Supporting Information

Exploring the Loading Capacity of Generation Six to Eight Dendronized Polymers in Aqueous Solution

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cphc_201600580_sm_miscellaneous_information.pdf

Contents
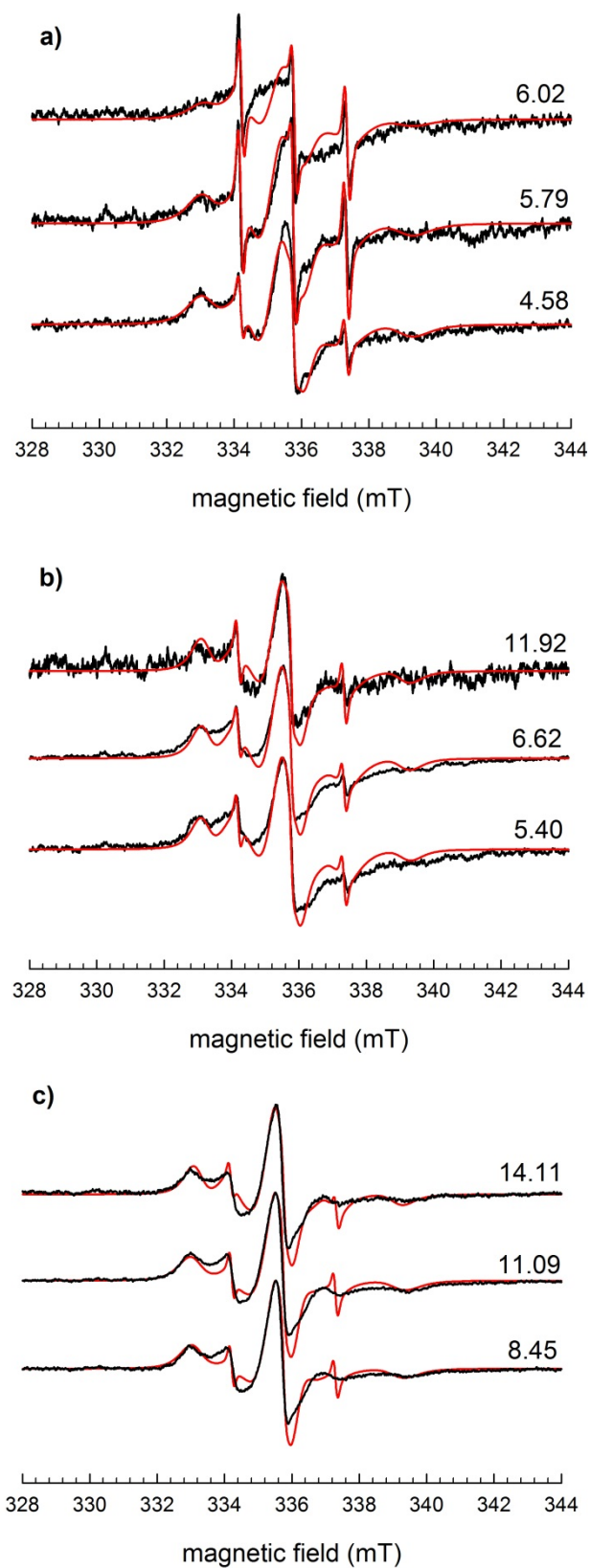
Figure S1. CW EPR spectra (black) and corresponding spectral simulation (red) for a) **PG6⁺**, b) **PG7⁺** and c) **PG8⁺** in solution with 16-DSA. The numbers on each spectra indicate the ratio of n(16-DSA)/n(DPs monomers) in 1wt% solution (see Table 1 and S1 for details on the calculations).
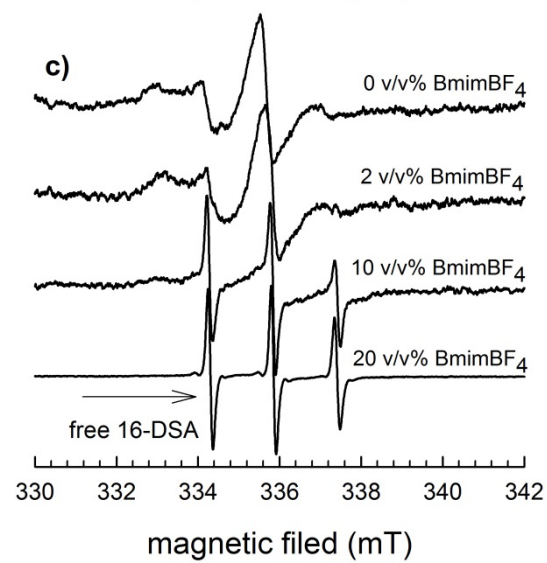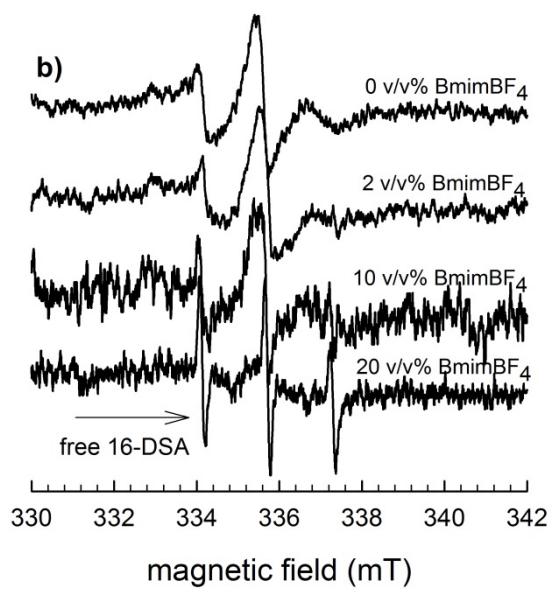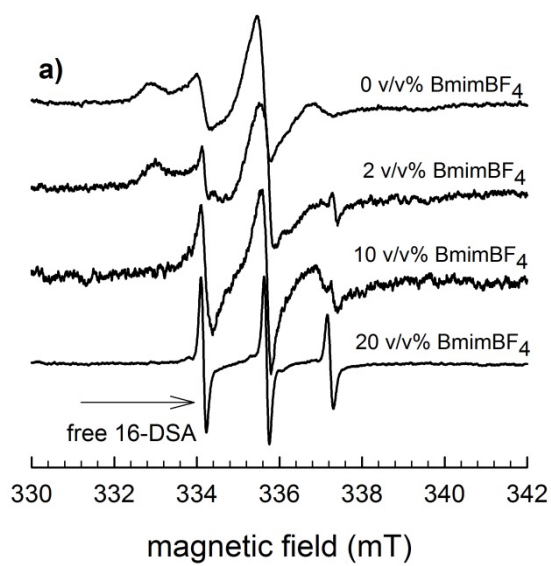
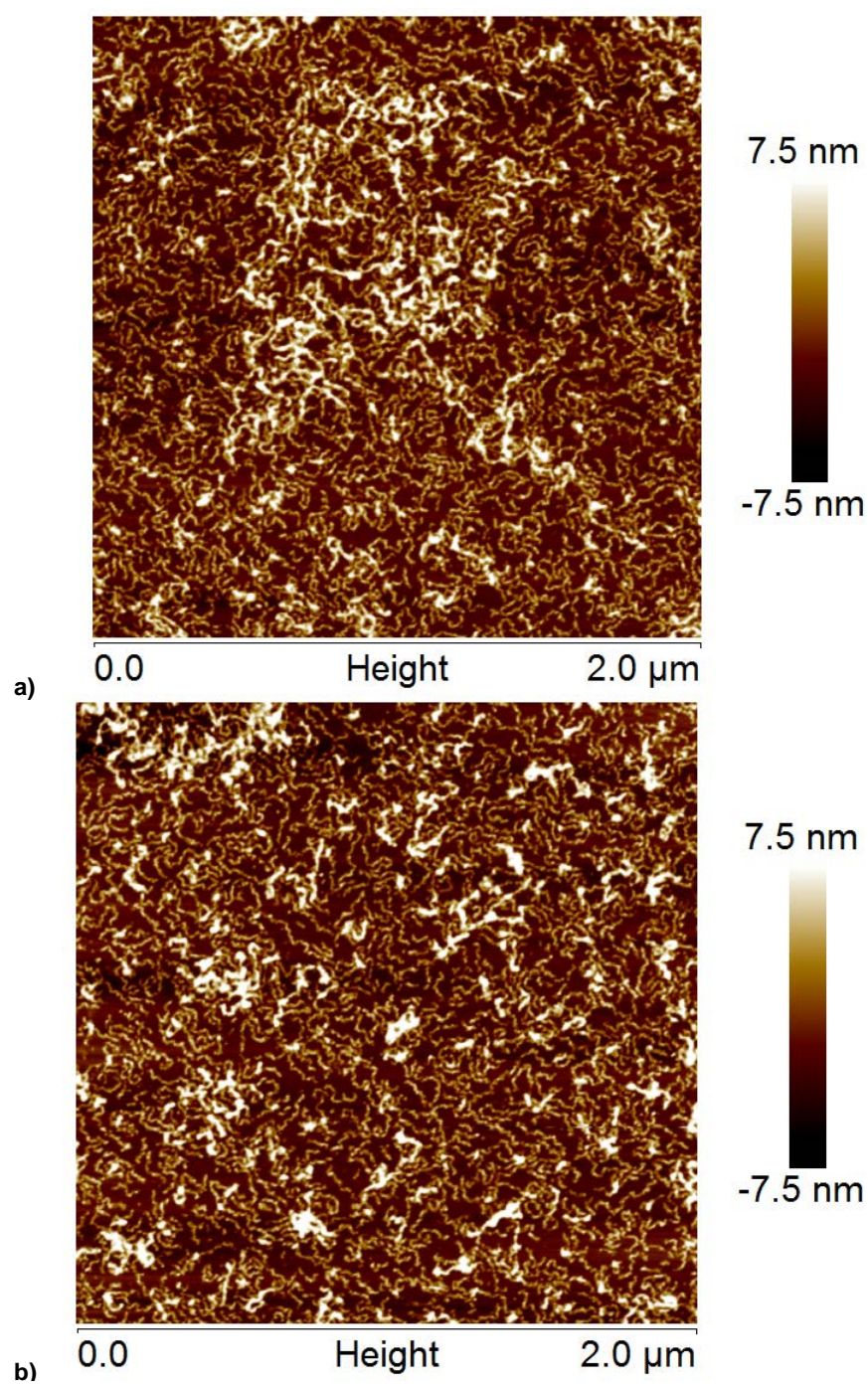**Figure S2.** Release of 16-DSA from a) **PG6⁺**, b) **PG7⁺** and c) **PG8⁺** by BmimBF₄.

Figure S3. AFM images of the a) **PG6+** and b) **PG7+**.

Table S1

Simulation parameters of 1mM concentration of 16-DSA and 1 wt% of the corresponding DPs. Spectra of 16-DSA incorporated in DPs were simulated with two spectral components (bound (slow) and unbound (fast)). g-tensor values for the unbound (fast) species were all identical g_{xx}=2.0087, g_{yy}=2.0065, g_{zz}=2.0029, as well as $A_{iso,fast}$=43.25 MHz. In the case 12:1 and 18:1 molar ratios, due to high local concentrations, a Heisenberg exchange term was applied to simulation.

	PG6$^+$	PG7$^+$	PG8$^+$
g_{iso}	2.0060	2.0060	2.0060
A_{iso}(MHz)	44.00	42.33	42.33
D_{xx}(MHz) *	1.99	3.59	1.59
D_{yy}(MHz) *	17.4	17.4	20.7
D_{xx}(MHz) *	11.8	11.7	11.8

* The tumbling rotation motion is not isotropic and it can be well characterized in terms of the effective rotational correlation time, which is defined as $\tau_c = 6^{-1}(D_{xx}D_{yy}D_{zz})^{-1/3}$, with D_{ii} denoting the principle components of the rotational diffusion tensor.

CHEMPHYSCHEM

Accepted Article

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This manuscript has been accepted after peer review and the authors have elected to post their Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: ChemPhysChem 10.1002/cphc.201600580

Link to VoR: http://dx.doi.org/10.1002/cphc.201600580

Exploring the loading capacity of generation 6 to generation 8 dendronized polymers in aqueous solution.

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Abstract: Aspects of size, structural (im)perfection, inner density, and guest loading capacity of dendronized polymers (DPs) of high generation g (6≤g≤8) in aqueous solution were studied using electron paramagnetic resonance (EPR) spectroscopy on amphiphilic, spin-labeled guest molecules. The presented results show that the interior of the charged DPs is strongly polar, especially in comparison to their lower generation analogues 1-4. This is a direct sign of large amounts of water penetrating the DP surface, which in return mirrors structural imperfections and much lower than theoretically achievable segmental densities of these high generation DPs. Images obtained with atomic force microscopy (AFM) reveal that the high g DPs show no aggregation and give further insights into structural (im)perfections. EPR-spectroscopic data further show that despite their structural imperfections, these DPs can bind and release large numbers of amphiphilic molecules. It can be concluded that attention should be paid to synthesis which needs to develop a protocol that avoids the relatively large amount of defects generated in the direct conversion of **PG4** to **PG6** which had to be employed here.

1. Introduction

Dendronized polymers (DPs) merge the well-studied classes of linear polymers[1] and dendrimers[2] as they have a dendritic side groups on every monomeric unit.[3-7] Depending on their generation g, these side groups can assume considerable molar mass and create a dense branch work around the main chain backbone. This branch work effectively defines a cylindrical volume around the backbone which for each g lends a certain thickness to the macromolecule[8-10] that goes along with a

defined "interior" and "exterior" of the DP. In addition, the main chain rigidity is tuned by g.[11] For higher g DPs the term "molecular object" was proposed.[12] DPs below the maximum generation g_{max}[13-16] can be swollen by solvent[17] or other guest molecules. Processes derived from these interactions alter the object's dynamics as well as the overall morphology.
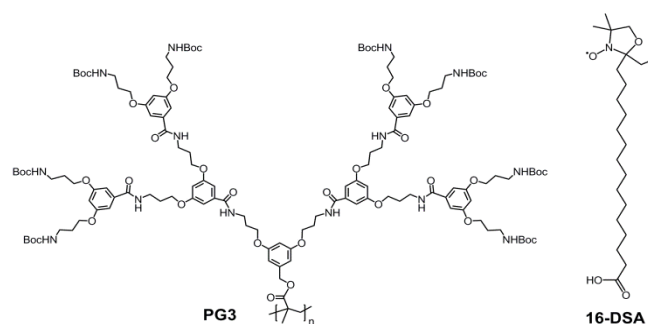

Figure 1. Chemical structure of the g=3 DP **PG3** with all the characteristics of the DPs **PG6**, **PG7** and **PG8** used in the present study except for the number of branch points (g) and of 16-doxyl stearic acid (16 DSA). Boc: *tert*-butyloxycarbonyl.

Several structural variants of DPs have been described over the years.[18] Our laboratory has concentrated on a series of DPs with PMMA backbone and pendent amide-based dendrons (see Figure 1 for the chemical structure of the g=3 representative **PG3**). The thicknesses of **PG1-PG6** in the unperturbed and solvent-free cylindrical states have been predicted by MD simulation to vary between D=2.3-11.8 nm.[8,9] Experimental thickness data from scanning (SEM) and transmission (TEM) electron microscopy using individualized, dried **PG2-5** chains on substrates such as mica or carbon film are in reasonable agreement.[10,11]

Diameters of charged g=1-4 DPs in aqueous medium were found to be similar to those of their dried neutral counter parts, suggesting water to be a poor solvent for the interior of DPs.[19] In a pulse electron paramagnetic resonance (EPR) study using charged, water-soluble DPs of the above kind up to g=4, with negatively charged, small molecule radicals (Fremy's salt

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dianions, FS), we were able to determine the distribution of FS self-assembled in the electrostatic potential of the surface of the respective cationic DPs.[19] These distributions reflected the cylindrical shape and the diameter expected for locally stiff cylinders and supported the idea of DPs (even as low as generation **PG3**) to be molecular objects with a largely environment-independent, persistent shape. In particular, the size of the ammonium-terminated DPs in solution turns increasingly similar to the size in the solid state (heights determined by tapping mode atomic force microscopy (AFM)) as the generation grows.

Using a combination of EPR spectroscopy and small molecule radicals and supporting methods such as AFM or TEM, our groups in recent years have also shown that DPs with g=4 dendron side chains and with a positively charged periphery, i.e., ammonium-capped surface shells, are capable of incorporating even more than 2 long chain fatty acids per repeat unit.[20] For DPs of $1 < g < 4$, we determined a long chain fatty acid (EPR spin-labeled stearic acid) loading capacity Φ to display an asymptotic increase with g as $\exp(\lambda g)$, with $\lambda = 1.27$, i.e. the capacity increases nearly with the square of the exponential g-dependence that is predicted from monomer mass alone (where one would expect $\lambda \approx \ln(2) \approx 0.69$). This dense guest molecule packing of one guest per ~1.5 nm^3 in charged **PG4**$^+$ emphasizes the molecular loading capacity of these state-of-the-art polymeric host systems.[21]. Large amounts and high densities of loaded molecules in small volumes are excellent characteristics of a carrier in polymer therapeutics. We have earlier developed the concept of a two-step packing technique as part of a *load-collapse-release cascade* that in principle should allow for transport of large amounts of drugs through body fluid within very small carrier volumes.[21] This idea and cascade was based on the fact that higher g DPs can be considered as molecular objects with a shape largely independent of their molecular environment.[11]

Fascinated by the prospect of creating molecular objects based on even larger single molecules than g=4 DPs, we started a research program aiming at DPs with g>5. In the present study, we specifically discuss the great potential for these high g DPs in the realm of interactions with, and loading, delivery, and release of small or intermediately sized molecules. During the synthesis, problems with main chain scission were encountered[22] which required modification of the previously applied protocol.[23] As a consequence, the high g DPs **PG6-PG8**

could in fact be obtained but only at the price of having more defects in their branch work than their lower g analogs. A direct comparison of DPs with g=1–5 and g=6–8 has, thus, to be seen under this limitation.

In this work, we use AFM images of the deprotected and thus charged derivatives of **PG6-PG8**, namely **PG6**$^+$-**PG8**$^+$, to qualitatively address potential aggregation or oligomer formation of high g DPs, which may be formed as a consequence of structural imperfections of polymer backbone and dendrons[24-26]. Furthermore, we focus on loading studies of **PG6**$^+$-**PG8**$^+$, performed with continuous wave (CW) EPR spectroscopy on nitroxide spin-labeled, polarity-sensitive fatty acid guest molecules in an aqueous environment. This method has already been used to determine the number of these guests taken-up by lower g DPs (e.g. > 2 fatty acids per repeat unit of **PG4**$^+$, see above) and thus sheds light on the aspect of dense packing in higher g congeners from a rather different vantage point – namely from potential guest molecules' point of view - than, e.g., SEM and TEM studies can offer.[10,12] Besides quantification of guest molecule incorporation, this method also provides information about the rotational motion of the guests and on changes in the polarity upon internalization from the aqueous phase into the DP interior. It can also allow insights into the effects of DP thickness and solvent properties.

In other words, it offers insights into the interior of DPs difficult to obtain otherwise.[19,20,21,27-28] The results will be discussed in terms of usable internal space, structural defects, and whether **PG6**$^+$-**PG8**$^+$ DPs are in the vicinity of g_{max}.

2. Results

2.1. EPR studies on charged, high g dendronized polymers. CW EPR spectroscopy and spin-labeled guests such as 4,4-dimethyl-oxazolidine-*N*-oxyl stearic acid (16-DSA)[20,21] can yield three kinds of information about host systems such as **PG6**$^+$-**PG8**$^+$: (a) the loading and release capabilities, (b) the level to which the guest molecules are immobilized by Coulomb and hydrophobic interactions with the charged DP host surfaces and their hydrophobic interior and (c) the hyperfine coupling (hfc) of the immobilized spectral components once guests have been up-taken as a sensor for polarity of the environment. This information provides, regarding (a), a lower bound for the capacity of a particular DP and thus insights into the tightness of the packing, regarding (b), facets of the mechanism of guest

internalization including the importance of the combination of Coulomb attraction and hydrophobic effect for an efficient up-take to occur, and, regarding (c), insights into the average polarity inside a DP in dependence of g and, thus, indirectly also into the impact of structural imperfection on the influx of water into the branches. Before entering the matter in detail it is noted that while the DP derivatives used for the EPR studies are charged rather than neutral (to ensure solubility in water), they are interesting in their own right and can nevertheless be considered useful models for neutral DPs, as has been shown for the lower generation DPs.[19,20,21] Their loading capacity (not the loading mechanism, though) should in a first approximation compare to the neutral analogs because the molar mass of the repeat units' terminal groups is virtually the same in both cases. Note that the trifluoroacetate counter ion of the charged series has a similar molar mass as the Boc-protecting group of the neutral series (113 Da and 102 Da, respectively).

Regarding point (a), loading and release studies have already been reported for the deprotected and thus charged g=1-4 DPs **PG1⁺**-**PG4⁺** with the same 16-DSA guests used in the present study.[19,21] It was found that e.g. **PG4⁺** can take up at least 2.2 such molecules per repeat unit (RU) if exposed to a 4-fold excess of 16-DSA per RU. To allow comparison of the present study with the previous one, we followed the same experimental protocols as in Ref. 21.[21] Typical stoichiometric ratios between 16-DSA and DP RU ranged between 6:1 and 18:1 (Table 1). Higher ratios could not be applied for solubility reasons and loading capacities therefore have to be considered lower bounds. While the obtained capacities (in numbers of 16-DSA per DP RU) of **PG6⁺**-**PG8⁺** increased with g from ~5, to 11.9 and 14.1, respectively (Figure S1, Table 1), they do not follow the exponential relation exp(λg), with λ = 1.27 found for the low g **PG1⁺**-**PG4⁺** series.[20] The increase is clearly less steep. Information regarding point (b) was obtained from a line shape analysis providing the ratio between broad and sharp lines, which are assigned to immobilized guest molecules (broad lines) and freely rotating (sharp lines) fatty acids (FAs). It was shown earlier that the broadening of the lines is associated with an anchoring of the fatty acids through their carboxylic acid function to the positively charged periphery of DPs and additional hydrophobic anchoring of the FA alkyl chains in the DP interior. Results obtained for the samples **PG6⁺**-**PG8⁺** (Figure 2) indicate a strong interaction of 16-DSA with DPs. Similarly as shown for the lower generations, for 16-DSA dissolved in the

presence of DPs **PG6⁺**-**PG8⁺**, a clear-cut decrease of rotational mobility (as evidenced by the rotational correlation time τ_c), compared to the free spin probe in water, can be observed through the asymmetric CW EPR spectra (especially the high and low field lines have lower intensities and are broader and slow-motion features appear as can be seen in Figure 2).

This restricted mobility indicates a strong interaction of DSA with the DPs. For example, **PG7⁺** in the presence of 12 equivalents of16-DSA per RU gave a ratio of immobilized to non-immobilized guests of 6.62:1 (Figure S1 (b)).

Table 1. Number of n(16-DSA) per n(DPs monomers) for the corresponding spectra in Fig S1.

	Stoichiometric ratios between 16-DSA and DP RU	n(16-DSA bound)/n(DP-RU)
PG6+	6:1	4.58
	9:1	5.79
	12:1	6.02
PG7+	9:1	5.40
	12:1	6.62
	18:1	11.92
PG8+	9:1	8.45
	12:1	11.09
	18:1	14.11

CW EPR on fatty acids becomes most powerful when combining the information of the number of immobilized fatty acids with information on their direct environment in the internalized state. This can be achieved when taking into account the hyperfine coupling (hfc) of 16-DSA which gives a more detailed view on the degree and state of internalization of the fatty acids.
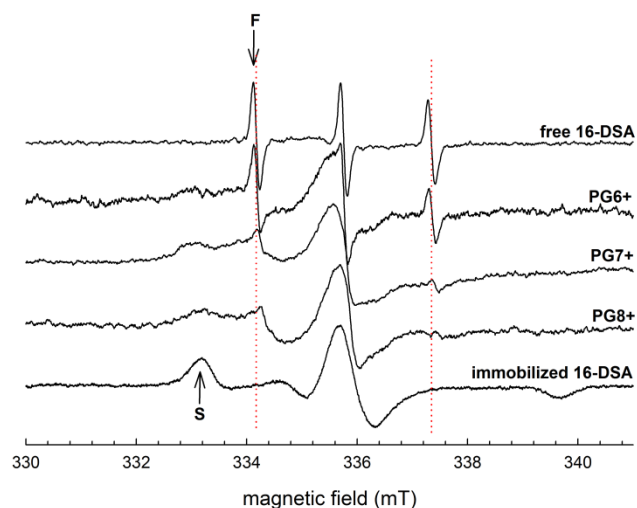
Figure 2. CW EPR spectra of 16-DSA in presence of different DP generations, free at RT (top) and completely immobilized at 80 K (bottom) 16-DSA in water. **S** and **F** indicate prominent spectral features in the contributions of two components if 16-DSA is bound (S-slow and F-fast, details in the text).

2.2. Microenvironment of the internalized fatty acids.

The hfc values of nitroxide radicals such as 16-DSA are an excellent measure of the local polarity as they are sensitive to even slight changes of polarity in the direct probe environment. Hence, in the present investigation, we used the hfcs to gain information about whether the guest molecules are internalized alone or together with water. Water up-take into charged DPs has been described in MD simulations[9] and could be a factor of increasing importance with increasing structural defects. In an earlier study it was observed that for DPs up to **PG4+** hfc values of 16-DSA decrease with increasing g from 1 to 4, indicating an increasingly less polar environment for the guests in the DP interior. Interestingly, in the present study the hfc value for **PG6+** was found to only slightly decrease compared to the value for the 16-DSA in neat water. Additionally for **PG7+** and **PG8+** the values remain unchanged at this level (see Table S1 in ESI). From the spectral simulations of the immobilized species one can deduce that the 16-DSA probe molecules actually strongly interact with the DPs and are in fact internalized: if they were only attached to the surface, the rotational motion of the long chain ends - where the NO moieties are situated – would by far not be as hindered as observed here. The rotational motion together with the rather large hfc (in comparison to the lower generation DPs studied earlier) is indicative of a dense yet highly polar (more polar than in **PG4+**) environment. This

suggests that for DSA the interior (close to the surface, though) of the host DPs becomes increasingly more polar, which in turn suggests that the structural defects enable considerable influx of water molecules.

Table 2. Number and total molar mass of up-taken 16-DSA guest molecules together with the molar mass per DP RU results in mass fractions of up-taken guest per DP RU.

DP	Number of up-taken 16-DSA	Molar mass of total guest [kDa]	Molar mass of DP RU including counter ions [kDa]	Mass fraction of up-taken guest to RU [%]
PG6	5.0	1.9	21	9
PG7	11.9	4.6	42	11
PG8	14.1	5.4	79	7

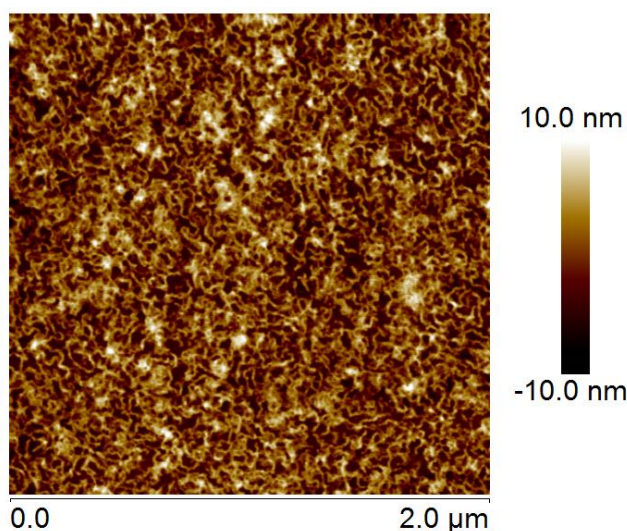## 2.3. AFM studies on, air-dried charged, high g dendronized polymers

Figure 3. AFM height image of **PG8+** on mica, deposited from aqueous solution.

A typical AFM image of **PG8+** adsorbed from aqueous solution is depicted in Figure 3. It shows a characteristic monolayer of single chains with occasional features of greater height. These features are often dot- or ball-like, sometimes more elongated, and may stem from polymer chains crossings (dots), duplexes or polymer chains in a second layer adhering to the chains in the first layer. AFM of **PG6+** and **PG7+** (Fig. S3) shows analogous features, though for these generations the adsorbate appears to

be more clearly separated into a first, quite dense adlayer and a second one, consisting of loosely deposited single chains. Likely, the apparently more pronounced interpenetration of these layers for **PG8⁺** is due to the larger corrugation of the polymer chains[23]. It can, however, be stated that for all generations many of the DP chains are clearly isolated and only few are associated with others, mostly in a loose and irregular fashion. This suggests that duplex structures or higher aggregates are unlikely to be present in significant concentrations in the solutions used for sample preparation.

3. Discussion

3.1. Host/guest interactions and structural imperfections.

PG6⁺-**PG8⁺** are derived from the neutral precursors **PG6**-**PG8** by reaction with neat trifluoroacetic acid, making them ammonium trifluoroacetate salts. The exponential increase of the loading capacity to the (assumed) cylindrical volume of the DPs reported for charged g=1-4 DPs, [20] apparently does not hold for g=6-8 DPs, as the data in Table 2 and Figure S1 suggest. The slope is less steep, which again is indicative of structural imperfections, making a direct comparison of the results on g=1-4 PG⁺'s and the g=6-8 PG⁺'s studied here impossible. For a better illustration of the number of 16-DSA molecules taken up per DP RU, Table 2 provides the mass fraction of total guest molecules per host RU. Thus, guest molecules can be loaded for up to 9% and 11% of the host mass for **PG6⁺** and **PG7⁺**(15% for **PG4⁺** [20]) and a further decreasing capacity of 7% when going from **PG7⁺** to **PG8⁺**. By nature of the applied method these estimates disregard eventual up-take of water, the impact that the kind of guest molecule may have and potential aspects as to where in the interior the guest molecules are located and whether this very location impacts the up-take. As a mechanism for internalization we propose that in the first step the negatively charged carboxylic acid groups electrostatically interact with the highly positively charged "surface" of the DPs, which leads to an initial immobilization. Once fixated in this way, the hydrophobic end of the 16-DSA molecule is taken up into the DP interior driven by the hydrophobic effect. The importance of the charge interaction has been supported before by use of methylated 16-DSA (thus, the corresponding ester) that was bound to much lower degrees by charged DPs.[19]

From the AFM images of samples deposited from aqueous solution (Fig. 3 and Fig. S3), it seems very likely that the

polymers of all three DP generations are present as unimers (at least to a very large degree) and that actually binding of fatty acids to individual polymers is observed, not binding to oligomers or aggregated polymers.

The hyperfine coupling (hfc) of the immobilized spectral components is a direct marker for the immediate nitroxide environment. Remarkably, the hfc extracted from spectral simulations indicates a much more polar environment for the DPs studied here (hfcs only slightly smaller than in bulk water) as compared to the lower generation charged DPs. The experimental facts that the number of 16-DSA per RU increases with generation, yet the relative mass (and volume) fraction of bound fatty acids decreases when going from g=4 to g=8 (see Table 1) and the high hfc can be reconciled assuming much more pronounced structural imperfections in the DPs so that more solvent can penetrate the charged DPs as already concluded in a previous paper[21]. One may speculate that backfolding of side chains the large dendritic RU rather than structural defects are responsible for the differences in microenvironment of 16-DSA when comparing binding to $g{\leq}4$ DPs (no or little imperfections) and states with binding to g>6 DPs. Such a backfolding should lead to denser, polymer-rich regions in the periphery, which should in return carry less solvent molecules (water) with them. Therefore, it is more likely that water swollen regions are the reason for the above described immobilization and microenvironment, in particular at the periphery, close to the charged DP surface, where the DSA molecules are most likely to reside being electrostatically attracted to the surface. This would lead to an extreme slowdown in rotational motion while the nitroxide moieties are still exposed to large quantities of water. In consequence, even though we reach large dendron generations with the three DPs studied here that are potentially close to the theoretical g_{max}, due to the above described structural imperfections these DPs can still be swollen by solvent and guest molecules.

The observed release capabilities by addition of 20 v/v% of an ionic liquid such as 1-butyl-3-methylimidazolium tetrafluoroborate (BmimBF₄) as release agent (Figure S2) confirm that nonetheless these structurally less perfect DPs are promising candidates for application in molecular transport, despite the fact that they cannot be strictly considered molecular objects and despite the fact that a clear-cut distinction of "interior" and "exterior" may not be possible in systems where solvent molecules can easily penetrate the exterior scaffolding. The

release of more than 90% of the 16-DSA molecules can be detected and is observable by the spectral signature of freely rotating 16-DSA, which is marked in Figure S2. This is in good agreement with the release properties found for the generation 1-4 analogues. The obtained results could indeed be expected since the dissolution rates (and high aqueous solubility) of all DP-generation analogues are similar, which should lead to similar release profiles. However, since the release capabilities have been explored in only limited conditions, further investigation under other conditions (temperature, pH, etc.) are necessary.

4.Conclusions

The EPR-active spin probes used in the loading studies clearly illustrate that the interior of the charged DPs is strongly polar, especially in comparison to their lower generation analogues. This is a direct sign of large amounts of water penetrating the DP surface, which in return mirrors the structural imperfections and much lower than theoretically achievable segmental densities of these very high generation DPs. From AFM images of the three charged high g DPs, we could furthermore show that the polymer chains are mostly present as unimers and that the EPR spectroscopic findings reflect the behavior of individual polymer chains. It seems like synthetic schemes need to be developed that avoid the relatively large amount of defects generated in the crucial step of direct conversion of **PG4** to **PG6**. Using simple CW EPR spectroscopy we have developed a straightforward tool to characterize the ensuing uptake and release properties and to gain insights into structural (im)perfections.

Experimental Section

Sample preparation: All samples for EPR spectroscopy were prepared as stated in Ref. [21]: the respective DP (1% wt) was dissolved in deionized water and solution of 16-DSA in ethanol (1% v/v of 200 mM).

EPR spectroscopy: Continuous wave (CW) EPR spectra were recorded on a MiniScope MS400 benchtop spectrometer (Magnettech, Berlin, Germany) working at X-band (ν_{mw}~9.4 GHz) at room temperature (293 K). A manganese standard reference, Mn^{2+} in ZnS (Magnettech, Berlin, Germany) was used to calibrate the magnetic field of the spectrometer. The microwave frequency was

recorded with a Racal-Dana frequency counter, model 2101 (Racal Instruments, Neu-Isenburg, Germany). A temperature control unit TC H03 (Magnettech, Berlin, Germany) with nitrogen gas flow was used to control the temperature within ±1 K. The sample volume (>10 μL) was always large enough to fill the complete resonator volume in the probehead. Changes in the spectral features after longer time periods were not detected.

Data analysis: The spectra were simulated with a custom-built program in MATLAB (The MathWorks, Inc.) using the Easyspin program package for the EPR[29].

AFM: Samples for AFM were prepared by immersion of a plate of mica (Plano GmbH, Wetzlar, Germany; ca. 7 mm x 7 mm, freshly cleaved on both sides) into a 1 % wt aqueous solution of DP to match the concentration used in the EPR experiments. After 5 min of gentle agitation, the mica was removed from the solution, rinsed with deionized water and affixed to a 12 mm diameter metal specimen disc (Ted Pella Inc., Redding CA, USA) by means of a small piece of double-sided tape. The sample was then dried in vacuum (10^{-2} mbar or lower) for at least 2 h before imaging. AFM was performed on a NanoScope IIIa (Digital Instruments, San Diego CA, USA) in tapping mode, using OMCL-AC160TS silicon cantilevers with a reflective aluminium coating (Olympus Corporation, Tokyo, Japan; typical resonance frequency 300 kHz, typical spring constant 26 Nm^{-1}).

Acknowledgements

[1] Paul J. Flory, "*Principles of Polymer Chemistry*", Cornell University Press, 1953.
[2] "*Dendrimers and other Dendritic Polymers*", J. M. J. Fréchet, D. A. Tomalia (eds), Wiley, Chichester, 2001.
[3] A. D. Schlüter, J. P. Rabe, *Angew. Chem. Int. Ed.* **2000**, *39*, 864-83.
[4] A. D. Schlüter, *Top. Curr. Chem.* **2005**, *245*, 151-91.
[5] H. Frauenrath, Progr. *Polym. Sci.* **2005**, *30*, 325-84.
[6] A. Zhang, *Prog. Chem.* **2005**, *17*, 157–71.
[7] B. M. Rosen, C. J. Wilson, D. A. Wilson, M. Peterca, M. R. Imam, V. Percec, *Chem. Rev.* **2009**, *109*, 6275–540.
[8] O. Bertran, B. Zhang, A. D. Schlüter, A. Halperin, M. Kröger, C. Alemán, *RSC Adv.* **2013**, *3*, 126-40.
[9] O. Bertran, B. Zhang, A. D. Schlüter, M. Kröger, C. Alemán, *J. Phys. Chem. B* **2013**, *117*, 6007-17.
[10] B. Zhang, R. Wepf, M. Kröger, A. Halperin, A. D. Schlüter, *Macromolecules* **2011**, *44*, 6785-92.
[11] A. D. Schlüter, A. Halperin, M. Kröger, D. Vlassopoulos, G. Wegner, B. Zhang, *ACS Macro Lett.* **2014**, *3*, 991-8.

[12] B. Zhang, R. Wepf, K. Fischer, M. Schmidt, S. Besse, P. Lindner, B. T. King, R. Sigel, P. Schurtenberger, Y. Talmon, Y. Ding, M. Kröger, A. Halperin, A. D. Schlüter, *Angew. Chem. Int. Ed.* **2011**, *50*, 737–40.

[13] P. G. deGennes, H. Hervet, *J. Phys. Lett.* **1983**, *44*, L351-XXX.

[14] D. A. Tomalia, *New J. Chem.* **2012**, *36*, 264-XXX.

[15] D. A. Tomalia, *Adv. Polym. Sci.* **2013**, *261*, 321-90.

[16] M. Kröger, A. D. Schlüter, A. Halperin, *Macromolecules* **2013**, *46*, 7550-64.

[17] A. Kröger, B. Zhang, C. Rosenauer, A. D. Schlüter, G. Wegner, *Colloid Polym. Sci.* **2013**, *291*, 2879-92.

[18] For example, see: A. Carlmark, C. J. Hawker, A. Hult, M. Malkoch, *Chem. Soc. Rev.* **2009**, *38*, 352-62; Y. Chen, X. Xiong, *Chem Commun.* **2010**, *46*, 5049-60; J. I. Paez, M. Martinelli, V. Brunetti, M. C. Strumia, *Polymers* **2012**, *4*, 355-95; A. Carlmark, E. Malmström, M. Malkoch, *Chem. Soc. Rev.* **2013**, *42*, 5858-79; R. Dong, Y. Zhou, X. Zhu, *Acc. Chem. Res.* **2014**, *47*, 2006-16.

[19] D. Kurzbach, D. R. Kattnig, B. Zhang, A. D. Schlüter, D. Hinderberger, *J. Phys. Chem. Lett. 2011*, *2*, 1583-87.

[20] D. Kurzbach, D. R. Kattnig, B. Zhang, A. D. Schlüter, D. Hinderberger, *Chem. Sci. 2012*, *3*, 2550-2558.

[21] D. Kurzbach, X. Zhang, B. Zhang, P. Arnold, D. Hinderberger, *Chem. Eur. J.* **2013**, *19*, 5602-5608.

[22] H. Yu, A. D. Schlüter, B. Zhang, *Helv. Chim. Acta* **2012**, *95*, 2399-410.

[23] H. Yu, A. D. Schlüter, B. Zhang, *Macromolecules* **2014**, *47*, 4127-4135.

[24] B. Zhang, H. Yu, A. D. Schlüter, A. Halperin, M. Kröger, *Nat. Commun.* **2013**, *4*, 1993-97.

[25] L. Shu, I. Gössl, J. P. Rabe, A. D. Schlüter, *Macromol. Chem. Phys.* **2002**, *203*, 2540-50.

[26] Y. Guo, J. D. Van Beek, B. Zhang, M. Colussi, P. Walde, A. Zhang, M. Kröger, A. Halperin, A. D. Schlüter, *J. Am. Chem. Soc.* **2009**, *131*, 11854-54.

[27] M. F. Ottaviani, G. Martini, L. Nuti, *Magn. Reson. Chem.*, **1987**, *25*, 897–904.

[28] B. R. Knauer, J. J. Napier, *J. Am. Chem. Soc.* **1976**, *98*, 4395–4400.

[29] S. Stoll, A. Schweiger, *J. Magn. Reson.* **2006**, *178*, 42-

ToC picture:

Nobody's perfect: Aspects of size, structural (im)perfection (see Figure), water content, and guest loading capacity of dendronized polymers (DPs) of high generation g ($6 \leq g \leq 8$) in aqueous solution were studied using AFM and electron paramagnetic resonance (EPR) spectroscopy on amphiphilic, spin-labeled guest molecules.

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