomer densities of G5 PPI dendrimers and water with shell width 0.5 Å; for comparison with G4 and solitary dendrimers5 G1 to G7 see Supplemental Material21 Section SVI.

That is, more water particles remain outside the dendrimers than if they were uniformly distributed. The same can be seen in the radial monomer density ($g_m$) Figure 12c which shows the distribution of monomers with respect to each dendrimer’s center of mass. The density is defined as the number of monomers ($N$) found within the shell of width $\Delta r$ starting at $r - \frac{\Delta r}{2}$ divided by the volume of this shell:

$$g_m(r) = \frac{N(r)}{\frac{4}{3} \pi (r + \frac{\Delta r}{2})^3 - \frac{4}{3} \pi (r - \frac{\Delta r}{2})^3}.$$  \hspace{1cm} (11)

It shows an increased overlap between dendrimers at higher concentrations, while the density peak of the water on the interface gradually recedes to more uniform levels. These observations lead to the conclusion that, although water is present in both domains, of the views presented in the introduction paragraph the one with water filling cavities between dendrimers is more accurate than the one with water filling cavities inside dendrimers.
IV. CONCLUSION

We examined the behavior of generation 4 and 5 poly(propylene imine) dendrimers in water at concentrations ranging from dilute to dendrimer melt, by performing coarse-grained simulations to compare with SANS experiments. The overlap concentration is said to be the point marking the transition between a dilute and semi-dilute solution. Calculations located them at 55% for $G_5$ and 68% for $G_4$. Indeed at 64% and up the average nearest neighbor dendrimer-dendrimer distance follows the limit for purely repulsive particles; the dendrimers cannot move farther apart. However as the overlap concentration is not a tipping point, the various properties—average nearest neighbor distance, radius of gyration, water content and interpenetration—steadily change with increasing concentrations.

From using experimental procedures with simulation calculations it follows that the interdendrimer distance calculated from $q_{\text{max}}$ with the traditional factor 1.22 is somewhat overestimated. On the same grounds the utilization of the decoupling approximation and constant form factor assumption to calculate apparent structure factors gives correct results only up to concentrations of about 20%. This is well below the overlap concentration, not because of form factor changes, they are small, but because the monomer positions of separate dendrimers are already correlated.

With increasing dendrimer concentrations and increased competition, the dendrimers’ volume diminishes. In the dilute situation there is quite some water inside the dendrimers. With increasing concentration the dendrimer volume decreases as water content decreases. Instead of being uniformly distributed, the solvent fills cavities between stacked dendrimers. Concurrently interpenetration of dendrimer branches does increase, but remains quite subdued. Only 15% external particles enter each dendrimers’ space. Each dendrimer remains a separate entity. Contrary to other publications, we found that when accounting for their size there is no difference between dendrimer generations $G_4$ and $G_5$ for both water penetration and dendrimer interpenetration. Together, this gives a clear molecular picture of PPI dendrimers from dilute solutions to melts in comparison with the available experimental data.
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REFERENCES

21See Supplemental Material at [URL will be inserted by AIP Publishing] for coarse-grained parameter values, detailed simulation input variables and initial states, volume and mass

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percentage conversions, relaxation times for radius of gyration, asphericity and rotation, average distance calculations from $q_{\text{max}}$, radius of gyration and convex hull results, and additional radial monomer density graphs.