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# CHARGE EFFECTS IN WATER SOLUTION OF A LOW GENERATION SODIUM CARBOXYLATE TERMINATED DENDRIMERS

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Abstract. Dendrimers, a class of novel nano-sized materials characterized by a highly ramified structure and a relevant number of functional surface groups, represent a versatile platform for a wide range of nanotechnology applications. The number and characteristic of their modifiable surface groups strongly influence the solution properties as well as some relevant processes involved in molecular recognition, signal processing as well as for binding various targeting or guest molecules. Owing to these properties dendrimers have attracted the interest of the researchers in the development of new prototypes implicated in many technological applications. However, despite of their recent extensive studies, their colloidal stability in solution environment have been relatively less investigated. With the aim to furnish useful insight about the inter-dendrimer interaction in solution, a Small Angle X-ray Scattering (SAXS) structural investigation on the sodium carboxylate terminated (-COO-Na) generations G1.5 of poly(amidoamine) dendrimers in water solution has been performed. The presence of a pronounced interference peak in the SAXS spectra in a wide range of concentrations gives evidence of a long range electrostatic interaction which has been ascribed to the ionisation of the surface carboxylate terminal end-groups. The experimental inter-dendrimer structure factor S(q) has been analysed in the framework of liquid integral equation theory. From that, we derived an effective inter-particle interaction composed of a screened Coulombic plus hard-sphere repulsion potential, which allows the estimation of the dendrimer effective surface charge $Z_{eff}$. The present analysis strongly supports the finding that colloidal stability and interaction of dendrimers in solution environment is strongly influenced by charge effects.

## 1. Introduction

Recently there has been increasing interest in the study of nanoparticles with novel structural and topological features for application in material science and biotechnology (Foster 2005). In addition to the chemical synthesis, the non-covalent (soft) interaction has been widely exploited for the design of functional materials with nano-scale organisation of structures (Hamley 2003; Hu *et al.* 2014; Lombardo *et al.* 2016a). Moreover, the hierarchical organization of nanostructures and their controlled properties opened up many possibilities for the design and device fabrication of innovative materials in a wide range of (nano-)technology applications (Nalwa 2004; Wegst *et al.* 2015; Pasqua *et al.* 2019).

However, it is necessary to achieve a deeper understanding of the main structural and dynamic properties of these nanoscale materials and their associated self-assembly processes before their transformation into functional nanodevices (Wang *et al.* 2009; Marguet *et al.* 2013; Liveri *et al.* 2018; Lombardo *et al.* 2019b). This allow to decipher the complexity of structure–function relationship, as well as to realize the practical utilization of those nanostructures (Calandra *et al.* 2010, 2015a,b; Zhang *et al.* 2017). One of the most studied smart nanomaterial in the field of nanotechnology is given by a special class of polymer-based nanoparticles, called dendrimers (Tomalia *et al.* 1985). This class of macromolecules present a regularly branched dendritic structures with well-defined number of end-groups, dimension and surface functionalization. Moreover the number and characteristic of the modifiable surface groups are the real responsible of much of the solution properties as well as for some relevant processes involved in molecular recognition, signal processing as well as for binding various targeting or guest molecules (Matthews *et al.* 1998; Abbasi *et al.* 2014). Moreover, the tunable interaction of dendrimers charged moieties (due to the presence of both internal and external chargeable groups) promises the possibility of controlling the dendrimer molecular conformation by varying the conditions of the solutions, and may lead to the creation of new types of molecular assemblies with controlled morphologies (*dendrimer-based assemblies*) (Bonaccorsi *et al.* 2009; Medina and El-Sayed 2009; Kono 2012; Lombardo 2014).

In this paper we discuss the results of a structural investigation in water solution of generation G1.5 poliamidoamine (Pamam) dendrimers by means of the Small-Angle X-ray Scattering (SAXS) technique. More specifically we point out to the important role of the charge ordering effects in water solution caused by the presence of chargeable internal and terminal groups in the macromolecule. The experimental structure factor is investigated in the framework of the Ornstein-Zernike integral equation and allow to obtain important information of the inter-dendrimer interaction in solution.

## 2. Experimental Section

**Materials.** Pamam dendrimers of generation G=1.5 ($M_w$=2935 g/mol) were purchased from Aldrich Chemical Co. As schematically shown in Figure 1, the investigated system consists of a tetrafunctional ethylenediamine core $[> NCH_2CH_2N <]$ and $[-CH_2CH_2(C = O)NHCH_2CH_2N <]$ spacers and is terminated at the final generation with 16 sodium carboxylate terminal groups ($COO^-$ $Na^+$) in average. The dendrimers were dispersed in deionised water, while the obtained solutions were filtered with Teflon filters (filter diameter was D=0.02 $\mu$m). The solutions were also checked by dynamic light scattering prior to SAXS measurements to remove the presence of possible aggregates in the system.

**Methods.** The Small Angle X-ray Scattering (SAXS) patterns have been recorded by a laboratory instrumentation consisting of a Philips PW X-ray generator (providing Cu K$\alpha$, Ni-filtered X-ray radiation of wavelength 1.5418 Å) with a Kratky-type small-angle camera in the “finite slit height geometry”. The range of scattering vector covered is 0.01 Å$^{-1}$ < q < 0.5 Å$^{-1}$. All measurements were carried out at the temperature of T = 20°C. The scattering intensities I(q) from the samples, detected by a gas-type linear detector, where corrected for the incident beam decay, sample thickness, and transmission. The background scattering from the solvent was also subtracted.

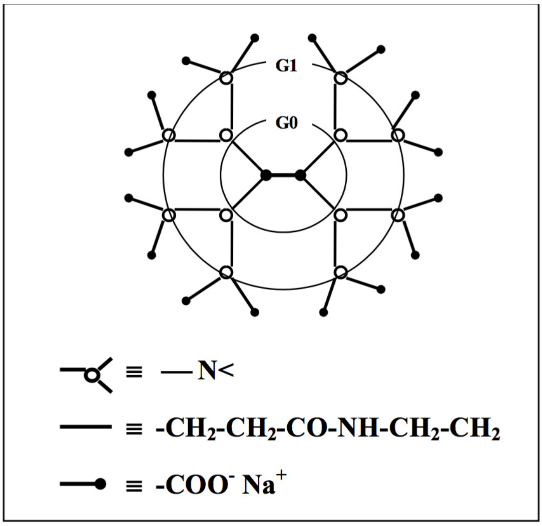

FIGURE 1. Schematic representation of a four-armed Pamam dendrimers. The precursor generations, G0 and G1, are identified to emphasize the iterative process of dendrimer synthesis.

## 3. Results and Discussion

In order to obtain valuable information about the structure and interaction of our investigated system a set of SAXS measurements has been carried out in the range of polymer mass fraction between $1.0 \leq c \leq 10.0$ wt%. In Figure 2 the SAXS spectra of the G1.5 Pamam dendrimers are presented for the two different mass fractions of c=5 wt% of and c=10 wt%. The figure evidence the presence of a pronounced interference peak which is indicative of a long range structural order in the system due to inter-particle interaction in solution.

Assuming the dendrimer solution as a monodisperse system, the SAXS scattering intensity I(q) can be expressed as a product of the form factor P(q), which contains information on the shape and dimension of the scattering particles and the structure factor S(q) describing the inter-particle interaction (Glatter and Kratky 1982):

$$I(q) = N(\Delta\rho)^2 P(q) S(q) \tag{1}$$

where N is the number density of the particles, and $\Delta\rho = (\rho - \rho_0)$ is the so-called "contrast" (i.e., the difference between the scattering length density of the particle $\rho$ and that of the solvent $\rho_0$). In the dilute region the inter-particle interaction can be neglected (i.e. S(q)=1), so that the analysis of scattering intensity I(q) can furnish direct information of morphological features of the scattering particles. The expression of the sphere form factor $P(q) = [3J_1(qR)/(qR]^2$ (where R is the sphere radius and J1(x) = [sin(x)-xcos(x)]/x2 is the first-order spherical Bessel function) has been used to fit our data for the concentration of c=1wt%, where inter-particles interference effects are assumed to be negligible.

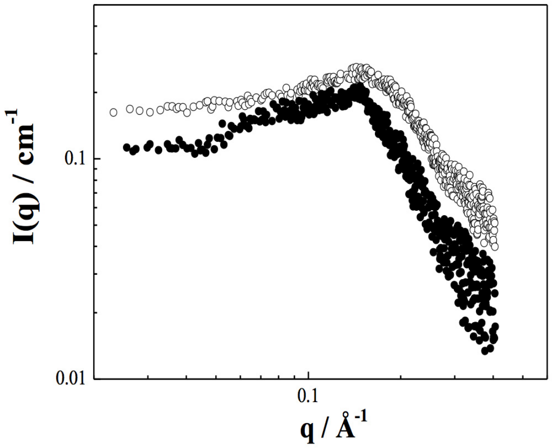

FIGURE 2. SAXS profiles of water solution of G=1.5 Pamam dendrimers for the two different concentration of c=5 Wt% (filled circles) and c=10 Wt% (hollow circles).

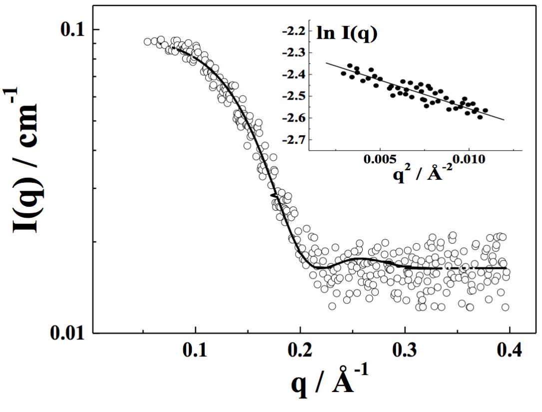

FIGURE 3. SAXS form factor data analysis for the water solution of Pamam dendrimers generation G=1.5 at the mass fraction of c=1 wt%. In the inset is shown the Guinier analysis for the evaluation of the radius of gyration $R_g$ for the same sample

Results of the fitting, as reported in Figure 3 for the sample at concentration c=1%, show that the scattering data are well reproduced in all the q range. Information about the dendrimer radius of gyration $R_g$ has also been obtained from the slope of $lnI(q)$ $vs$ $q^2$ in the so called Guinier region (i.e. for $qR_g \ll 1$), where the particle form factor can be expressed as

$$P(q) \cong P(0)exp\left(-\frac{q^2R_g^2}{3}\right) \tag{2}$$

The results of the form factor data analysis for all the studied concentrations are summarized in the above inset of Figure 3 . The obtained results in the dilute regime furnish an average values of R=9.4 Å for the sphere radius and of $R_g$=8.6 Å for the radius of gyration. At higher concentration (i.e. for $c \geq 2\%$) a source of uncertainty in the determination of $R_g$ is connected with the presence of the structure factor S(q) contribution to the SAXS spectra. The existence of dendrimer chargeable groups leads also to inter-molecular interactions which may be strong enough to influence both the long range structural and dynamic properties of the dendrimer solutions. The observation of the structure factor peak at the higher concentrations, in fact, can be mainly ascribed to a partial ionisation of the dendrimer carboxilate ($COO^-Na^+$) terminal groups (Micali *et al.* 1998). The generated dendrimer charge in water solution exert a long range electrostatic repulsive interaction, leading to a consequent ordering effect throughout the system. Intermolecular interactions between dendrimers in solution has been recently investigated by means of Small-Angle Neutron Scattering (SANS) technique upon the addition of acid (Scherrenberg *et al.* 1998; Nisato *et al.* 1999). In that case the long range interaction potential has been ascribed to the protonation of the amino end groups ($NH_2$) in the integer generation dendrimers. The investigation of the inter-particles interactions and the main effects of their synergistic combination represent a fundamental aspect in the study of the complex phenomena in many-body systems of soft nanomaterials (Lombardo *et al.* 2004b; Walkey and Chan 2012; Ji and Walz 2015; Brown *et al.* 2018). The unique and emerging properties of novel nanomaterials is in fact a direct consequence of specific combination of forces, that play a crucial role in determining the morphology and architecture as well as the functions of materials (Calandra *et al.* 2004b; Northrop *et al.* 2009; D'Angelo *et al.* 2017). The study of this topic, represent then a first important step for the understanding of the complex cooperative behaviour in nano-materials and their correlated properties, as demonstrated by different investigations (Calandra *et al.* 2000; Mallamace *et al.* 2001; Longo *et al.* 2006; Wang *et al.* 2014). In our case, indirect information about the inter-dendrimers interaction can be obtained by the analysis of the SAXS structure factor S(q). A schematic representation of the model adopted for the inter-dendrimer interaction is shown in Figure 4 .

According to this model a hard sphere type repulsive component for the potential has been adopted to represent the close contact inter-dendrimer interaction. Far from the close contact, the dendrimers (which are approximated as impenetrable spheres of diameter $\sigma$=2R whose charge $Z_0 \cdot e$ is distributed on the surface) interact via a screened Coulombic repulsive potential given by (Hunter 1986).

$$U(r) = \frac{Z_0e^2}{4\pi\varepsilon(1+\kappa\sigma)^2}\frac{e^{-\kappa(r-\sigma)}}{r} \tag{3}$$

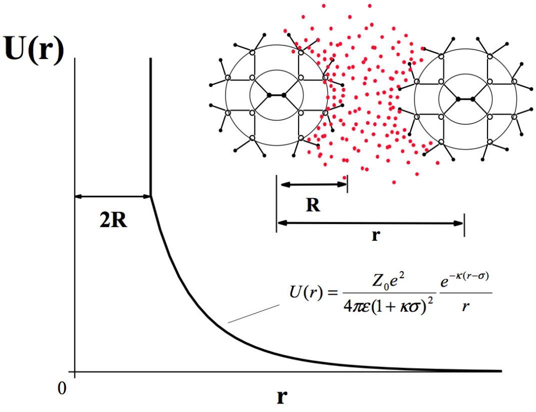

FIGURE 4. Schematic representation of the adopted inter-dendrimer interaction potential

Here $\kappa$ is the Debye-Huckel inverse screening length which is determined, at a given temperature T, by the ionic strength I of the solvent (in mol/l), according to the following relation:

$$\kappa = \sqrt{\frac{8\pi e^2 N_a I}{\varepsilon K_B T \times 10^3}} \tag{4}$$

Here $e$ is the unit of electron charge, $K_B$ the Botzmann constant, $N_a$ the Avogadro number, $\varepsilon$ the dielectric constant. Different solutions schemes, deduced from the statistical mechanics of simple liquids, and based on the solution of the Ornstein-Zernike equation (OZ), has been proposed in the past years to calculate SANS and SAXS intensities in charged macromolecular solutions (Hansen and McDonald 1986). According to this schemes the equilibrium structural properties of a macroion solution are computed by numerical methods starting from the knowledge of some structural parameters, such as the particles concentration C (in mol/l), the effective charge in Ze and the particle diameter $\sigma$=2R. In our specific case we have investigated the observed inter-dendrimer structure factors S(q), obtained by SAXS experiments, in terms of the *hypernetted chain* approximation scheme (HNC) (Hansen and McDonald 1986). The structure factor for a system of interacting particles can be written as

$$S(q) = 1 + \int_0^\infty 4\pi^2 \rho_C [g(r) - 1] \frac{sin(qr)}{(qr)} dr \tag{5}$$

where $\rho_C = C/M$ is the particle number density (number of particles per unit volume). This last relation provide a way to relate the structure factor S(q) with the radial pair correlation function g(r) (i.e. the probability that two particles stay at distance r in the system), and then, by means of the HNC scheme, to the interparticle potential U(r).

In Figure 5 the numerical structure factor S(q) computed according the HNC scheme has been compared with the experimental structure factor obtained from SAXS spectra

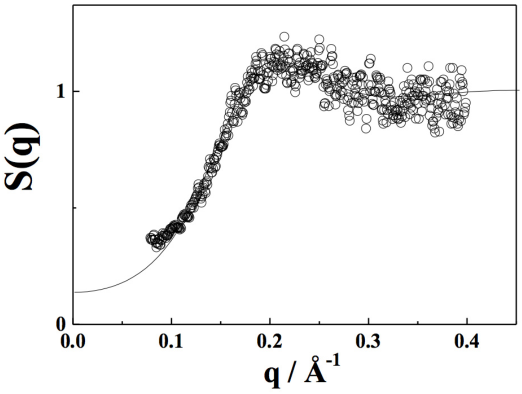

FIGURE 5. Comparison between the experimental structure factor S(q) (for the sample at polymer mass fraction of c=10 Wt%) and the one computed by means of the adopted model.

for the sample at mass fraction of W=10%. More specifically data analysis according the adopted theoretical model consider the experimental structure factor S(q) which is obtained by dividing the SAXS spectra at the higher concentrations by the form factor P(q) relative to the SAXS intensity profile of the sample at mass fraction of c=1 wt% (for which the contribution of the structure factor is assumed equal to S(q)=1). From the figure it is clear how the adopted model reproduce quite satisfactorily the experimental results with an average dendrimer effective charge of $Z_eff = 10.5 \pm 1.0$ (in unit of electron charge e).

From the finding our obtained results we can deduce that the carboxilate terminal groups of Pamam half integer dendrimers in water solution are partially dissociated (COO- Na+). In particular an average number of 10.5 over the 16 total carboxylic groups per dendrimer (for the G=1.5 generation) realize this ionization. This result show that the generation g=1.5 Pamam dendrimers present in water solution a degree of ionisation which is around 65%. In this respect the condensed counterions at the surface of the dendrimer preserve, with the neutralizing action of their charge compensation, the local electroneutrality within the dendrimer in solution. The consequent long range electrostatic repulsive interaction lead to an ordering effect throughout the system.

A rather different result has been obtained in our previous SAXS investigation of the half integer sodium carboxylate terminated Pamam dendrimers of generation G3.5 (Lombardo 2009). In that case, in fact, analysis of the SAXS experiments in a wide range of concentrations revealed a weaker ionisation process due to the dendrimer chargeable carboxylic end-groups which was less than 10% in methanol solution (Micali *et al.* 1998) and about 40% in water solution (Lombardo 2009). The difference in the detected degree of ionisation state a different screening efficiency in solution of Pamam dendrimers of generation G1.5 with respect to generation G3.5. This effect can be traced back to a different surface charge density (number of surface charge moieties per dendrimer surface unit) of the two species. In this sense the congestion of the charged surface moieties with increasing dendrimer generation promote a sensitive localization of $Na^{+}$ counterions in the vicinity of dendrimer surface. This circumstance confirms the finding that that great part of structural and interaction properties of starburst dendrimers in solution is regulated by the electrostatic nature of the interactions of the dendrimer chargeable moieties with the solvent molecules. The dendrimers surface charge represent an important parameter that regulate the interaction with biological components of living systems. Recent investigations have shown how the effective dendrimers charge represents a crucial control parameter that plays an important role in the self-assembly processes involving dendrimer-(bio-)membrane interaction (Lombardo *et al.* 2016b, 2018). Size, concentrations and surface functionality (and their charge expression in solution) are the main control parameters which determine the fundamental interaction between nanostructured materials and biological systems, as demonstrated in different research investigations employing smart nanomaterials (Calandra *et al.* 2004a; Kiselev *et al.* 2008; Albanese *et al.* 2012; Kiselev *et al.* 2013; Dai *et al.* 2017; Kiselev and Lombardo 2017; Lombardo *et al.* 2019a). In this perspective, novel nanostructured systems (nanoparticles) has been recently developed by exploiting the biological microenvironment (such as ionic strength, pH, enzyme concentration) to enhance the therapeutic actions in diseased tissues (Lombardo *et al.* 2004a; Bourgaux and Couvreur 2014; Ma and Zhao 2015; Scialabba *et al.* 2017). Alternatively, key parameters in biological systems can be controlled by external stimuli (such as temperature, light, magnetic triggers) (Mura *et al.* 2013; Molla *et al.* 2015). However, although external stimuli responsive systems are easier to be controlled, they may present major problems and critical issues connected with their safe use in biological systems, as in the case of the exposure to electromagnetic fields that may have sensitive influence on cell components (Calabrò *et al.* 2013; Thakur and Sahu 2016; Calabrò and Magazù 2018a,b,c).

The main finding of our investigation evidence that together with the size and topology, the dendrimers effective charge (and surface functionality) regulate the dendrimer electrostatic interaction in solution environment. This highly controllable parameter allows a wide range of biochemical and nanotechnology applications of dendrimers-based nanostructures. For example, electrostatic conjugation of chemical species into the dendrimer surface gives rise to the development of new prototypes that can function as detecting, targeting, or imaging agents (Tomalia *et al.* 1985; Matthews *et al.* 1998; Abbasi *et al.* 2014). Moreover, the mechanisms of interaction between dendrimers and bio-memmbranes, strongly depend on the force balance between charged dendrimers and the charged (or zwitterionic) lipids of the bio-membranes ( Figure 6A). This interaction highlights some critical issues connected with possible disruptive effect of polymer–based (soft) nanoparticles during the interaction with biological membranes and that may be associated with unwanted cytotoxicity effects of the nanoparticles/dendrimers inclusion process (Lombardo *et al.* 2016b, 2018).

Particularly interesting is the electrostatic interactions between charged dendritic nanoparticles and polyelectrolytes systems, as the corresponding complex formation represents a basic nano-platform in many biological systems (Tomalia *et al.* 1985; Matthews *et al.* 1998; Abbasi *et al.* 2014). For example, Miura *et al.* (1999), evidenced the prominent role of the surface charge in regulating the binding processes between carboxyl-terminated dendrimers and oppositely charged polyelectrolyte. Moreover, some fluorescence spectroscopy studies evidenced the important role of charge interaction in dendrimers binding with double-stranded DNA (Örberg *et al.* 2007) and human serum albumin (Giri *et al.* 2011).

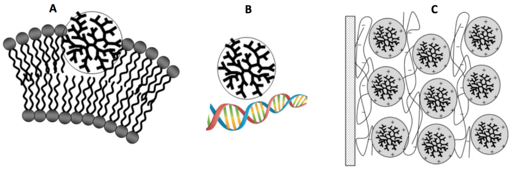

FIGURE 6. Dendrimer interaction at the nanoscale level. Dendrimers-lipid membrane interaction (A), dendrimer-DNA interaction (B) and dendrimer-containing layer-by-layer (LbL) nano-assemblies (C).

Recently both hyperbranched polymeric nanostructures and dendrimers have been employed as efficient templates for construction of organic/inorganic hybrid nanomaterials (Bonaccorsi *et al.* 2013a,b; Huang *et al.* 2014). In this case, the presence of a highly branched polymer topology provides a complementary steric stabilization that induces an enhanced colloidal stability within the complex synthesis environment, thus facilitating the self-assembly process involved during the synthesis protocols (Bonaccorsi *et al.* 2013a,b; Huang *et al.* 2014). The presence of charged functional groups in the dendrimer's exterior also permits the addition of suitable moieties (such as folate and/or antibodies) that can actively target specific diseases and/or improve drug delivery in tumor targeting strategies. For example, nucleic acids usually form complexes with the charged surface of most dendrimers while (under physiological conditions) polyamidoamine (PAMAM) dendrimer-DNA complexes ( Figure 6B), called dendriplexes, bind to the charged surface of the cell membranes (Mendes *et al.* 2017). Finally, a widely used configuration in medicine and biotechnology is given by the dendrimer-based Layer-by-Layer (LbL) nanostructures, constructed using positively charged dendrimers combined with polyanions (or negatively charged dendrimers and polycations). Alternatively, oppositely charged dendrimers may be used for self-assembly of dendrimer LbL films without the intervention of polymers ( Figure 6C). Synthetic dendrimer-containing layer-by-layer (LbL) assemblies have been developed for biosensing, controlled drug release, and bio-imaging applications (Sato and Anzai 2013).

## 4. Conclusions

With the aim to furnish useful insight about the inter-dendrimer interaction in solution a Small Angle X-ray Scattering (SAXS) structural investigation on the sodium carboxylate terminated (-COO-Na) generations G1.5 of poly(amidoamine) dendrimers in water solution has been performed. The presence of interference peaks in the SAXS spectra of a water solution of generation g=1.5 Pamam dendrimers has been ascribed to the long range intermolecular electrostatic interaction caused by the presence of charged moieties in the system. The experimental inter-dendrimer structure factor S(q), analysed in the framework of current models for charged systems in solution, allowed us to model inter-dendrimer interaction potential as well as to obtain important information about the dendrimer effective charge $Z_{eff}$ (degree of ionisation). The finding of the obtained results point out the important role of the dendrimer effective charge in regulating, through the modulation of the electrostatic interaction, main part of their structural properties in solution. As size, concentrations and surface functionality (and charge expression) represent crucial control parameters which determine the fundamental interaction between nanostructured materials and biological systems, the study of the inter-particles interaction represent the first step in the optimization of the targeting effect in nano-biology and its modulation through endogenous and external stimuli. In this respect further experiments are in progress in our laboratories in order to better clarify the role of the dendrimer charge in different solvent conditions (such as solvent quality, pH, ionic strength), as well as to investigate the relevant parameters that regulate the interactions of dendrimers in different biological microenvironment.

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