

# Visualization and Characterization of Poly(amidoamine) Dendrimers by Atomic Force Microscopy

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Poly(amidoamine), or PAMAM, dendrimers in the G5–G10 range were imaged by tapping mode atomic force microscopy (AFM). Individual dendrimer molecules could be clearly observed in the AFM images, which showed that these dendritic particles appear to be monodispersed, dome-shaped, and randomly distributed on the mica surface. Molecular diameter and height can also be measured from each particle's profile section from the AFM line scans. The diameter and height data were used to calculate the molecular volume of each single molecule. Absolute molecular weight and polydispersity were then estimated for each dendrimer generation. The calculated molecular weights for G5–G8 were in very good agreement with theoretical values. In addition, lower generation dendrimers, such as G4, were also studied by AFM. Although individual molecules of G4 dendrimer could not be imaged, the surface morphology of G4 dendrimer films on mica at different surface densities was observed by AFM.

## Introduction

Poly(amidoamine), or PAMAM, dendrimers are a class of synthetic, highly branched, spherical molecules with defined chemical functionality.<sup>1</sup> These water-soluble macromolecules are uniform in size, with a high density of primary amino groups mostly in close proximity to the surface.<sup>2</sup> The “dendrimer” name reflects the ordered, branching treelike structure of these novel polymers.<sup>3</sup> They are synthetically grown from a small core molecule, and each complete iterative reaction sequence results in a new dendrimer “generation” with a larger molecular diameter, twice the number of reactive surface sites, and approximately twice the molecular weight of the preceding generation.<sup>4</sup> The defined size, structure, and large number of surface functional groups of PAMAM dendrimers have enabled these polymers to be employed as substrates for the attachment of antibodies, contrast agents, radionuclides, etc., for use in various biological/medical applications. Recent research has shown that PAMAM dendrimers can be used as DNA-transfer vectors for gene transfection.<sup>5,6</sup>

With these important potential applications, there is a need for analytical techniques that help answer a number of fundamental questions concerning dendrimer characteristics and properties, e.g., their dimensions, uniformity of size and shape, degree of rigidity, etc.<sup>7–9</sup> Atomic force

microscopy (AFM) offers this possibility, since it provides high-resolution imaging and measurement of surface topography and properties on the molecular (or even atomic) scale.<sup>10</sup> It has been shown to be very useful in gaining insight into the properties of nanostructures.<sup>11</sup>

In recent years, there have been several studies of surface morphology of PAMAM dendrimers by AFM.<sup>12–17</sup> Watanabe and Regen<sup>12</sup> used an electrostatic layer-by-layer deposition technique to fabricate self-assembled films from alternating molecular layers of oppositely charged PAMAM dendrimers and the low molar mass compound K<sub>2</sub>PtCl<sub>4</sub>. They observed linear growth of the film thickness with each new layer, which is consistent with multilayer ordering. The surface morphologies of high-generation G9.5 dendrimer molecules have been studied by Badyal et al.<sup>13</sup> Tapping mode AFM unveiled a complex network of randomly intertwined PAMAM dendrimer microfibers. Tsukruk et al.<sup>14–16</sup> systematically studied assembled films of dendrimers in monolayers or multilayers on a solid surface. Examples of undistorted and compressed dendritic macromolecules within monolayers and multilayers films were presented. Also the interfacial behavior of dendrimers was compared to the surface states predicted by molecular dynamics simulations. Crooks et al. recently published a short communication reporting individual G4 and G8 PAMAM dendrimers adsorbed on a Au(111) substrate

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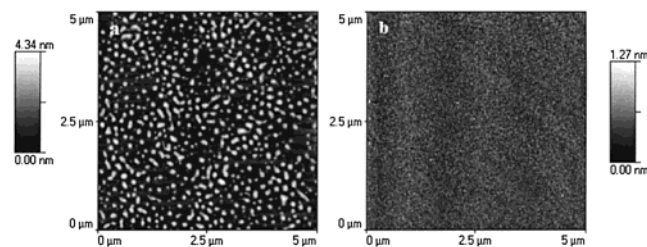
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**Figure 1.** Tapping mode AFM images of G4 PAMAM dendrimers on mica surface at different concentrations: (a) 0.1% w/w; (b) 0.01% w/w.

visualized by AFM.<sup>17</sup> However, they did not systematically study a full series of dendrimer generations.

In this study, we have chiefly focused on the molecular properties of PAMAM dendrimers as imaged by AFM, i.e., their size, shape, and rigidity after deposition on a mica surface. A new method of calculating the molecular weight of macromolecules by AFM techniques has also been developed.

### Experimental Section

**Materials.** Ethylenediamine (EDA) core poly(amidoamine) dendrimers (generations 4–10) were obtained from Dendritech Inc. (Midland, MI) as methanol solutions. After thorough drying, the solids were weighed and then redissolved in distilled water to produce stock solutions of 0.1% (w/w). The solutions were stored at 4 °C until use.

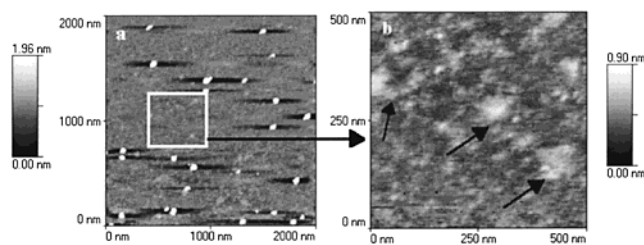
**Sample Preparation.** Ultrathin films of G4 dendrimer were prepared by spin-coating a solution of known concentration on freshly cleaved mica surfaces, which were then air-dried at room temperature. Film samples of each generation were similarly prepared. The concentrations of dendrimer solution that were used in each experiment are described in the appropriate figures.

**AFM Techniques.** The samples on mica were examined with a TopoMetrix 2000 Discoverer Instrument, using tapping mode under ambient conditions. Silicon tapping probes having a spring constant of ca. 30 N/m with about 5–10 nm radius were used for tapping scans. A precisely calibrated 7 μm scanner (*x*, *y*, and *z* directions) by TopoMetrix was used in every experiment. Image analysis was performed with TopoMetrix SPMLab 3.06.06 software. Particular experimental conditions will be more fully described in the next section. Each sample was examined at least twice under the same conditions, and the images were found to be very reproducible.

### Results and Discussion

PAMAM dendrimers with surface amino groups are easily spread on a mica surface to form films.<sup>14</sup> However, we found that the film uniformity is mostly determined by the concentration of the dendrimer solution, regardless of generation. Figure 1a,b shows this effect using a G4 dendrimer, in which the dendrimer is deposited on mica surface by spin-coating. The same spin-coating procedure was used for two different concentrations (2000 rpm for 2 min). The AFM image in Figure 1a of the film deposited from the higher concentration (0.1% w/w) shows randomly deposited globular structures (aggregates) of different sizes. In contrast, the AFM image (Figure 1b) of the film deposited from the lower concentration (0.01% w/w) shows a very uniform and flat surface. This indicates that dendrimer molecules first tend to form a densely packed film on a mica surface in order to maintain lower surface tension. Excess dendrimer molecules then aggregate on the surface of the dendrimer film to produce the globular structure seen in Figure 1a.

However, we also found that it is very difficult to image individual molecules of the G4 dendrimer (the theoretical size of G4 is about 4.5 nm), even by casting films from a very low concentration solution. This is shown in Figure



**Figure 2.** Tapping mode AFM images of G4 PAMAM dendrimers on mica surface at different magnifications. Concentration: 0.001% w/w. Key: (a) 2000 nm × 2000 nm; (b) 500 nm × 500 nm.

**Table 1. Selected Properties of Poly(amidoamine) PAMAM Dendrimers (Ethylenediamine Core)**

generation	measd <sup>a</sup> diameter (nm)	no. of surface groups	molecular weight
5	5.4	128	28 826
6	6.7	256	58 048
7	8.1	512	116 493
8	9.7	1024	233 383
9	11.4	2048	467 162
10	13.5	4096	934 720

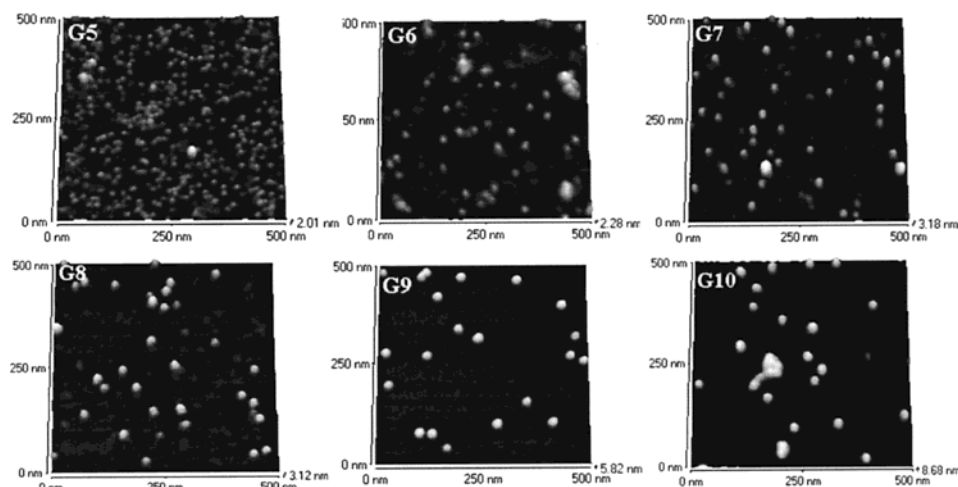
<sup>a</sup> Light scattering.

2a,b. As the dendrimer concentration is lowered to 0.001% w/w, the cast G4 film is no longer uniform and flat. Individual dendrimer molecules are not seen, but instead the dendrimers aggregate to give a patchy film (Figure 2a). The surface morphology can be seen much more clearly at the higher magnification shown in Figure 2b. Except for some regions indicated by arrows, most of the surface consists of small domains of different sizes. If the concentration is lowered to  $1 \times 10^{-4}\%$  or even  $1 \times 10^{-5}\%$  w/w, very similar images are obtained. Again, the surface morphology is not at all uniform, and individual G4 dendrimer molecules cannot be observed. These AFM images give further evidence that low-generation members of the dendrimer homologous series exist as open and platelike molecules in which the dendrimer branches can relatively easily interpenetrate each other and establish intermolecular interactions.<sup>18</sup>

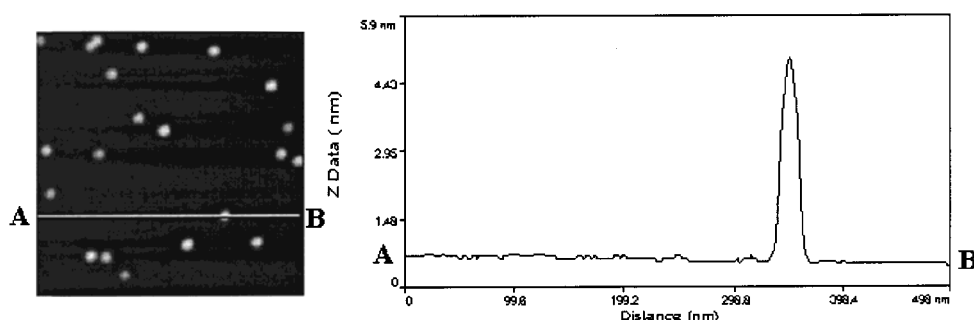
We have found, however, that AFM can be used to image individual dendrimers if they are larger than G4 (see Figure 3). The six samples from G5 to G10 were prepared by using dilute solution concentrations (0.001% w/w) and film forming conditions as for G4, i.e., spin-coating on a mica surface and drying at room temperature for 1 h. In Figure 3, one can see that the images for G5 and G6 generations have more particles than higher generations, due to their lower molecular weight (i.e., at the same weight concentration, their solutions contain more molecules than those of the higher generations). Selected properties of generation 5–10 PAMAM dendrimers are compared in Table 1: the diameter of G5–G10 increases from 5.4 to 13.5 nm, the number of surface functional groups increases from 128 to 4096, and the molecular weight increases from 28 826 to 934 720.

In the AFM images of Figure 3, one can see many separate and randomly deposited globular particles on the mica surface. In each image, the particles appear to be substantially uniform in size; i.e., they are essentially monodisperse. This is not surprising if each of the bright spots represents a single dendrimer molecule. There are a few large irregular clusters in the G6 image, perhaps caused by dendrimer aggregation. Using computer-imag-

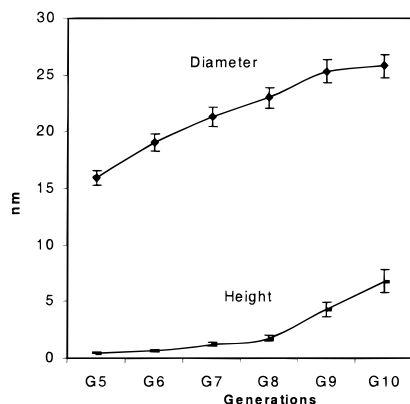
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**Figure 3.** Tapping mode AFM 3D images of PAMAM dendrimer molecules from G5 to G10. Dendrimer molecules (0.001% w/w) are deposited on the mica surface by spin coating.

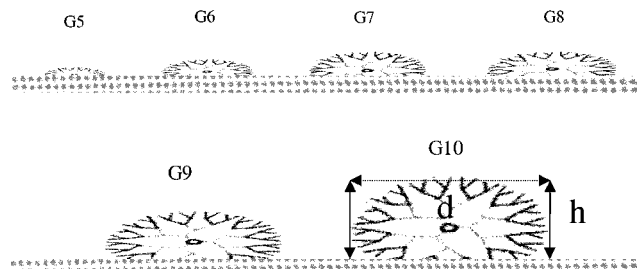


**Figure 4.** Profile section of G9 dendrimer on the mica surface.



**Figure 5.** Mean dendrimer diameter and height measured by AFM from particle profiles, as a function of generation. The error bars for AFM data represent one standard deviation.

ing analysis (TopoMetrix SPMLab 3.06.06),<sup>19</sup> profile data for each dendrimer molecule can be obtained as shown in Figure 4. A plot of the calculated mean diameters and heights as a function of generation (Figure 5) demonstrates that the measured diameters are always larger than the heights, indicating that the dendrimer molecules are no longer spherical but instead dome-shaped when deposited on a mica surface. It also shows that the diameter from G5 to G9 increases slowly with increasing generation, with the curve flattening out as it approaches G10. In contrast, the height from G5 to G8 increases slowly with increase in generation, with the height increasing more rapidly after G8. These results suggest that the molecular rigidity



**Figure 6.** Cartoon model of PAMAM dendrimers after drying on a mica surface.

of dendrimers increases dramatically with increasing generation, as expected from surface group dense-packing considerations.<sup>1</sup> It is also evident that the surface adsorption forces between the mica surface and individual dendrimers decreases with increasing generation, as predicted from Mansfield's model of dendrimer surface adsorption (see Figure 6).<sup>20</sup>

In fact, it has observed previously that measured diameters of dendrimer molecules by AFM are much larger than the theoretical values, which indicates that the dendrimers "spread out" and flatten on the surface.<sup>16,17</sup> We think that there are three major factors that may cause this deformation. First, the unique architecture and chemical structure of PAMAM dendrimers result in these macromolecules that are not solid balls but instead they are relatively "open" and hence soft materials. It is expected that the rigidity will increase substantially with increasing generation number.<sup>8</sup> Therefore, when deposited on solid substrates, they tend to deform to different degrees

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**Table 2.** Comparison of the Mean Molecular Weight Calculated by AFM and Theoretical Molecular Weight of PAMAM Dendrimers

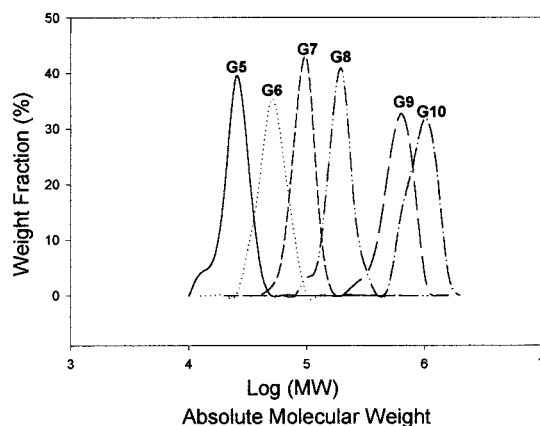
PAMAM dendrimers	no. of molecules	MW (AFM) measd	MW theoretical	rel error (%)	polydispersity
G5	48	27 900	28 826	-3.2	1.05
G6	48	56 000	58 048	-3.5	1.06
G7	61	120 000	116 493	3.0	1.05
G8	59	242 000	233 383	3.7	1.06
G9	52	616 000	467 162	31.9	1.08
G10	47	1 200 000	934 720	28.4	1.07

as a result of the interplay between their inherent rigidity and surface energetics from the interaction between the dendrimer molecules and the mica surface. Secondly, full generation PAMAM dendrimer molecules have a positively charged surface (in neutral, aqueous solution), while mica surfaces have the opposite polarity. Finally, we believe that the AFM tip can influence the sample surface morphology. This occurs when the tip scans a soft sample, even when using tapping mode. Unfortunately, there is currently no established method to explore how an AFM tip acts on soft molecules to induce apparent size changes.<sup>21</sup>

Figure 6 shows models representing single, isolated dendrimer molecules from G5 to G10 after drying on a mica surface. On the basis of the dome shape (i.e., a spherical cap), molecular volume can be calculated from the following equation:<sup>19</sup>

$$V = \frac{1}{6}\pi h(h^2 + \frac{3}{4}d^2)$$

Here  $h$  is the height and  $d$  is the diameter of the cap. For each generation, molecular volume can be calculated using measured diameter and height from each particle's profile. If the density of the dried dendrimer molecules is taken to be 1.0 g/cm<sup>3</sup>, an absolute molecular weight can be estimated, as shown for each generation in Table 2. The distribution of molecular weights of each generation was determined from measurements on about 50 molecules for each generation, with results shown in Figure 7 (the weight fraction is the percent dendrimer in each interval of molecular weight under consideration). On the basis of these distributions, the polydispersity index ( $M_w/M_n$ ) of G5–G10 can be calculated, with results also shown in Table 2.<sup>22</sup> They are all less than 1.08, which means that the particle size distribution is very uniform for each generation. Comparing the calculated and theoretical values, as well as FT-ICR MS values<sup>23</sup> for molecular weight, we found that there is very good agreement for G5–G8 but not for G9 and G10. The discrepancies for G9

**Figure 7.** Absolute molecular weight distribution as measured by AFM.

and G10 are probably due to a breakdown of the assumption that the deposited molecular shape is that of a spherical cap (see Figure 6).

### Conclusion

We have discussed the surface morphologies of PAMAM dendrimers from G4 to G10 by AFM. Experiments have shown that it is very difficult to get single molecular images of dendrimers smaller than G5, due to their lower rigidity, the smaller number of surface functional groups, and the ability of the branches of lower generations to easily interpenetrate one another. Tapping mode AFM has successfully imaged individual dendrimer molecules of the higher generations (G5–G10) spread on a mica surface. The image data have been used to determine the diameter and height of individual macromolecules, from which absolute molecular volumes and weights are estimated. The molecular weight results from G5 to G8 are in very good agreement with the theoretical values but not for the higher generations (G9 and G10). The discrepancy for these higher generations is probably due to the breakdown of the assumed shape model (that of a spherical cap) as a result of their increased rigidity. These experiments have demonstrated that AFM is not only useful in imaging dendritic macromolecules, but also can be useful in estimating important numerical molecular parameters, such as volume and molecular weight, which are not easily obtained by other techniques.

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