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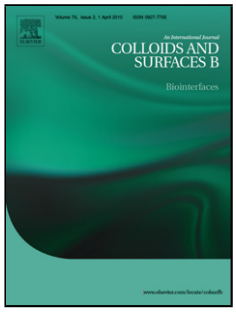

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FACTORS AFFECTING INTERACTIONS BETWEEN SULPHONATE-TERMINATED DENDRIMERS AND PROTEINS: A THREE CASE STUDY

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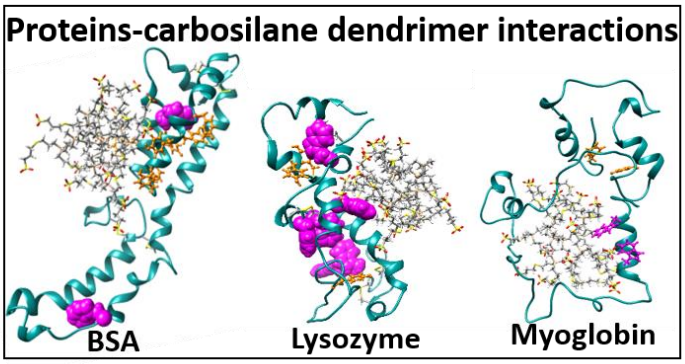
Graphical abstract



Highlights

- Sulphonate-terminated carbosilane dendrimers interact with proteins.
- Interactions greatly depend on the protein itself.
- High dendrimer generations and concentrations and acidic pHs favor interactions.
- Computer molecular modeling endorses experimental results.

ABSTRACT

This work proposes a deep study on the interactions between sulphonate-terminated carbosilane dendrimers and proteins. Three different proteins with different molecular weights and isoelectric points were employed and different pHs, dendrimer concentrations and generations were tested. Variations in fluorescence intensity and emission wavelength were used as protein-dendrimer interaction probes. Interaction between dendrimers and proteins greatly depended on the protein itself and pH. Other important issues were the dendrimer concentration and generation. Protein-dendrimer interactions were favored under acidic working conditions when proteins were positively charged. Moreover, in general, high dendrimer generations promoted these

interactions. Modeling of protein-dendrimer interactions allowed to understand the different behaviors observed for every protein.

Key words: sulphonate-terminated carbosilane dendrimers; protein-dendrimer interaction; fluorescence; quenching; computer modeling; molecular dynamics

1. Introduction

Dendrimers are synthetic macromolecules whose structure is constituted by layers, called generations, where functional groups or ligands with different biological activities can be introduced [1]. This structure lets differentiate four regions in dendrimers: the encapsulated core, which is surrounded by a singular micro-environment created by the flexible branches of the dendrimer; the own branches; the refuge formed by the cavities bordered by the branches; and the multivalent surface which delimits the macroscopic properties of the dendrimer [2]. This structure has allowed to use dendrimers for biomedical applications, both in the diagnosis and treatment of diseases as cancer, for genes and drugs delivery, molecular recognition, and, also, in the development of chemical sensors and enzymatic catalysis [1,2].

Interactions between proteins and poly(amidoamine) (PAMAM) dendrimers have been observed and explored [3-6]. Ottaviani *et al.* [3] studied the interaction of low and high-generations PAMAM dendrimers with selected amino acids (acidic, basic, neutral(zwitterionic)-polar, and low-polar) and proteins (acidic and basic isoelectric points). They observed that the binding of proteins and amino acids with dendrimers was predominantly promoted when both hydrophobic and electrostatic interactions were present. On the other hand, Nowacka *et al.* [4] studied the (end-group)-dependence of

PAMAM dendrimers on the interaction with bovine insulin protein finding that the interaction was mainly electrostatic.

Carbosilane dendrimers present a more hydrophobic skeleton than further studied PAMAM dendrimers [7]. This skeleton, conformed by very strong Si-C bonds, makes them to have a high kinetic and thermodynamic stability [8]. Moreover, carbosilane dendrimers are more spherical than PAMAM and this fact make easier the synthesys of higher dendrimer generations without increasing the repulsion between end groups [9]. These characteristics make them very attractive for many applications involving the interaction with biomolecules. There are some works showing the interaction of proteins with carbosilane dendrimers. In 2007, Chonco *et al.* [10] studied the interaction between ammonium-terminated carbosilane dendrimers, phosphorothiate oligodeoxynucleotides (ODNs) and bovine serum albumin (BSA), observing that the dendrimer/ODN dendriplex stability depended on electrostatic interactions and that dendrimer protected ODN from BSA interaction. At the same time, Shcharbin *et al.* [11] studied the interaction between the same kind of dendrimers, short ODN and BSA, finding again that the dendriplex formation considerably decreased the interaction of ODNs with the protein. Later, Pedziwiatr *et al.* [12] studied the interaction between ammonium-terminated carbosilane dendrimers and BSA by fluorescence quenching. These publications confirmed the potential of this kind of dendrimers for drug delivery. Additionaly, Montealegre *et al.* [13] proposed the use of carboxylate-terminated carbosilane dendrimers as nanoadditive in electrokinetic chromatography (EKC) to improve protein separations. Moreover, high antiviral activity of carbosilane dendrimers was related to their capacity to strongly interact with some viral and/or cell proteins [14]. Nevertheless, no work has still characterized the interactions between sulphonate-terminated carbosilane dendrimers and different proteins. Protein-dendrimer interactions

can be significantly different from one protein to another due to the heterogenic and complex nature of proteins that can present very different molecular weights, isoelectric points, structure, etc. For that reason, a comprehensive study of these interactions should involve the study and comparison of the interactions of dendrimers with different proteins and the characterization of these interactions. These studies are of great interest in order to find potential applications of carbosilane dendrimers, e.g. in protein sample preparation.

Protein sample preparation is a key step in the analysis of proteins from complex samples e.g. vegetable samples. Protein sample preparation mainly involves the extraction and purification/enrichment of proteins. Both steps are time-consuming and usually require the use of high amounts of solvents that make the process tedious, expensive, non-sustainable and not friendly with environment [15-17]. More suitable procedures are needed at this regard and new materials, as dendrimers, could be a key for the advancement in this area. In fact, dendrimers, in general, could play an important role since it has been demonstrated its interaction with proteins. These interactions could be the base for the designing of supports to specifically retain proteins from a matrix. This approach would be more sustainable and cheaper than usual methods.

The aim of this work was to explore the interactions between sulphonate-terminated carbosilane dendrimers and three different proteins and to identify the main factors affecting these interactions. Modeling of protein-dendrimer interactions with every protein at different pHs will enable to understand the singularities of interactions and the potential of this material in protein sample preparation.

2. Materials and methods

2.1. Chemicals and samples

All chemicals and reagents were of analytical grade. Water was daily obtained with a Milli-Q system from Milipore (Bedford, MA, USA). Tris(hydroxymethyl)aminomethane (Tris) and hydrochloric acid (HCl) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide, trifluoroacetic acid (TFA), bovine serum albumin (BSA), lysozyme from chicken egg white and myoglobin from equine heart were acquired at Sigma-Aldrich (Saint Louis, MO, USA). Sulphonate-terminated carbosilane dendrimers were prepared according to a method described in literature [18]. Dendrimer structures from first to third generation are displayed in **Figure 1**. Computer model for the second generation dendrimer is shown in **Supporting Information (Figure S1)**.

2.2. Methods

All fluorescence measurements were performed in a spectrofluorometer RF-1501 (Shimadzu, Kioto, Japan). It was fixed a λ_{exc} of 279-281 nm and λ_{em} was measured from 290 to 400 nm. Dendrimer solutions at three different pHs (water (pH 6.5), 0.1% TFA (pH 1.8), and 5 mM Tris-HCl (pH 9.0)) were prepared. These solutions were added to three different standard proteins (BSA, lysozyme and myoglobin) at concentrations of 0.10, 0.35, and 0.30 μM, respectively. Blanks with the same concentration of dendrimer, in absence of protein, and at the three pHs were also measured and final fluorescence intensity of mixtures were obtained by subtracting blanks signals. Stern-Volmer equation was applied to analyze fluorescence quenching in proteins:

$$I_0/I = 1 + K_{SV}[Q]$$

where I_0 is the fluorescence intensity of proteins without dendrimer, I is the fluorescence intensity of proteins at the different dendrimer concentrations, K_{SV} is the Stern-Volmer constant and [Q] is the dendrimer concentration. K_{SV} corresponding to the interaction between proteins and dendrimers was presented when there was a linear relationship between Io/I and the [Q].

All data were expressed as mean ± standard deviation of 4 measurements corresponding to two independent samples measured in duplicate.

2.3. Modeling of protein-dendrimer interactions

3D computer models of dendrimer structures were created using a dendrimer builder, as the implemented in the Materials Studio software package from BIOVIA (formerly Accelrys). GAFF force field (Generalized Amber Force Field) [19] was used for parameterization of dendrimers. AM1-BCC technique [20] was employed for calculation of dendrimer atoms partial charges. For this purpose, the Antechamber suite, part of the AMBER 14 software [21], was used. This tool was also employed for assigning force field atom types. QM calculations, needed for partial charges derivation, and calculation of missing force field parameters were done using GAMESS software [22]. Regarding proteins, the following experimentally determined (X-RAY difraction) protein structures were used: BSA (PDB: 4F5S) [23], lysozyme (PDB: 4RLM) [24], myoglobin (PDB: 3RGK) [25]. Furthermore, force field ff14SB was employed for simulations of all proteins.

On the other hand, the *pmemd.cuda* module from AMBER 14 package was employed for Molecular Dynamics simulations [26]. Moreover, the initial configurations of the protein/dendrimer complexes were created using UCSF Chimera

software which was also used for final visualizations [27]. Please see **Supporting Information** for more details.

3. Results and discussion

Interactions between sulphonate-terminated carbosilane dendrimers and proteins were monitored by measuring the intrinsic fluorescence intensity and emission wavelength of proteins. Fluorescence quenching studies are very powerful to explore the accessibility of fluorescence protein probes (mainly tryptophan (Trp) residues) to a quencher. Fluorescence measurements provide information on the molecular environment in the vicinity of fluorescence sites of the molecule [28]. Therefore, variations on fluorescence intensity and maximum emission wavelength could be due to a protein unfolding or to its binding to other molecules. In fact, changes on the fluorescence intensity (due to protein unfolding or binding to other molecules) are protein-dependent and could result in an increased or a decreased fluorescence intensity depending on the protein. Nevertheless, a red-shift in the maximum emission wavelength is always caused by an increasing Trp accessibility [29]. To explore the dependence of these interactions on the protein, three different standard proteins covering different molecular weights and isoelectric points were selected. BSA is a 66.5 kDa protein with acidic isoelectric point (pI = 4.7) and two Trp residues (Trp-134 and Trp-213). Lysozyme is a basic protein (pI = 11.35) with a molecular weight of 14.3 kDa and two dominant Trp residues (Trp-62 and Trp-108). Myoglobin presents an intermediate isoelectric point (pI = 6.8), a molecular weight of 17.8 kDa and two Trp residues (Trp-7 and Trp-14). Sulphonate-terminated dendrimers from first to third generation, 1G (8 functional groups), 2G (16 functional groups), and 3G (32 functional groups), were employed. Sulphonate-terminated dendrimers remain deprotonated and negatively charged at all pHs.

3.1. Study of BSA – sulphonate-terminated dendrimer interactions

Figure S2 in Supporting Information and **Figure 2.A** show the variation of the fluorescence intensity and the maximum emission wavelength of a BSA solution at different pHs (pH 1.8, 6.5 and 9.0) and concentrations of sulphonate dendrimers at three different generations (1G, 2G, 3G). Intrinsic fluorescence of BSA decreased when increasing the dendrimer concentration at all pHs and for all dendrimer generations. When Stern-Volmer equation was used to analyze fluorescence quenching, a linear relationship between the fluorescence intensities ratio (I_o/I) and the dendrimer concentrations was observed at acidic and neutral pH conditions (see **Figure 2.B**). This behavior is common in efficient quenchers [28]. Stern-Volmer constants (K_{SV}) were calculated from the slope of the Stern-Volmer lines and data were grouped in **Table 1**. Stern-Volmer constant informs about the accessibility of the chromophore to the quencher [28]. K_{SV} increased along with the dendrimer generation at acidic and neutral pH, which meant that the accessibility of Trp residues to the dendrimer was greater for the 3G dendrimer than for the 2G and 1G dendrimers. Nevertheless, at neutral pH, K_{SV} constants were lower than the observed at acidic pH which could be explained due to a higher denaturation of BSA and a greater exposition of Trp residues at acidic pH than at pH 6.5. In the case of basic pH, the behavior was totally different observing an initial increase in the fluorescence intensity at low dendrimer generations followed by a decreasing intensity (except for the 3G dendrimer) and it was not possible to apply Stern-Volmer equation. Taking into account just the decreasing part, it is interesting to observe that the decreasing ratio was lower with higher dendrimer generations which could mean a greater accessibility of Trp to the 1G dendrimer than to the 2G or 3G dendrimers. Probably, the reduction of the cationic zone near the Trp when going from

pH 6.5 to 9.0 resulted in an increase in dendrimer-protein repulsion for higher generations of anionic dendrimers (see **Figure S3 in Supporting Information**).

The decrease in fluorescence intensity came along with a wavelength shift at all pHs. This shift in the emission wavelength could be attributed to a structural change in the vicinity around Trp residues promoted by its interaction with the dendrimer. In all cases, an initial blue-shift in wavelength (higher energy) at low concentrations of dendrimer was followed by an increasing wavelength (lower energy) at higher dendrimer concentrations. A similar behavior was observed by Moriyama et al. [30] when studying the interactions of BSA with low and high concentrations of ionic surfactants. A decreasing emission wavelength could indicate that Trp residues environment could become more hydrophobic upon the binding of small amounts of dendrimer. Taking into account that BSA possesses two Trp residues (Trp-134, located in the surface, and Trp-213, buried within the protein), it is likely that a conformational change of the protein might have buried the exposed Trp residue within the protein structure. Nevertheless, when increasing the dendrimer concentration, Trp residues could become more exposed since, according to Moriyama et al. [30], BSA helical structure abruptly decreases. The higher exposition of Trp would explain the increasing maximum emission wavelength at higher concentrations. Contribution to this wavelength variation of the two different Trp residues present in BSA is probably different. In fact, it is likely that the environment of Trp-134 was more affected when adding the dendrimer than that of Trp-213 [30, 31].

Despite the fact that all these results could demonstrate the interaction of sulphonate-terminated dendrimers with BSA, there is no information on the nature of these interactions. Modeling of the interactions between BSA and sulphonate carbosilane dendrimers could be helpful for this task. Modeling at acidic pH was

performed using BSA fragment (118 – 260) since it contains the two BSA fluorophores (Trp-134 and Trp-213) (see **Figure S4 in Supporting Information**). Trp-134 is located in the surface of the molecule in a more hydrophilic environment, while Trp-213 is placed in a hydrophobic cavity [31]. At pH 1.8, where the protein is more unfolded, the modeling showed the possibility of formation of two complexes, Complex I and Complex II (see **Figure 3**), between BSA and the dendrimer. In both cases, dendrimer interacted with BSA near the Trp residues, partially blocking the emission of fluorescence. Since at this pH, BSA is positively charged (estimated net charge = + 97, estimated net charge of the BSA fragment 118-260 = + 25) and the dendrimer is negatively charged, the electrostatic interaction could play an important role here in the complexation process. Additionally, since 3G dendrimer has a higher negative charge than 2G and 1G dendrimers, the interaction with 3G dendrimer could be stronger being this the reason why there is a greater decrease in fluorescence intensity and a higher Stern-Volmer constant.

At neutral and basic pHs, BSA (pH > pI = 4.7) presented a negative net charge of - 17 and - 22, respectively. Nevertheless, modeling showed some local cationic sites (see **Figure S3 in Supporting Information**) that, from an electrostatic point of view, could bind anionic molecules, such as sulphonate dendrimers. This fact could explain the decreasing fluorescence intensity observed at these pHs for BSA. Nevertheless, these binding sites were not so close to Trp residues (~10 Å) as they were at acidic pH which could explain the lower reduction in fluorescence intensity and the lower K_{SV} values obtained at neutral pHs. Additionally, other sites where the dendrimer could eventually bind the protein, for example, by hydrophobic or Van der Waals interactions, could occur. At basic pH, and unlike acidic and neutral pHs, the decrease in fluorescence intensity was sharper for the 1G dendrimer than for the 2G and 3G

dendrimers. This could be attributed to the difficulty (steric and electrostatic impediments) to reach the local positive sites of BSA that are hidden and surrounded with rather anionic environment. These sites are more accessible to low dendrimer generations (small and flexible) than to higher dendrimer generations (bigger, more rigid and more negatively charged). Since, BSA is less negatively charged at neutral pHs, the interaction with local positive sites is more favored and there are more positively charged sites than at basic pH. Monitoring of distance between the dendrimer and BSA during the simulations confirmed creation of stable dendrimer/protein complexes and, in relevant cases, the ability of dendrimer to interact with some Trp residues (see **Figure S5 in Supporting Information**).

3.2. Study of lysozyme – sulphonate-terminated dendrimer interactions

Lysozyme is a 4.6 times smaller size protein than BSA. It presents a higher number of Trp residues and it shows a net positive charge at all tested pHs. **Figure 2S in Supporting Information** and **Figure 4.A** show the variations in fluorescence intensity and maximum emission wavelength of lysozyme, respectively, when different concentrations of 1G, 2G, and 3G dendrimers were added at the three tested pHs. At acidic pH and for the three generations, there was an abrupt decrease in the fluorescence intensity up to a dendrimer concentration of 12, 6, and 3 μM for 1G, 2G, and 3G dendrimers, respectively. Higher dendrimer concentrations did not affect lysozyme fluorescence. This behavior contrasted with BSA that showed a continuous fluorescence decrease. This difference might be due to the differences in size and net charge of these two proteins. While BSA is a relatively large protein (66.5 kDa) with a high positive net charge (+ 97), lysozyme is smaller (14.3 kDa) and presents a much lower positive net charge (+ 17). Thus, lysozyme becomes saturated at smaller concentrations of dendrimer than BSA. Application of the Stern-Volmer model to the decreasing plots

(see **Figure 4.B**) enabled to calculate K_{SV} (see **Table 1)**. According to K_{SV} values, there were more interactions and/or stronger interactions of dendrimers with Trp residues in lysozyme than in the case of BSA. This difference could be related to the better steric (shape/size) conditions for complexation in lysozyme than in BSA and, also, to the significantly higher number of relatively well accessible Trp residues in lysozyme comparing to BSA (6 vs 2). Moreover, the dendrimer-Trp residues interactions increased with the dendrimer generation, like with BSA, since higher dendrimer generations favored electrostatic and Van der Waals interactions between dendrimer and protein. This seems to be more significant for lysozyme since bigger dendrimers may influence simultaneously the surrounding of different Trp residues than smaller dendrimers. At neutral and basic pHs, there was not a significant variation in fluorescence intensity, especially at higher dendrimer concentrations. This earlier protein saturation could be mainly related to a smaller positive protein net charge (+8 at neutral pH vs +17 at pH 1.8). Regarding emission wavelengths, it was not observed a significant variation. These results could suggest that interactions between lysozyme and dendrimer at neutral and basic pHs, if any, were very weak.

Modeling enabled to observe an electrostatic interaction between lysozyme (positively charged) and the dendrimer (negatively charged) at acidic pH. Indeed, lysozyme yielded two stable complexes (**Figure 5**) in which dendrimer interacted close to Trp (hydrophobic) residues blocking the emission of fluorescence. At neutral and basic pHs, modeling showed that the dendrimer is able to create three stable complexes with lysozyme but they are likely to be less stable due to the smaller positive charge of the protein at these pHs in comparison to low pH (see **Figure 5**). More folded and rigid structure at neutral and basic pHs can also induce lower accessibility of Trp residues. Monitoring of the distance between the dendrimer and lysozyme during computer

simulations confirmed the establishment of stable dendrimer/protein complexes and, in relevant cases, the ability of dendrimer to interact with some Trp residues (see **Figure S6 in Supporting Information**).

3.3. Study of myoglobin – sulphonate-terminated dendrimer interactions

Myoglobin is a protein with a molecular weight between those of lysozyme and BSA and with an isoelectric point close to 7. A singular feature of myoglobin is its heme group. This heme unit is attached to the apomyoglobin at neutral and basic pHs. Nevertheless, at acidic pH, this group is not attached and the apomyoglobin behaves as an ordinary protein [32]. **Figure 2S in Supporting Information** and **Figure 6.A** show the variation of the fluorescence intensity and maximum emission wavelength, respectively, at the three tested pHs for the three dendrimer generations. At acidic pH, there was a sharp decrease in fluorescence intensity up to a dendrimer concentration of 12, 6, and 3 μM for 1G, 2G, and 3G dendrimers, respectively, followed by a smoother decrease at higher dendrimer concentrations. This behavior is similar to the observed with BSA and lysozyme at acidic pH. According to K_{SV} constants, calculated using decreasing points (see Stern-Volmer representation in **Figure 6.B**), presented in **Table 1**, there is a more significant interaction between dendrimer and Trp residues (and/or their surrounding) in myoglobin than in BSA or lysozyme at low pH, which is probably related to the excellent accessibility of Trp residues and the overall cationic character including Trp residues nearby the area (see **Figure S7 in Supporting Information)**. Also, the high flexibility of this molecule at low pH enhances the creation of very stable complexes which are likely more stable than in the case of BSA or lysozyme (see **Figure S8 in Supporting Information**). Moreover, these interactions increased with the dendrimer generation as previously observed for BSA and lysozyme. Regarding emission wavelengths, they showed a sharp decrease followed by a smooth increase at

increasing dendrimer concentrations. This behavior could be due, like in BSA, to a change in the exposure of Trp residues, in this case, at protein:dendrimer molar ratios of 1:8, 1:4, and 1:2 for 1G, 2G, and 3G, respectively. Since, apomyoglobin possess an exposed Trp residue (Trp-7) and a buried Trp residue (Trp-14), a conformational change of the protein might have buried the exposed Trp residue within the protein structure [33].

Unlike previous proteins, myoglobin showed a huge increase in emitted light intensity at neutral and basic pH when adding the dendrimer. This increase in fluorescence intensity came along with a significant increase in emission wavelength (increase in the released energy). In order to explain this singular behavior, it is important to take into account that apomyoglobin, at basic and neutral pH, is attached to the heme group and that it has been documented an energy transfer from Trp residues to the heme group when they are close [29]. The increase in fluorescence intensity observed when adding the dendrimer could be explained if the dendrimer was binding very close to the heme group. This binding would decrease the energy transfer from Trp residues to the heme group increasing the intensity of emitted light by Trp.

Modeling of myoglobin-dendrimer interactions at acidic pH is shown in **Figure S8 in Supporting Information**. As mentioned above, we can see that at this pH the dendrimer strongly interacted with the apomyoglobin (heme group is not attached) stabilizing its conformation and creating a stable complex. Simulation demonstrated also the possibility of a very good contact of Trp residues with dendrimer, which is most likely the main reason for such high K_{SV} value (see **Figure S9 in Supporting Information**).

Regarding neutral and basic pHs, four potential binding sites in myoglobin were tested although only in two cases, stable complexes were formed (Complex I and II, see

Figure 7). Complex I binding was mainly based on hydrophobic interactions, as we can deduce from the tight clasp of the hydrophobic inner part of the dendrimer and the protein surface, while terminal sulphonate groups were more involved in Complex II binding. Unlike acidic pH, there was no distinct change in protein structure as a result of the dendrimer binding at neutral and basic pHs (see **Figure S10 in Supporting Information**). Monitoring of the distance between the dendrimer and myoglobin during the computer simulations confirmed the creation of stable dendrimer/protein complexes and, in relevant cases, the ability of dendrimer to interact with Trp residues or the heme group (see **Figure S11 in Supporting Information**).

4. Conclusions

No generalized behavior was observed when studying the interactions between sulphonate-terminated carbosilane dendrimers and proteins. Acidic pHs favored the interactions between proteins and dendrimers. Since, at this pH, all proteins are positively charged and dendrimer negatively charged, electrostatic forces probably play an important role in these interactions. Also, higher flexibility of protein molecules at acidic conditions supports stronger protein-dendrimer interactions. Moreover, accessibility of Trp residues is better at low pH as indicated by experimental results and clearly showed using simulated protein structures. Strength of interactions could not be correlated with the protein molecular weight or charge observing a higher affinity of myoglobin to the dendrimer followed by lysozyme and BSA. In all cases, the strength of the interaction increased with the dendrimer generation at acidic pH.

Most significant differences on protein-dendrimer interactions were observed at neutral and basic pHs. Despite its negative net charge, BSA seemed to interact with the

dendrimer by electrostatic forces due to local positive sites. In the case of lysozyme, no quenching was detected at pH 7.4 and 9.0, however, the ability of dendrimer to bind lysozyme was clearly demonstrated using computer modeling. Comparing to low pH case, the protein-dendrimer interaction should be weaker since the estimated protein net charge at low pH is +17, while at pH 7.4 and 9.0 is +8. Myoglobin resulted in a huge increase in fluorescence intensity attributed to interactions with dendrimer near the heme group that affected the energy transference established between Trp residues and the heme group. Interactions between proteins and sulphonate-terminated carbosilane dendrimers, especially at acidic pH, could be the base for proposing a new approach in protein sample preparation based on the application of dendrimers for the selective retention of proteins in a matrix. Nevertheless, further studies are still required for their efficient and reliable application. These studies could be the aims of further researches.

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Figure Captions

Figure 1. Chemical structure of the first, second and third generation of the sulphonate-terminated carbosilane dendrimer.

Figure 2. Variation in BSA maximum emission wavelength observed when adding different dendrimer concentrations at pHs 1.8, 6.5, and 9.0 **(A)** and Stern-Volmer representation obtained at pHs 1.8 and 6.5 **(B)**. Three dendrimer generations are

presented: 1G, 2G, and 3G. Error bars show the standard deviation corresponding to two independent samples measured in duplicate.

Figure 3. Structures of Complex I and II obtained by modeling the interaction between BSA and sulphonate-terminated carbosilane dendrimer at pH 1.8.

Figure 4. Variation in lysozyme maximum emission wavelength observed when adding different dendrimer concentrations at pHs 1.8, 6.5, and 9.0 **(A)** and Stern-Volmer representation obtained at pH 1.8 **(B)**. Three dendrimer generations are presented: 1G, 2G, and 3G. Error bars show the standard deviation corresponding to two independent samples measured in duplicate.

Figure 5. Structure of the different complexes formed between lysozyme and sulphonate-terminated carbosilane dendrimer at acidic pH (two complexes) and at neutral and basic pHs (three complexes).

Figure 6. Variation in myoglobin maximum emission wavelength observed when adding different dendrimer concentrations at pHs 1.8, 6.5, and 9.0 **(A)** and Stern-Volmer representation obtained at pH 1.8 **(B)**. Three dendrimer generations are presented: 1G, 2G, and 3G. Error bars show the standard deviation corresponding to two independent samples measured in duplicate.

Figure 7. Structure of the two complexes formed between sulphonate-terminated dendrimer and myoglobin obtained by simulation at neutral and basic pHs.

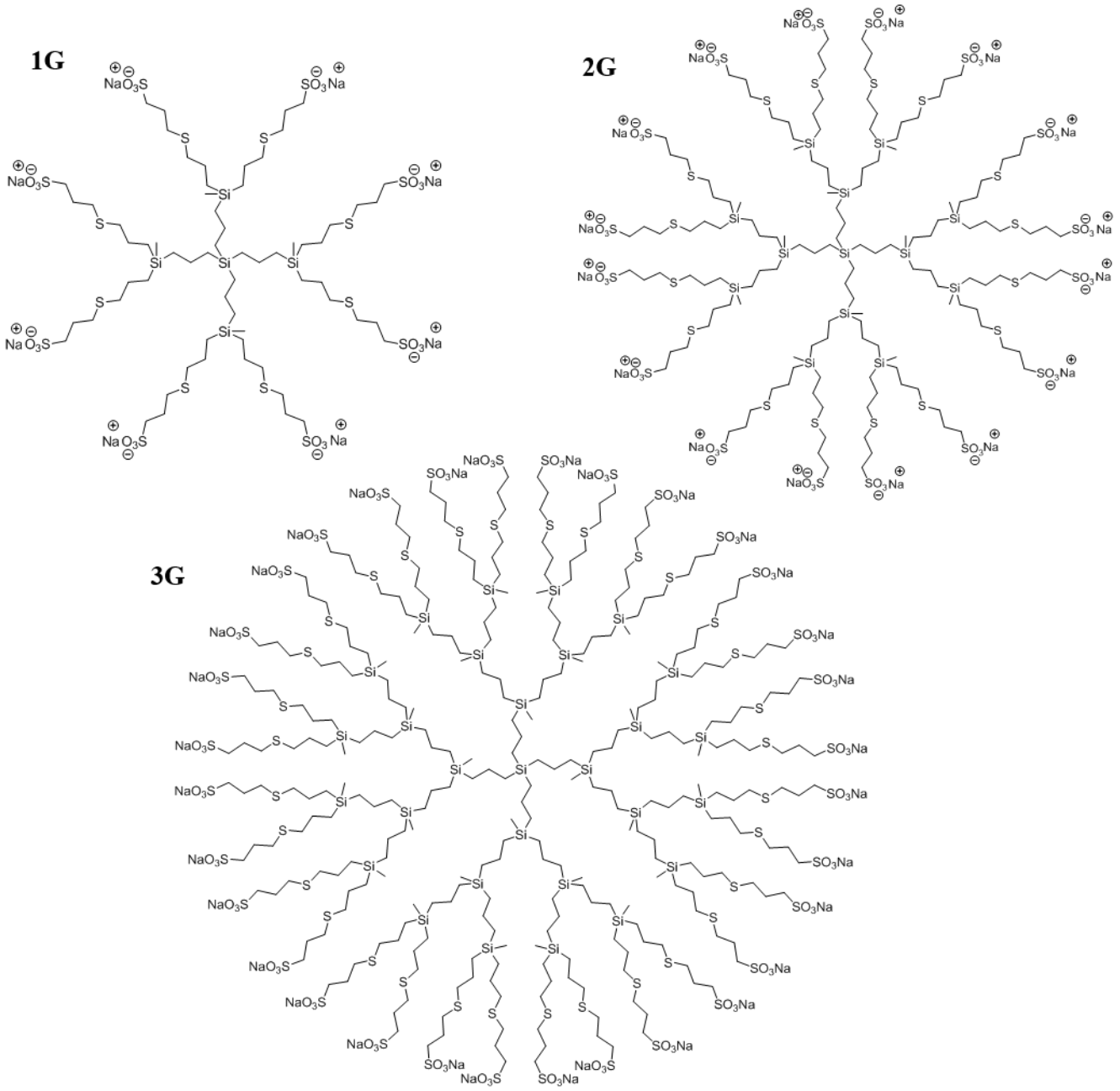

Figure 1

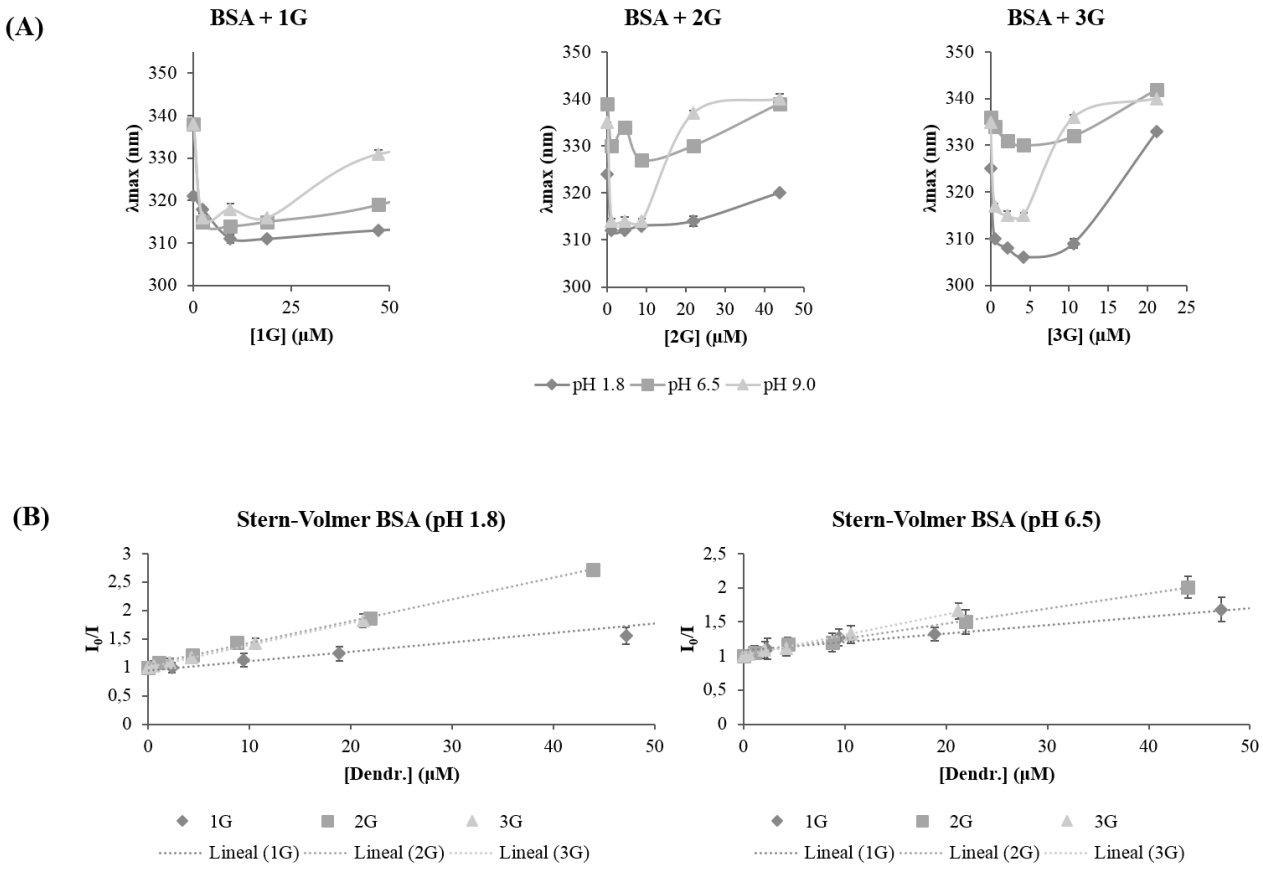

Figure 2

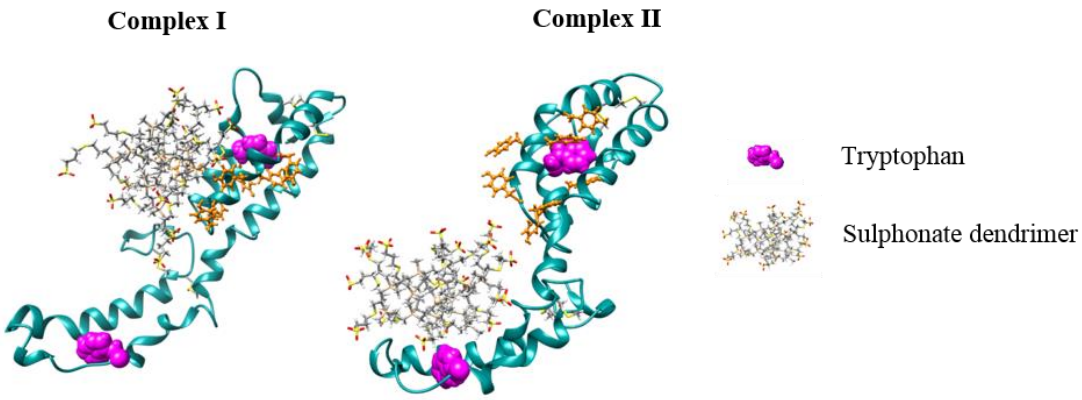

Figure 3

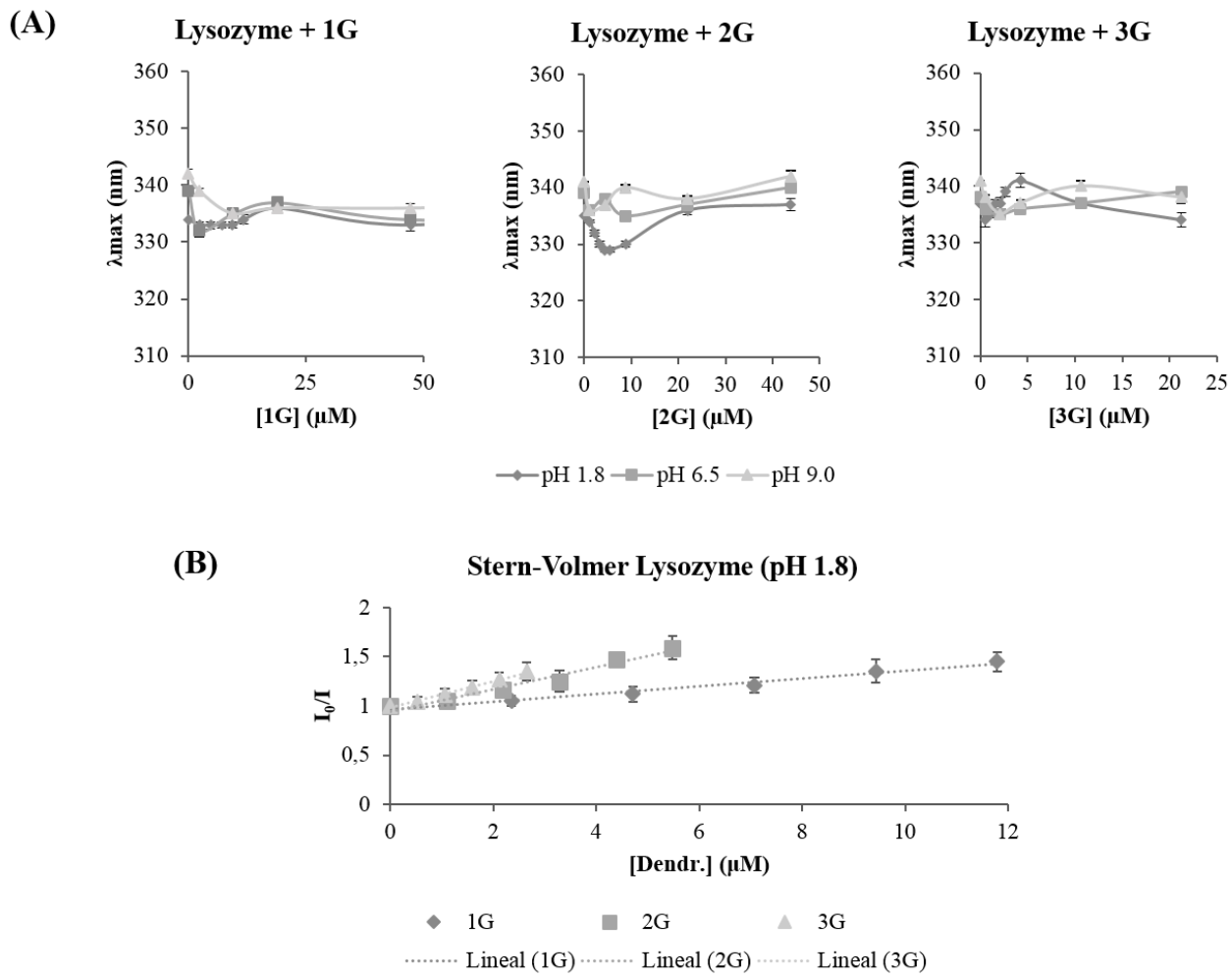

Figure 4

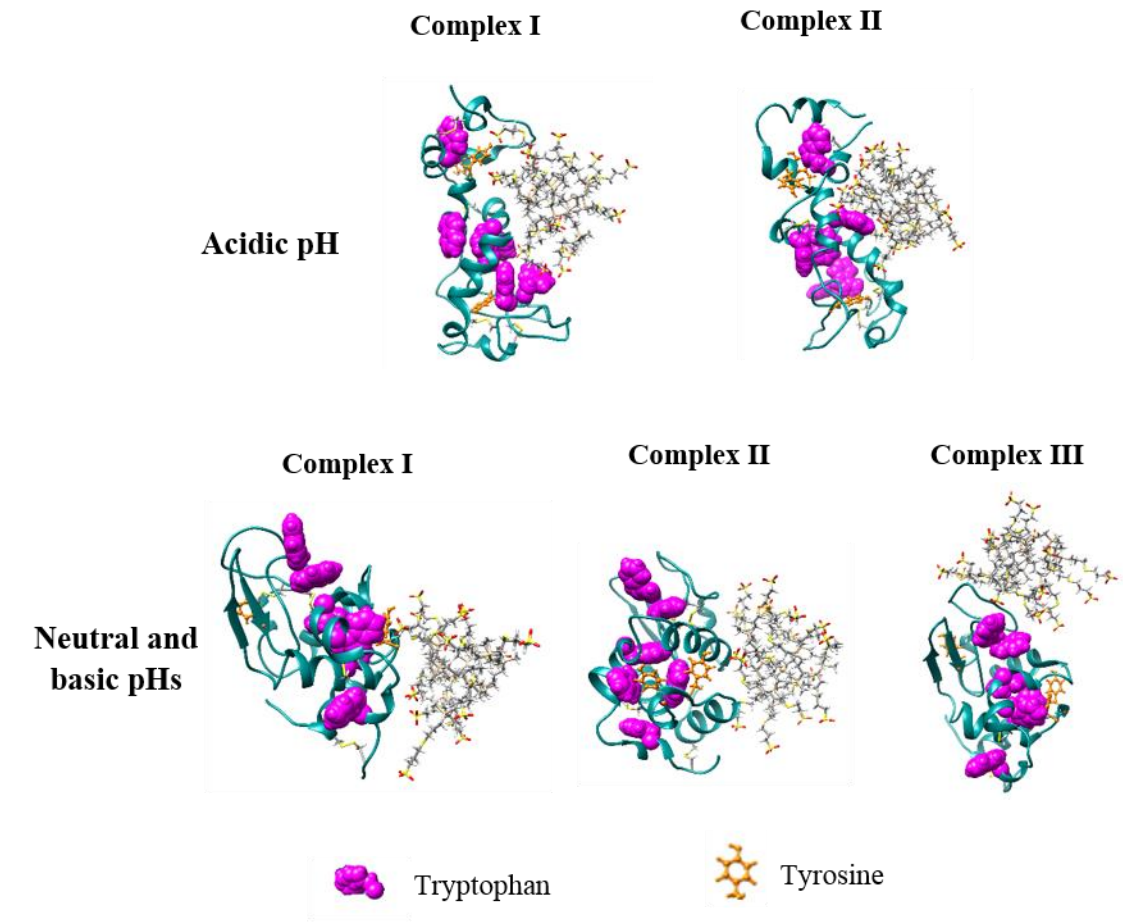

Figure 5

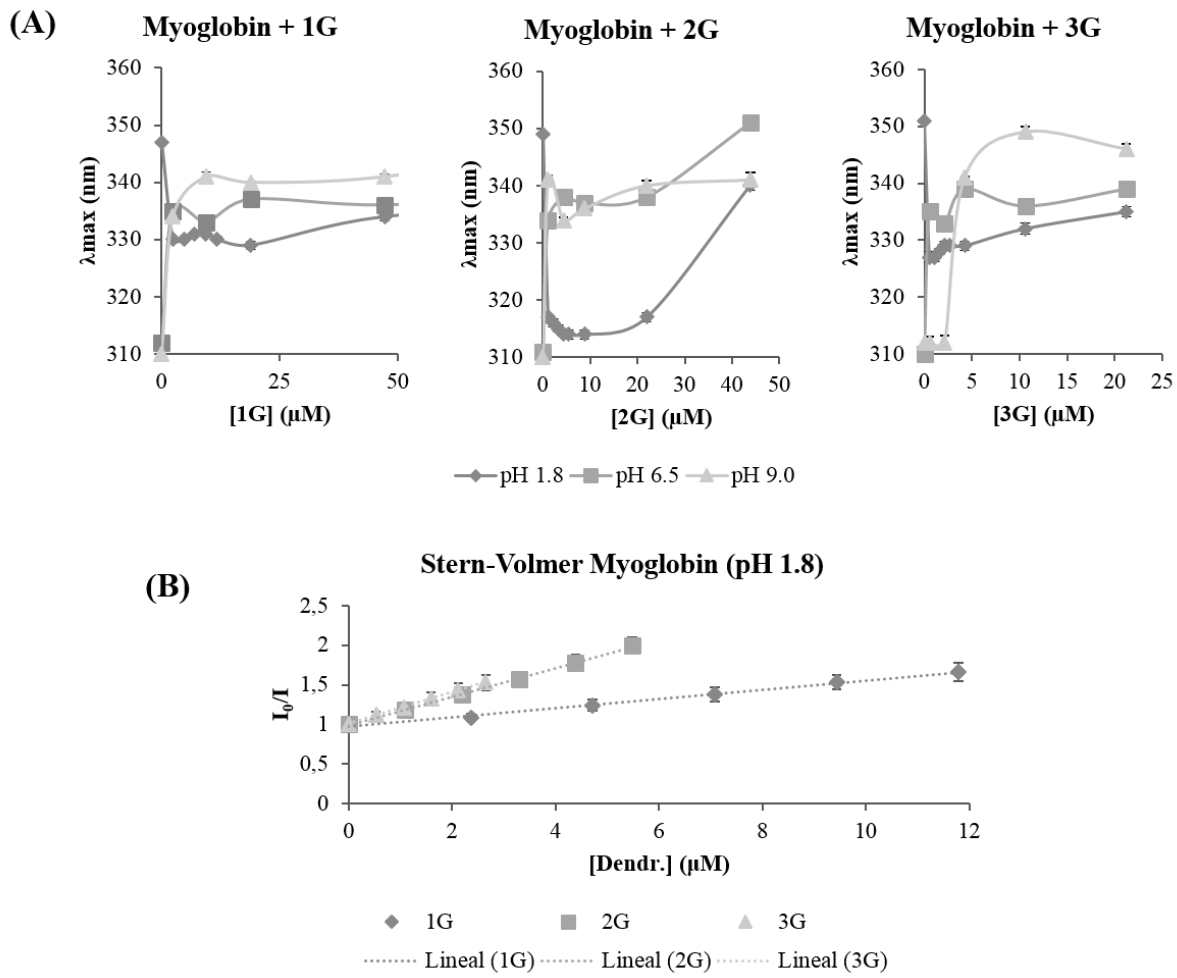

Figure 6

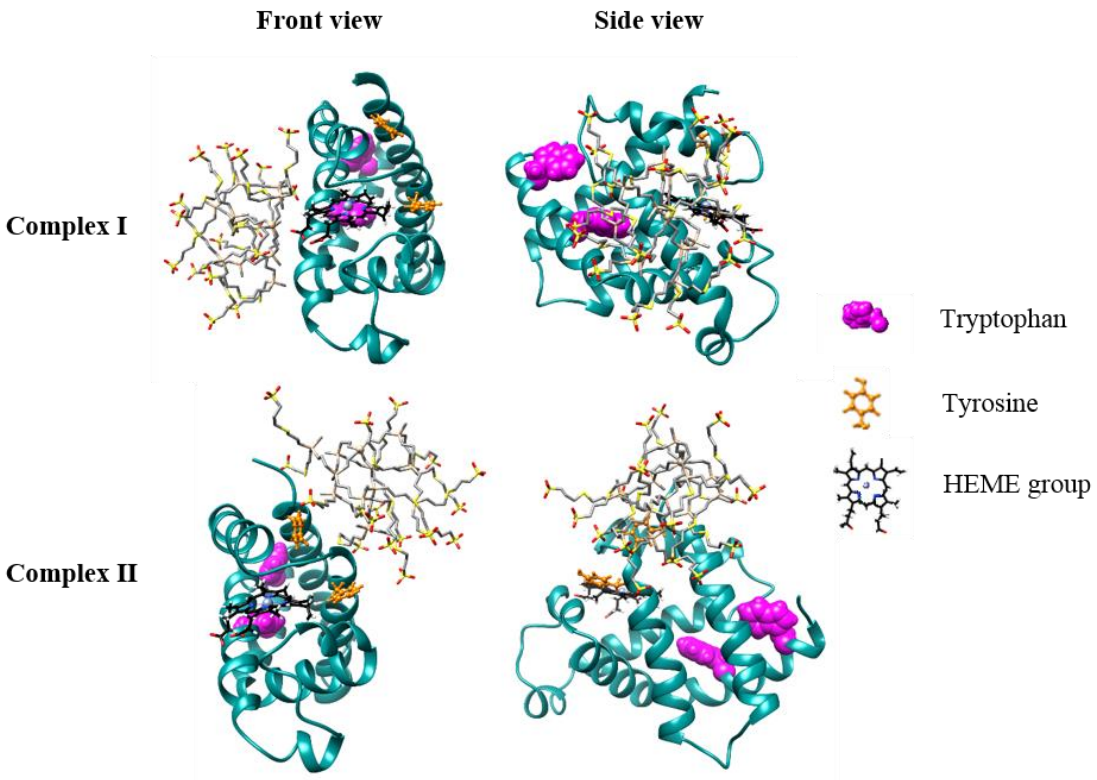

Figure 7

Table 1. Stern-Volmer constants corresponding to the quenching of proteins fluorescence by sulphonate-terminated carbosilane dendrimers at three different pHs (1.8, 6.5, and 9.0). All data were expressed as mean ± standard deviation corresponding to two independent samples measured in duplicate.

Protein	**Dendrimer generation**	**Molecular weight (kDa)**	**Stern-Volmer constant (K_{SV}) (M^{-1} x 10^3)**		
			pH = 1.8	**pH = 6.5**	**pH = 9.0**
BSA	1G	2.12	16.4 ± 1.2	12.7 ± 1.0	NQ
66.5 kDa	2G	4.56	37.4 ± 5.7	22.8 ± 6.1	NQ
pI = 4.7	3G	9.43	38.3 ± 6.0	31.3 ± 5.3	NQ
Lysozyme	1G	2.12	39.2 ± 3.3	NQ*	NQ
14.3 kDa	2G	4.56	112.4 ± 9.2	NQ	NQ
pI = 11.35	3G	9.43	134 ± 13	NQ	NQ
Myoglobin	1G	2.12	58.1 ± 3.7	NQ	NQ
17.8 kDa	2G	4.56	187 ± 13	NQ	NQ
pI = 6.8	3G	9.43	195 ± 12	NQ	NQ

* No quenching.