Supporting Information

Control over the Electrostatic Self-assembly of Nanoparticle Semiflexible Biopolyelectrolyte Complexes

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A. Scattering Experiments:

1. Static Light Scattering (SLS)

In SLS experiments the scattering intensity is measured as a function of the scattered wavevector, q, given by $q=(4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index of the solvent (1.34 for water at 25 °C), and θ is the scattering angle.^{1,2} In our experiments, θ was varied between 30° and 130°, which corresponds to scattering wave vectors, q, in the range from 7.4×10⁻³ to 2.6×10⁻² nm⁻¹. The measurements used a 3D DLS spectrometer (LS Instruments, Fribourg, Switzerland) equipped with a 25mW HeNe laser (JDS uniphase) operating at λ =632.8 nm, a two channel multiple tau correlator (1088 channels in autocorrelation), a variable-angle detection system, and a temperature-controlled index matching vat (LS Instruments). The scattering spectrum was measured using two single mode fibre detections and two high sensitivity APD detectors (Perkin Elmer, model SPCM-AQR-13-FC). Solutions were filtered through 0.2 µm Cellulose Millipore filter into the cylindrical scattering cell.

In Static Light Scattering (SLS) experiments, the excess of scattered intensity is measured with respect to the solvent. The absolute scattering intensity (i.e., the excess Rayleigh ratio in cm-1) can be deduced by using a toluene sample reference for which the excess Rayleigh ratio is well-known ($R_{toluene}$ =1.3522×10⁻⁵ cm⁻¹ at 633 nm):

$$R_{solute}(cm^{-1}) = \frac{I_{solution} - I_{solvent}}{I_{toluene}} \times \left(\frac{n}{n_{toluene}}\right)^2 \times R_{toluene}(cm^{-1})$$
(1)

where $I_{solution}$ is the scattering intensity of the solution sample, $I_{solvent}$ the scattering intensity of the solvent, and $n_{toluene}$ is the refractive index of the toluene ($n_{toluene}=1.49$ at 20°C).

A Virial expression for the osmotic pressure can be used in dilute regime to deduce the following relationship:

$$\frac{KC}{R_{solute}(q,C)} = \frac{1}{M_W} \left[1 + q^2 \frac{R_G^2}{3} + \dots \right] + 2A_2 Q(q,C)C + \dots$$
(2)

where M_W is the weight-average molecular weight and R_G the radius of gyration of the molecular species. The function Q(q,C) is approximately unity for flexible polymer chains but not for spheres, and Q(0,C) is equal to 1 in any case. C is the solute concentration, and A_2 is the second Virial coefficient, which describes the solute-solvent interactions. The scattering constant is $K=4\pi^2n^2(dn/dC)^2/N_A\lambda^4$ where dn/dC is the refractive index increment and N_A is Avogadro's number.

The dn/dC of chitosan and SiNPs in 0.3 M CH₃COOH/ 0.2 M CH₃COONa solutions were measured at room temperature using a Mettler Toledo Portable Lab refractometer over a concentration range varying from 10^{-4} to 5×10^{-3} g/cm³ and from 10^{-3} to 5×10^{-2} g/cm³ for chitosan and silica, respectively. Values of 0.195 cm³/g and 0.066 cm³/g were obtained for chitosan and SiNPs, respectively. For Poly-L-lysine (PLL) in the presence of 0.2 M KBr, one obtains dn/dC=0.1645 cm³/g.

2. Dynamic Light Scattering (DLS)

In DLS the experimental signal is the normalized time autocorrelation function of the scattered intensity:¹⁻³

$$g^{(2)}(q,t) = \frac{\left\langle I(q,0)I(q,t)\right\rangle}{\left\langle I(q,0)\right\rangle^2} \tag{3}$$

The latter can be expressed in terms of the field autocorrelation function or equivalently in terms of the autocorrelation function of the concentration fluctuations, $g^{(1)}(q,t)$, through:

$$g^{(2)}(q,t) - 1 = \alpha + \beta |g^{(1)}(q,t)|^2$$
(4)

Where α is the baseline (varying between 1×10^{-4} and 2×10^{-4} depending on the scattering angle and/or the system) and β the coherence factor, which in our experiments is varying between 0.7 and 0.9 depending on the samples. The normalized dynamical correlation function, $g^{(1)}(q,t)$, of polymer concentration fluctuations is defined as:

$$g^{(1)}(q,t) = \frac{\left\langle \delta c(q,0) \delta c(q,t) \right\rangle}{\left\langle \delta c(q,0)^2 \right\rangle}$$
(5)

Where $\delta c(q,t)$ and $\delta c(q,0)$ represent fluctuations of the polymer concentration at time t and zero, respectively.

In our experiments, some solutions were characterized by a single relaxation mechanism with a characteristic relaxation time inversely proportional to q^2 . For these solutions we have also adopted the classical cumulant analysis:⁴

$$\ln g^{(1)}(q,t) = k_0 - k_1 t + \frac{k_2}{2} t^2 + \dots$$
(6)

Where $k_1=1/\langle \tau_c \rangle$ and k_2/k_1^2 represents the polydispersity index. The extrapolation of $(\tau_c q^2)^{-1}$

to q=0, where τ_c is the average relaxation time of $g^{(1)}(q,t)$, yields the mutual diffusion coefficient D. The latter is related to the average apparent hydrodynamic radius, R_H , of the scattered species through the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi \eta_s R_H} \tag{7}$$

Where k_B is the Boltzmann constant, η_s the solvent viscosity (0.89 cP for water at T=25 °C), and T the absolute temperature. From the polydispersity index k_2/k_1^2 one can determine the hydrodynamic radius width distribution.¹⁻⁴ Indeed, the second cumulant k_2 provides a quantitative measure for the polydispersity of the diffusion coefficient distribution function, σ_D , which is defined as:

$$\sigma_{D} = \frac{\sqrt{\langle D^{2} \rangle - \langle D \rangle^{2}}}{\langle D \rangle} = \sqrt{\frac{k_{2}}{k_{1}^{2}}}$$
(8)

The size (R_H) polydispersity, σ_R , can be calculated from the polydispersity of the diffusion coefficients σ_D .

$$\sigma_{R} = \frac{\sqrt{\langle R_{H}^{2} \rangle - \langle R_{H} \rangle^{2}}}{\langle R_{H} \rangle}$$
(9)

We also used the Contin method based on the inverse Laplace transform of $g^{(1)}(q,t)$,⁵ especially for solutions characterized by several relaxation mechanisms. If the spectral profile of the scattered light can be described by a multi-Lorentzian curve, then $g^{(1)}(q,t)$ can be written as:

$$g^{(1)}(q,t) = \int_{0}^{\infty} G(\Gamma) \exp(-\Gamma t) d\Gamma$$
(10)

Where $G(\Gamma)$ is the normalized decay constant distribution.

3. Scattering Length Densities Calculated for Small-angle Neutron and X-ray (SANS and SAXS)

Components	Specific volume	Calculated SLD-	Calculated SLD-
	(cm^{3}/g)	$SANS/\times 10^{10} \text{ cm}^{-2}$	$SAXS/\times 10^{10} \text{ cm}^{-2}$
H ₂ O	1	-0.56	9.37
D_2O	0.9058	6.36	9.31
Chitosan	0.478	2.47 for C ₆ H ₁₁ NO ₄	18.7
		$(in H_2O)$	
		5.32 for $C_6H_7NO_4D_4$	
		$(in D_2O)$	
Silica	0.4545	3.47	18.5

Table SI-1 Scattering length densities SLDs per unit volume calculated for SANS and SAXS. The specific volumes of the different components are also indicated.⁶

B. Single solute solutions characterization:

In this paragraph, we present characterization performed on each component (chitosan and SiNPs) before mixing to ascertain their dimensions and initial dispersion state in the solvent used.

1. First Consider Simple Dilute Chitosan Solutions:

The data obtained at low q using SLS and corresponding to large spatial scales can be fitted by a Zimm law as:

$$\frac{KC_{chitosan}}{R(q, C_{chitosan})} = \frac{1}{M_w} \left(1 + q^2 \frac{R_G^2}{3} \right) + 2A_2 C_{chitosan}$$
(11)

A classical graphical technique for simultaneously extrapolating the SLS data in dilute regime to both zero scattered wave vector and zero concentration is the Zimm plot, from which weight average molar mass, radius of gyration, and second Virial coefficient A₂ can be determined. This is achieved by plotting KC_{chitosan}/R as a function of $q^2+10C_{chitosan}$ as shown in Figure SI-1 where the prefactor of C_{chitosan} is chosen equal to 10 to give a convenient spacing of the data points on the graph. From the best fits to the data, one obtains R_G=66±5 nm and M_W= 313±20 kDa, which corresponds to a contour length L_c=943 nm. The small positive value obtained for the second Virial coefficient, A₂=(2.5±0.3)×10⁻⁴ cm³g⁻²mol, indicates that the 0.3 M CH₃COOH / 0.2 M CH₃COONa aqueous medium is a good solvent for chitosan.



Fig. SI-1 Zimm plot of chitosan at four different concentrations (0.1, 0.2, 0.3, and 0.4 g/l) with 0.3 M CH₃COOH and 0.2 M CH₃COONa at T=20°C.

In the dilute regime the scattered electric field autocorrelation function $g^{(1)}(q,t)$ is clearly monomodal, as illustrated by Figure SI-2. From the relaxation time, which varies as q^{-2} , one obtains the apparent cooperative diffusion coefficient of chitosan chains. Using the Stokes-Einstein relation (7) one obtains an apparent hydrodynamic radius of R_H =44 nm. The value of the ratio R_G/R_H ~1.5 is characteristic of the semi-rigid polymer-like conformation of chitosan.³ In the inset is also presented the results obtained by applying the Contin procedure to our data. One obtains a relatively narrow single distribution indicating that chitosan chains are welldispersed in the buffer at pH=4.5. Moreover the polydispersity index has been calculated using the cumulant procedure and one obtains $k_2/k_1^2=0.24$ confirming the trend observed using the Contin method.



Fig. SI-2 Scattered electric field autocorrelation function, $g^{(1)}(q,t)$, at $\theta=90^{\circ}$ for a C_{Chitosan}=0.1 g/l chitosan solution in 0.3 M CH₃COOH and 0.2 M CH₃COONa at T=20°C. The normalized distribution of scattered intensity as a function of the size obtained with the Contin method is presented in the inset.

Figure SI-3 displays the scattering pattern of a 0.1 g/l chitosan solution obtained by coupling SLS (low-q data) and SANS measurements. We normalized, for both techniques, the scattered intensity by the corresponding contrast term. Then assuming that 0.1 g/l is a concentration low enough to consider the solution as dilute, the result is $M_{W\times}P(q)$, where P(q) is the form factor. The gap between the q-ranges for the SLS and the SANS data, comprised between 3×10^{-3} and 10^{-2} Å⁻¹, is relatively important. Nevertheless, these experiments would give us a good direct estimation of the chitosan persistence length that is anyway determined at higher q.

The plot as a function of q exhibits three domains: i) a low-q smooth variation with measurable zero-q limit (I(q=0)), analogous to a Guinier regime; ii) an intermediate polymer coil regime in which the q-dependence of the scattered intensity can be described by a power law with exponent close to -2, like in Gaussian coils; and finally iii) a q^{-1} domain at higher q characteristic of rigid rodlike behaviour for distances smaller than the persistence length L_p . This suite of variations can be fitted satisfactorily to the form factor of a wormlike chain model with no excluded volume interactions (this is justified by the small value of the second Virial coefficient A_2 determined above in the presence of a 0.2 M excess of salt). We used an expression⁷ based on the expressions derived by Burchard and Kajiwara for rodlike structures⁸ in which the form factor calculated by Sharp and Bloomfield⁹ for finite wormlike chains of contour length L_c is used at low q:

$$P(q) = \left[\frac{2[\exp(-x) + x - 1]}{x^{2}} + \left[\frac{4}{15} + \frac{7}{15x} - \left(\frac{11}{15} + \frac{7}{15x}\right)\exp(-x)\right]\frac{2L_{p}}{L_{c}}\right] \times \exp\left[-\left(\frac{2qL_{p}}{\alpha}\right)^{\beta}\right] + \left[\frac{1}{2L_{c}L_{p}q^{2}} + \frac{\pi}{qL_{c}}\right] \times \left[1 - \exp\left[-\left(\frac{2qL_{p}}{\alpha}\right)^{\beta}\right]\right]$$
(12)

with $x=L_cL_pq^2/3$ and valid for $L_c>4L_p$. Values of the empirical parameters $\alpha=5.53$ and $\beta=5.33$, which contribute to the balance between the low-q and the large-q terms, have been optimized in ref 7. Eq 12 does not account for excluded volume interactions, which are anyway negligible in the SANS q-range where L_p is determined.^{10,11}



Fig. SI-3 Combined SLS and SANS spectra for a 0.1 g/l chitosan solution. To superimpose both techniques we plot $M_WP(q)$ as a function of q. The continuous line represents the fit of the data with eq 12.

The fit of the data (see Figure SI-3) yields M_W =301K, in good agreement with the value determined using the Zimm analysis, L_c =1116 nm corresponding to the theoretical value of a single strand chitosan chain and L_p =9 nm: here L_p is approximately equal to the intrinsic persistence length, the electrostatic additive contribution to the persistence length being negligible in the presence of 0.2 M of salt. This value is close to that previously reported.¹² It is determined directly by SANS for the first time.

2. Dilute SiNP Solutions:

Electrophoretic Mobility:

Electrophoretic mobility μ was measured with a Malvern ZetaSizer apparatus. Measurements are based on the laser Doppler velocimetry principle yielding the velocity of the complexes submitted to an alternating electrical field of 125 V/cm oscillating at a frequency of 20 Hz.





The results shown in Figure SI-4 revealed that the iso-electric point (IEP) of SiNPs (Ludox AM) is located around pH~2, which corresponds to literature data.^{13,14} These particles carry a pronounced negative surface charge over the entire pH range above IEP due to the substitution of tetravalent silicium by trivalent aluminium ions as was also observed in previous studies.^{13,14} To derive the electrical charge per SiNP, Z_{eff} , we choose the expression of electrophoretic mobility derived from the Hückel model : $Z_{eff}=6\pi\mu\eta_s R_H=\mu k_B T/D$,¹⁵ where D is the diffusion coefficient of SiNP and η_s the viscosity of the solvent. We obtain $Z_{eff}=25$ elementary charges per SiNP at pH = 5.

DLS and SAXS:

SiNPs:

The SiNPs were characterized by DLS and SAXS in the same experimental conditions for a concentration $C_{SiNP}=3$ g/l. The correlation function $g^{(1)}(q,t)$ depicts a monoexponential cooperative relaxation mode (see Figure SI-5), with a relaxation time inversely proportioned to q^2 , corresponding to $R_{H,app}=11.7\pm1.5$ nm. The narrow size distribution obtained by applying the Contin procedure to our data shows that the SiNPs are monodisperse in size (see inset of Figure SI-5). This is corroborated by the value of the polydispersity index, 0.1, obtained using the cumulant method. Also the DLS experiments performed at different aging times during several months show no time evolution of the system. Thus, SiNPs are well dispersed and stable in solution and show no tendency to aggregation with time.



Fig. SI-5 Scattered electric field autocorrelation function, $g^{(1)}(q,t)$, at $\theta=90^{\circ}$ for a C_{SiNP}=3 g/l SiNPs solution in 0.3 M CH₃COOH and 0.2 M CH₃COONa at T=20°C. The normalized distribution of scattered intensity as a function of the size obtained with the Contin method is presented in the inset.

Figure SI-6 shows the SAXS spectra obtained for the same solution at large qs. For dilute solutions and/or for large qs, it is generally assumed that the scattering is arising from isolated non-interacting NPs, with a structure factor $S(q) \sim 1$. The data can be fitted satisfactorily by means of the form factor expression derived for hard spheres of radius R:

$$P(q) = 9 \times \left[\frac{\sin(qR) - qR\cos(qR)}{(qR)^3}\right]^2$$
(13)

The form factor oscillations, damped by the size distribution, are well reproduced with I(q) calculated as indicated in the main text. The SiNPs solution is well represented by a suspension of hard spheres with R=9.2 nm, and σ =0.12.

The extrapolation of the SAXS scattered intensity to zero-q allows us to determine a weight-average molecular weight for the NPs, one obtains $M_W=(3\pm0.2)\times10^6$ g/mol.



Fig. SI-6 Variation of the SiNPs form factor, P(q), with q obtained using SAXS measurements for a C_{SiNP}=5 g/l solution at T=20°C. The continuous red line corresponds to the best fit of the data using the form factor expression derived for hard spheres of radius R=9.2 nm with σ =0.12.

SAXS Characterization of the Nanorods:

High-q data have been fitted by a Guinier expression for the form factor of the nanorod section:

$$VP(q) = \frac{\pi S}{q} \exp\left(\frac{-q^2 r_c^2}{2}\right)$$
(14)

Where V is the volume of the scattered object.

For the rods, we used the classical form factor derived for cylinders:

$$P(q)_{cylinder} = \int_{0}^{\pi/2} \left[\frac{\sin\left(q\frac{L}{2}\cos\alpha\right)}{q\frac{L}{2}\cos\alpha} \frac{2J_{1}(qR\sin\alpha)}{qR\sin\alpha} \right]^{2} \sin\alpha d\alpha$$
(15)

where α is the angle between the axis of symmetry of the cylinder and q, and J₁ the first-order Bessel function.

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