Characterization of Starburst Dendrimers by the EPR Technique. Copper(II) Ions Binding Full-Generation Dendrimers

M. Francesca Ottaviani,*,† Fabio Montalti,† Nicholas J. Turro,‡ and Donald A. Tomalia§

Department of Chemistry, University of Florence, 50121 Florence, Italy, Department of Chemistry, Columbia University, New York, New York 10027, Michigan Molecular Institute, Midland, Michigan 48640

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Cu(II) was used as a probe to investigate the structure and ion binding ability of full-generation poly(amidoamine) starburst dendrimers (*n*SBDs). Computer-aided analysis of the EPR spectra provided information on the formation of copper complexes in various internal or external locations of the dendrimers, as well as the *n*SBD structure as a function of the size (generation) of the dendrimers, pH, temperature, Cu(II) concentration, and aging of the samples. At low pH, Cu^{2+} competes with protons for binding with external amino groups, which become available for complexation at pH > 3.5. The $Cu(H_2O)_6{}^{2+}$ complexes are localized in proximity to the SBD surface, where structural modifications of the water solution prevented the occurrence of a freezing transition. A portion (about 20%) of $Cu(H_2O)_6{}^{2+}$ resides in the water pools in the open structure of the earlier generation dendrimers (*G* < 4) and are capable of undergoing a freezing transition. Progressive penetration of Cu^{2+} ions into the SBD structure occurs with an increase in pH and gives rise to the formation of complexes with various amino groups of the SBD structure. Between pH 4 and 5, the EPR spectra clearly show the superposition of three components corresponding to (a) a $Cu(H_2O)_6{}^{2+}$ complex, (b) a complex with two surface NH_2 groups, and (c) a complex with two surface NH_2 groups and two internal NR_3 groups ($Cu-N_4$). A fraction of the third component increases with an increase in generation. At pH ≥ 6 the $Cu-N_4$ complex is the only species present in the Cu(II)−*n*SBD solution. The low mobility of this complex supports the hypothesis that this complex is located in the external layers of the SBDs, which become densely packed in the later generations. At higher pH, Cu^{2+} migrates to the internal SBD structure and the complexes show higher mobilities. The dendrimers decomposed upon aging and decomposition was almost complete for the earlier generation dendrimers at pH > 5.5 and at 60 days after preparation.

Introduction

Electron paramagnetic resonance (EPR) has been used recently to characterize a novel class of macromolecules termed starburst dendrimers.[1−6] The dendritic macromolecules are obtained by covalently attaching monomers in radially branched layers, termed generations, to a central core.[7−11] Interest in these novel polymers resides not only in their inherently novel structural features but also in their potential applications to various scientific and industrial fields, such as catalysis, or as carriers of drugs, medication, biomolecules, or biomacromolecules into biostructures, or as models of supramolecular biological and colloidal structures, and as new materials for technical and industrial applications. When amidoamine units are used as repeating monomers in the dendrimers, starting from an ammonia core, the resulting polyamidoamine dendrimers[7] are termed PAMAM-SBDs or, more simply for the purposes of this work, SBDs. The external surface of SBDs may be terminated with amine groups, producing the so-called full-generation SBDs (termed *n*SBDs), or with carboxylate groups, producing the so-called half-generation SBDs (termed *n*.5-SBD).

EPR studies have proved to be very informative in determining the binding and structural properties of host molecules and surfaces in the case of various spin labels and spin probes as guest molecules.[12] In particular, we have investigated properties of *n*.5-SBDs by using nitroxide radicals[1−4] or paramagnetic

metal ions.[5,6] For example, nitroxide radicals with an attached carbon chain of different lengths were shown to be valuable probes for identifying hydrophilic and hydrophobic interacting and binding sites at the surface of the dendrimers.[1] When the chain length became long enough to ensure self-aggregation, different supramolecular structures were formed between *n*.5-SBDs and surfactant aggregates.[2−3] Interactions of anionic and cationic surfactants with the *n*.5-SBD surface were also investigated by fluorescence spectroscopy by means of pyrene probes,[13] leading to information comparable to that provided by EPR of nitroxide surfactants. When the nitroxide group is attached to a phenanthroline ligand of a $Ru(phen)_3{}^{2+}$, interaction with the surface of sodium alkyl sulfate micelles[14] was much stronger than the interactions with the *n*.5-SBD surface,[4] despite demonstrated similarities between anionic micelles and half-generation dendrimers.[15] However, the nitroxide group is apparently too large to enter the internal dendrimer structure, and therefore, EPR only monitors directly the occurrence of interactions between polar nitroxide groups and polar or charged groups at the SBD external surface. Conversely, paramagnetic metal ions may enter the dendrimer structure and bind to both internal and external ligand groups.[5−6] Use of both Cu(II) and Mn(II) as probes of interacting abilities of various ligand sites of host molecules provides complementary information, i.e., solution EPR spectra of Mn(II) exhibit a marked dependence on environmental conditions, whereas the spectra of Cu(II) ions provide information on the complexes directly formed with surface ligand groups.[16−19] It is well-known that the interacting ability of Mn(II) toward amino, amide, or carboxylate groups is much lower than the complexing ability of Cu(II) toward the same ligands; in particular, the dependence of the EPR

* To whom correspondence should be addressed at Department of Chemistry, Via Gino Capponi, 9, 50121 Firenze - Italy.
† University of Florence.
‡ Columbia University.
§ Michigan Molecular Institute.
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parameters of cupric complexes on coordinating atoms, geometry, and charge has been widely investigated.[20–21]

For the most part, past work has focused on the EPR spectra of copper complexes of biological interest.[22–23] Evaluation of magnetic parameters (main components of the **g** tensor for the Zeeman interaction between the electron spin and the magnetic field and of the **A** tensor for hyperfine interactions between the electron spin and the nuclear spin) at room and low temperatures provides information on the number and type of ligand groups, as well on the structure and dynamics of the complexes. Extended theories allow a detailed analysis of EPR line shape,[24–25] providing information on the mobility of the complexes and on the covalency of the coordinate bonding.[26] The study carried out on the complexes formed between Cu^{2+} and $n.5$-SBDs is consistent with the following conclusions:[5]

(1) Different complexes are formed with carboxylate and amino groups as a function of generation, pH, and temperature with the results indicating an equivalence between the Cu(II) complexes formed with the $n.5$-SBD surface and complexes with biomacromolecules, such as proteins or enzymes of similar structures.

(2) Ions are distributed in different locations inside and outside the dendrimer structure.

(3) The morphology of the dendrimers changes with the increase in generation, in line with results from molecular simulation of SBD structures.[27] Simulation indicates a modification in dendrimer morphology from an open, disklike-shaped structure, for the so-called earlier generation dendrimers (generation = G < 4), to a closed, densely packed, and spherical structure for the so-called later generation dendrimers ($G \geq 4$). Morphology variations from earlier to later generations have also been confirmed by photophysical measurements.[28]

(4) The dendrimer structure is homogeneous and stable with temperature.

Both surface carboxylate groups and internal amino groups are available for complexation in half-generation dendrimers. Conversely, full-generation dendrimers have only different types of amino groups. Indeed, Mn(II) has not shown any interaction with nSBD,[6] and therefore, Mn(II) is not a good probe of the interacting abilities of full generation dendrimers. In this study we analyzed complexes formed between Cu(II) and ligand groups of the nSBD as a function of the following parameters: (a) generation; (b) pH; (c) temperature; (d) concentration; (e) aging of the samples.

Analysis of the spectra has been carried out by computation of the spectra. The signals of powder material (frozen solutions) were computed using program CU23 (kindly provided by Prof. M. Romanelli of the University of Florence, Italy). For room and intermediate temperatures, no computing programs were available to include different relaxation mechanisms affecting EPR line shape and to extract the magnetic parameters.[29] Therefore we used a program by Schneider and Freed,[30] which holds better for nitroxide radicals. This program provided computed line shapes that fitted well the experimental signals and allowed for evaluation of the mobility and magnetic parameters in good agreement with magnetic parameters extracted from computation of low-temperature spectra. Where two or more spectral components contribute to the overall EPR signal, subtraction of the single components (experimental or computed) allows for analysis of the spectra, also leading to the evaluation of the fractions of Cu(II) ions contributing to each component.

Experimental Section

The nSBDs were synthesized by means of the procedure described elsewhere.[7] A thorough purification was accom-

plished by reprecipitation from water solutions. This study reports on results obtained from solutions of 3SBD, 5SBD, and 7SBD (the number refers to n, the generation of the SBD, throughout the paper). These SBDs were dissolved in bidistilled Millipore water at concentration 0.3 M in surface NH_2 groups SBD-NH_2. Unless otherwise specified, concentrations of the SBD are expressed in surface groups. The SBD solutions were stored under nitrogen at 278 K and diluted as necessary. Portions of this solution were added to aqueous solutions of $Cu(NO_3)_2 \cdot 3H_2O$ (Merck, used as received). Final concentrations were 0.2 M SBD-NH_2, and between 20 mM and 1 mM Cu^{2+}. Sample preparation and EPR measurements were repeated several times to confirm the reproducibility of the results. The pH of the samples was modified by addition of different amounts of $HClO_4$ (Merck, starting concentration of 1 M). The starting pH of the Cu^{2+}-nSBD samples was about 10.5. Measurements of pH were made on a pH meter (Sentron 2001).

The EPR spectra were recorded on a Bruker 200D interfaced to a PC outfitted with Stelar software system. Temperature control was achieved with a Bruker 100/700T unit.

Magnetic parameters were measured by field calibration with diphenylpicrylhydrazyl (DPPH, g = 2.0036). Accuracy in determining magnetic parameters, correlation time for motion, and relative percentages of the spectral component was 5%, as obtained from fitting between the experimental and computed signals.

Results and Discussion

Parameters which caused the formation of different Cu(II) complexes were mainly the dendrimer generation and the pH of the solution. Changes in the concentrations of both Cu(II) and the dendrimers' surface groups did not provide any evident line shape variations of the EPR spectra, when the ratio between the SBD-NH_2 groups and the Cu^{2+} ions was larger than 10. At concentrations of 0.2 M SBD-NH_2 and 20 mM Cu^{2+} the spin–spin interactions among Cu^{2+} ions which were localized in the same dendrimer and in close sites became discernible in the EPR spectra in the form of expected line broadening (results not shown).[31]

Identification of types of complexes of Cu^{2+}, i.e., the coordinating ligands and structure of the coordination center, was established by evaluating the magnetic parameters from the spectra at room and low temperatures.

(1) Identification of the Spectral Components. Figure 1 reports EPR experimental spectra (full lines) of solutions of 7SBD (0.2 M) and Cu^{2+} (1 mM) at various pH, and at 298 K (Figure 1a) and 130 K (Figure 1b). The dashed lines superimposed on the experimental spectra are the computed signals. Experimental and computed EPR spectra of Cu^{2+} (1 mM) in 3SBD solutions (0.2 M) at various pHs are reported in Figure 2 (Figure 2a, 298 K; Figure 2b, 130 K). The main features of the spectra are as follows:

(a) The spectra are typical of Cu^{2+} complexes with an elongated octahedral structure (square planar, with $d_{x^2-y^2}$ ground level) and an almost axial symmetry ($g_{zz} > g_{xx} \approx g_{yy}$).

(b) The spectra at low temperature are typical of Cu^{2+} in a glasslike environment, meaning that the Cu^{2+} solutions did not show a freezing transition at a specific temperature but a progressive quenching of motion.

(c) Either at lower or at higher pH, the spectra of 7SBD solutions are clearly constituted by one component possessing different magnetic parameters at lower and higher pH. The signal, which is predominant at lower pH, was henceforth called spectrum A at low temperatures and spectrum a at high temperatures, whereas the signal at higher pH was termed
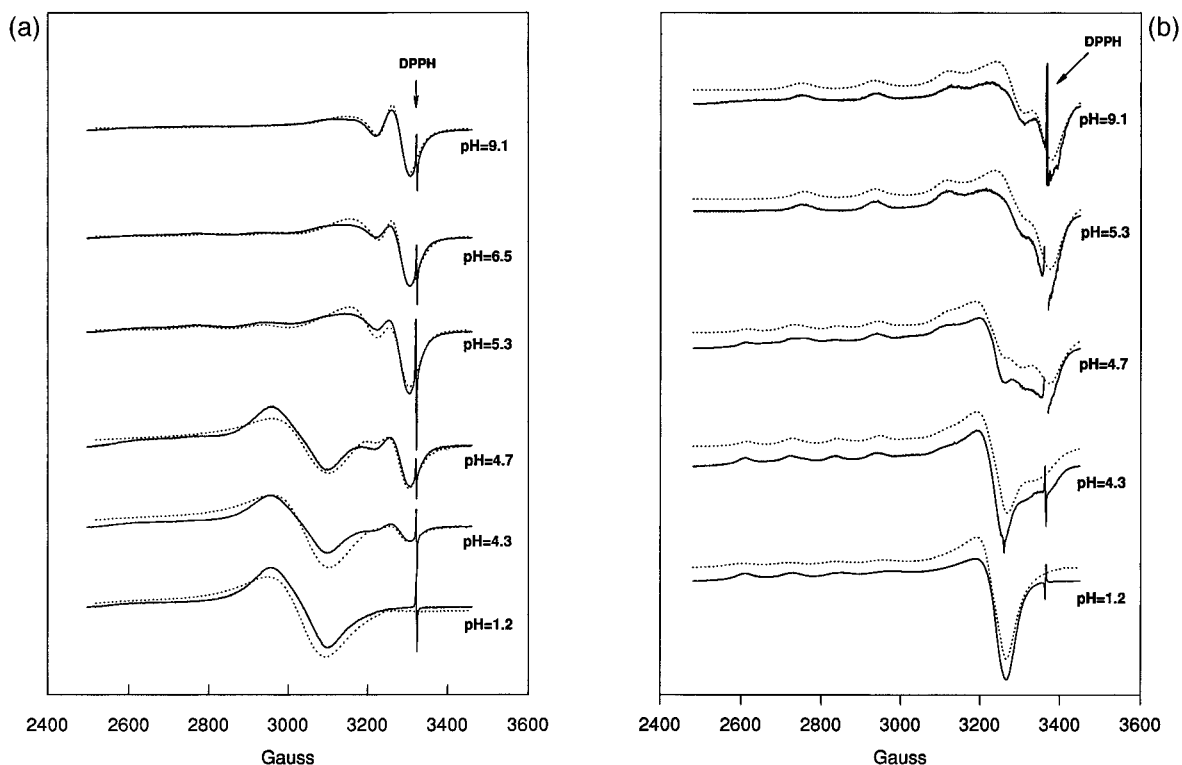
Figure 1. EPR experimental (full lines) and computed (dashed lines) spectra of solutions of 7SBD (0.2 M) and Cu^{2+} (1 mM) at various pH: (a) at 298 K; (b) at 130 K.
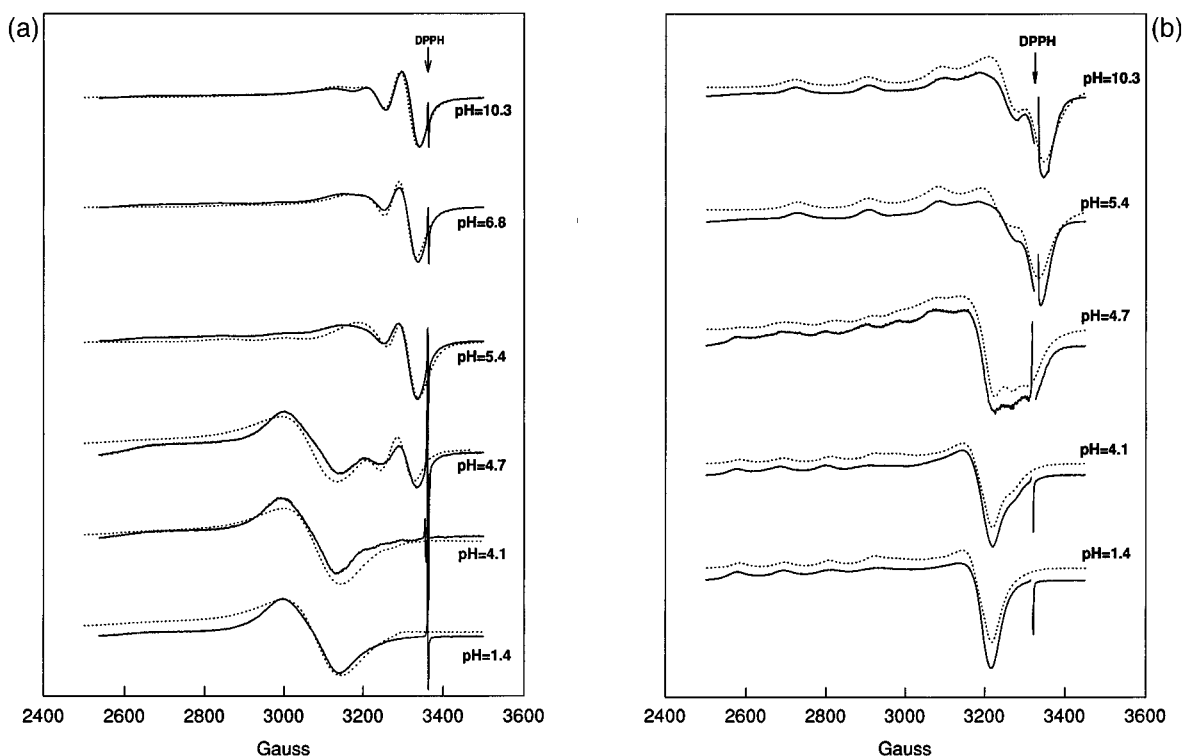

Figure 2. EPR experimental (full lines) and computed (dashed lines) spectra of Cu^{2+} (1 mM) in 3SBD solutions (0.2 M) at various pH: (a) at 298 K; (b) at 130 K.

spectrum B at low temperature and spectrum b at high temperature. Conversely, the spectra at intermediate pH (4 < pH < 5) are complicated by the superposition of two or more components. Subtraction of signals at lower and higher pH from the signal at intermediate pH allowed the identification of a third component, termed signal C at low, and c at high temperatures, respectively.

(d) Both signal a and signal b at room temperature do not show resolution of the four hyperfine lines.

(e) Low-temperature and low-pH spectra of 3SBD solutions show, upon subtraction of signal A, a further component constituted by a broad single line, which is termed signal D.

Examples of computed components a, b, and c and A, B, C, and D are shown in Figure 3, indicating the magnetic and mobility parameters used for computation. Magnetic parameters evaluated from both room- and low-temperature spectra are the components of the **g** tensor for coupling between the electron spin and magnetic field and components of the **A** tensor, for

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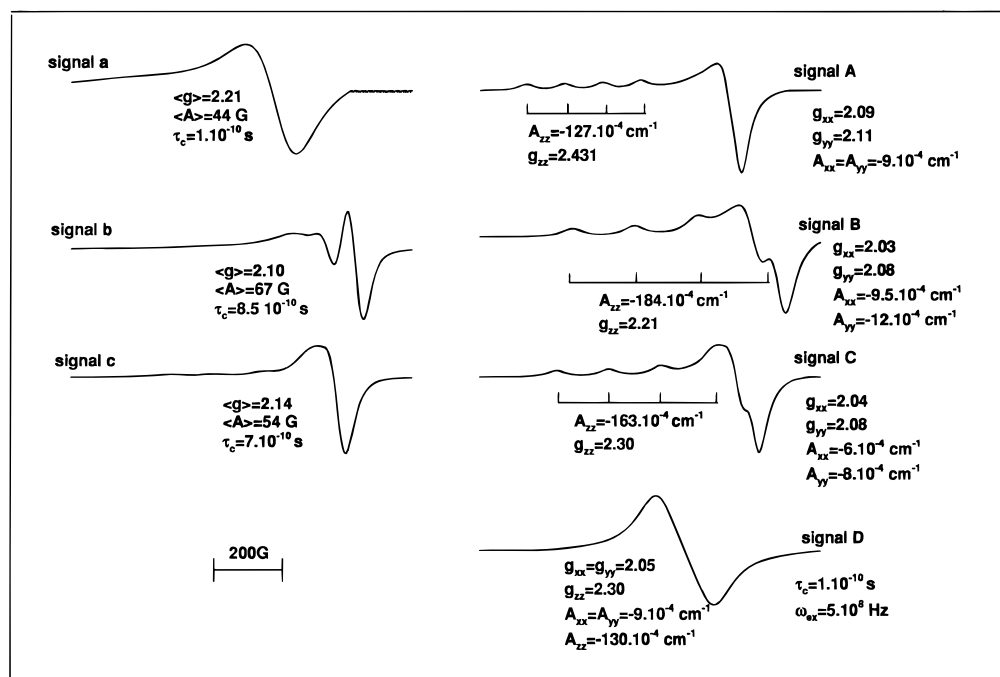
Figure 3. Examples of the computed components a, b, and c and A, B, C, and D, indicating the magnetic and mobility parameters used for the computation.

hyperfine coupling between the electron spin and nuclear spin ($I = {}^3/_2$). However, the magnetic parameters relevant to room-temperature spectra are $\langle A \rangle = (A_{xx} + A_{yy} + A_{zz})/3$ and $\langle g \rangle = (g_{xx} + g_{yy} + g_{zz})/3$, which are reported in Figure 3. The mobility parameter, which was evaluated from computation of the spectra at room temperature, is the correlation time for reorientation diffusion of the Cu(II) complexes, τ_c. A Brownian diffusion was assumed to modulate components of the **g** and **A** tensors. Therefore, the diffusion coefficient D is related to the correlation time of motion by the equation $D = 1/(6\tau_c)$. The assumption that Brownian reorientational diffusion governed the relaxation process is an approximation, since τ_c for Cu(II) systems may be the Debye reorientation time or the pseudoreorientation time (governed by interconversion of the Jahn−Teller distortion axis).[31] For signal D a further parameter is indicated in Figure 3, i.e., the exchange frequency ω_{ex} which measures the Heisenberg spin−spin exchange interactions.[32] These interactions occur in the case of Cu^{2+} ions that are close to each other (distance < 14 Å). The parameters reported in Figure 3 are consistent with the following findings:

(1) Signals a and A are characteristic of $Cu(H_2O)_6^{2+}$ complexes. However, larger A_{zz} and lower g_{zz} have been found by other researchers for $Cu(H_2O)_6^{2+}$.[18c,31,33,34] Kivelson and Neiman[26] and Faber and Rogers[35] indicate that a larger covalency of the coordinative bonding reduces the magnitude of the g factors. On the other hand, the increase in the hyperfine coupling parameters corresponds to a larger spin density localized on the copper nucleus as is expected for lower electronegativity of the ligands. At low pH, the amino groups at the surface are totally protonated[6] and the water molecules localized close to the surface are affected by the surface charge. As a result, the spin density of Cu(II) was largely delocalized onto the water ligands in the hydration layers of the SBD surface, with a consequent increase in the bond ionicity. The low value of the hyperfine coupling constant of signal a, together with broadening of the lines, was responsible for the lack of resolution of the four hyperfine lines.

Figure 4 reports, in a two-dimensional projection of a piece of the structure of the nSBDs, the proposed location of the $Cu(H_2O)_6^{2+}$ complex responsible for signals a and A. It is

noteworthy that copper ions coordinate to half-generation dendrimers even at pH 5, since carboxylate groups at the surface form stable complexes with Cu^{2+}. Conversely, Cu^{2+} competes with H$^+$ for interaction with amino groups of full-generation dendrimers, but protonation of the SBD-NH$_2$ did not allow for copper complexation at low pH.

(2) To identify the complexing site of Cu^{2+} ions responsible for signals b and B, the following issues are considered:

(a) The magnetic parameters of signals b and B are comparable to the parameters found for Cu^{2+} complexing four amino groups in the internal structure of the half-generation dendrimers.[5]

(b) A deviation from axial symmetry was found for signal B in the full-generation dendrimers with respect to half-generation dendrimers,[5] that is, $|g_{xx} - g_{yy}| = 0.005$ for n.5-SBDs and $|g_{xx} - g_{yy}| = 0.04$ for nSBDs. In ref 5 the authors suggest that Cu^{2+} coordinates four almost equivalent NR$_3$ groups in the internal structure of the n.5-SBD.[5] Conversely, the larger value of $|g_{xx} - g_{yy}|$ in the full-generation dendrimers suggested that the four amino ligands were not all the same.

(c) Binding ability toward Cu(II) of the different amino groups in the nSBD structure is expected to follow the trend NH$_2$ > NR$_3$ > NHCO. Therefore, Cu^{2+} should preferentially coordinate the NH$_2$ groups;

(d) Slow motion conditions ($(1−3) \times 10^{-9}$ s $< \tau_c < 5 \times 10^{-8}$ s) were found for signal b, indicating that the Cu(II) are localized in a region in which the degrees of freedom of the copper complexes are quite low. The more external the shell of the nSBD, the more packed the dendrimer structure as revealed by molecular simulation.[27]

On the basis of the above consideration, we postulate that the Cu(II) ions responsible for signals b and B are localized in the internal structure of the SBD structure, immediately below the external layer ($-CH_2CH_2NH_2$ groups), and are bound to two NH$_2$ groups and two NR$_3$ groups. Figure 4 also shows the proposed location of Cu(II) for the formation of the complex responsible for signals b and B.

(3) The parameters of signal C are intermediate between signal A and signal B. In the case of the n.5-SBDs, it is
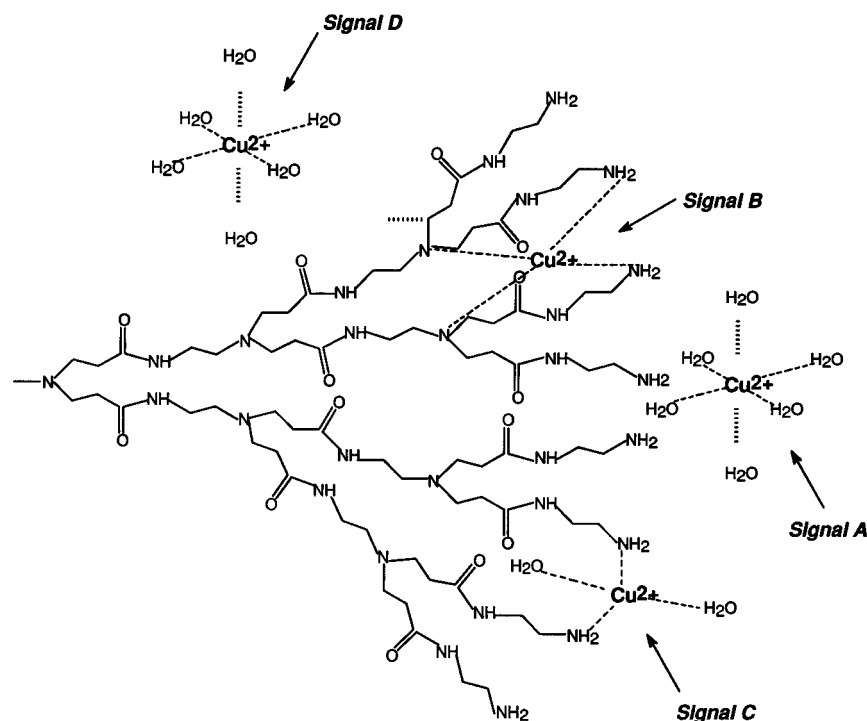
Figure 4. Two-dimensional projection of a piece of the structure of nSBDs. Some of the possible locations of Cu^{2+} for formation of the various complexes are shown schematically in this projection by the dashed lines. Specific binding of a single complex is not to be implied by the dashed lines. For example, bidentate and higher order structures with different binding strengths should be considered.

suggested that a stable complex of Cu^{2+} forms with two carboxylate groups and two internal NR_3 groups.[5] The measured parameters are between the parameters for copper interacting with carboxylate and water ligands and the parameters for copper interacting with four amino ligands. The analogy between the $Cu^{2+}-n.5$-SBD system and the $Cu^{2+}-n$SBD system is straightforward, that is, the intermediate condition for Cu^{2+} complexation between four water ligands and four amino ligands is two water ligands and two amino ligands. The parameters reported in the literature for similar Cu^{2+} complexes are in good agreement with this assumption.[16−19] Figure 4 shows the proposed location of Cu^{2+} for the formation of the complex responsible for signals c and C.

(4) Signal D arose from Cu^{2+} salt which segregated from the solution upon the freezing transition.[32] The portion of solution which underwent the freezing transition did not show the modifications of rheological properties of water usually found in the vicinity of charged or polar surfaces.[34,36−39] Because of segregation, Cu^{2+} ions are in proximity of one another leading to spin−spin broadening.

(2) Spectral Modification as a Function of pH. Modifications of the EPR spectra shown in Figures 1 and 2 as a function of pH are summarized as follows:

(i) Signal A parameters were moderately affected by the variation of pH. For instance, A_{zz} of 7SBD samples decreased from 119 to 109 G, whereas g_{zz} values increased from 2.421 to 2.425, by increasing the pH from 2 to 4. As suggested above (point 1), the decrease in A_{ii} reflects a decrease in spin density on the copper nucleus. Hence, the increase in pH favored Cu^{2+}_{aq} to approach the protonated SBD surface, leading to a larger delocation of spin density onto the water ligands. This also corresponds to a progressive destabilization of $Cu(H_2O)_6^{2+}$ with the increase in pH, since the Cu^{2+} ions start interacting with the amino groups.

(ii) The range of existence of signal A reflected the protonation behavior of the dendrimers.[6] At pH = 4−5 the NR_3 groups are almost all deprotonated and the NH_2 groups start

deprotonating. Therefore, the electrostatic repulsion between the positively charged dendrimers and the Cu^{2+} ions is responsible for the formation of $Cu(H_2O)_6^{2+}$. As soon as deprotonated NH_2 groups become available by increasing the pH, signals a and A disappear and signals b and B and c and C contribute to the overall EPR signals.

(iii) Signals c and C have a short range of existence as a function of pH.

(iv) Signal b shows a decrease in the correlation time for motion with an increase in pH; correspondingly, the A_{zz} component increases for both signals b and B and g_{zz} decreases. For instance, $\tau_c = 1.7 \times 10^{-9}$ s and $A_{zz} = 180$ G were calculated at pH = 5.3 for 7SBD samples, whereas $\tau_c = 4.5 \times 10^{-10}$ s and $A_{zz} = 191$ G were obtained at pH = 9.1. We hypothesize that the Cu^{2+} ions transferred from the external SBD surface to the internal structure with the increase of pH. Therefore, variation of both magnetic parameters and mobility of copper ions reflects the progressive penetration of Cu^{2+} internally to the dendrimers and the stabilization of the $Cu-N_4$ chromophore. The lower mobility at lower pH is a consequence of the location of Cu^{2+} in the most congested area of the dendrimers, that is, close to the surface NH_2 groups. Hence, mobility increased when Cu^{2+} migrated with an increase in pH to an intermediate distance between the NH_2 groups and the NR_3 sites.

(v) Signal D was present in the spectra of 3SBD samples from the lower pH to pH ∼ 7. At larger pH values deprotonation of the amino groups led to almost all the Cu^{2+} ions localizing in the vicinity of the SBD surface. Perturbation of the rheological properties of the liquid in the vicinity of the dendrimer surface accounts for the formation of a glasslike structure of the solution at low temperature.

The combined procedure of the addition of different signals at the proper relative intensity ratios to reproduce the experimental spectra, and the subtraction of experimental spectra constituted by a single component from the experimental spectra constituted by two or three components allowed the evaluation of the relative percentages of the various components at each

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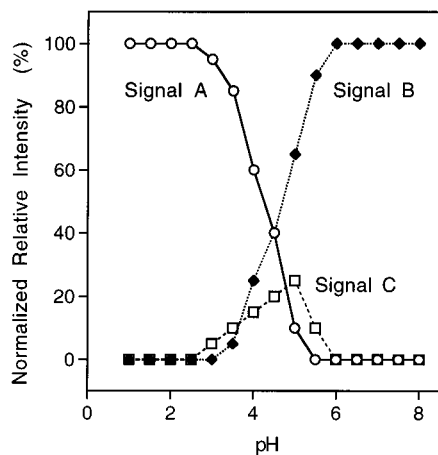
Figure 5. Variation of the percentages of the signals A, B, and C of Cu^{2+} (1 mM) in 7SBD solutions (0.2 M) as a function of pH.

TABLE 1: Relative Percentages of the Spectral Components for 3-, 5-, and 7SBDs at Three pHs

G	pH	% signal A	% signal B	% signal C	% signal D
3	4.2	65		15	20
5		65	20	15	
7		55	30	15	
3	4.7	30	25	25	20
5		30	50	20	
7		30	50	20	
3	5.3		80		20
5			90	10	
7			100		

TABLE 2: A_{zz}, G^{zz}, and τ_c Values Obtained from the Computation of Signals B and b for 3- and 7SBDs at Three pHs

G	pH	A_{zz} ($\times 10^{-4}$ cm^{-1})	g_{zz}	τ_c ($\times 10^{-10}$ s)	G
3	5.3	−185	2.22	8.5	3
7		−186	2.22	17.0	7
3	6.5	−186	2.22	4.0	3
7		−189	2.21	8.0	7
3	9.0	−189	2.20	3.0	3
7		−195	2.19	4.5	7

pH. Figure 5 reports variation of the percentages of signals A, B, and C of 7SBD samples as a function of pH. Signal B gradually increased in intensity between pH 3.5 and pH 5.5 at the expense of signal A, and simultaneously, signal C gained in intensity up to 20% and, then, rapidly disappeared. Therefore, the Cu-N$_2$O$_2$ chromophore responsible for signal C may be considered to be a transient species formed by copper ions while transferring from the external surface to the internal SBD structure.

(3) Variation of Generation. By comparing the spectra of 7SBD samples (Figure 1) with the spectra of 3SBD samples (Figure 2), it is evident that the line shape variations as a function of pH are comparable in the two cases. However, the magnetic and mobility parameters evaluated from spectral computation, such as the relative percentages of the various components, changed as a function of generation (at a constant pH).

Tables 1 and 2 reports some parameters describing the differences among the different generations. Table 1 reports the relative percentages of the spectral components for 3-, 5-, and 7SBD at pH = 4.2, 4.7, and 5.3. The reported percentages further demonstrate that large spectral variations, i.e., transformation of the observed signals, occurred within a small variation of pH. The main variations as a function of generation are (a) the presence of signal D in the "earlier" generation 3SBD, whereas the same signal was not recognizable in the spectra of "later" generation SBDs (5SBD and 7SBD), (b) the increase of signal B intensity from earlier to later generations, mainly in substitution of signal D, and (c) further increase in signal B intensity from 5- to 7SBD at the expense of signal A or signal C. However, both signals A and C underwent rather small changes in relative intensity as a function of generation. Therefore, the main spectral variations were found from earlier to later generations and arose from the morphology of the dendrimers.[27] Earlier generation dendrimers possess an open structure in which large water pools can be hosted. The portion of Cu^{2+} solution localized in the middle of these pools is not significantly affected by the presence of the SBD surface and retains the rheological properties of the Cu^{2+} solutions in the absence of dendrimers. Consequently, solutions of earlier generations undergo a freezing transition with a decrease in temperature, giving rise to signal D. Conversely, the closely packed structure of later generation dendrimers does not permit the freezing of the solution, since all the Cu^{2+} is localized in the vicinity of the SBD surface.

Table 2 reports the A_{zz}, and the g_{zz} values, together with correlation times for motion, which were obtained from the computation of signal B and signal b for 3SBD and 7SBD at pH = 5.3, 6.5, and 9. As was discussed above, an increase in pH led to an increase (as absolute value) in A_{zz} and a decrease in τ_c, reflecting the increased stability of the Cu-N$_4$ chromophore because the Cu^{2+} ions were localized more internally in the SBD structure. The change of generation from 3 to 7 also led to an increase in A_{zz}, which was larger at larger pH, whereas the mobility decreased, and this decrease was larger at lower pH. These results are in line with changes in morphology from the earlier generation to the later generation SBDs. Quenching in the mobility of the Cu(II) complex arose from trapping of Cu^{2+} ions in the congested external layer of the later generation SBDs. This effect mainly occurs at pH = 5−6, whereas at higher pH, the Cu^{2+} ions localized more deeply in the SBD structure and, consequently, the changes of structural packing from earlier to later generations are less effective.

(4) Variation in Temperature. Figure 6 shows the EPR experimental spectra of 7SBD solutions (0.2 M) containing Cu^{2+} at 0.1 mM concentration and pH = 7, recorded as a function of temperature in the range 213−338 K. Mobility increased with an increase in temperature. The spectrum at 338 K was simulated with $\tau_c = 2 \times 10^{-10}$ s, whereas the mobility at 298 K was 4 times lower. The decrease in line width with an increase in temperature, in the range of high temperatures, indicated that the modulation of the magnetic tensor components via diffusion reorientation of the complexes in solution is the main relaxation mechanism.[25d,e,40] Enhanced resolution of the hyperfine structure allowed for evaluation of the isotropic hyperfine constant as 75 G at 338 K. It is noteworthy that the spectrum at 228 K is equivalent to the spectrum at 130 K. Therefore the mobility of the complex Cu−N$_4$ is completely quenched at 228 K.

The quenching of mobility occurred at higher temperatures for 7SBD samples than it occurred for 3SBD samples. For instance, the spectrum of 7SBD at T = 268 K in Figure 6 is equivalent to the spectrum of 3SBD at T = 262 K (same pH) and indicated low mobility of the complexes, whereas the spectrum of 7SBD at T = 278 K is equivalent to the spectrum of 3SBD at T = 268 K, and the complexes still show rather high mobilities. This effect is in line with the results described in the previous paragraphs, that is, the complexes that are formed in the packed structure of 7SBD are characterized by low mobility and rapid quenching of mobility upon a decrease in temperature. This achievement was described for signals b and

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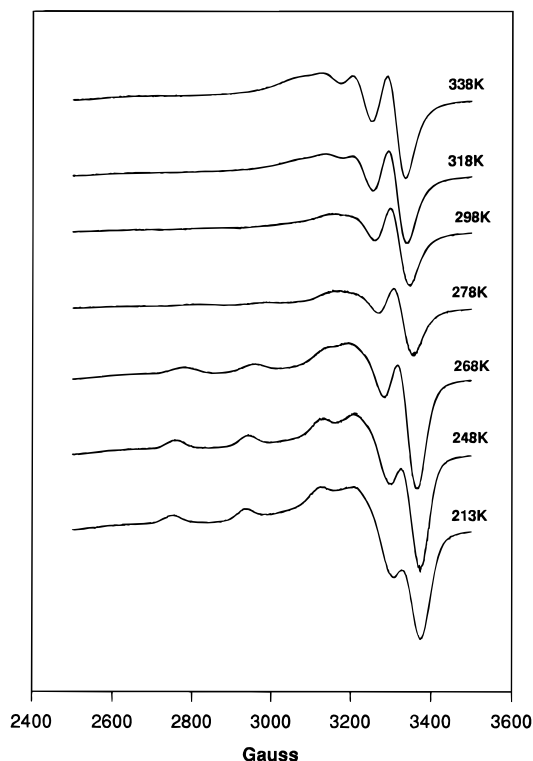
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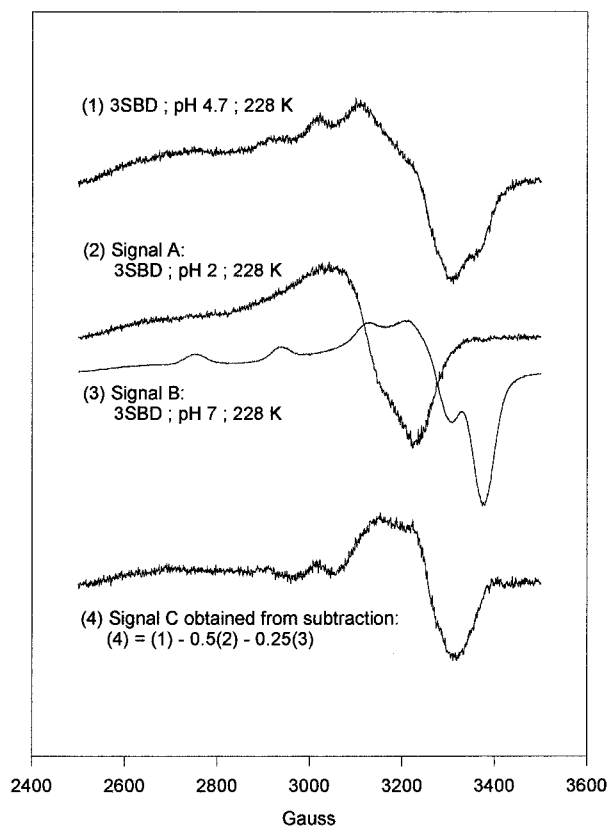
Figure 7. Signals A, B, and C at the same temperature (228 K) for Cu^{2+} (1 mM) in 3SBD solutions (0.2 M) at three different pH values. Signal C was obtained upon subtraction of signals A and B from the experimental spectrum at pH 4.7.

B. Figure 7 shows the three components, that is, signals A (for simplicity we did not consider signal D, which is 20% of signal A reported in the figure), B, and C, at the same temperature (228 K) for 3SBD samples at three different pH values. Signal

C was obtained upon subtraction of signals A and B from the experimental spectrum at pH 4.7, at the relative intensities reported in Table 1. Both signals A and C show that a large portion of the corresponding Cu(II) complex retained fast mobility (τ_c in the range $(1-3) \times 10^{-9}$ s). In particular, signal A achieved mobility quenching as detected at 130 K, at about 208 K. The location of Cu^{2+} for the formation of the three different complexes accounted for the differences in the mobility variations as a function of temperature. Ions trapped in the dendrimer structure to form the $Cu-N_4$ chromophore are moving slowly at room temperature and their mobility is completely quenched at 228 K. Conversely, ions remaining in the hydration layers at the dendrimer external surface are fast moving at room temperature, but their mobility is completely quenched at 208 K.

(5) Effect of Aging. Stability of Cu−*n*SBD solutions over time depends on (a) the time period of aging, (b) the generation of the dendrimer, and (c) the pH of the solution. All the samples provided well-reproducible EPR spectra during the first month after sample preparation. However, beginning with the 40−50th days after preparation, a progressive line shape variation occurred for many samples. Spectral variations were permanent and constant after about 2 months. Figure 8 shows two examples of EPR spectra of 60-day aged samples. Figure 8a shows the spectra at 130 K of Cu^{2+} solutions containing 3SBD and 7SBD at pH 4.7. The full lines represent the spectra of aged samples compared with the spectra of freshly prepared samples (dotted lines). It is shown that signals B and C increased at the expense of signal A for 3SBD, whereas almost no variations of line shape were found for 7SBD. More significant spectral variations were found at pH 7, as shown in Figure 8b for 3SBD and 7SBD. Computer-aided analysis of the spectra indicated the presence of two main components whose g_{zz} and A_{zz} parameters are reported in the figure. Comparison of the spectra in Figure 8b with the spectra of freshly prepared samples at similar pH values reported in Figures 1 and 2 indicate the following results:

(1) The spectral components of aged samples showed an increase in $|A_{zz}|$ when compared to signal B. This increase was larger for 3SBD than for 7SBD.

(2) The magnetic parameters were similar to those measured for ethylenediamino complexes of Cu^{2+}.[20−23]

(3) The spectra of samples at room temperature were consistent with fast mobility of the Cu(II) complexes.

Therefore, we hypothesize that samples at larger pH (pH > 5.5) partially depolymerized upon aging, and the fragments produced, structures probably related to ethylenediamine or ethyldiamine, which formed low molecular weight chelate complexes with the Cu(II) ions. Decomposition was almost complete for earlier generation dendrimers, whereas later generations partially retained their structure. The Cu(II) ions, however, penetrate the SBD structure to reach the innermost region in which the dendrimer branches have larger freedom of motion than in the congested external layers.

Conclusions

The present study completes the investigation of the complexation behavior of Cu(II) and Mn(II) ions in the presence of polyamidoamine starburst dendrimers (SBDs) by means of computer-aided analysis of the cw-EPR spectra.[5,6] The carboxylate groups at the surface of the half-generation SBDs (*n*.5-

Characterization of Starburst Dendrimers

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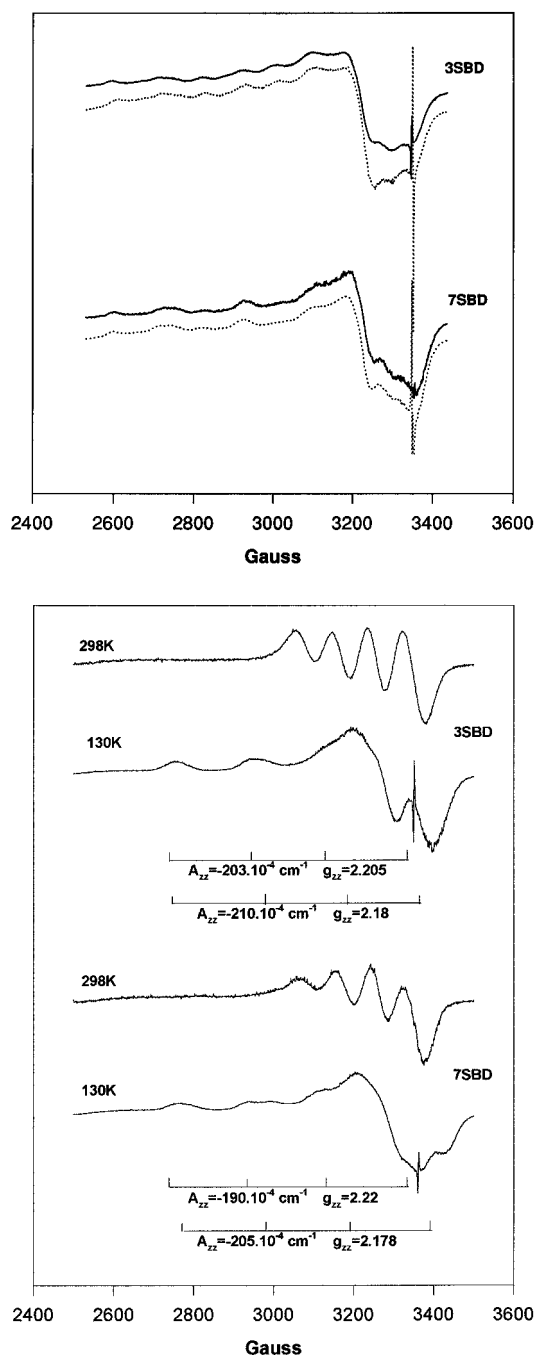
Figure 8. Two examples of EPR experimental spectra of 60-day aged samples (full lines, EPR spectra of aged samples; dotted lines, EPR spectra of freshly prepared samples): (a) Cu^{2+} (0.1 mM) in solutions of 3SBD and 7SBD (0.2 M) at 130 K and pH 4.7; (b) Cu^{2+} (0.1 mM) in solutions of 3SBD and 7SBD (0.2 M) at 298 and 130 K at pH 7.

SBDs) are available for complexation to both Cu^{2+} and Mn^{2+} over a large range of pH.[5,6] Conversely, the amino groups at the surface of the full-generation dendrimers (nSBD) prevent the interaction with Mn(II)[6] and allow for Cu^{2+} complexation only at pH > 3.5. Identification of the complex responsible for each spectral component was accomplished by referring to previous literature, based mainly on Cu^{2+} complexing biomolecules.[22,23] An intermediate species, existing only between pH 4 and 5 at a maximum relative fraction of about 25%, was formed by Cu^{2+} coordinating two external NH_2 groups. The Cu^{2+} ions may penetrate the SBD structure but are localized in the packed external structure of the SBDs, showing complete quenching of motion at 228 K. Only at high pH are the Cu^{2+} ions allowed to enter the internal SBD structure.

As for the n.5SBD−Cu(II) system,[5] EPR analyses provided information on the morphology changes from the open structure of the earlier generation dendrimers ($G < 4$) to the packed external structure of the later generation dendrimers ($G \geq 4$). The earlier generation dendrimers differ from the later ones; thus (a) the presence of a fixed fraction of solution (about 20%) which underwent freezing transition and (b) a smaller relative fraction of $Cu−N_4$ complex at constant pH, e.g. only 25% of $Cu−N_4$, was found at $G = 3$ and pH = 4.7, whereas the calculated fraction of $Cu−N_4$ was 50% at $G = 5$ and $G = 7$ and at the same pH], (c) faster mobility of the complexes, and (d) almost complete decomposition of the nSBD in the constituent monomers at pH > 5.5 60 days after preparation.

It should be noted that the dendrimer samples employed in this investigation are of high purity[41] as determined by several analytic techniques. Perhaps the most convincing demonstration of sample purity was achieved employing matrix-assisted laser desorption mass spectrometry, which show the samples to be highly monodisperse through generation 7, the highest generation employed in this investigation.

In summary, Cu^{2+} ions have shown to be good probes in investigating the structure and dynamics of the nSBDs and have demonstrated that the interacting abilities of these macromolecules resemble the complexation behavior of Cu^{2+} in the presence of biomolecules, such as proteins and enzymes.

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