

Paramagnetic NMR Investigation of Dendrimer-Based Host-Guest Interactions

Fei Wang¹, Naimin Shao¹, Yiyun Cheng^{1,2*}

1 Shanghai Key Laboratory of Regulatory Biology, School of Life Sciences, East China Normal University, Shanghai, People's Republic of China, **2** Shanghai Key Laboratory of Magnetic Resonance, Department of Physics, East China Normal University, Shanghai, People's Republic of China

Abstract

In this study, the host-guest behavior of poly(amidoamine) (PAMAM) dendrimers bearing amine, hydroxyl, or carboxylate surface functionalities were investigated by paramagnetic NMR studies. 2,2,6,6-Tetramethylpiperidinyloxy (TEMPO) derivatives were used as paramagnetic guest molecules. The results showed that TEMPO-COOH significantly broaden the ¹H NMR peaks of amine- and hydroxyl-terminated PAMAM dendrimers. In comparison, no paramagnetic relaxation enhancement (PRE) was observed between TEMPO-NH₂, TEMPO-OH and the three types of PAMAM dendrimers. The PRE phenomenon observed is correlated with the encapsulation of TEMPO-COOH within dendrimer pockets. Protonation of the tertiary amine groups within PAMAM dendrimers plays an important role during this process. Interestingly, the absence of TEMPO-COOH encapsulation within carboxylate-terminated PAMAM dendrimer is observed due to the repulsion of TEMPO-COO⁻ anion and anionic dendrimer surface. The combination of paramagnetic probes and ¹H NMR linewidth analysis can be used as a powerful tool in the analysis of dendrimer-based host-guest systems.

Citation: Wang F, Shao N, Cheng Y (2013) Paramagnetic NMR Investigation of Dendrimer-Based Host-Guest Interactions. PLoS ONE 8(6): e64722. doi:10.1371/journal.pone.0064722

Editor: Bing Xu, Brandeis University, United States of America

Received: December 20, 2012; **Accepted:** April 18, 2013; **Published:** June 10, 2013

Copyright: © 2013 Wang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors are thankful for financial support from the Science and Technology of Shanghai Municipality (11DZ2260300), the Talent Program of East China Normal University (No.77202201), and the Program for New Century Excellent Talents in University of Ministry of Education of China on this project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: yycheng@mail.ustc.edu.cn

Introduction

Dendrimers are a new class of nanoscopic containers and delivery devices [1,2,3,4]. They have unique molecular architectures, spherical shapes, interior cavities, excellent monodispersity, and high density of surface functionalities [5,6,7,8,9,10,11]. Dendrimers have high solubility in various organic solvents and water, allowing miscellaneous modifications on dendrimer surface and in-depth characterization by different techniques [4]. These physicochemical properties render dendrimers the applications in host-guest systems [12,13]. Tomalia *et al.* first reported the diffusion of aspirin molecules into the interior cavities of poly(amidoamine) dendrimers by NMR relaxation measurements [14]. Meijer *et al.* synthesized a dendritic box by modification of poly(propylene imine) (PPI) dendrimer surface with 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids and the dendritic box was able to encapsulate guest molecules such as Rose Bengal and *p*-nitrobenzoic acid [15,16,17]. The release of *p*-nitrobenzoic acid from the dendritic box can be triggered by removing Fmoc protection, while Rose Bengal cannot diffuse out of the dense shell due to its larger size than *p*-nitrobenzoic acid [17]. In a separate study by the Meijer group, palmitic acid modified PPI dendrimers were successfully used for the extraction of anionic xanthene dyes from aqueous solution [18]. These pioneer studies significantly promote the interests of scientific community in dendrimer-based host-guest systems [12,19,20,21].

Our group systematically investigated PAMAM and PPI dendrimer-based host-guest systems using several NMR techniques in the past five years [21,22,23,24,25,26]. NMR techniques

have proved to be powerful and informative tools in the characterization of dendrimers and dendrimer/guest complexes [12]. Dendrimers have successive repeat units, well-defined branched structures, highly symmetric frameworks, and spherical shapes like proteins [27]. Repeat unit means rapid chemical shift assignment of the dendrimer protons and much simplified NMR spectrum [12]. Highly symmetric framework and well-defined branched structures allow us to predict the location and orientation of guest molecules within the interior pockets of dendrimers by nuclear Overhauser effect spectroscopy (NOESY) [22,25]. Spherical shape encourages us to characterize the size and morphology of dendrimers and dendrimer/guest complexes or aggregates using diffusion NMR techniques such as pulsed gradient spin echo (PGSE) and diffusion ordered spectroscopy (DOSY) [26,28]. Besides, the rapid technological development in high-field NMR spectroscopy and use of cryogenic probe system significantly increase the sensitivity in NMR analysis, and reduce the time and sample concentration needed in an NMR experiment.

When a paramagnetic probe such as nitroxide spin label is in spatial proximity with a host molecule, magnetic dipolar interactions between the unpaired electrons of the paramagnetic probe and the nucleus of interest will result in an increase in nuclear relaxation rates, which is defined as paramagnetic relaxation enhancement (PRE) effect [29]. In comparison with NOE, where the effect is limited to a short range within 5–6 Å, the PRE method can be used to get distance information in the range of 15–24 Å for nitroxide spin label, owing to the large magnetic moment of the unpaired electron [30]. Distance between the spin

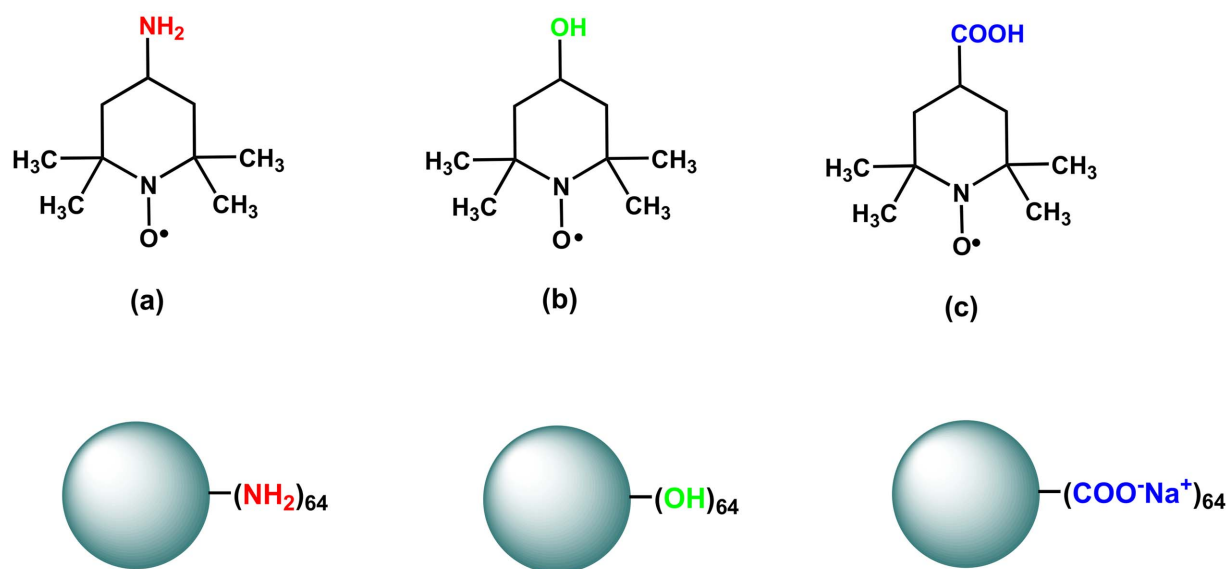


Figure 1. Molecular structures of the three nitroxide spin probes (a) TEMPO-NH₂ (b) TEMPO-OH (c) TEMPO-COOH and structure chart of PAMAM dendrimers with three types of surface groups.
doi:10.1371/journal.pone.0064722.g001

label and the nucleus of interest can be determined from the increased R_2 relaxation rates, allowing quantitatively interpretation of the PRE effect [31]. As a result, PRE NMR is becoming a sensitive and powerful tool to produce long-range site-specific constraints that can complement NOE constraints [29,32].

In dendrimer-based host-guest systems, if a paramagnetic probe is encapsulated within the dendrimer interior pockets, the PRE effect will lead to decreased NMR signal intensities and increased peak linewidths of dendrimer protons close to the paramagnetic probe in a ^1H NMR spectrum, which is an important supplement to the NOESY techniques in the investigation of dendrimer-based host-guest systems [29]. Previously, Newkome *et al.* investigated dendrimer structure by different NMR techniques using paramagnetic cobalt (II) as a probe [33,34]. In this study, 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) derivatives were used as paramagnetic guests (**Fig. 1**). PAMAM dendrimers bearing hydroxyl, primary amine, and carboxylate groups were used as model hosts (**Fig. 2**). Though the interactions of TEMPO derivatives and PAMAM dendrimers were previously investigated by Turro and Tomalia *et al.*, they use an electron spin resonance (ESR) technique to analyze the dendrimer/TEMPO complexes [35,36]. The major goal of this study is to reveal the host behaviors of PAMAM dendrimers with different surface functionalities by PRE NMR.

Experimental

Materials

Generation 4 (G4) ethylenediamine (EDA)-cored and primary amine-terminated PAMAM dendrimer (G4-NH₂), G4 EDA-cored and hydroxyl-terminated PAMAM dendrimer (G4-OH), and G3.5 EDA-cored and carboxylate-terminated PAMAM dendrimer (G3.5-COONa) were purchased from Dendritech (Midland, MI). 4-amino-TEMPO (TEMPO-NH₂) and 4-carboxy-TEMPO (TEMPO-COOH) were purchased from Sigma-Aldrich (St. Louis, MO). 4-hydroxy-TEMPO (TEMPO-OH) was obtained from Aladdin Chemistry Co. Ltd (Shanghai, China). Deuterium oxide (D₂O) was purchased from Beijing Chongxi High-Tech Incubator Co. Ltd. (Beijing, China). Dioxane and deuterated dimethyl

sulfoxide (d₆-DMSO) were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). Dendrimers were received in methanol and the solvents were distilled to obtain the products as white gels. All other chemicals were used as received without further purification.

Sample preparation

G4-NH₂, G4-OH, and G3.5-COONa were dissolved in D₂O at a concentration of 20 mg/mL. TEMPO-NH₂, TEMPO-COOH, and TEMPO-OH were dissolved in d₆-DMSO at a concentration of 5 mg/mL. The stock solutions were stored at 4°C before NMR studies. The dendrimer/TEMPO complex solutions were prepared at different TEMPO/dendrimer molar ratios in 500 μL D₂O and d₆-DMSO mixtures (V/V, 80/20). Dendrimer concentrations in the complex solutions are fixed at 1 mg/mL and the molar ratios of TEMPO/dendrimer are 0, 1, 2, 4, 8, 12, 16, 24, 32, 40, 48, and 64, respectively. Each sample contains a certain amount of dioxane which was used as internal standard. The samples were sonicated for 2 h before NMR analysis and the pH condition of each sample was measured immediately after the NMR experiments.

NMR analysis

^1H NMR spectra of the PAMAM dendrimer and TEMPO complex solutions were obtained on a Varian 699.804 MHz NMR spectrometer at 298.2 ± 0.1 K. The peak linewidths in ^1H NMR spectrum were measured using the VNMRJ software.

Results and Discussion

Interactions of TEMPO-NH₂ and TEMPO-OH with three types of PAMAM dendrimers

The host-guest chemistry of TEMPO-NH₂ and TEMPO-OH with PAMAM dendrimers bearing amine, hydroxyl, and carboxylate groups were investigated by ^1H NMR. G4-NH₂, G4-OH, and G3.5-COONa PAMAM dendrimers were used because these dendrimers have the same numbers of surface functionalities (64) and similar molecular size. Besides, these PAMAM dendrimers

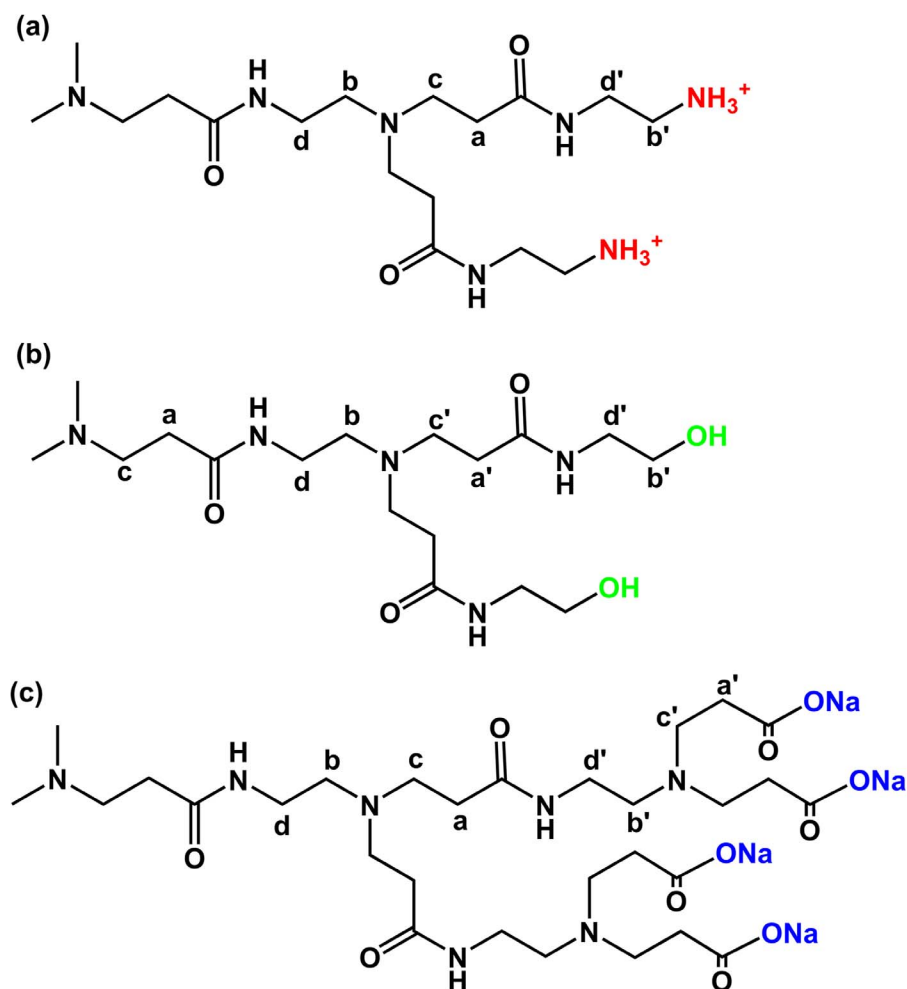
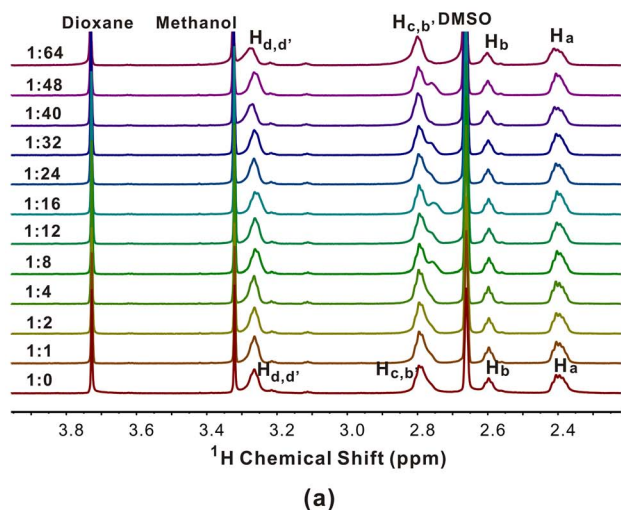


Figure 2. Molecular structures and proton labeling of PAMAM dendrimers. Amine-terminated (a), hydroxyl-terminated (b) and carboxylate-terminated (c) PAMAM dendrimers. doi:10.1371/journal.pone.0064722.g002

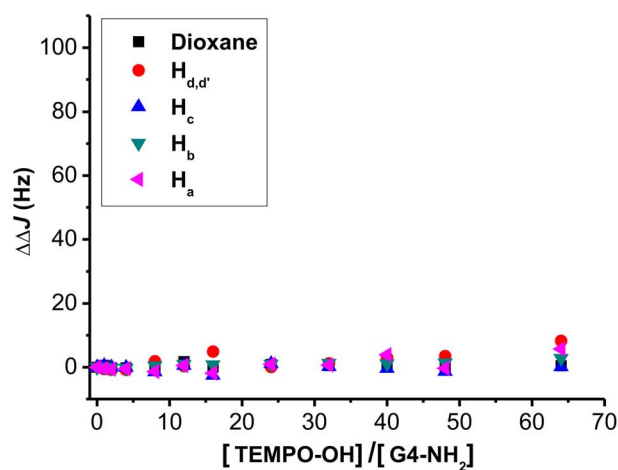
were reported to have interior hydrophobic pockets and open surface structures, which are excellent candidates in host-guest systems [3,5]. As shown in Fig. 3, G4-NH₂ has four ¹H NMR peaks: H_{d, d'} at 3.26 ppm, H_{c, b'} at 2.79 ppm, H_b at 2.60 ppm, and H_a at 2.40 ppm, respectively. The peak at 3.72 ppm corresponds to the internal standard dioxane, while peaks at 3.32 ppm and 2.66 ppm arise from the residual methanol and DMSO, respectively. Both the chemical shift and linewidth of the G4-NH₂ peak were scarcely affected by the addition of TEMPO-OH. Previous studies have demonstrated that ionic binding of anionic guests on the surface of amine-terminated PAMAM dendrimer shifts the resonance signals of dendrimer surface (H_{b'} and H_{d'}) to higher frequencies, and hydrophobic encapsulation of guests within dendrimer interior pockets shifts the signals of dendrimer interior (H_{a~d}) to lower frequencies. [12] The absence of H_{a~d} signal shifts in Fig. 3 indicates the absence of ionic binding and hydrophobic encapsulation. In addition, TEMPO-OH is a paramagnetic guest molecule. If TEMPO-OH molecules are encapsulated within the G4-NH₂ interior cavities via hydrophobic or ionic interactions, the PRE effect will lead to significant enhancement of linewidths of G4-NH₂ resonance signals. However, no significant variation in linewidth of G4-NH₂ signals during the addition of TEMPO-OH in Fig. 3b suggests the absence of TEMPO-OH encapsulation within G4-NH₂. Similarly,

when TEMPO-NH₂ was added into G4-NH₂, no significant change in the ¹H NMR spectrum of G4-NH₂ was observed (Fig. S1). The only difference is the slight shifts of H_{b'} and H_{d'} to lower frequency when increasing TEMPO-NH₂/G4-NH₂ molar ratio. During the addition of TEMPO-NH₂ into G4-NH₂, the pH value of the dendrimer solution increases from 8.72 to 9.59 when the molar ratio of TEMPO-NH₂/G4-NH₂ reaches 64. Deprotonation of the NH₃⁺ groups (pKa~10.0) on G4-NH₂ surface well explains the slight shifts of H_{b'} and H_{d'} signals in Fig. S1. The absence of G4-NH₂ linewidth variation during TEMPO-NH₂ titration also confirms the absence of TEMPO-NH₂ encapsulation within G4-NH₂.

Similar phenomena are observed between TEMPO-NH₂ (TEMPO-OH) and G4-OH (G3.5-COONa) (Fig. 4 and Fig. S2). These results suggest that no host-guest interaction occurs between TEMPO-NH₂ (TEMPO-OH) and the three types of PAMAM dendrimers. TEMPO-NH₂ (171 Da) and TEMPO-OH (172 Da) have dendrimer pocket-matched sizes. Our previous studies have reported successful encapsulations of much larger guests such as sodium dodecyl sulfate (288 Da), dexamethasone 21-phosphate (516 Da), and Congo red (696 Da) within G4-NH₂ PAMAM dendrimer [21,25,37]. Therefore, the failure of TEMPO-NH₂ and TEMPO-OH encapsulation within PAMAM dendrimers should not be attributed to size limitations of the guests, but probably to



(a)



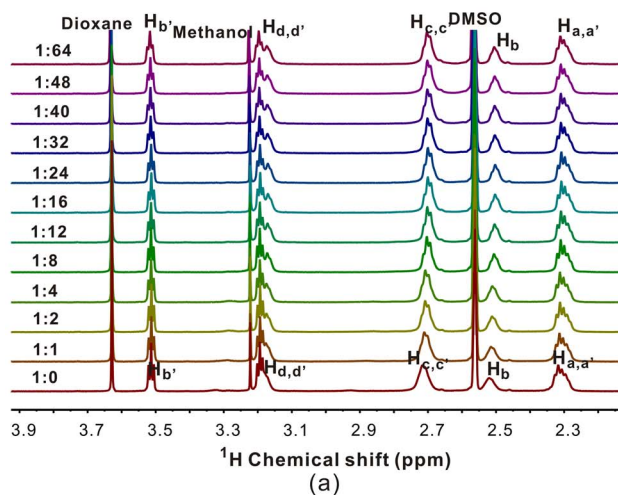
(b)

Figure 3. The effect of TEMPO-OH on the signal intensity of G4-NH₂. (a) ¹H NMR spectra of the G4-NH₂/TEMPO-OH complexes in D₂O/d₆-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-OH and G4-NH₂ ranges from 0 to 64. (b) The linewidth variations of G4-NH₂ peaks in ¹H NMR spectra during the titration experiment. doi:10.1371/journal.pone.0064722.g003

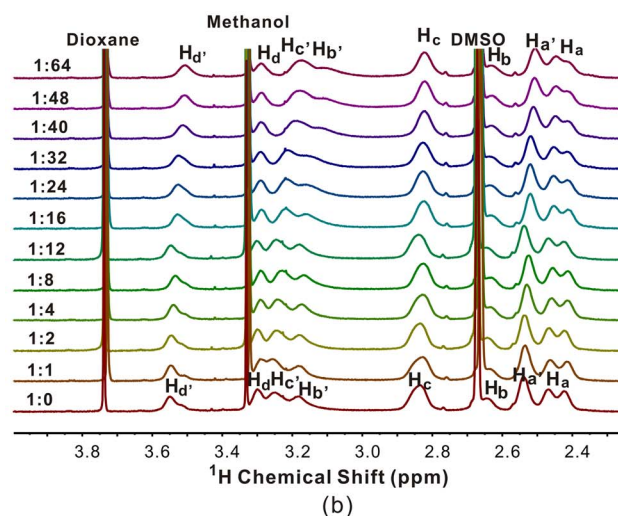
the reason that PAMAM dendrimer pockets are not hydrophobic enough to encapsulate TEMPO-NH₂ or TEMPO-OH in d₆-DMSO/D₂O (20%/80%, V/V) via hydrophobic interactions.

Interactions of TEMPO-COOH with G4-NH₂ and G4-OH PAMAM dendrimers

As shown in Fig. 5, signal intensities of all the G4-NH₂ resonance signals (H_{a-d}, H_b, and H_{d'}) are gradually decreased with increasing TEMPO-COOH/G4-NH₂ molar ratios. Also, the linewidths of the signals are much broader after the addition of TEMPO-COOH molecules. This phenomenon can be recognized as an evidence of TEMPO-COOH encapsulation within G4-NH₂. One may argue that decreased molecular mobility or increased molecular size also contribute to increased peak linewidth. In the system of G4-NH₂ and TEMPO-COOH, this effect is not significant as compared to the PRE effect. Take G4-NH₂ for an example, its diffusion coefficient is slightly changed after the addition of 8 or 16 molar ratios of TEMPO-COOH (Fig. S3). When more TEMPO-COOH were added (molar ratio of 32 or



(a)



(b)

Figure 4. The effect of TEMPO-NH₂ on the signal intensity of G4-OH and G3.5-COONa. ¹H NMR spectra of the G4-OH/TEMPO-NH₂ (a) and the G3.5-COONa/TEMPO-NH₂ (b) complexes in D₂O/d₆-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-NH₂ and dendrimer ranges from 0 to 64. doi:10.1371/journal.pone.0064722.g004

64), the peak intensities of G4-NH₂ are extremely low due to the PRE effect and the diffusion coefficients of G4-NH₂ are not available using pulsed gradient spin-echo (PGSE) NMR. In this case, 32 molar ratios of deoxycholate which was proved to interact with G4-NH₂ were used instead of TEMPO-COOH. [24] Again, only a slight change (~3%) on the diffusion coefficient of G4-NH₂ was observed before and after the addition of deoxycholate (Fig. S3). These results suggest that the increased peak linewidths in Fig. 5 are mainly caused by the PRE effect of TEMPO-COOH encapsulated within G4-NH₂ pockets rather than the increased dendrimer size after TEMPO-COOH addition.

Besides variations in signal intensity and linewidth, shifts of H_b and H_{d'} resonance signals to higher frequencies in Fig. 5 were observed. This is due to ionic interactions between deprotonated TEMPO-COOH and cationic charged G4-NH₂ surface [38,39]. The pH value of TEMPO-COOH/G4-NH₂ solution decreases to 5.61 when the molar ratio of TEMPO-COOH and G4-NH₂ reaches 64. As the pK_a values of surface amine and interior tertiary amine groups of PAMAM dendrimers are around 10.0 and 6.5 respectively [40]. All the surface amine groups and partial

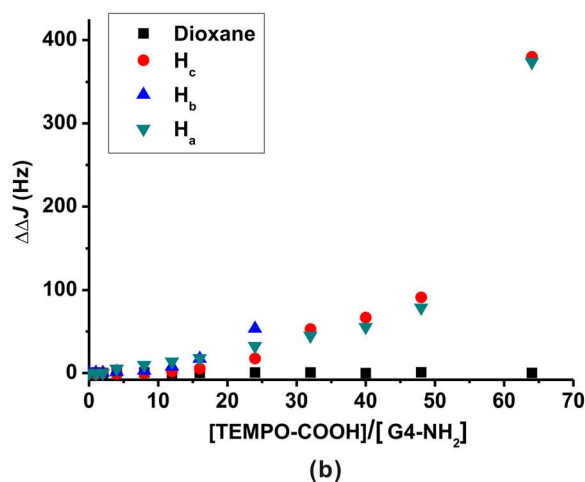
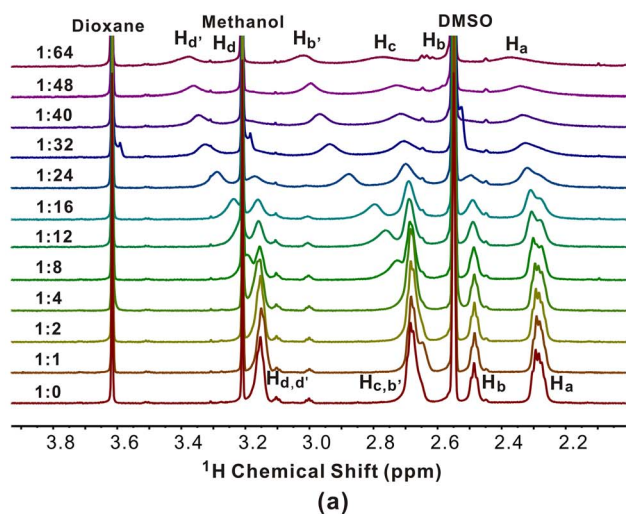


Figure 5. Increased concentration of TEMPO-COOH decreases the signal intensity of G4-NH₂. (a) ¹H NMR spectra of the G4-NH₂/TEMPO-COOH complexes in D₂O/d₆-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-COOH and G4-NH₂ ranges from 0 to 64. (b) The linewidth variations of G4-NH₂ peaks in ¹H NMR spectra during the titration experiment.
doi:10.1371/journal.pone.0064722.g005

interior tertiary amine groups of G4-NH₂ should be positively charged when the molar ratio of TEMPO-COOH and G4-NH₂ is above 40 (pH 6.81). In such a complex solution, TEMPO-COOH is negatively charged and can be either bound on the surface or encapsulated within the interior of G4-NH₂ via ionic interactions. To exclude the possibility that the decreased signal intensity and the broadened peak linewidth are caused by amine protonation rather than the PRE effect, we titrated G4-NH₂ with acetic acid. As shown in Fig. S4, almost no change in signal intensity and peak linewidth of H_{a-d} are observed. The changes in linewidths of H_{b'} and H_{d'} in Fig. S4 can be explained by exchanges of NH₃⁺ and NH₂ on G4-NH₂ surface during the addition of acetic acid. We also used NOESY to prove the encapsulation of TEMPO-COOH within G4-NH₂. As TEMPO-COOH is a paramagnetic molecule, the PRE effect broadens both the G4-NH₂ and the TEMPO-COOH peaks as shown in Fig. 5 which makes it difficult to detect the cross-peaks of between G4-NH₂ and TEMPO-COOH using a NOESY method. In this case, we treated the TEMPO-COOH with 12 N HCl to scavenge the TEMPO radical. The resulting

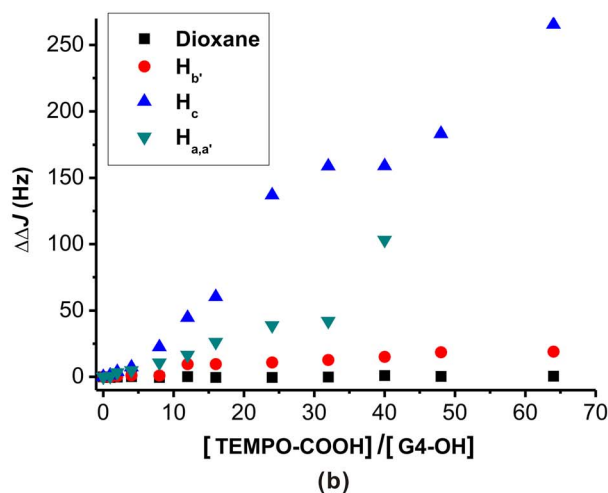
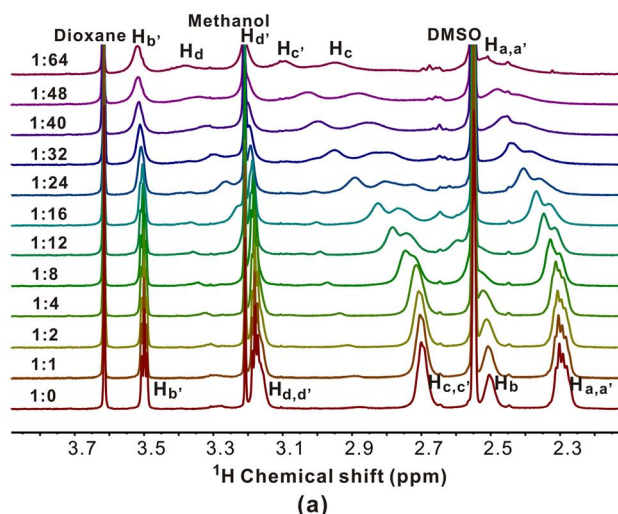


Figure 6. The effect of TEMPO-COOH on proton signal intensity of G4-OH. (a) ¹H NMR spectra of the G4-OH/TEMPO-COOH complexes in D₂O/d₆-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-COOH and G4-OH ranges from 0 to 64. (b) The linewidth variations of G4-OH peaks in ¹H NMR spectra during the titration experiment.
doi:10.1371/journal.pone.0064722.g006

guest was complexed with G4-NH₂ at a molar ratio of 32:1 and the complex was analyzed by ¹H-¹H NOESY at a mixing time of 300 ms. As shown in Fig. S5, weak NOE cross-peaks between the methyl groups of the guest and the methylene protons (H_{a, c}) of G4-NH₂ were observed, indicating the encapsulation of the TEMPO-COOH derivative within G4-NH₂. This result indicates that the PRE NMR method can be used as an alternative method to NOESY in the investigation of inclusion structures. The PRE method using ¹H NMR to probe host-guest interaction is more facile and sensitive than the NOESY method.

When G4-OH was titrated with TEMPO-COOH or acetic acid, the protonation of the interior tertiary amine groups of G4-OH also caused the broadening of resonance signals for adjacent methylene protons such as H_{a-d} (Fig. 6 and Fig. S6). This makes it difficult to analyze the PRE effect in the system. However, the variations of linewidth for H_{b'} in the samples titrated with TEMPO-COOH are much higher than that in the samples titrated with acetic acid (Fig. S6), indicating the presence of PRE effect in TEMPO-COOH/G4-OH. Since the surface of G4-OH is non-charged, no ionic interaction occurs between TEMPO-

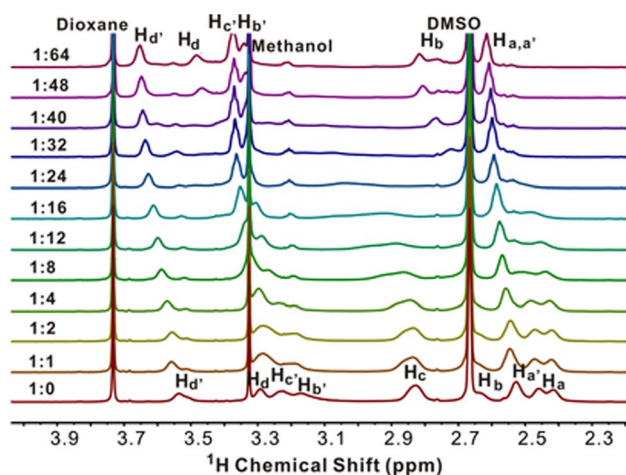


Figure 7. ^1H NMR spectra of G3.5-COONa/TEMPO-COOH complexes. The molar ratio of TEMPO-COOH and G3.5-COONa ranges from 0 to 64. The solvent is $\text{D}_2\text{O}/d_6\text{-DMSO}$ (80/20, V/V). doi:10.1371/journal.pone.0064722.g007

COOH and G4-OH surface. TEMPO-COOH molecules should be at least encapsulated within the outermost layer of G4-OH dendrimer. Interior methylene protons (H_{a-d}) of G4-OH dendrimer exhibit significant higher frequency shift, while surface methylene protons of G4-OH such as H_b , and H_d are slightly shifted, suggesting quaternization of the interior tertiary amine groups of G4-OH (pH ranges from 8.11 to 5.10). The formation of inclusion complexes of TEMPO-COOH with G4-NH₂ or G4-OH should be driven by ionic interactions rather than hydrophobic interactions. This property is distinct from previous dendrimer/guest inclusion complexes such as PAMAM dendrimers with mycophenolic acid, [12] sodium deoxycholate and sodium dodecyl sulfate, [24] and phenylbutazone [22], in which the encapsulations are driven by hydrophobic interactions.

Interaction of TEMPO-COOH with G3.5-COONa

As shown in Fig. 7, titration of G3.5-COONa with TEMPO-COOH leads to higher frequency shifts of all the dendrimer protons. During this period, the pH value of the complex solution gradually decreases from 9.04 to 5.45, which means partial protonation of interior tertiary amine groups within G3.5-COONa. Interestingly, the signal intensities of peaks $\text{H}_{a'}$, b' , c' , d' increase during the addition of TEMPO-COOH. Meanwhile, the linewidths of these peaks become lower. This result is not in accordance with those observed in TEMPO-COOH/G4-NH₂ and TEMPO-COOH/G4-OH. This phenomenon can be explained by the equilibrium equation in Fig. S7. Due to the presence of carboxylate groups on G3.5-COONa surface, the tertiary amine group located on the outermost layer has a much higher pK_a value (8.0~9.0) than the interior tertiary amine groups (pK_a~6.5). [41] Considering the initial G3.5-COONa solution has a pH value around 8.7, the exchange equilibrium between the anion (A) and the zwitterion (B) in Fig. S7 caused the peaks of $\text{H}_{a'}$, b' , c' , d' broaden in ^1H NMR spectrum. With the increase of TEMPO-COOH concentration, the pH value of the G3.5-COONa decreases to 6.0 and increased percent of the surface tertiary amines are protonated. The zwitterion (B) is the major form in the solution at high molar ratios of TEMPO-COOH and G3.5-COONa. Therefore, the peaks $\text{H}_{a'}$, b' , c' , d' become narrow with the addition of TEMPO-COOH. This phenomenon is also observed when amino acids were titrated with acid. [42] In the

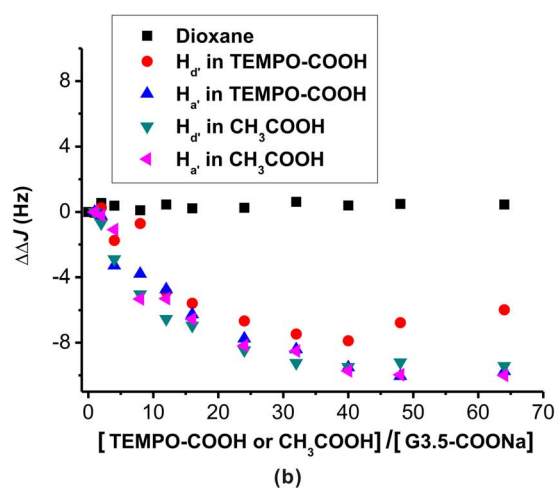
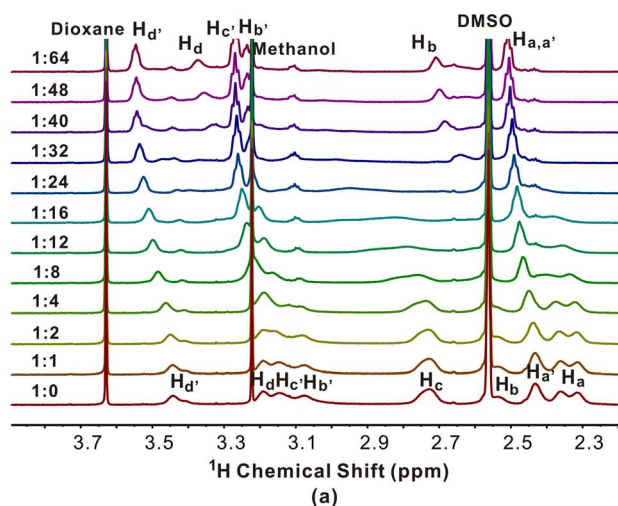


Figure 8. Comparison of TEMPO-COOH and acetic acid on the signal intensity of G3.5-COONa. (a) ^1H NMR spectra of the G3.5-COONa/acetic acid complexes in $\text{D}_2\text{O}/d_6\text{-DMSO}$ solutions (80/20, V/V), the molar ratio of acetic acid and G3.5-COONa from 0 to 64. (b) The linewidth variations of G3.5-COONa peaks in ^1H NMR spectra during the titration of G3.5-COONa with TEMPO-COOH and acetic acid. doi:10.1371/journal.pone.0064722.g008

case of interior methylene protons such as H_{a-d} , the interior tertiary amine (pK_a~6.5) is not protonated at the beginning, and start to protonate after the addition of TEMPO, the exchange between protonated and non-protonated state caused the peaks H_{a-d} adjacent to these tertiary amine groups becoming broader. Therefore, the linewidth of the peaks is controlled by exchange modulation of the tertiary amine groups rather than the PRE effect. To prove this speculation, we titrated G3.5-COONa dendrimer with acetic acid. As shown in Fig. 8, the same trend for linewidth of G3.5-COONa peaks was observed when this dendrimer was titrated with TEMPO-COOH and acetic acid. During this period, no PRE effect was observed between the protons ($\text{H}_{a'}$, H_d) and the unpaired electron in the nitroxide radical (Fig. 8b). The failure of TEMPO-COO⁻ within G3.5-COONa cavities is probably due to the repulsion of TEMPO-COO⁻ ions and the anionic G3.5 surface.

Conclusions

In this study, we investigated the host-guest chemistry of PAMAM dendrimers with amine, hydroxyl, and carboxylate surface functionalities and TEMPO radicals with amine, hydroxyl, and carboxyl groups using ^1H NMR titration experiments. PRE effects were observed in TEMPO-COOH/G4-NH₂ and TEMPO-COOH/G4-OH complexes. The inclusion complexes of TEMPO-COOH and G4-NH₂ (G4-OH) are driven by ionic interactions rather than hydrophobic interactions. In other dendrimer/TEMPO systems, only protonation or deprotonation of the surface functionalities and interior tertiary amine groups occur. The absence of TEMPO-COOH encapsulation within G3.5-COONa is due to the repulsion of TEMPO-COO⁻ anion and the negatively charged G3.5-COONa surface. This study proved that PRE NMR is a powerful method in the investigation of dendrimer-based host-guest interactions.

Supporting Information

Figure S1 ^1H NMR spectra of the G4-NH₂/TEMPO-NH₂ complexes in D₂O/d6-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-NH₂ and G4-NH₂ ranges from 0 to 64 (a). The linewidth variations of G4-NH₂ peaks in ^1H NMR spectra during the titration experiment (b). (TIF)

Figure S2 ^1H NMR spectra of the G4-OH/TEMPO-OH (a) and the G3.5-COONa/TEMPO-OH (c) complexes in D₂O/d6-DMSO solutions (80/20, V/V), the molar ratio of TEMPO-OH and dendrimer ranges from 0 to 64. The linewidth variations of G4-OH and G3.5-COONa peaks in the ^1H NMR spectra during the titration experiment are shown in (b) and (d), respectively. (TIF)

Figure S3 Diffusion coefficients of G4-NH₂ in the absence and presence of TEMPO-COOH at a molar ratio of 8 and 16 (a). Diffusion coefficients of G4-NH₂ in the absence and presence of deoxycholate at a molar ratio of 32 (b). ^1H NMR spectra of G4-NH₂ before and after the addition of deoxycholate (c). (TIF)

Figure S4 ^1H NMR spectra of G4-NH₂ titrated by acetic acid, the molar ratio of acetic acid and G4-NH₂ ranges from 0 to 64. (TIF)

Figure S5 ^1H - ^1H NOESY of TEMPO-COOH derivative and G4-NH₂ at a molar ratio of 32:1. The mixing time is 300 ms. (TIF)

Figure S6 (a) ^1H NMR spectra of G4-OH titrated by acetic acid, the molar ratio of acetic acid and G4-NH₂ ranges from 0 to 64. (b) The linewidth variations of G4-OH peaks (H_b) in ^1H NMR spectra during the addition of TEMPO-COOH or CH₃COOH. (TIF)

Figure S7 The protonation and deprotonation equilibrium of tertiary amine groups and surface carboxylate groups of G3.5-COONa. (TIF)

Abstract S1 Graphical abstract.
(DOC)

Text S1 The method of ^1H - ^1H NOESY spectrum of the TEMPO-COOH derivative and G4-NH₂ complex. (DOC)

Author Contributions

Conceived and designed the experiments: YYC. Performed the experiments: FW NMS. Analyzed the data: YYC FW NMS. Contributed reagents/materials/analysis tools: YYC. Wrote the paper: YYC FW NMS.

References

- Tomalia DA (2012) Dendritic effects: dependency of dendritic nano-periodic property patterns on critical nanoscale design parameters (CNDPs). *New J Chem* 36: 264–281.
- Tomalia DA (2010) Dendrons/dendrimers: quantized, nano-element like building blocks for soft-soft and soft-hard nano-compound synthesis. *Soft Matter* 6: 456–474.
- Tomalia DA (2005) Birth of a new macromolecular architecture: dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. *Prog Polym Sci* 30: 294–324.
- Aulenta F, Hayes W, Rannard S (2003) Dendrimers: a new class of nanoscopic containers and delivery devices. *Eur Polym J* 39: 1741–1771.
- Cheng YY, Zhao LB, Li YW, Xu TW (2011) Design of biocompatible dendrimers for cancer diagnosis and therapy: current status and future perspectives. *Chem Soc Rev* 40: 2673–2703.
- Astruc D, Ornelas C, Ruiz J (2008) Metalloenyl dendrimers and their applications in molecular electronics, sensing, and catalysis. *Acc Chem Res* 41: 841–856.
- Menjoge AR, Kannan RM, Tomalia DA (2010) Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. *Drug Discov Today* 15: 171–185.
- Caminade AM, Majoral JP (2010) Dendrimers and nanotubes: a fruitful association. *Chem Soc Rev* 39: 2034–2047.
- Caminade AM, Turrin CO, Majoral JP (2008) Dendrimers and DNA: combinations of two special topologies for nanomaterials and biology. *Chem Eur J* 14: 7422–7432.
- Caminade AM, Laurent R, Majoral JP (2005) Characterization of dendrimers. *Adv Drug Delivery Rev* 57: 2130–2146.
- Caminade AM, Ouali A, Keller M, Majoral JP (2012) Organocatalysis with dendrimers. *Chem Soc Rev* doi: 10.1039/C2CS35030K: ASAP.
- Hu JJ, Cheng YY, Ma YR, Wu QL, Xu TW (2009) Host-guest chemistry and physicochemical properties of dendrimer-mycophenolic acid complex. *J Phys Chem B* 113: 64–74.
- Astruc D, Boisselier E, Ornelas C (2010) Dendrimers designed for functions: from physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine. *Chem Rev* 110: 1857–1959.
- Naylor AM, Goddard III WA, Kiefer GE, Tomalia DA (1989) Starburst dendrimers. 5. molecular shape control. *J Am Chem Soc* 111: 2339–2341.
- Jansen JFGA, de Brabander-van den Berg EMM, Meijer EW (1994) Encapsulation of guest molecules into a dendritic box. *Science* 266: 1226–1229.
- Jansen JFGA, Peerlings HWI, de Brabander-van den Berg EMM, Meijer EW (1995) *Angew Chem Int Ed* 34: 1206.
- Jansen JFGA, Meijer EW (1995) *J Am Chem Soc* 117: 4417.
- Baars MWPL, Froehling PE, Meijer EW (1997) *Chem Commun*: 1959.
- Boisselier E, Liang L, Dalako-Csiba M, Ruiz J, Astruc D (2010) Interactions and encapsulations vitamins C, B₃, and B₆ with dendrimer in water. *Chem Eur J* 16: 6056–6068.
- Boisselier E, Ornelas C, Pianet I, Aranzaca JR, Astruc D (2008) Four generations of water-soluble dendrimers with 9 to 243 benzoate tethers: synthesis and dendritic effects on their ion pairing with acetylcholine, benzyltriethylammonium, and dopamine in water. *Chem Eur J* 14: 5577–5587.
- Yang K, Weng L, Cheng YY, Zhang HF, Zhang JH, et al. (2011) Host-guest chemistry of dendrimer-drug complexes. 6. fully acetylated dendrimers as biocompatible drug vehicles using dexamethasone 21-phosphate as a model drug. *J Phys Chem B* 115: 2185–2195.
- Zhao LB, Wu QL, Cheng YY, Zhang JH, Wu JH, et al. (2010) High-throughput screening of dendrimer-binding drugs. *J Am Chem Soc* 132: 13182–13184.
- Wang MM, Gong XL, Hu JJ, Yu YH, Chen Q, et al. (2011) Understanding the binding interactions between dendrimer and 18 common amino acid by NMR techniques. *J Phys Chem B* 115: 12728–12735.
- Yang K, Cheng YY, Feng XY, Zhang JH, Wu QL, et al. (2010) Insights into the interactions between dendrimers and multiple surfactants: 6. Formation of miscellaneous mixed micelles revealed by a combination of ^1H NMR, diffusion, and NOE analysis. *J Phys Chem B* 114: 7265–7273.
- Fang M, Zhang J, Wu QL, Xu TW, Cheng YY (2012) Host-guest chemistry of dendrimer-drug complexes: 7. formation of stable inclusions between acetylated dendrimers and drugs bearing multiple charges. *J Phys Chem B* 116: 3075–3082.
- Fang M, Cheng YY, Zhang JH, Wu QL, Hu JJ, et al. (2010) New insights into interactions between dendrimers and surfactants. 4. Fast-exchange/slow-exchange transitions in the structure of dendrimer-surfactant aggregates. *J Phys Chem B* 114: 6048–6055.

27. Esfand R, Tomalia DA (2001) Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discov Today* 6: 427–436.
28. Feng XY, Cheng Y, Wu QL, Zhang JH, Xu TW (2011) Stimuli Response of Cystamine-Core Dendrimer Revealed by Diffusion and NOE NMR Studies. *J Phys Chem B* 115: 3777–3783.
29. Clore GM, Iwahara J (2009) Theory, practice, and applications of paramagnetic relaxation enhancement for the characterization of transient low-population states of biological macromolecules and their complexes. *Chem Rev* 109: 4108–4139.
30. Pintacuda G, John M, Su XC, Otting G (2007) *Acc Chem Res* 40: 206.
31. Furtado F, Galvosas P, Stallmach F, Roland U, Kärger J, et al. (2011) Paramagnetic relaxation enhancement (PRE) as a tool for probing diffusion in environmentally relevant porous media. *Environ Sci Technol* 45: 8866–8872.
32. Xue Y, Podkorytov IS, Rao DK, Benjamin N, Sun H, et al. (2009) Paramagnetic relaxation enhancements in unfolded proteins: theory and application to drkN SH3 domain. *Protein Sci* 18: 1401–1424.
33. Epperson JD, Ming LJ, Baker GR, Newkome GR (2001) Paramagnetic Cobalt(II) as an NMR Probe of Dendrimer Structure: Mobility and Cooperativity of Dendritic Arms. *J Am Chem Soc* 123: 8583–8592.
34. Epperson JD, Ming LJ, Woosley BD, Baker GR, Newkome GR (1999) NMR study of dendrimer structures using paramagnetic cobalt(II) as a probe. *Inorg Chem* 38: 4498–4502.
35. Ottaviani MF, Turro NJ, Jockusch S, Tomalia DA (1996) Aggregational process of the positively charged surfactants CTAC and CAT16 in the presence of starburst dendrimers: an electron paramagnetic resonance spectroscopic study. *Colloids and Surf A* 115: 9–21.
36. Ottaviani MF, Andechaga P, Turro NJ, Tomalia DA (1997) Model for the interactions between anionic dendrimers and cationic surfactants by means of the spin probe method. *J Phys Chem B* 101: 6057–6065.
37. Cheng YY, Li YW, Wu QL, Xu TW (2008) New insights into the interactions between dendrimers and surfactants by two dimensional NOE NMR spectroscopy. *J Phys Chem B* 112: 12674–12680.
38. Hu JJ, Cheng YY, Ma YR, Wu QL, Xu TW (2009) Host-guest chemistry and physico-chemical properties of dendrimer-mycophenolic acid complexes. *J Phys Chem B* 113: 64–74.
39. Hu JJ, Xu TW, Cheng YY (2012) NMR insights into dendrimer-based host-guest systems. *Chem Rev* 112: 3856–3891.
40. D'Emanuele A, Attwood D (2005) Dendrimer-drug interactions. *Adv Drug Delivery Rev* 57: 2147–2162.
41. van Duijvenbode RC, Rajanayagam A, Kopoer GJM (2000) Synthesis and protonation behavior of carboxylate-functionalized poly(propyleneimine) dendrimers. *Macromolecules* 33: 46–52.
42. Cohen EA, Shiller AM, Chan SI, Manatt SL (1975) The ^{14}N NMR linewidth versus pH profiles for several amino acids. *Org Mag Reson* 7: 605–609.