

Film Structures of Poly(Amido Amine) Dendrimers with an Azacrown Core and Long Alkyl Chain Spacers on Water or Ag Nanoparticle Suspension

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Newly designed poly(amido amine) dendrimers, which have an azacrown core, hexyl spacers, and methyl ester terminals (aza-C6-PAMAM dendrimer), were spread at the air–water and air–silver nanoparticle suspension interfaces, and their film structures were examined by surface pressure–area (π -A) and surface potential–area (ΔV -A) isotherms and epifluorescence microscopy. It was revealed that generation (G) 1.5 aza-C6-PAMAM dendrimer on a water subphase formed homogeneous film with face-on configuration, and this configuration was maintained during compression. On the other hand, a G2.5 dendrimer film on the air–water interface took initially homogeneous and face-on configuration that was followed by the conformational change during compression. Using a silver nanoparticle suspension as subphase, G1.5 film was significantly reinforced, and the partial collapse (cracks) in the film appeared as network texture. For a G2.5 dendrimer film, the π -A and ΔV -A isotherm properties were similar to that on the water subphase except for the collapsed film; small spots instead of cracks were formed under the film after collapse. These effects of the silver nanoparticle may be due to the formation of a dendrimer/silver nanoparticle composite. The formation process of the nanocomposite film was verified by UV–vis spectroscopy. For the G1.5 dendrimer, silver clusters and nanoparticles adsorbed to the dendrimer film after spreading and formed a small amount of aggregates. During compression, the aggregation proceeded even at low surface pressure. For the G2.5 dendrimer, a dendrimer/nanoparticle composite was also formed after spreading. However, with the initial compression, the absorption bands of clusters, nanoparticles, and aggregate increased together. Upon further compression, while the bands of cluster and nanoparticles decreased, the bands of aggregate still increased. These results suggest that the G2.5 dendrimer covered the cluster and nanoparticles more efficiently than the G1.5 dendrimer did because of the larger molecular size.

Introduction

Dendrimers are a new class of polymers that have many symmetric branches, terminal groups, and a monodisperse molecular weight. These characteristics are valuable in applications such as catalysts, sensors, drug delivery, and reaction matrixes.^{1–6} Many investigations of dendrimers have been developed not only in solutions but also at interfaces. Especially, Langmuir and Langmuir–Blodgett films of amphiphilic dendrimers are considered to be effective architectures in material science, e.g., molecular recognition,⁷ optical engineering devices,⁸ and building blocks for self-assembly.^{9,10} On such applications, the

efficiency of dendrimer films should be influenced by its chemical structure because the structure could regulate the location of functional groups in the film. Thus, the investigation of film structure related to the dendrimer conformation has attracted attention in the studies at interfaces.

Meanwhile, to fabricate highly functional ultrathin films, hybridization of a Langmuir film has been examined by means of many amphiphilic and hydrophobic compounds. In such studies, the Langmuir film was adsorbed with counter-charged ions, small compounds, polymers, proteins, and nanoparticles.^{11,12} Especially, the hybridization of Langmuir film with metal nanoparticles is important in materials science because the films of metal nanoparticles are very useful for optical and electrical applications.¹³ Although nanofabrication of dendrimers with metal nanoparticles is already reported in solution,¹⁴ film formation of such nanocomposites is just at its infancy.^{15,16}

In the present study, film structures of amphiphilic dendrimers and adsorption behaviors of metal nanoparticles on the film were examined by surface pressure–

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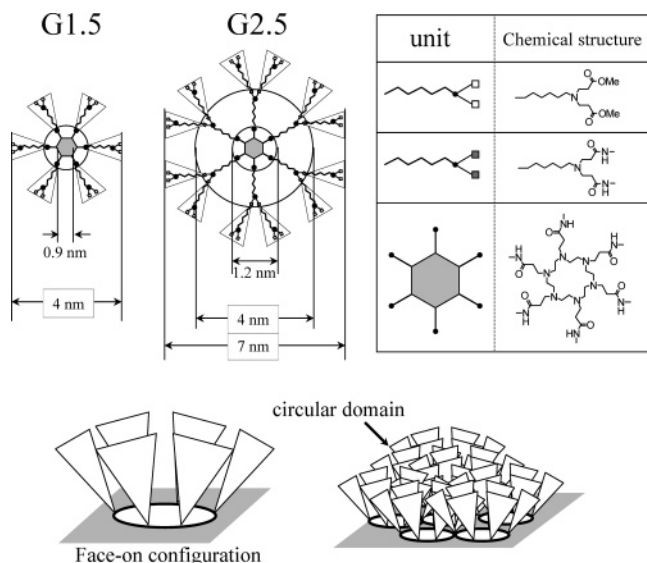


Figure 1. Chemical structure of aza-C6-PAMAM dendrimers and their domain at the air–water interface.

area (π -A) isotherm and surface potential–area (ΔV -A) measurements, epifluorescence microscopy, and UV–vis spectroscopy, and effects of this adsorption were discussed. The Ag nanoparticles used in this study were protected with 3-mercaptopropionic acid (MPA) to stimulate an interaction with tertiary amine groups in the poly(amido amine)-type (PAMAM) dendrimer. Acid–base interaction has been reported about adsorption of PAMAM dendrimer onto a MPA self-assembled monolayer (SAM).¹⁷ The aza-C6-PAMAM dendrimers used in this study were methyl ester-terminated PAMAM dendrimers of generation (G) 1.5 and 2.5¹⁸ having an azacrown core and longer repeating units than commercially available PAMAM dendrimers. Because the peripheral methyl ester groups are hydrophobic but the azacrown core is hydrophilic, the aza-C6-PAMAM dendrimers could behave as an amphiphilic compound. Furthermore, azacrown derivatives with long alkyl chains were reported to form monolayers on water subphase and adsorbed acidic compounds.¹⁹ Thus, the azacrown core is expected to be an efficient adsorption site. The long spacer should allow conformational change of the dendrimer and ensure its flexibility when adsorbed on the nanoparticle surface. Thus, the aza-C6-PAMAM dendrimer is assumed to be suitable for hybridization with nanoparticles at the air–water interface.

Experimental Section

Chemicals. Aza-C6-PAMAM dendrimers (G1.5 and G2.5, methyl ester-terminated) (Figure 1) were synthesized as previously reported.¹⁸ AgNO₃, MPA, NaBH₄, and CHCl₃ of highest purity available were purchased from Aldrich Co. or Tokyo Kasei Co. and used without further purification. Ultrapure water with resistivity of 18 M Ω ·cm and surface tension of 72.6 mNm⁻¹ at 20.0 \pm 0.5 $^{\circ}$ C was used as the subphase throughout the experiments. Water was obtained from a Modulab 2020 water purification system (Continental Water Systems Corp., San Antonio, TX). The aza-C6-PAMAM dendrimers were solved in CHCl₃ (0.2 mM for G1.5, 0.1 mM for G2.5).

Synthesis of Silver Nanoparticles Capped with 3-Mercaptopropionic Acid (Ag-MPA). 0.106 g (1 mM) of MPA was dissolved in 50 cm³ of water. 0.113 g (3 mM) of NaBH₄ was added in the MPA solution at 0 $^{\circ}$ C. AgNO₃ solution (0.340 g (2 mM) in

50 cm³ of water) cooled to 0 $^{\circ}$ C was poured at once into the MPA/NaBH₄ solution at 0 $^{\circ}$ C and stirred vigorously for 5 min. After mixing, black precipitate immediately appeared. The suspension was kept under mild stirring for 2 h at room temperature. Subsequently, the suspension was left at rest to separate dark-brown precipitate from colorless supernatant. The supernatant was removed by decantation, and the precipitate was washed three times by 30 cm³ of water. The precipitate was silver nanoparticles with a diameter of 5–10 nm, which was determined by transmission electron microscopy (TEM). The precipitate was dispersed in water and resulted in a homogeneous dark-yellow suspension (\sim 10 mM/Ag). This stock suspension was diluted with water to the desired concentration. The concentration of Ag-MPA suspension used as the subphase in a Langmuir trough was 0.05 mM (as Ag atom), and the pH was 5.8–6.3.

Transmission Electron Microscopy. TEM observation was performed using a Hitachi H-7000 instrument at an acceleration voltage of 100 kV. Ag-MPA (20 cm³) suspension was put into a Petri dish (diameter = 8 cm), and the dendrimer solution (20 mm³ of a G1.5 solution, 10 mm³ of a G2.5 solution) was spread on the Ag-MPA suspension. The Petri dish was covered and left at rest for 0.5 h. Then, a copper grid (Nissin EM300 mesh) covered with carbon film was horizontally put on the interface and picked up. The grid was dried in vacuo and then observed by TEM.

Surface Pressure–Area and Surface Potential–Area Isotherms. Simultaneous measurements of π -A isotherms and ΔV -A^{20,21} were carried out by using a Kibron μ Trough S (Kibron Inc., Helsinki, Finland). The sensitivity of the Wilhelmy plate for π -A isotherms was \pm 0.01 mNm⁻¹. The surface potential was measured by using the vibrating capacitor method. The bottom of the trough was coated with gold and used as a reference electrode. The vibrating electrode was placed at 1–2 mm above the subphase with the sensitivity of the electrode \pm 1 mV, and the surface potential was set to 0 mV before spreading the dendrimer solution. The dendrimer solution (50 mm³ of a G1.5 solution, 20 or 60 mm³ of a G2.5 solution) was spread on water or the Ag-MPA suspension subphase. After keeping 0.5 h for solvent evaporation, π -A and ΔV -A isotherms were measured simultaneously. The compression rates were 0.10 nm²/molecule/min for G1.5 and 0.40 or 0.10 nm²/molecule/min for G2.5 (20 or 60 mm³ spread, respectively).

Epifluorescence Microscopy. Epifluorescence micrographs of Langmuir films on different subphases were taken with an Olympus IX-FLA epifluorescence microscope (Olympus America Inc., Melville, NY). An Optronix Magnafire TM CCD camera (Meyer Instruments, Houston, TX) was used to detect the fluorescence emission excited by UV light. The Langmuir trough is a Kibron μ Trough S, which is equipped with a quartz window (located in the center of the trough) for in situ spectroscopic measurements. Dendrimer solutions (1 cm³) were mixed with a 0.1 mM CHCl₃ solution of 5-(N-octadecanoyl)-aminofluorescein (20 mm³ for G1.5, 10 mm³ for G2.5). The molar ratio of dendrimer/fluorophore was 100:1 in the mixture. The compression speeds of dendrimer films on water or Ag-MPA suspension were same as in the π -A and ΔV -A measurements. After keeping 0.5 h for solvent evaporation, the epifluorescence micrographs were taken at different surface pressures with the π -A isotherm simultaneously recorded.

UV–vis Spectroscopy. UV–vis absorption spectra of the Ag-MPA suspensions at various concentrations were measured by using a Perkin-Elmer UV–vis–NIR spectrometer Lambda 900 with a quartz cuvette of 1 cm path length. Absorption spectra of Langmuir films on a water subphase were recorded with a Hewlett-Packard 8452A diode array spectrophotometer (Hewlett-Packard, Wilmington, DE) and can be slid over a KSV minitrough (7.5 \times 37.5 cm) (KSV Instrument, Helsinki, Finland). The minitrough is equipped with a quartz window (located in the center of the trough) for in situ spectroscopic measurements. After the absorption spectra of the subphase (water or the Ag-MPA suspension) without films were measured and used as a background (background-A), the dendrimer solution was spread.

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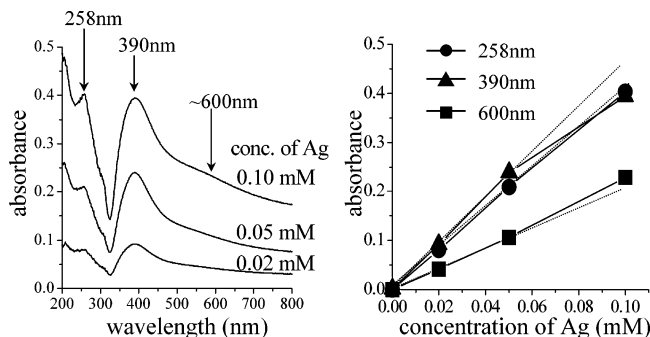


Figure 2. UV-vis spectra and absorbance plots of Ag-MPA suspensions as a function of Ag concentration.

The differential absorption spectra of Langmuir films were recorded for 40 min using a background-A. The absorption spectra of films after 40 min were newly measured as backgrounds (background-B). Then, the differential absorption spectra of the compressed films were recorded using the background-B. The barrier speed on the compression process was 10 mm/min for spread of both a G1.5 solution (150 mm³) and a G2.5 solution (50 or 100 mm³).

Results and Discussion

Characterization of MPA-Capped Ag Nanoparticles. Silver nanoparticles synthesized by reduction of AgNO₃ were capped by MPA to stabilize the dispersion and to make them water soluble. The obtained nanoparticles were mainly 5–10 nm in diameter and contained minor amount of larger particles. The similar heterogeneous distribution was reported for nanoparticles capped by thiosalicylic acid.²² MPA-capped Ag nanoparticles (Ag-MPA) formed dark-brown precipitate at high concentration and neutral pH. The precipitation should be due to the low solubility of nanoparticles. The pH-dependent association and dissociation of MPA-capped Au nanoparticles, depending on the degree of protonation of carboxylic groups of MPA, have been reported.²³ When the dark-brown precipitate was dispersed at lower concentrations in water, a transparent-yellow suspension was obtained. The yellow Ag-MPA suspension showed three UV-vis absorption bands above 200 nm (Figure 2). A band at 258 nm corresponds to the small Ag clusters, a 390 nm band is a surface plasmon absorption band of nanoparticle, and a broad band at 600 nm is assigned to aggregate of nanoparticles.^{24,25} It was confirmed from Figure 2 that the absorbance of these bands obeyed the Beer-Lambert law below 0.05 mM Ag concentration. Thus the Ag-MPA concentration of 0.05 mM was applied for the subphase used in further Langmuir film studies.

Hybridization of Dendrimer with Ag-MPA at Air-Water Interface. At 0.5 h, after spreading the dendrimer, an orangy film was observed at the air-water interface. This film was transferred to a TEM grid by the Langmuir-Schaefer method (horizontal transfer) and observed by TEM. The TEM images were shown in Figure 3. For both G1.5 and G2.5 dendrimer films, dispersed and aggregated Ag-MPAs were observed. Because the Ag-MPAs were not transferred to the grid without dendrimers, it was concluded that the dendrimer/Ag-MPA composites were formed at the interface.

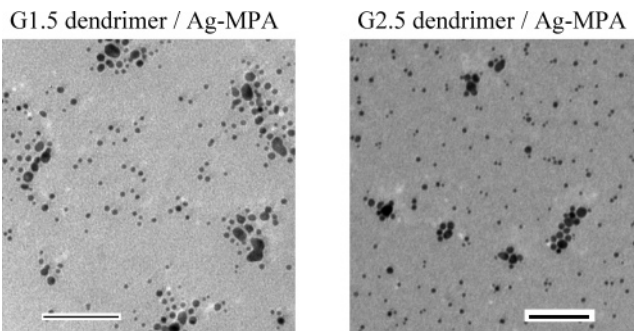


Figure 3. TEM images of dendrimer/Ag-MPA composite film. Scale bars correspond 100 nm.

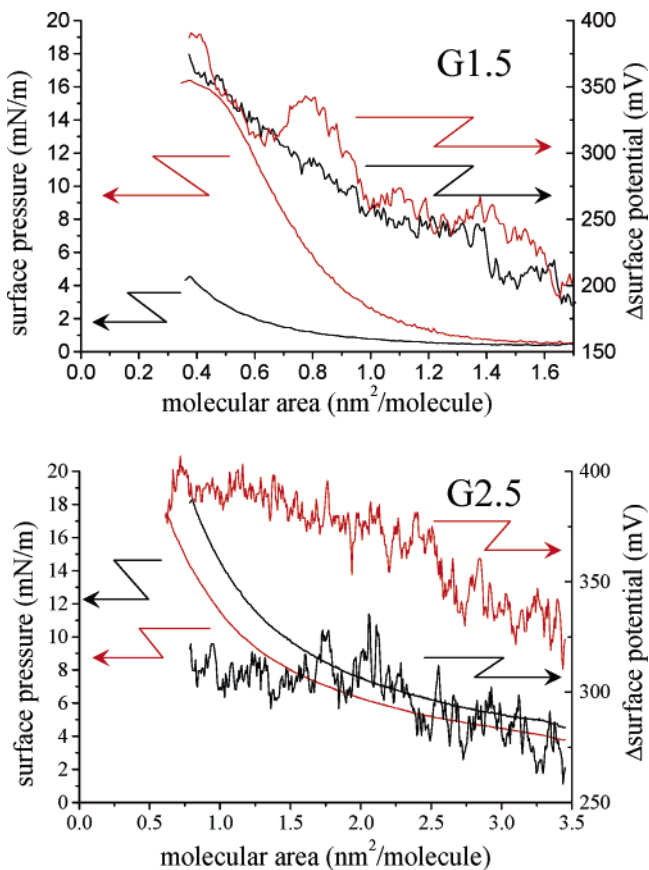


Figure 4. Surface pressure and surface potential-area isotherms of aza-C6-PAMAM dendrimers at air-subphase interfaces. Black line: on water; red line: on Ag-MPA suspension.

Surface Pressure and Surface Potential-Area Isotherm Profiles of Dendrimer Films and Their Hybrids with Ag-MPA at the Air-Water Interface. The π -A and the ΔV -A isotherms of G1.5 or G2.5 dendrimer films on the water or Ag-MPA suspension were shown in Figure 4. The surface pressure of the G1.5 dendrimer film on the water subphase was rather low at whole surface area, and the phase transition and occupied molecular area were not clearly determined. This suggests that the G1.5 dendrimer film is in gas to liquid states at 0.4 nm²/molecule. The surface potential increased simply during compression down to 0.4 nm²/molecule. This means that the vertical component of the dipole moment of the molecules increased as the molecular area was compressed. This change of surface potential has already been reported for an amphiphilic dendrimer with long alkyl chains in its periphery.^{20,21} The molecular structures of aza-C6-PAMAM dendrimers are axially symmetrical for

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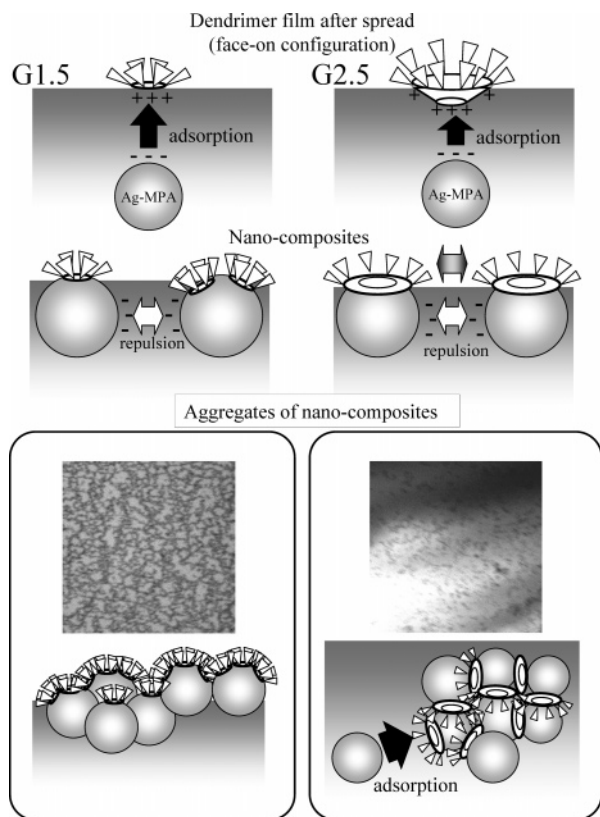


Figure 5. Schematic illustration of the formation of aza-C6-PAMAM dendrimer/Ag-MPA composites and composite aggregates. Fluorescence microscopic images ($500 \mu\text{m} \times 500 \mu\text{m}$) were taken after film collapse.

the center of azacrown core, and thus, the dipole moment should radiate from the center to the branching terminals of the molecule. Therefore, the increasing surface potential suggests that the dendron moieties in the dendrimer stand up from subphase with compression of the molecular area. Then the dendrimer in the film took face-on configuration, as illustrated in Figure 1.

The π - A and ΔV - A isotherms of the G1.5 dendrimer film were also measured on Ag-MPA suspension and compared with that on water, as shown in Figure 4. The surface pressure on the Ag-MPA suspension was much higher than that on the water. Thus, it was suggested that the composite film of Ag-MPA and dendrimer was more stable on the subphase. The driving force of the adsorption must be electrostatic interaction between COO^- groups of the negatively charged Ag-MPA and partially protonated amine groups of the positively charged dendrimer. Such electrostatic interaction between MPA SAM and PAMAM dendrimer has been reported,¹⁷ and the adsorption of negatively charged compounds to azacrown derivatives was also reported.¹⁹ Because the Ag nanoparticles in this study were larger than G1.5 dendrimer (Figure 1), the adsorbed Ag nanoparticle behaved as a larger component in the film, which raised the surface pressure. Furthermore, electrostatic interaction between Ag-MPAs could also contribute to a higher surface pressure because the COO^- groups of Ag-MPA should repulse each other (Figure 5). Despite the higher surface pressure of the G1.5 dendrimer film on the Ag-MPA suspension compared to the one on water, the surface potentials on both subphases showed similar behavior. From this result, the orientation process of molecular conformation was assumed to resemble both subphases. While the surface potential of the dendrimer film on the water increased

smoothly, that on the Ag-MPA suspension increased with fluctuation. This phenomenon suggests that the former film is homogeneous but the latter film consists of domains. We believe that the Ag-MPA and the dendrimer formed a stable composite at low surface pressures and the domains of the composite still remained after compression (This assumption will be discussed later relating to epifluorescence microscopy).

The π - A isotherms of G2.5 dendrimer films on two subphases were higher than those of G1.5 dendrimer films. The larger size of the G2.5 dendrimer molecule should contribute to the higher molecular area, and the longer repeating units could make the dendrimer films stable. While the G1.5 dendrimer film was mechanically strengthened by the hybridization with Ag-MPA, the π - A isotherm of the G2.5 dendrimer film did not change or became slightly lower by the existence of Ag-MPA. This means that the Ag-MPA/G2.5 dendrimer composite film was less compact or rough than the pure G2.5 dendrimer film. Different from the G1.5 dendrimer, the repeating units of the G2.5 dendrimer were long enough to protect Ag-MPA (Figure 5).

In the larger molecular area down to $2.5 \text{ nm}^2/\text{molecule}$, the surface potentials of the G2.5 dendrimer and its hybrid with Ag-MPA increased linearly (similar to that of the G1.5 dendrimer). However, the increase changed at 2.0 – $2.5 \text{ nm}^2/\text{molecule}$. At the smaller molecular area ($<2.0 \text{ nm}^2/\text{molecule}$), the surface potential curve was a plateau on the water subphase or moderately increasing on the Ag-MPA subphase. The variation of the surface potential suggests the variation of dendrimer conformation in the dendrimer films. As mentioned above, the face-on configuration would result in the increasing surface potential. The plateau observed on the surface potential profile implies that the conformational change of dendrimer was accomplished.²⁰ The dendron moieties in the dendrimer could not vary their conformation anymore. The moderate increase in the surface potential of the G2.5 dendrimer film on the Ag-MPA subphase implied that, with increasing surface pressure, hybridized dendrimers gradually varied their conformation because of the electrostatic interaction of tertiary amine groups in a dendrimer with carboxylate groups in underlying Ag-MPA.

Visual Observation of Dendrimer Films and Their Hybrids with Ag-MPA at Air–Water Interface. The fluorescence micrographs of the dendrimer films on water and Ag-MPA suspension were observed and shown in Figure 6. G1.5 dendrimer film on water without Ag-MPA consisted of small circular spots at low surface pressure after spreading. When the film was compressed, these spots were fused each other at 1 mN m^{-1} , and the film became homogeneous around 4 mN m^{-1} . The light spots in the fluorescence image mean that the fluorescent probes were in the spots. Thus the G1.5 dendrimer molecules might assemble to small domains with fluorescent probes in the film after spreading on the water subphase (Figure 1). The intensity of fluorescence referred to the density of dendrimers, and dark areas in the background were voids of the film. (Such voids have been reported for polymerizable amphiphilic PAMAM dendrimer film²⁰).

With compression, these domains fused to form the homogeneous film. This transition confirms the face-on configuration of dendrimer, which grows up with film compression. Although it was reported that the azacrown derivatives with long alkyl chains could take the edge-on configuration and display elongated and growing domains on water subphase,²⁶ the present dendrimer formed only the face-on configuration. This difference is assumed to be due to steric hindrance and less hydrophobicity of

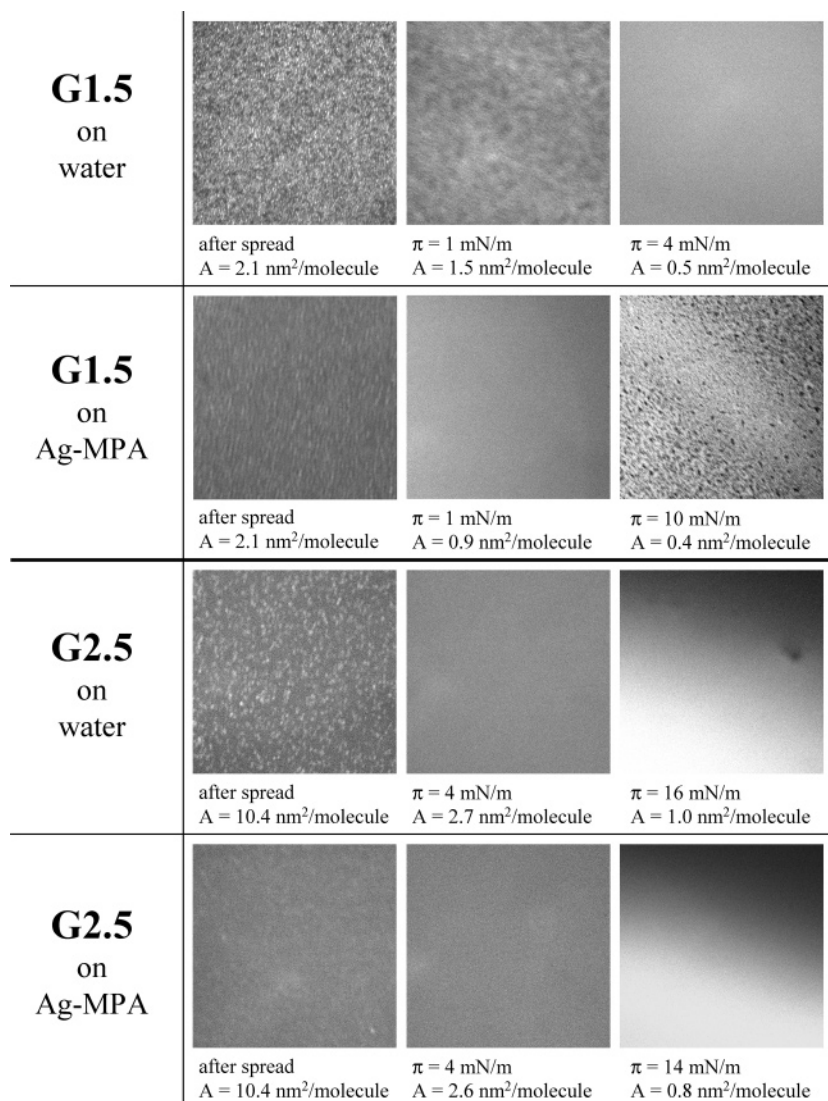


Figure 6. Fluorescence microscopic images ($500 \mu\text{m} \times 500 \mu\text{m}$) of aza-C6-PAMAM dendrimers at air–subphase interfaces.

dendron moieties in the dendrimer. While the significant role of hydrogen bonding of peripheral groups to form edge-on configuration was reported for the OH-terminated amphiphilic PAMAM dendrimer,²¹ terminal groups of the present dendrimer do not hydrogen bond.

On the Ag-MPA subphase, the G1.5 dendrimer film also consisted of small circular spots after spreading the dendrimer. During compression, the spots fused and formed a homogeneous film at 1 mN m^{-1} . This transition happened at low surface pressure in comparison with the case of the film on the water subphase without Ag-MPA. At higher surface pressure (10 mN m^{-1}), small dark spots appeared. As the surface pressure increased, the dark spots increased in number and finally formed the network texture. It is suggested that the circular spots after spreading are domains of dendrimers, which fuse each other during the structural change of dendrimers to be the face-on configuration (similar to the film structure at low surface pressure on water subphase). The dark spots at high surface pressure were assumed to be aggregates of dendrimer/Ag-MPA composites. Although the dendrimer molecules can bind Ag-MPA at low surface pressure, the Ag-MPA does not quench the fluorescence

in a homogeneous film. The aggregates at high surface pressure have no or low fluorescence because the aggregated Ag-MPA might exclude the fluorescent probes or behave as quenchers. This quenching could be explained by the energy-transfer mechanism from the fluorescent probe to the metal nanoparticle.²⁷ The aggregates would take metallic character and stock the energy from the fluorescent probes. Although exclusion of the fluorescent probes by crystallization of film matrix has also been reported,²⁸ the present dendrimer is hardly crystallized.

The G2.5 dendrimer film on a water subphase displayed circular spots after spreading. The film became homogeneous at a surface pressure of 4 mN m^{-1} . At high surface pressure (16 mN m^{-1}), cracks appeared along the dark stripes in the homogeneous film. As the surface pressure increased until the collapse, the stripes became bright lines at the order of $100 \mu\text{m}$, and many cracks were formed. At low surface pressure, the film texture of the G2.5 dendrimer on a water subphase resembled that of the G1.5 dendrimer. The face-on configuration was assumed to compose of circular spots after spreading, and homogeneous film was formed at 4 mN m^{-1} from the same reason as in the case of the G1.5 dendrimer. However, the

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structure of the G2.5 dendrimer film at high surface pressure was quite different from that at low surface pressure. The stripe structures appeared clearly, and the cracks assembled in lines. These stripes were wrinkles of the film and indicate that the G2.5 dendrimer film was rather rigid. The large G2.5 dendrimer molecule could form a film of well-defined stripe structure at high surface pressure because the long dendron moieties assembled in a thick and rigid film. The G1.5 dendrimer was so small that the film was thin and easily collapsed at low surface pressure on water subphase. For the G2.5 dendrimer, the stripe structure appeared at around $11\text{--}14\text{ mN m}^{-1}$ in the fluorescence micrographs, but the inflection point of surface potential was 8 mN m^{-1} . This gap was due to the qualitative determination from visual observation of the stripe structure on fluorescence microscopy.

On the Ag-MPA subphase, the G2.5 dendrimer film displayed unclear spots after spreading and became homogeneous at 4 mN m^{-1} , but the film structure changed to the stripes at 14 mN m^{-1} . This process was similar to that of the G2.5 dendrimer film on a water subphase. The presence of Ag-MPA favored the formation of a homogeneous film of the G2.5 dendrimer at low surface pressure. (This tendency was also observed in the G1.5 dendrimer films.) When the Ag-MPA bound, the dendrimer molecules covered Ag-MPA (Figure 5) because the azacrown core and tertiary amines in repeating units could bind to the Ag-MPA. Such covering by the G2.5 dendrimer is stronger than that by the G1.5 dendrimer owing to the additional binding of amide groups in repeating units and a large molecular area comparable to that of Ag-MPA. The dendrimer/Ag-MPA composite is protected from aggregation by electrostatic repulsion between COO^- groups on the bare surface of Ag-MPA, which is not covered by dendrimer. The stripe structure at high surface pressure is due to the same reason, which was discussed above for the G2.5 dendrimer on water subphase.

The significant difference of aggregation between G1.5 and 2.5 dendrimers is that dark particles appeared after collapse of the G2.5 dendrimer film and they floated under the surface. The dark particles are aggregates of the dendrimer/Ag-MPA composites. However, different from the floated aggregates of the Ag-MPA/G2.5 dendrimer composite, the aggregates of the Ag-MPA/G1.5 dendrimer composite stayed in the film (Figure 5) as the G1.5 dendrimer film formed on Ag-MPA subphase. These behaviors suggest that the aggregates of the Ag-MPA/G2.5 dendrimer composites became more hydrophilic than the G2.5 dendrimer itself. It is assumed that the hydrophilicity is due to the aggregate structure, where some Ag-MPA molecules aggregate by the intermediation of G2.5 dendrimer. The hydrophilic moiety of G2.5 dendrimer is large enough to be a binder of Ag-MPA. Thus, the surface of aggregates was hydrophilic. On the other hand, for the G1.5 dendrimer, hydrophilic moiety is so small that dendrimers could form dense packing on Ag-MPA.

Formation Process of Dendrimer/Ag-MPA Composites and Their Aggregates. To verify the formation process of dendrimer/Ag-MPA composites and their aggregates at the air-water interface, differential UV-vis absorption spectra of G1.5 and G2.5 dendrimer films on a Ag-MPA subphase were measured as a function of time after spreading and were shown in Figure 7. No additional bands in the region of $200\text{--}800\text{ nm}$ were observed, except three bands originated from Ag-MPA because no absorption bands existed in the G1.5 and G2.5 dendrimer films on a water subphase.

With time, the absorption bands at 258 and 390 nm decreased without wavelength shift, and a band at about

600 nm increased with the shift, as seen in differential absorption spectra and plots of differential absorbance vs time in Figure 7. As described above, the absorption bands at 258 and 390 nm correspond to isolated clusters and nanoparticles, respectively. The differential absorbance decrease of these bands means that both clusters and nanoparticles of Ag-MPA in subphase bind, as aggregates, with dendrimer at the interface. The assembling to aggregates should result in the increase of an absorption band at 600 nm.^{25,29} This band was broad but red-shifted with time. This indicates that the assembling to aggregates with larger sizes proceeded. These transitions were accomplished within about a half an hour.

When the spread amount of the G2.5 dendrimer was doubled, the differential absorbance of three bands increased double, indicating a proportional relation of spread amounts of dendrimer to the adsorption amounts of Ag-MPA. On the other hand, although the spread amount of the G1.5 dendrimer was $30 \times 10^{-9}\text{ mol}$, the absorbance was comparable to that in the case of the $5 \times 10^{-9}\text{ mol}$ G2.5 dendrimer. This suggests that the amount bound to the G1.5 dendrimer was much less than that to the G2.5 dendrimer. Not only the azacrown core, but also tertiary amine groups in the G2.5 dendrimer should be positively charged and effective as binding sites for negatively charged Ag-MPA (Figure 5). Thus, a smaller amount of Ag-MPA could bind to the film of the G1.5 dendrimer, which has less binding sites in the molecule.

After the dendrimer films were spread and kept for 40 min, the films were compressed. As shown in Figure 8, the differential absorption bands at 258, 390, and 600 nm in the G1.5 dendrimer film became high during compression of the film. This suggests that the density of the Ag-MPA at the interface increased. The increase in differential absorbance at 600 nm was higher than those of the other bands. This means that the aggregation progressed during film compression.

For the G2.5 dendrimer film, the differential absorbance changed differently from the G1.5 dendrimer. At a large molecular area ($>6.5\text{ nm}^2/\text{molecule}$), the differential absorbance at 258, 390, and 600 nm increased together with compression. This suggests that the adsorbed Ag-MPA does not give rise to the aggregation. With further compression at a smaller molecular area than $6.5\text{ nm}^2/\text{molecule}$, the differential absorbance of the three bands decreased, but the absorbance at 600 nm increased again from $5.0\text{ nm}^2/\text{molecule}$. This change indicates that isolated nanoclusters and nanoparticles started aggregation at $6.5\text{ nm}^2/\text{molecule}$. Slight decrease of the differential absorbance at 600 nm in $6.5\text{--}5.0\text{ nm}^2/\text{molecule}$ range might suggest less density of newly formed aggregates, although heterogeneity of the whole film can be also assumed for such slight decrease. This transition occurred at very low surface pressure ($6.5\text{ nm}^2/\text{molecule}$ corresponds to $2\text{--}3\text{ mN m}^{-1}$). At this surface pressure, because the film was just homogeneous in the fluorescence microscopy (Figure 6), the heterogeneity described above should be below the resolution of the epifluorescence microscope. At $3.5\text{--}3.0\text{ nm}^2/\text{molecule}$, the differential absorbance at 258 and 390 nm bands stopped the decrease and increased again. This indicates that the increase of Ag-MPA density at the interface caused the rise of the differential absorbance.

When the spread amount of the G2.5 dendrimer was doubled, the increase of differential absorbance at 390 and 600 nm remarkably started from 2.5 to $3.0\text{ nm}^2/$

(29) Tao, A.; Kim, F.; Hess, C.; Goldberger, J.; He, R.; Sun, Y.; Xia, Y.; Yang, P. *Nano Lett.* **2003**, *3*, 1229.

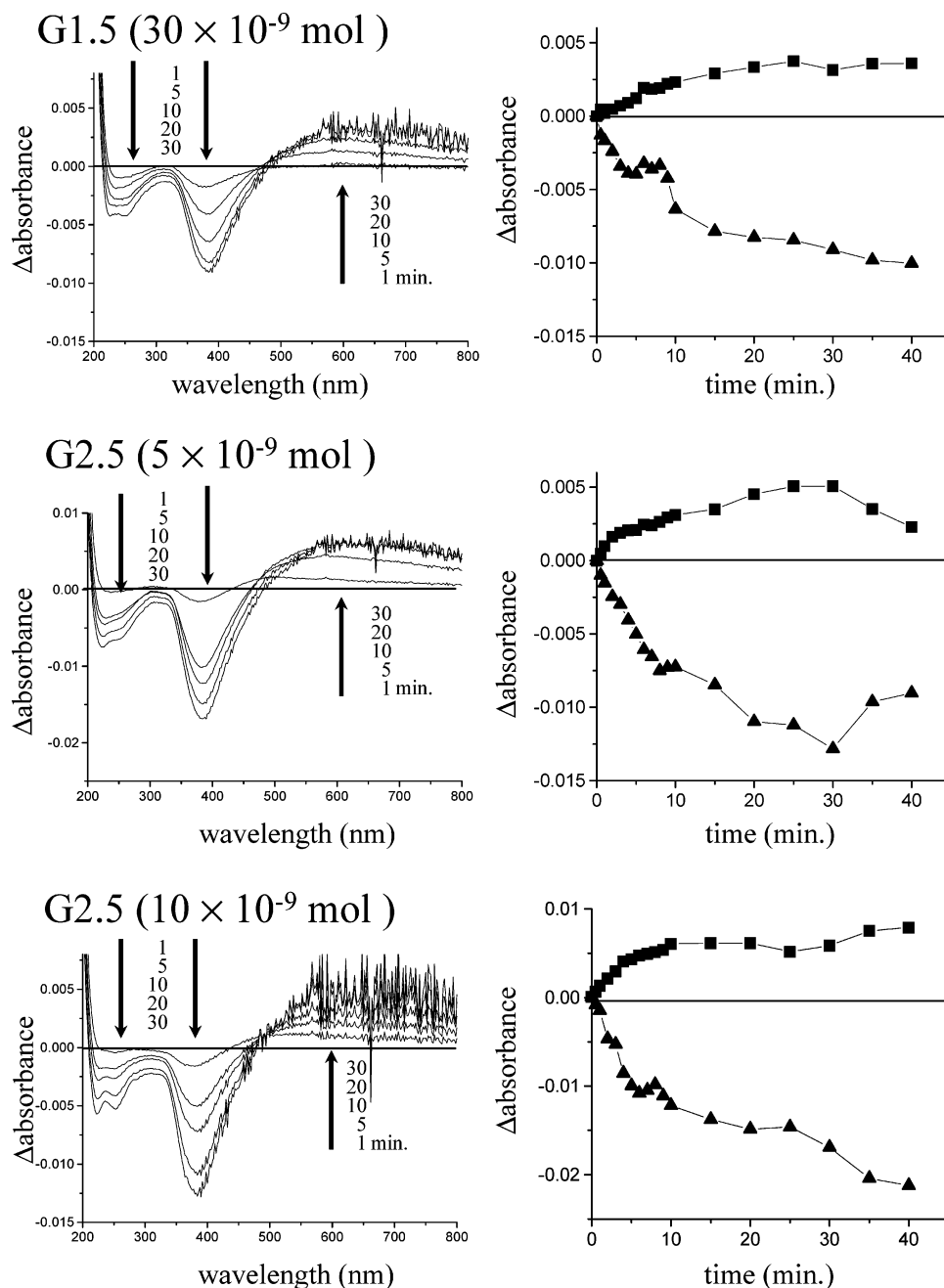


Figure 7. Differential UV-vis absorption spectra and differential absorbance plots of aza-C6-PAMAM dendrimer/Ag-MPA systems without compression after spreading: ▲, 390 nm; ■, 600 nm.

molecule on the compression process, as seen in UV-vis spectra in Figure 8. This corresponds to the increase, which happened at 3.5–3.0 nm²/molecule in the case of 5×10^{-9} mol spread.

It should be noted that the differential absorbance of the G1.5 dendrimer film at 600 nm increased more than that of the G2.5 dendrimer film. That is, the Ag-MPA tended to aggregate more easily on the G1.5 dendrimer film than on the G2.5 dendrimer film. The driving force of aggregation at the compressed situation should be van der Waals interaction or hydrogen bonding between Ag-MPA superior to electrostatic repulsion. The G1.5 dendrimer could suffer this interaction easily because of their smaller molecular size or less covering than the G2.5 dendrimer. The aggregation occurred even at very low surface pressure, 2–3 mN m⁻¹, which corresponds to 1.6 nm²/molecule. On the other hand, a large G2.5 dendrimer molecule covers the surface of Ag-MPA and

prevents the aggregation of Ag-MPA. However, the aggregation of Ag-MPA is possible by the intermediation of G2.5 dendrimer. The comparative structures of aggregates are schematically illustrated in Figure 5.

Conclusions

Molecular conformation of two different generation (G1.5 and G2.5) poly(amido amine) dendrimers, which have an azacrown core and hexyl spacers (aza-C6-PAMAM dendrimer) at the air–water interface, were investigated by using surface pressure and surface potential–area isotherm measurements and epifluorescence microscopy. The surface pressure and potential of the G1.5 dendrimer increased with the decrease in the molecular area during compression. The surface pressure of the G2.5 dendrimer increased, but the surface potential leveled off after reaching an area of 2.0–2.5 nm²/molecule. These results suggested that the G2.5 dendrimer film accomplished their

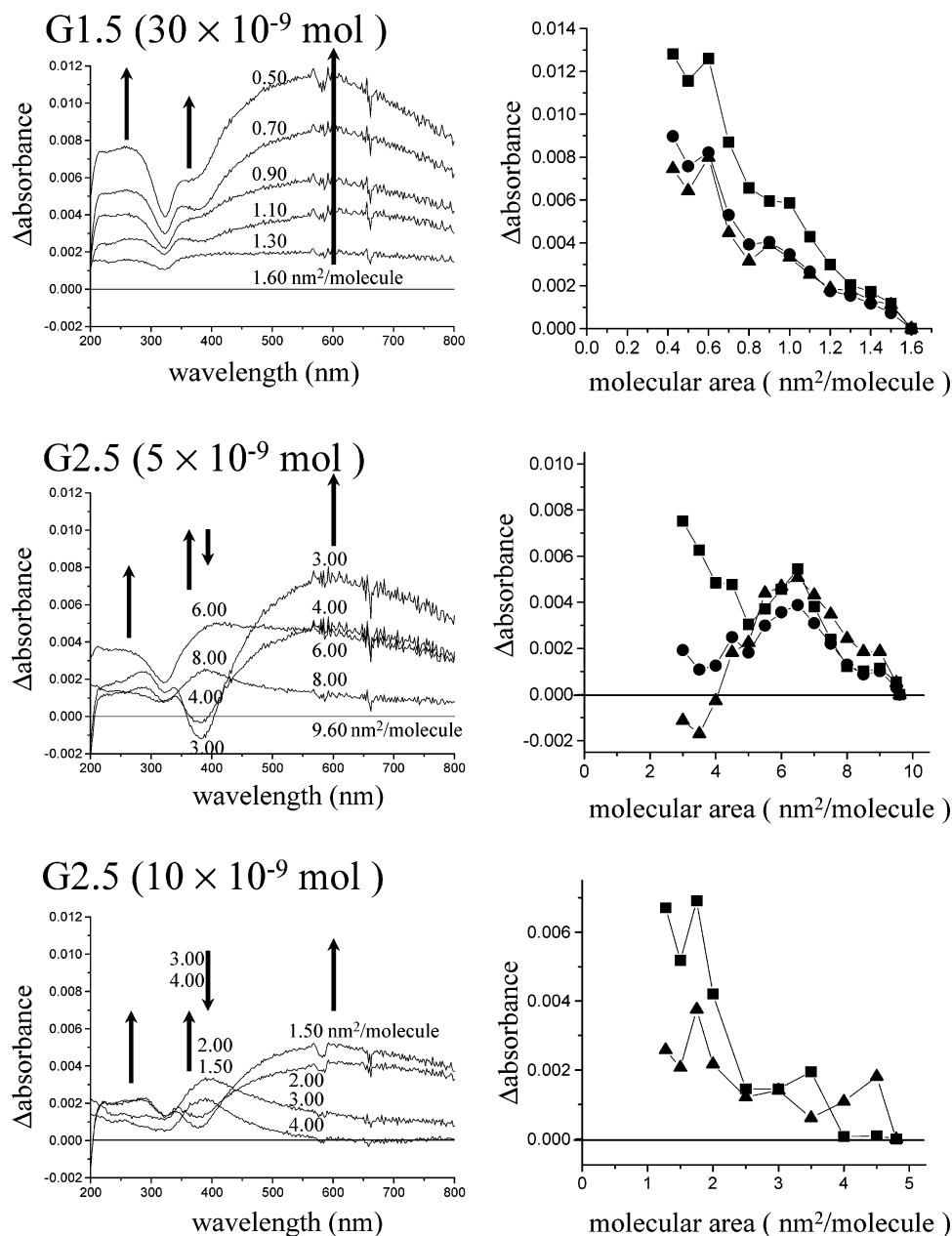


Figure 8. Differential UV-vis absorption spectra and differential absorbance plots of aza-C6-PAMAM dendrimer/Ag-MPA systems during compression: ▲, 390 nm; ■, 600 nm.

structural variation. Similar difference between dendrimers was observed by epifluorescence microscopy. After spreading, G1.5 and G2.5 dendrimers showed circular spots at the air-water interface. During compression, the G1.5 dendrimer film became homogeneous, while the G2.5 dendrimer film was homogeneous at low surface pressure but formed stripes at high surface pressure. The first circular spots were assumed to be domains of dendrimer molecules in the film. The change from the circular spots to the homogeneous film suggested that the molecules had a face-on configuration. During compression, domains fused, and the films became homogeneous. The formation of stripes in the G2.5 dendrimer film may be related to the leveling off of the surface potential described above.

When silver nanoparticles protected by 3-mercaptopropionic acid (Ag-MPA) were dissolved in subphase, the surface pressure of the G1.5 dendrimers became higher by the presence of silver nanoparticles, but that of G2.5 was similar to that without Ag-MPA. The behavior of

surface potential for both dendrimers was not different from the case without Ag-MPA. The epifluorescence microscopy revealed that, after a homogeneous film of dendrimer and Ag-MPA was formed at the air-water interface, dark spots appeared. For the G2.5 dendrimer, after collapse, dark spots flowed under the film. The dark spots may be aggregates of dendrimer/Ag-MPA composites.

The process of aggregate formation was investigated by UV-vis spectroscopy. After spreading the dendrimer on the Ag-MPA subphase, the surface plasmon band of the Ag-MPA nanoparticles (390 nm) decreased, and the absorption band at 600 nm increased. This means that Ag-MPA adsorbed to the dendrimer and formed aggregates of dendrimer/Ag-MPA composite. During compression of the G1.5 dendrimer film, the absorption bands increased together, and the increase in differential absorbance at 600 nm was higher than that of the other band, indicating the formation of the aggregates of Ag-MPA. On the other

hand, during compression of the G2.5 dendrimer film, the bands at 390 and 600 nm behaved differently from those of the G1.5 dendrimer. The difference suggested that the G2.5 dendrimer protected Ag-MPA more effectively than the G1.5 dendrimer.

The dendrimers used in this study could efficiently bind with silver nanoparticles and form homogeneous films. The aggregates of the nanocomposites varied their hydrophilicity, depending on the generation of dendrimer,

because of the size ratio of interior binding site to the molecule.

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