SAXS and SANS Investigation of Synthetic Cholesteric Liquid-Crystal Polymers for Biomedical Applications

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Abstract: Multifunctional cholesteric liquid-crystal polymer, designated [PTOBEE]₁₀-NH₂, has been synthesized, from precursor PTOBEE [C₂₆H₂₀O₈]_n previously reported, by functionalization with amine groups. Cholesteric PTOBEE has been shown to be able to entrap DNA, being tested as non-viral vector in gene therapy, including cationic monomeric surfactants in their formulation. The direct interaction between cationic cholesteric liquid-crystal polymer PTOBEE-NH₂, with anionic commercial [Poly-C-Poly-G], is proposed as new formulation for biomedical applications. The interaction mechanism is studied in three different volume ratios: (1:2), (1:1) and (2:1), respectively. Their structures, studied by SAXS at ESRF, provide information about the complexes size and shape. Based on preliminary neutron scattering experiments, showing sufficient contrast (scattering length density difference) between cholesteric PTOBEE-NH₂ (1.5 to 1.9×10^{10} /cm²) and polynucleotide [PolyC-PolyG] (3.32×10^{10} /cm²), contrast variation SANS experiments were performed at NIST, with a wavelength of $\lambda = 8 \text{ \AA}$, using different H₂O:D₂O mixtures (i.e., contrasts) to match the cholesteric polymer (44.5%:55.5%), the polynucleotide (65% or 70%):(35% or 30%) or the whole complex (30%:70%), being able to “observe separately” both component structures within the complexes. SANS results agree and complement the information obtained by SAXS. Different TWO SHELL molecular models are proposed for the interaction by using a combination of model-fitting (FISH).

Key words: (Cholestericliquid-crystal polymer/polynucleotide) complex, gene therapy, non-viral vector, SANS/SAXS, molecular models.

Nomenclature

CLCP: Cholesteric liquid crystal polymer

Greek letters

λ : Wavelength radiation

θ : The scattering angle

1. Introduction

1.1 Aperiodic Crystals Cholesteric Liquid-Crystal DNA

At the end of autumn of 1944, Schrödinger, completed in Dublin a small book: What is Life? The Physical Aspect of the Living Cell [1].

Referring to the nature of the chromosome material,

he introduced the concept of an “aperiodic crystal” to define a gene or perhaps the whole fiber of the chromosome, as the carrier of life, able to grow in aggregates bigger and bigger “without the clumsy resource of repetition” in three directions[2].

The concept of “aperiodic crystal” was surprisingly premonitory, nine years before the crystallographic x-ray diffraction patterns proved the long range order of DNA fibers (main constituent of chromosome material), Fig. 1, explained in terms of a helical structure by Franklin [3] and Wilkins [4], opened the way to the double helix by Watson and Crick [5], in Fig. 2a, with periodic order of the sugar-phosphate backbone, every 34 Å, along the helix and the aperiodic distribution of the complementary base pairs. From the biological point of view this absence of periodic order is convenient to endow DNA of their capacity to store

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Fig. 1 X-Ray diffraction pattern of a DNA oriented fiber under physiological conditions, called “Photograph 51”.

Fig. 2 (a) DNA structure according to Ref. [5]; (b) polynucleotide PolyC-PolyG and (c) section view of PolyC-PolyG and its amphiphilic nature, according to Ref. [6].

genetic information that comes coded by the peculiar sequence of base pairs along the structure.

Fig. 2b shows the molecular model of double stranded PolyC-PolyG from their nucleotide C-G bases, marked within the ellipse. Cross view in Fig. 2c indicates the hydrophilic and hydrophobic regions, according to Hammouda [6].

In 1984 a new ordering of matter appeared, not crystalline but with discrete diffraction patterns of extraordinary quality, quasiperiodic crystal [7]

In 1988, Ringsdorf [8] established a parallelism between the behavior of liquid crystals in materials science and lipids in life science, due to their liquid-crystalline nature, both being self-organizing systems.

In 1992, the International Union of Crystallography redefined the concept of crystal as: "Any solid which has a diffraction pattern essentially discrete" (Fourier space) [9]. Since then, the CRYSTAL FAMILY has included: -Periodic and -Aperiodic crystals.

Liquid crystals belong to the second group. With varying temperature they do not directly go from the crystalline state into the melt, instead, they go through an intermediate mesophase state, which combines order of perfect crystals and mobility of liquids.

Liquid crystal DNA possesses a cholesteric

mesophase, with special array of nematic planes, containing complementary base pairs, stacked in a super structure with chiral helical symmetry of charge distribution [10-12], whose morphology is shown in Fig. 3.

1.2 Synthetic Cholesteric Liquid Crystal Polymers (CLCP)

Multifunctional cholesteric liquid crystal polymer, designated [PTOBEE]₁₀-NH₂, in Fig. 4, has been synthesized by us [13], from precursor PTOBEE [C₂₆H₂₀O₈]_n, as previously reported [14,15], by functionalization with amine end groups. The amphiphilic nature of their monomers makes them polymerize along helical chains. Both CLCP have been characterized as thermotropic (mesophases self associate under the effect of temperature) and also behave as lyotropic, when dispersed in solution, self-organizing their mesophases both by the effect of varying temperature and concentration, adopting different conformations depending on the solvent.

Due to their lyotropic behaviour, cholesteric precursor PTOBEE has been found to entrap smaller molecules inside and to interact with liquid crystalline biomacromolecules, such as nucleic acids and lipids (both neutral and cationic). The structures of the

Fig. 3 Cholesteric morphology of liquid crystal DNA.

Fig. 4 Molecular model of Poly[PTOBEE]₁₀-NH₂. Sectional view at lower right hand corner.

complexes were analyzed by simultaneous SAXS/WAXS with synchrotron radiation monochromatic beam ($\lambda = 1.5 \text{ \AA}$) on the X33 camera of the EMBL (DESY, Hamburg) [16-18]. Both linear detectors were calibrated using Tripalmitin. Data were normalized for incident intensity and analyzed by using the programs SAPOKO and OTOKO, included in Primus [19].

1.3 Non-Viral Vectors

The introduction of exogenous genetic material in cells is a key stage in the development of basic research in cellular biology. The term “transfection” is used to indicate the transfer of DNA into the nuclei of cells of higher organisms. This technique provides an indispensable tool for a growing number of researchers [20].

The direct application of this technology in living organisms opens many possibilities, among which, gene therapy and vaccines using DNA.

Viral vectors for DNA transfection attracted the attention of specialists in gene therapy and consisted the majority of the clinical trials, although with low efficiency levels.

However, non-viral vectors seem to have more success as transfecting agents because they are easier to manage and possess a bigger range of applications. The exponential development in this field reveals a promising future for non-viral vectors as a logical alternative to viral vectors for gene therapy, providing similar advantages and eliminating the inherent inconveniences to the virus. The non-viral term is a concept that includes a great variety of non related structures, including conjugated polylysine, lineal and ramified polycations, complete or fractured dendrimers, nanospheres, polysaccharides, cationic liposomes.

The development of new non-viral vectors requires synthetic molecules that can bind polynucleotide fragments (the therapeutic agent) and transfect cells, but not stimulate an immune response.

Because of the common cholesteric nature with DNA, formulations based on synthetic cholesteric liquid crystal polymers, including cationic monomeric surfactants, were developed in our lab to entrap an anionic plasmid of DNA. The complexes have been tested successfully as non-viral vectors in gene therapy, both *in vitro* and *in vivo* [21, 22].

In this work, a new formulation with cationic PTOBEE-NH₂ able to directly interact with polynucleotides of increasing complexity, is proposed with potential application as non-viral vector.

A combined SAXS and Cold Neutron SANS study of the complex formed between PTOBEE-NH₂ and commercial polynucleotide [PolyC-PolyG], much more simple than plasmid DNA, is performed, in three different volume ratios, in order to determine how this affects their shape and size. Taking advantage of the difference in neutron scattering length densities (NSLD), of the two components, based on preliminary neutron scattering experiments, contrast variation SANS experiments performed at NIST, permit to “distinguish separately” both component structures within the complexes. This was done without any selective deuteration, just using different H₂O:D₂O mixtures (i.e., contrasts) to match the cholesteric polymer, the polynucleotide or the whole complex, providing complementary information to that obtained by SAXS. Different two shell molecular models are proposed for the interaction between cholesteric PTOBEE-NH₂ and [PolyC-PolyG] by using a combination of model-fitting (FISH).

2. Experiments

2.1 Materials

Cholesteric PTOBEE-NH₂ was suspended in Tris Acetate-EDTA buffer (0.04M TRIS, 0.001M EDTA) and complexed with commercial [PolyC-PolyG], in three different mass fractions: (1:2), (1:1) and (2:1) in volume, by mixing and digesting with TAE for 12 h in a swinging shaker.

2.2 SAXS Experiments

SAXS data were collected at the ESRF, at room temperature, with a monochromatic beam at $\lambda = 0.9795$ Å. A 2D detector was placed at 6 m from the sample. The spectra were normalized and converted to 1D profiles with Fit2D [23]. Example data are shown in Fig. 5.

2.3 SANS Experiments

SANS data were collected at the NG7 beam line at NIST. The beam was monochromatic at $\lambda = 8$ Å. The 2D detector was placed at 15 m, 13 m and 4 m from the sample, to give a Q range of $(0.0009 \text{ nm}^{-1} < Q < 0.025 \text{ nm}^{-1})$, $(0.0025 < Q < 0.02)$ and $(0.006 < Q < 0.08)$ respectively, Q being the scattering variable $Q = (4\pi/\lambda) \cdot \sin(\theta/2)$ where θ is the scattering angle. Neutron focusing lenses were used in conjunction with the 15 m configuration.

The spectra were corrected and converted to 1D profiles using data reduction software running under IGOR Pro. [24]. The calculated neutron scattering length densities of the CLCP and polynucleotide are given in Table 1.

3. Results and Discussion

Information about size [R_g (Guinier Plot)] and shape of the complexes between [PolyC-PolyG] and PTOBEE-NH₂ in the three studied proportions was estimated from the SAXS data using plots of $\ln I(Q)$ versus Q^2 and $I(Q)$ versus $Q \cdot R_g$, Fig. 5. The results are given in Table 2 and hint at a change in morphology with increasing nucleotide content.

However, because the electron densities of the polymer and polynucleotide are so similar, the SAXS data only reflects the overall structure of the complex.

Preliminary SANS experiments performed on the LOQ beam line at ISIS had suggested that there should be sufficient contrast between PTOBEE-NH₂ and [PolyC-PolyG] to undertake a contrast variation SANS study. However, the same data also indicated that it would be necessary to measure to much lower Q values.

This is why SANS measurements were performed at NIST.

Table 3 shows the first results obtained by SANS of the new synthesized polymer, PTOBEE-NH₂ at pH = 7.2. The number average relative molecular mass estimated by the Guinier approximation was ≈ 581 monomers per chain.

In Table 4, comparison of the R_g calculated from SANS is given for cholesteric PTOBEE-NH₂ and the precursor PTOBEE when suspended in different solvents (D₂O and ethanol) using different fitting programs and model.

A decrease in the R_g value of precursor PTOBEE is observed in Table 4, when amine end groups enter contribute to form PTOBEE-NH₂, both when dispersed in D₂O and in ethanol-d₆. The presence of nitrogen atoms in this formulation is able to alter the amphiphilic self-aggregation of PTOBEE, either by the introduction of positive charge or by formation of H-bridges with neighboring oxygen atoms or by both, causing reduction of its volume.

The percent change between PTOBEE and PTOBEE-NH₂ R_g , smaller in ethanol-d₆ than in D₂O implies less shrinkage in the medium with the lower dielectric constant, and would reinforce the formation of H-bridges.

The lower contrast between the polymer and ethanol-d₆ than between the polymer and D₂O may explain the difference between the R_g value obtained for each solvent.

In order to “see” all the components of the complex structures, without any selective deuteration, three

Table 1 Calculated NSLD values for PTOBEE-NH₂ and [PolyC-PolyG].

Material	NSLD/(cm ²)
PTOBEE (precursor)	1.927E + 10
PTOBEE-NH ₂	1.887E + 10
[POLY(C) – POLY(G)]	3.320E + 10
D ₂ O	6.361E + 10
H ₂ O	-0.561E + 10
Ethanol-d ₆	0.624E + 10

Fig. 5 SAXS patterns obtained for [PolyC-PolyG:PTOBEE-NH₂] complex at ESRF.

Table 2 Structural features of complexes [PolyC-PolyG : PTOBEE-NH₂]by SAXS at ESRF.

	Complex proportion	R_g (nm) Guinier	Shape	Slope
			$I(Q)$ versus $Q \cdot R_g$	$\ln I(Q)$ versus $\ln Q$
PolyC-PolyG		18.78	rod	-1.57
PolyC-PolyG:PTOBEE-NH ₂ (D4)	(1:2)	33.89	sphere	-2.64
PolyC-PolyG: PTOBEE-NH ₂ (D5)	(1:1)	34.24	sphere	-2.64
PolyC-PolyG: PTOBEE-NH ₂ (D6)	(2:1)	31.02	disk	-2.31

Table 3 Structural information by SANS for cholesteric PTOBEE-NH₂.

	Molecular mass polymer (g/mol)	Molecular mass monomer (g/mol)	N° monomers
PTOBEE-NH ₂ at pH = 7.20	267, 330 Guinier approximation	460	581

Table 4 Estimation of R_g from SANS for cholesteric: PTOBEE-NH₂ and precursor PTOBEE, in different solvents using different fitting programs [19, 25, 26].

Polymer	Primus (Rod particles) (nm)	Primus (Globular particles) (nm)	Sandra (Guinier approx. for spheres) (nm)	Sandra (Debye Gaussian Coil Z-average R_g (nm))
PTOBEE in D ₂ O	12.0	17.5	18.2	37.5
PTOBEE-NH ₂ in D ₂ O	8.12	11.0	12.92	15.0
PTOBEE in Ethanol-d ₆	10.5	15.7	22.46	30.0
PTOBEE-NH ₂ in Ethanol-d ₆	8.27	12.6	18.59	20.0

different contrasts volume fractions H₂O:D₂O were used to match the CLC polymer (44.5%:55.5%), the polynucleotide (65% or 70%):(35% or 30%), and the whole complex (30%:70%), respectively.

Fig. 6 exhibits the SANS spectra of complex [(PolyC-PolyG):(PTOBEE-NH₂)] in the studied proportions: (a) (1:2), (b) (1:1) and (c) (2:1) for each of the three contrast.

In Table 5, R_g is evaluated for each component from their Guinier plot. Although the size is still increasing in the analysed Q range, we take these results as valid since they are in agreement with the previous SAXS measurements, at higher Q values.

In the $0.005 < Q < 0.1$ range, with the detector placed at 4 m and 16 m, the R_g values of isolated polynucleotide PolyC-PolyG (not complexed) and

Fig. 6 SANS spectra of complex [(PolyC-PolyG): (PTOBEE-NH₂)] in proportion: (a) (1:2), (b) (1:1) and (c) (2:1).

isolated polymer PTOBEE-NH₂ are similar, about 14 nm.

Since R_g of isolated [PolyC-PolyG] in the $0.002 < Q < 0.02$ range (SANS, 13 m) is 53 nm, similar to R_g of [PolyC-PolyG] forming part of the complex, it seems to be totally complexed in the studied mixtures. In the same Q range, average R_g of the complex seems to increase with PolyC-PolyG proportion. R_g of the polymer within the complex seems to decrease with increasing proportion of polynucleotide. A proposed model for complex D4 (PolyC-PolyG:PTOBEE-NH₂)

(1:2) would be a two sell model with the excess polymer in the outer shell, decreasing its R_g and the nucleic acid added within the core.

In complex D6, with twice the proportion of (PolyC-PolyG), a possible model would be PTOBEE-NH₂ surrounded by the polynucleotide rigid rods.

3.1 Porod Plot

Porod plot, $\log(Q)$ versus $\log Q$, corresponding to complex (PolyC-PolyG:PTOBEE-NH₂) in the three studied compositions, are given in Fig. 7, each with the three contrasts.

Complex D4, with double polymer content, is seen in Fig. 7a to adopt a mass fractal network, while the polymer would show rough interface of fractal dimension and the polynucleotide would appear as collapsed polymer coils. The molecular model should also include the attractive potential between negatively charged nucleic acid molecules and positively charged polymer chains.

A variety of experimental studies have established conclusively that orientationally ordered states, and in certain materials even density modulations, develop in the vicinity of the free surface [27].

According to the Porod plot, with increasing PolyC-PolyG content, complex [PolyC-PolyG: PTOBEE-NH₂] (1:1), D5, Fig. 7b would exhibit a smooth surface, with polymer mass fractal morphology and the polynucleotide still adopting collapsed polymer coils conformation.

With twice the content innucleic acid, the complex [PolyC-PolyG:PTOBEE-NH₂] (2:1), D6, Fig. 7c, would still appear as a smooth surface, the polymer as mass fractal and the PolyC-PolyG, in excess, would be present as rigid rods.

3.2 Fitting Models

With help of the FISH model-fitting program [28], the SANS spectra of Complexes [PolyC-PolyG: PTOBEE-NH₂] have been adjusted to different models

Table 5 Comparative R_g values from SAXS and SANS for the complexes and the isolated components.

Detector to sample distance Technique	15 m			13 m			4 m/16 m
	SANS			SANS/SAXS			
	0.0009 < Q < 0.002 range			0.002 < Q < 0.02 range			0.005 < Q < 0.1
	R_g (nm)	R_g (nm)	R_g (nm)	R_g (nm)	R_g (nm)	R_g (nm)	
	Complex	PTOBEE-NH ₂ in complex	PolyC-PolyG in complex	Complex	PTOBEE-NH ₂ in complex	PolyC-PolyG in complex	
(PolyC-PolyG:PTOBEE-NH ₂) (1:2)	D4	245		48	55	50.6	33.89
(PolyC-PolyG:PTOBEE-NH ₂) (1:1)	D5	144		58	56	54.4	34.24
(PolyC-PolyG:PTOBEE-NH ₂) (2:1)	D6	106		62	47	-	31
PolyC-PolyG isolated $M_w = 300.000$; 694 (g/mol/monomer)						53	18.8 ESRF/SAXS 14 NIST/SANS
PTOBEE-NH ₂ isolated $M_w = 267.260$; 460 (g/mol/monomer)							13 ISIS/SANS 14.3 NIST/SANS

(a)

(b)

(c)

Fig. 7 Porod plots of all the complexes [(PolyC-PolyG): (PTOBEE-NH₂)] in proportion: (a) (1:2), (b) (1:1) and (c) (2:1).

with excellent GOF (goodness of fit or χ^2), in accordance to the previous results.

The polymer in complex D4 (1:2) with double the proportion of the nucleic acid could be adjusted, to a Schultz Polydisp Two Shell Hard Sphere model, as a spherical core-shell (“onion skin”) $P(Q)$ allowing for a polydisperse core and a fixed shell thickness. The fitting, in Fig. 8a, shows an overall radius $R^2 = 83.5$ nm.

Complex D4 also fitted a Schultz Polydisp Two Shell *Hard Sphere model with an overall radius $R^2 = 71$ nm (Fig. 8b).

Fig. 8 Fitting with FISH of: $\log(Q(\text{\AA}^{-1}))$ versus $I(Q)$ in arbitrary units (a.u.) for complex D4 [PolyC-PolyG:PTOBEE-NH₂] with (1:2) to a Schultz Polydisp Two Shell *Hard Sphere model: (a) polymer, (b) complex and (c) PolyC-PolyG fitting a Schultz Polydisp Two Shell * H-P for charged colloids model.

The SANS spectrum of PolyC-PolyG in complex D4 can be adjusted to a Schultz Polydisp Two Shell * H-P model, in APPENDIX I, with an overall radius $R^2 = 59$ nm. This model incorporates a Hayter-Penfold type $S(Q)$ structure factor for charged colloids, shown in Fig. 8c. It is interpreted as due to the higher negative charge per monomer (2 according to the adjustment) than the positive charge of cholesteric polymer per monomer.

The D4 model could be explained as a double shell with the nucleic acid in the core, surrounded by the complexed interface and the excess polymer placed in the outer shell. The whole shape is approaching a sphere, in agreement with the SAXS data in Fig. 5. Throughout this paper, statistical error bars correspond to one standard deviation.

Polynucleotide PolyC-PolyG in complex D6: (PolyC-PolyG:PTOBEE-NH₂) (2:1) in double proportion to the polymer fits a Two Shell Ellipsoid model, shown in Fig. 9a, with a radius = 369 nm and a shell thickness of 790 nm. The polynucleotide in excess, probably appearing as rigid rods based on the Porod plot, would be located in the outer part of shell, imposing an ellipsoidal shape to the whole complex, in agreement with the SAXS results in Fig. 5, where the whole complex exhibited a disk shape.

Complex D6 can be adjusted to a two-shell model, with an overall R^2 radius of 39 nm and 3% polydispersity, Fig. 9b. It also fits a Schultz Polydisperse Spheres* Fractal Plus Porod, see Appendix I, with an average particle radius R_{BAR} of 6.2 nm, a roughness size 0.27 nm (should be < 0.3), and a primary particle radius of 5 nm.

Polymer in complex D6 matched a Schultz Polydisp Spheres* Fractal Plus Porod Scatter, in agreement with its Porod plot previously shown, with an average particle radius R_{BAR} of 10 nm, a particle polydispersity 0.1 % and a fractal dimension 0.2 nm, as shown in Fig. 9c. The polymer would be located in the core surrounded by the complexed interface with the polynucleotide in excess in the outer shell.

(a)

(b)

(c)

Fig. 9 Fitting of: $\log(Q(\text{\AA}^{-1}))$ versus $I(Q)$ (a.u.) for complex D6 [PolyC-PolyG:PTOBEE-NH₂] with (2:1); (a) PolyC-PolyG to a Two Shell Ellipsoid model; (b) Complex fitting to a two shell model and (c) Polymer matches a Schultz Polydisp Spheres* Fractal plus Porod Scatter model.

(a)

(b)

(c)

Fig. 10 (a) Fitting of $\log(Q(\text{\AA}^{-1}))$ versus $I(Q)$ (a.u.) for Complex D5 adjusted to a surface fractal model; (b) Polymer two shell model and (c) Fitting of PolyC-PolyG in complex D5 to a Schultz Polydisp Two Shell * H-P Charged Sphere.

Complex D5 with equal volume fraction for both components can be adjusted to first order, to a Surface Fractal model with 3.14 fractal dimension, in Fig. 10a, while the polymer approaches a two-shell model with an overall R_2 equal to 36 nm, Figure 10b. Once again the nucleic acid follows a Schultz Polydisp Two-Shell * H-P charged sphere $S(Q)$ structure factor, in Figure 10c, with an overall radius of 22 nm, probably in the form of collapsed polymer coils, in agreement with Porod results.

4. Conclusions

We may conclude that SANS results are compatible with those obtained from SAXS.

The H_2O/D_2O proportions used as deuterated solvents, allowed us to distinguish between the whole complex, the cholesteric liquid-crystal polymer and the nucleic acid in each sample.

In the studied Q ranges the aggregates size increased.

The R_g value, about 14 nm, of isolated polynucleotide PolyC-PolyG (not complexed) and isolated polymer PTOBEE-NH₂ are similar, and their molecular structures are also similar.

The R_g of polymer within the complex seems to decrease with increasing nucleic acid fraction.

A proposed model for complex (PolyC-PolyG):(PTOBEE-NH₂) in (1:2) proportion, designated D4, would be a Schultz Polydisp Two Shell * Hard Sphere model, as a spherical core-shell ("onion skin") $P(Q)$ allowing for a polydisperse core and a fixed shell thickness, with the nucleic acid in the core surrounded by a complexed interface and the excess polymer residing in the outer shell. SANS spectrum of PolyC-PolyG in complex D4 can be adjusted to a Schultz Polydisp Two Shell * H-P model. This model incorporates a Hayter-Penfold type $S(Q)$ function for charged colloids, interpreted as due to the negative charge per monomer, higher than the positive charge of cholesteric monomer. The shape of the whole complex D4 approaching a sphere is in agreement with

the SAXS data.

For the complex (PolyC-PolyG):(PTOBEE-NH₂) in (2:1), named D6, a possible model would be a Two Shell Ellipsoid model with PTOBEE-NH₂ in the core surrounded by the interface complex. The polynucleotide in excess, probably in the form of rigid rods according to the Porod plot, would be located in the outer part of the shell, imposing an ellipsoidal shape to the whole complex, in agreement with the SAXS results, whereby the whole complex exhibited a disk shape.

Complex D5, with (1:1) proportion, in volume, between both components can be adjusted to a Surface Fractal with 3.14 fractal dimension. The polymer would approach a two-shell model and again the nucleic acid follows a Schultz Polydisp Two Shell * H-P charged sphere $S(Q)$ model, probably as collapsed polymer coils, in agreement with Porod results.

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Appendix

Schultz Polydisp * H-P

This is a spherical core-shell (onion skin) P(Q) model allowing for a polydisperse core and a fixed shell thickness. The polydispersity function is a Schultz distribution (M. Kotlarchyk and S-H Chen, *J. Chem. Phys.* 79 (1983) 2461-2469), which is similar to a log-normal distribution but easier to code. The model also incorporates a Hayter-Penfold type (J.B. Hayter and J. Penfold, *Mol. Phys.* 42 (1981) 109-118) S(Q) function for charged colloids, and a floating background value.

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1	10	1	RHO1-RHO2	NSLD difference between core-shell
2	10	2	R1	
3	10	1	RHO2-RHO3	NSLD difference between shell-solvent
4	10	2	R2=R1+R3	Overall radius
5	2	2	R3	Shell thickness
6	6	11	SCHZ.SCALE 10E-24	Volume fraction of particles . (RHO1-RHO3) ² f RHO's in cm ⁻²
7	6	12	RBAR	Average core radius
8	6	13	R-SHIFT	
9	6	14	SIG/(RB-R0)	Core polydispersity% / 100
10	23	1	H-P S(Q) R	S(Q) interactionradius
11	23	2	CHARGE	Charge per "particle"
12	23	3	AKK SCREEN	Inverse Debye screening length
13	23	4	VOL FRAC	S(Q) volume fraction
14	3	11	BKG A	Flat (Q-independent) background

"Schultz Polydisp Spheres * Fractal Plus Porod"

This is a model for a P(Q) of polydisperse but homogeneous (ie, not core-shell) spheres with surface scattering, coupled with the P(Q) for a fractal aggregate comprised of smaller 'primary' particles. The polydispersity function is again a Schultz distribution. There is no colloidal S(Q) in this model because of the fractal contribution.

1	6	11	SCHULTZ SCA	10E-24 . Volume fraction of particles . (RHO1-RHO3) ² for RHO's in cm ⁻²
2	6	12	RBAR	Average particle radius
3	6	13	R0-SHIFT	
4	6	14	SIG/(RB-R0)	Particlepolydispersity %/100
4	13	1	FRACTAL D	Fractal dimension (should be <3)
5	13	2	AGG SIZE	Aggregate size
6	13	3	"RADIUS"	Primary particle radius
7	3	11	BKG A	Flat (Q-independent) background
12	12	11	Porod scale	Porod constant
13	12	12	sigma	Interfacial roughness (width of interface)