

Negatively Charged Gold Nanoparticles “Control” Double-Stranded DNAs Spatial Packing

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Abstract

We report here on new optic and small-angle X-ray scattering (SAXS) measurements concerning the formation of the dispersions from double-stranded (ds) DNA molecules, doped with negatively charged gold nanoparticles (nano-Au). The nano-Au fixation nearby the surfaces of linear ds DNA in solution of high ionic strength (0.3 M NaCl) and subsequent phase exclusion of (ds DNA-nano-Au) complexes from solution containing poly(ethylene glycol) results in decrease in the amplitude of abnormal negative band in the circular dichroism spectra of the formed cholesteric liquid-crystalline dispersions (CLCD). Besides, doping of linear ds DNA with nano-Au and phase exclusion of the formed (ds DNA-nano-Au) complexes does not accompanied by alteration in the standard structural parameters obtained from SAXS data which reflect local ordering of ds DNA molecules, but results in the decrease in the amplitude of the characteristic Bragg maximum. Our experimental data, supplemented by a simple model numeric computations of screened (in the water-salt solution of high ionic strength) electrostatic energy for ds DNA molecules and negatively charged but polarizable nano-Au, suggest that doping of ds DNA with negative charged nano-Au results in an appearance of a population of “modified” by nano-Au ds DNA molecules. These molecules, in contrast to the free ds DNA molecules, lose an ability to form spatially twisted structure at phase exclusion and instead of ordered spatial structure of ds DNA CLCD only random disordered aggregates are obtained.

Keywords: Phase exclusion of linear DNA; Liquid-crystalline dispersions; Negatively charged gold nanoparticles; Circular dichroism; Small-angle X-ray scattering

List of Abbreviations: ds DNA: double-stranded DNA; nano-Au: gold nanoparticles; PEG: poly(ethylene glycol); DNA CLCD: DNA cholesteric liquid-crystalline dispersion; CD: circular dichroism; SAXS: small-angle X-ray scattering

Introduction

During the last few years double-stranded (ds) DNA spatially twisted (cholesteric) liquid-crystalline dispersions (CLCD) doped with metallic nanoparticles (such as gold nanoparticles (nano-Au) or cobalt ferrite nanoparticles) have attracted a lot of experimental and theoretical attention motivated by their potential applications and nontrivial biological consequences [1-6]. It is well established that the physicochemical properties of ds DNA CLCD reflect some properties of these macromolecules in biological objects such as chromosomes of primitive organisms (for instance, the chromosomes of the Dinoflagellate) and DNA-containing viruses [7,8]. Hence, doping ds DNA CLCDs with nano-Au is of interest to both biologists and researchers in the area of nanotechnology. Indeed, a study of the effect of nano-Au on the properties of DNA CLCD may be useful for establishing the reasons for the genotoxicity of nano-Au [9-11]. Nanotechnologically, the treatment of DNA liquid-crystalline dispersions by nano-Au may lead to the formation of new materials with unique properties [12].

The properties of linear single-stranded and ds DNA molecules containing of positively charged nano-Au on their surfaces were described in the pioneer contributions by Mirkin [13] and Alivisatos [14].

The goal of our study is to reveal the phenomena caused by the negatively charged nano-Au action on linear ds DNA molecules. It seems constructive to pose a few questions and to answer them on a general level. Namely, we tried to answer the following questions.

1. Whether nano-Au is coordinated with linear ds DNA molecules in colloid solutions?
2. Whether (linear ds DNA-nano-Au) complexes can form CLCD as result of their exclusion from polymer-containing solution?

Materials and Methods

Preparation of nano-Au and determination their average size

Colloid solutions containing nano-Au of two sizes were used in this study. Solutions (hydrosols) containing nano-Au with the average diameters of 2 and 15 nm were prepared by reduction of HAuCl_4 according to the previously described procedures. Specifically, 2 nm nano-Au with the numerical concentration of $C_N = 3.5 \times 10^{15}$ particle ml^{-1} were obtained by Duff method [15]. 15 nm nano-Au ($C_N = 3.5 \times 10^{12}$ particle ml^{-1}) were synthesized according to Frens citrate protocol [16]. The numerical concentrations of all nano-Au samples were calculated using the material balance (provided that Au is reduced completely). All nanoparticles were negatively charged. Their ξ -potentials at neutral pH values were approximately -40 mV.

The average sizes (diameters) of the nano-Au in stock preparations were verified by transmission electron microscopy. Micrographs of nanoparticles were obtained using a Jem-100CX electron microscope (Jeol, Japan).

Reagents and preparation of ds DNA CLCDs

ds DNA and chemicals: A calf thymus depolymerized ds DNA (Sigma, USA) with a molecular mass of $\sim (0.6\text{--}0.8) \times 10^6$ Da after additional purification was used.

DNA concentration in the water-salt solutions was determined spectrophotometrically using the known value of the molar extinction coefficient ($\epsilon_{\text{max}} = 6,600 \text{ M}^{-1} \text{ cm}^{-1}$).

Poly (ethylene glycol) (PEG; Serva, Germany; molecular mass of 4,000 Da) sample was used without additional purification.

Formation of ds DNA CLCDs: Standard CLCDs of ds linear DNA molecules were prepared according to the technology of intensive mixing (stirring) of PEG-containing water-salt solution with water-salt ds DNA solution. All details of this technology can be found in [8].

Measurements

The absorption spectra were taken by Cary 100 Scan (Varian, USA) spectrophotometer. The circular dichroism (CD) spectra were recorded using a SCD-2 portable dichrometer (produced by Institute of Spectroscopy of the Russian Academy of Sciences, Moscow-Troizk) [17]. The CD spectra were represented as a dependence of the difference between the intensities of absorption of left- and right-handed polarized light (ΔA ; $\Delta A = (A_L - A_R)$) on the wavelength (λ).

Structure analysis of the DNA phases by SAXS

Pellets (~ 3 mg) obtained by slow speed centrifugation ($5,000 \text{ rev. min}^{-1}$, 40 min, 4°C ; centrifuge K-23, Germany) of the DNA CLCD particles as well as the dispersion particles, formed by ds DNA molecules doped with nano-Au (~ 2 and 15 nm) and condensed in PEG-containing water-salt solution, were analyzed by small-angle X-ray scattering (SAXS). The measurements were carried out on a laboratory diffractometer Amur-K using a Kratky-type (infinitely long slit) geometry in the range of the momentum transfer $0.12 < s < 10.0 \text{ nm}^{-1}$, where $s = 4\pi\sin\theta/\lambda$, 2θ is the scattering angle and $\lambda = 0.1542 \text{ nm}$ is the X-ray wavelength. The experimental scattering profiles were corrected for the background scattering from the solvent, and preliminary processed using standard procedures [18].

The repeating distances of the periodical motifs in the crystalline regions ($\bar{d} = 2\pi/s_{\text{max}}$), corresponding to the peak position (s_{max}) on the scattering patterns, were calculated using program PEAK [18].

The mean long-range order dimension, L , (the size of crystallites) and the degree of disorder in the system (Δ/\bar{d}) were calculated using the standard equations as described in [19].

Results

The principal scheme of the ds DNA CLCD formation as well as the main factors which influence this process are presented in Figure 1. This Figure allows one to explain briefly the principle used for phase exclusion of ds DNA molecules doped with the negatively charged nano-Au. Solution of linear ds DNA was doped with nano-Au (~ 2 nm or 15 nm) and stored within 1 h at room temperature. Then the final composition was mixed under stirring with solution of PEG. As a result, the phase exclusion of (ds DNA-nano-Au) complexes, if they are formed, takes place. After 1 h of additional exposition, the CD spectra of obtained mixtures were measured. (One can add that the process of phase exclusion of linear ds DNAs from water-salt polymer-containing solutions is known since Lerman's experiments as " ψ -condensation" (ψ is the acronym for *polymer-and-salt-induced*) [20,21]. In the case of linear, rigid, low molecular mass ds DNA (molecular mass of about $(0.6\text{--}0.8) \times 10^6$ Da) phase exclusion of these molecules from PEG solution is accompanied by the formation of CLCD [8].

Figure 2 shows that the formation of ds DNA CLCD is accompanied by an appearance of intense (abnormal) negative band in the CD spectrum (curve 1) located in the region of absorption of nitrogen bases ($\lambda \sim 270 \text{ nm}$). The appearance of this band univocally testifies the spatially twist of neighboring "quasinematic layers" in particles of DNA dispersion [22]. The negative sign of the abnormal band in the CD spectrum proves the left-handed twist of the right-handed DNA molecules (B-form) in CLCD particles. The amplitude of the CD band depends upon the nano-Au concentration in solution (curves 2-4). The higher the nano-Au concentration, the greater the reduction in abnormal band in CD spectra of dispersions of DNA.

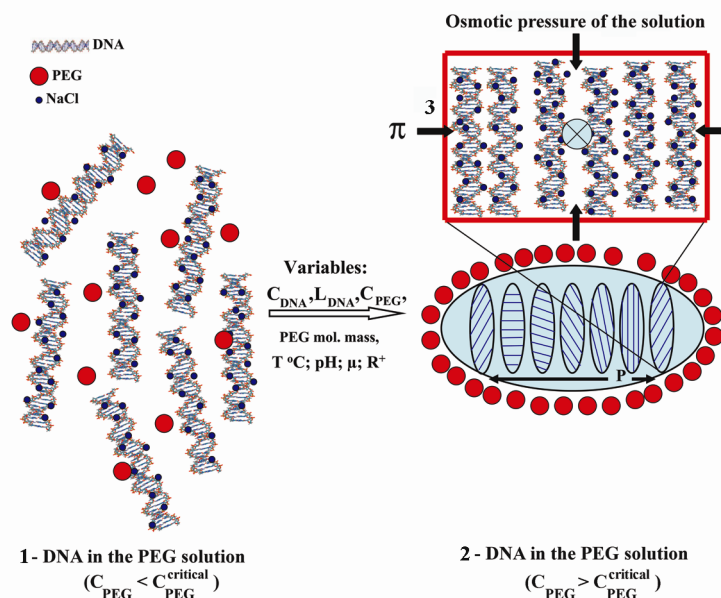


Figure 1: Schematic diagram of formation of CLCD of ds DNA molecules (1). Mobility of DNA molecules in quasimematic layers (3) attaches liquid properties to the formed particle (2), and the ordered location of DNA gives crystal properties, i.e. liquid-crystalline packing is typical of particles. Dispersion particle exists only at a definite osmotic pressure (π) of the polymer-containing solution, and it cannot be “taken into hands”

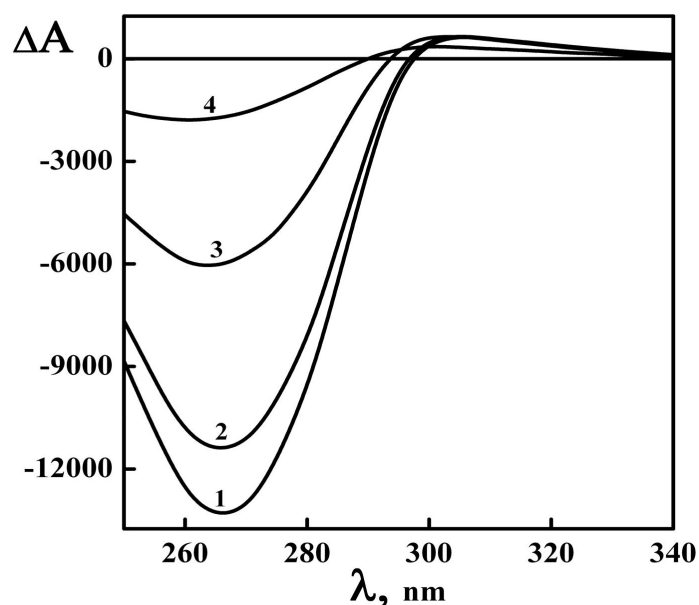


Figure 2: CD spectra of dispersions formed in water-salt PEG-containing solution by ds DNA molecules doped with nano-Au (the diameter of the nano-Au is ~ 2 nm)
 1 - $C_{Nano-Au} = 0$, 2 - $C_{Nano-Au} = 0.57 \times 10^{-8}$ M,
 3 - $C_{Nano-Au} = 1.72 \times 10^{-8}$ M, 4 - $C_{Nano-Au} = 3.59 \times 10^{-8}$ M.
 $C_{DNA} = 30 \mu\text{g ml}^{-1}$, $C_{PEG} = 170 \text{ mg ml}^{-1}$, 0.3 M NaCl + 0.002 M Na^+ -phosphate buffer.
 $\Delta A = (A_L - A_R) \times 10^{-6}$ optical units; $l = 1$ cm.

Besides, any possible aggregation of independent nano-Au outside of particles of CLCD can not induce the change in the real value of the abnormal band in the CD spectrum in the region of absorption of DNA nitrogen bases.

The decrease in the amplitude of the CD band (Figure 2) shows definitely that the negatively charged nano-Au (~ 2 nm) must interact with free, linear ds DNA molecules to induce the changes in the optical activity of the formed CLCD particles.

The CLCDs were formed as well by linear ds DNA molecules doped with 15 nm nano-Au. The CD spectra of these CLCDs are shown in Figure 3 (curves 2-5). One can see again that there is a dependence of spectral effect upon concentration of nano-Au. The higher the concentration of nano-Au in solution, the greater the reduction in intense negative band in CD spectra of DNA dispersions. Besides, comparison of Figure 3 to Figure 2 and their legends shows that 15 nm nano-Au induce the spectral changes approximately 10 times more effective than 2 nm nano-Au.

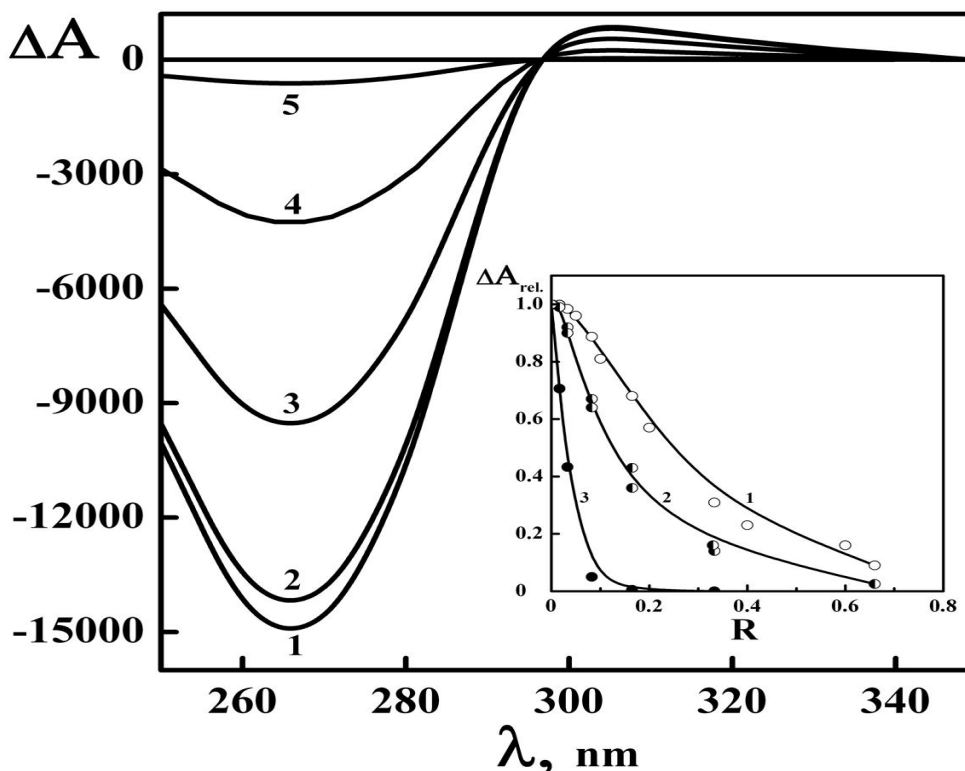


Figure 3: CD spectra of dispersions formed in water-salt PEG-containing solution by ds DNA molecules doped with nano-Au (the diameter of the nano-Au is ~ 15 nm)

1 - $C_{\text{Nano-Au}} = 0$, 2 - $C_{\text{Nano-Au}} = 0.0125 \times 10^{-8}$ M,

3 - $C_{\text{Nano-Au}} = 0.0614 \times 10^{-8}$ M, 4 - $C_{\text{Nano-Au}} = 0.127 \times 10^{-8}$ M,

5 - $C_{\text{Nano-Au}} = 0.309 \times 10^{-8}$ M.

$C_{\text{DNA}} = 30 \mu\text{g ml}^{-1}$, $C_{\text{PEG}} = 170 \text{ mg ml}^{-1}$, 0.3 M NaCl + 0.002 M Na^+ -phosphate buffer.

$\Delta A = (A_L - A_R) \times 10^{-6}$ optical units; $l = 1$ cm.

Inset: the dependence of the relative amplitude band ($\lambda = 270$ nm) in the CD spectra of the CLCDs formed by linear ds DNA molecules doped in solutions of high ionic strength (~0.3) with nano-Au of different sizes versus R value.

1 - DNA + 2 nm nano-Au; 2 - DNA + 5 nm nano-Au; 3 - DNA + 15 nm nano-Au.

R is the ratio of the number of nano-Au to the number of ds DNA molecules in solution.

The CD spectra in Figures 2 and 3 demonstrate clearly that the negatively charged nano-Au in solution of high ionic strength must undoubtedly be bound to initial ds DNA molecules to induce the changes in the abnormal optical activity of their CLCD particles. The binding process depends definitely on the size of nanoparticles. Namely, the greater the size of nanoparticles, the more effective changes in the abnormal optical activity of the CLCD particles. This statement is supported by the inset in Figure 3, which shows that the efficiency of the drop in the amplitude of the abnormal band in the CD spectra of ds DNA CLCDs (formed as a result of phase exclusion of these molecules doped with nano-Au of various sizes) is changed in the following order: 15 nm > 5 nm > 2 nm ([4] and our work in progress).

To get more information on the properties of condensed phase formed by ds DNA molecules doped with nano-Au we have applied the SAXS method.

Experimental scattering intensity profiles from the cholesteric phase of free, linear ds DNA (curve 1, control) and the phases formed by the linear ds DNA doped with 2 nm nano-Au (curves 2-4) are presented in Figure 4. Scattering profile from the cholesteric phase formed by free, linear ds DNA molecules (curve 1) demonstrates the characteristic Bragg peak typical for densely packed ds DNA molecules in CLCD particles with standard structural parameters (Table 1).

The broad Bragg peaks in all cases reflect a local ordered arrangement of the DNA molecules. The twisting of ds DNA molecules cannot be detected directly by X-ray studies, which merely seem to conform that the arrangement of the molecules over small regions containing only tens of molecules.

Figure 4 (curves 2-4) shows that doping of linear ds DNA with nano-Au and phase exclusion of the formed (ds DNA-nano-Au) complexes from PEG-containing solution does not accompanied by alteration in the characteristic structural parameters which reflect local ordering of ds DNA molecules, but results in the decrease in the amplitude of the Bragg maximum. The higher the concentration of nano-Au in solution, the greater the decrease in the Bragg maximum.

All presented optical and SAXS data speak in favor of binding of negatively charged nano-Au to the linear ds DNA molecules.

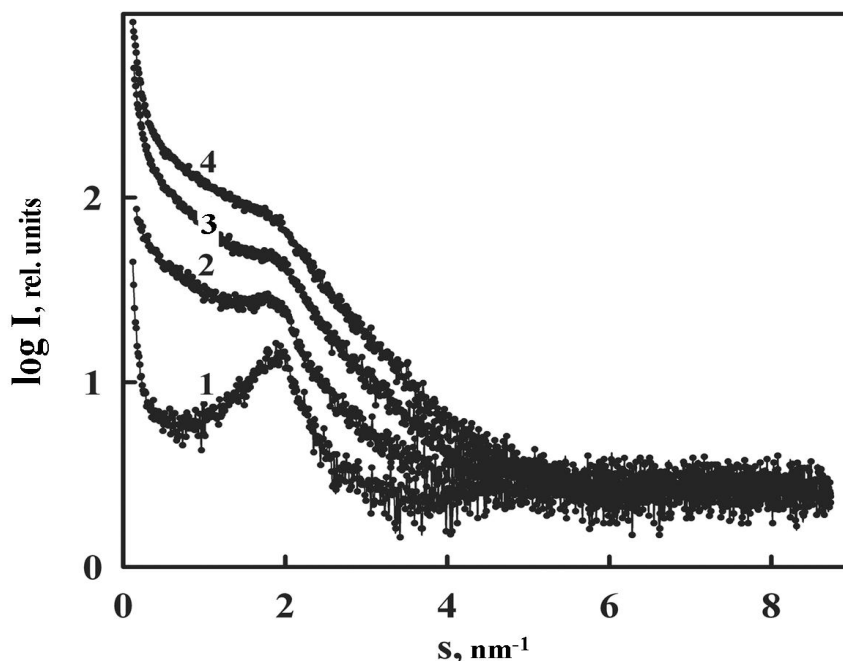


Figure 4: Experimental SAXS curves of phases obtained from the DNA CLCD (curve 1, control) and of phases formed in the PEG-containing water-salt solution by ds DNA molecules doped with nano-Au (curve 2-4)

Specimen 1 – cholesteric liquid-crystalline phase obtained as a result of low-speed centrifugation of the particles of DNA CLCD

formed in the water-salt of PEG ($C_{\text{DNA}} = 30 \mu\text{g ml}^{-1}$, $C_{\text{PEG}} = 170 \text{ mg ml}^{-1}$, $0.3 \text{ M NaCl} + 0.002 \text{ M Na}^+$ -phosphate buffer, $C_{\text{Nano-Au}} = 0$);

Specimen 2 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au ($C_{\text{DNA}} = 30 \mu\text{g ml}^{-1}$, $C_{\text{PEG}} = 170 \text{ mg ml}^{-1}$, $0.3 \text{ M NaCl} + 0.002 \text{ M Na}^+$ -phosphate buffer, $C_{\text{Nano-Au}} = 0.57 \times 10^{-8} \text{ M}$);

Specimen 3 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au ($C_{\text{DNA}} = 30 \mu\text{g ml}^{-1}$, $C_{\text{PEG}} = 170 \text{ mg ml}^{-1}$, $0.3 \text{ M NaCl} + 0.002 \text{ M Na}^+$ -phosphate buffer, $C_{\text{Nano-Au}} = 1.72 \times 10^{-8} \text{ M}$);

Specimen 4 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au ($C_{\text{DNA}} = 30 \mu\text{g ml}^{-1}$, $C_{\text{PEG}} = 170 \text{ mg ml}^{-1}$, $0.3 \text{ M NaCl} + 0.002 \text{ M Na}^+$ -phosphate buffer, $C_{\text{Nano-Au}} = 3.59 \times 10^{-8} \text{ M}$);

The diameter of the nano-Au is $\sim 2 \text{ nm}$

Specimen of DNA phase	$s_{\text{max}}^2, \text{ nm}^{-1}$ ($\pm 0.1 \text{ nm}^{-1}$)	$\bar{d}, \text{ nm}$ ($\pm 0.1 \text{ nm}$)	$L, \text{ nm}$ ($\pm 3.0 \text{ nm}$)	Δ/\bar{d}	$S,$ (relative units)
1	1.9	3.3	20.0	0.14	0.0070
2	1.9	3.3	23.0	0.12	0.0047
3	1.9	3.2	23.0	0.12	0.0034
4	1.9	3.3	21.0	0.13	0.0027

Table 1: The structural characteristics of the phases obtained from the DNA CLCD particles and particles formed by ds DNA molecules, doped with nano-Au (the diameter of the nano-Au is $\sim 2 \text{ nm}$)

Here: s_{max} – wave vector ($s_{\text{max}} = 4\pi\sin\theta/\lambda$; 2θ – scattering angle, λ – radiation wavelength equaling 0.1542 nm);

\bar{d} – interplane distance; L – size of crystallites; Δ/\bar{d} – degree of disordering; S – area under the Bragg peak.

Specimens 1-4 – see Figure 4

Discussion

A few remarks are necessary at the beginning. One can stress [8] that the efficiency of the ds DNA molecules ordering in the CLCD particles depends on the parameters of their secondary structure. The phase exclusion only of ds DNA molecules that possess linear, rigid (or semi-rigid) secondary structure results in the obtaining of the CLCD particles. Flexible single-stranded DNA molecules as well as ds DNA molecules with modified by the bulky chemical or biologically active compounds lose their ability to form the CLCD particles [8]. However, these molecules can form the unordered aggregates as a result of their phase exclusion from the polymer-containing solutions.

The particles of ds DNA liquid-crystalline dispersions formed as a result of the phase exclusion from water-salt (0.3 M NaCl) PEG-containing solution (Figure 1) have a size of about 500 nm ; each particle contains approximately 10^4 DNA molecules [8].

Taking into account the technology of obtaining these particles one can say that minimization of the excluded volume induces unidirectional alignment of 10^4 ds DNA molecules in a form of particles with lateral hexagonal order, which corresponds to the optimal solution to pack a great number of linear, rigid DNA molecules into a reduced volume. Despite of the close packing linear high-molecular mass ds DNA molecules in the hexagonal array, the structure of formed particles does not correspond exactly to structure of a true crystal. In this structure one can define three potential series of molecular or “quasinematic” [23] layers, accor-

ding to the three main directions of the hexagonal structure. In these layers linear ds DNA molecules are lying in the plane of layers whose thickness is close to the interhelix spacing. Double-stranded DNA molecules possess some disorder around their positions; they can slide and bent with respect of each other, as well as they can rotate around their longitudinal axes.

In the case of helical (chiral) molecule such as ds DNA, their unidirectional alignment, and hence, spatial organization, competes with the tendency of helices to form spatially twisted structures. The main effect of chirality is that the high molecular mass DNA molecules (or their “quasinematic layers” [24]) do not tend to pack parallel to their neighbors, but rather at a minor angle with respect to their neighbors. Chirality results in a macroscopic twist in the orientation of ds DNA molecules with a characteristic spatial pitch (P). (For ds DNA molecules close packing is important electrostatic interaction between these molecules because of high density of fixed surface charges (backbone phosphate groups and adsorbed counter ions). Chiral interactions are likely to be dominated by electrostatics.)

The experiments showed that the ds DNA liquid-crystalline particles are characterized by spatially twisted structure with “quasinematic layers” spaced by 2.9 to 5.0 nm, depending on the osmotic pressure of the PEG solution; each following layer of ds DNA molecules in a particle of CLCD is rotated by a certain angle ($\sim 0.5^\circ$) with respect to the previous one [8].

Among the interactions that have been involved to explain the alignment of linear ds DNA molecules are:

1. Long-range attractive dispersion (van der Waals) interactions between ds DNA molecules;
2. Short-range repulsive interactions whose origin in the steric (chiral) structure of ds DNA molecules;
3. Direct Coulomb interactions which usually take the form of dipole-quadrupole interactions between electrically neutral ds DNA molecules [25].

Up to now no consensus has been reached to exactly explain which microscopic interaction between ds DNA molecules dominates in producing the CLCD particles.

Besides, spatial structure of ds DNA CLCD particles is influenced by varying the properties both of ds DNA molecules and the polymer (PEG) outside particles, as well as the preparation method, i.e. the boundary conditions at the CLCD particle surface. The surface boundary conditions will tend to influence the orientation of ds DNA molecules near the surface and the aligning effect may propagate inside the particle. In general, there will be competition between the molecular orientation of DNA molecules induced by surface boundary condition, the effects of ordering on the liquid crystal itself due to the DNA molecules trying to arrange parallel to each other and the disordering effect of spatial twist and temperature [26].

The existence, shape and size of the CLCD particles results from a fine balance between their bulk free energy and their surface free energy. The bulk free energy tends to increase the size of CLCD particles, whereas the surface free energy, which depends strongly on the surface tension between the cholesteric and isotropic DNA phases, tends to reduce the size of CLCD particles [27].

Hence, there is a whole set of various physical factors which influence the mode of ds DNA molecules packing in the CLCD particles as well as their spatial structure [28].

There are a very important, for our consideration, theoretical statements, a namely, counterions bound to the ds DNA influence the efficiency of condensation of these molecules [29] and especially, “the macroscopic properties of the cholesteric ds DNA phase are affected by the pattern of counterion adsorption” [30,31].

With these remarks in mind one can return back to Figure 2 and Figure 3. From the theoretical point of view [22] the amplitude of the abnormal band in the CD spectrum of CLCD particles depends on their concentration, on their size and on long-range order of the chromophores of ds DNA molecules (nitrogen bases). (Because nitrogen bases are rigidly fixed in the secondary structure of linear ds DNA molecules, it means that the amplitude of abnormal band in the CD spectrum depends on long-range order of these molecules.)

Taking into account the used fixed (standard) technology for obtaining all ds DNA CLCDs, one can say that the amplitude of the abnormal band is related with extent of twist of ds DNA “quasinematic layers” in the spatial structure of CLCD particles [22].

However, the peculiarities of the secondary structure of linear ds DNA molecules and pattern of fixation of various compounds nearby the surfaces of the neighboring molecules define the mode of ds DNA’s “recognition”, which is necessary for formation of spatially twisted structure at phase exclusion.

If negatively charged nano-Au are fixed by this or that way nearby surfaces of neighboring ds DNA molecules [5], one can suppose that the peculiarities of the formed (ds DNA-nano-Au) complexes differ from the that of linear ds DNA molecules. In this case the mode of the “recognition” of the (ds DNA-nano-Au) complexes can have a dramatic effect on the properties of spatial structure formed at phase exclusion, and on the value of an abnormal band amplitude in the CD spectrum. One can expect that the higher the extent of fixation of nano-Au, the lower the amplitude of the band in the CD spectrum.

Hence, the simplest explanation for the decrease in the amplitude of abnormal band in the CD spectra (Figures 2 and 3) looks like so [22]: “the decrease in the amplitude of abnormal bands in the CD spectra is related to the change in the extent of helical twist of ds DNA “quasinematic layers” in structure of CLCD particles”. This change means the unwinding of helical structure of ds DNA CLCD particles, induced by alteration of structural parameters of linear ds DNA molecules as a result of fixation of nano-Au nearby ds DNA surfaces before the phase exclusion.

From this point of view, the results presented in Table 1 are of interest. These results demonstrate that despite of increase in concentration of nano-Au added to linear ds DNA, the characteristic structural parameters (s_{\max} , d - and L - values), which describe short-range order of neighboring ds DNA molecules, are practically constant for all formed phases. We do not see any changes in the structural properties of ds DNA molecules forming all phases, which scatter the X-rays. This contradicts the simplest explanation for the CD band change formulated above. Table 1 shows that for all obtained phases the areas under the Bragg peaks are diminished.

Taking into account that we have used fixed concentration of ds DNA molecules ($C_{\text{DNA}} \sim 3.75 \times 10^{-8}$ M) and standard procedure to obtain all samples for the CD and the SAXS studies, as well as standard conditions to perform the SAXS experiments, one can accept (after [30]) that the area under the Bragg peak is related to the concentration of free, linear ds DNA molecules in the solution, whose secondary structure "suitable" for formation of the CLCD at the phase exclusion [7,8]. In the frame work of this hypothesis, line 1 in the Table 1, which reflects the properties of CLCD formed by free, linear, initial ds DNA molecules, means that 100 % of these DNA molecules are capable of forming the CLCD. Its characteristic structural parameters obtained from SAXS data are shown in Table 1 (line 1) and optical property is expressed as the negative abnormal band in the CD spectrum (Figure 2, curve 1).

Table 1 shows that the characteristic structural parameters (s_{\max} , d - and L - values) that reflect the peculiarities of the secondary structure of ds DNA molecules as well as the distance between these molecules in the formed condensed phases are not altered, i.e. the liquid-crystalline phases are formed by ds DNA molecules with *constant initial secondary structure*.

Because the structural parameters of all phases formed after doping of ds DNA molecules with nano-Au and their phase exclusion are not changed (except the areas under the Bragg peak (S)), one can suppose that the doping of linear ds DNA molecules with nano-Au results in an appearance of a "population" of such molecules, which are not capable of forming the CLCD. Instead, they form aggregates, "invisible" by SAXS.

Indeed, it is not excluded that process of doping, accompanied by fixation of nano-Au nearby surfaces of ds DNA molecules, is characterized by specific mode of distribution of nano-Au between ds DNA molecules and, probably, by modification of their secondary structure [32]. So, an increase in nano-Au concentration results in decrease in concentration of free, linear ds DNA molecules capable of forming CLCD. It means that the second (and so on) line in Table 1 with not altered characteristic structural parameters (but diminished the area under the Bragg peak) reflects decrease in concentration namely of free ds DNA molecules, which is proportional to decrease in the area under the Bragg peak (in comparison to the area under the Bragg peak for initial ds DNA molecules). Thus, using this hypothesis, one can obtain the concentration of free, linear ds DNA molecules indeed forming the CLCD (C_{DNA} ; Table 2, column 4) despite of addition of nano-Au to initial ds DNA solution. The difference between the initial concentration of ds DNA molecules (Table 2, line 1, column 4) and the obtained value can evaluate the concentration of linear ds DNA molecules bounded with nano-Au (C'_{DNA} ; Table 2, column 5). This allows one to estimate the value of the "binding constant" (K_b) of nano-Au with ds DNA molecule. Its value is equal to about $3.5 \times 10^7 \text{ M}^{-1}$. Because the concentrations of nano-Au are known, one can estimate R-value (the ratio of the number of nano-Au particles to the number of DNA molecules modified by nano-Au calculated according to the SAXS data (Table 2, column 8)).

Specimen of DNA phase	S, (relative units)	% of DNA molecules forming CLCD particles	C_{DNA} (M)	C'_{DNA} (M)	$C_{\text{Nano-Au}}$ (M)	ΔA_{270} (optical units)	R
1	2	3	4	5	6	7	8
1	0.0070	100	3.75×10^{-8}	0	0	-13330×10^{-6}	0
2	0.0047	67.14	2.52×10^{-8}	1.23×10^{-8}	0.57×10^{-8}	-11420×10^{-6}	0.464
3	0.0034	48.57	1.82×10^{-8}	1.93×10^{-8}	1.72×10^{-8}	-6060×10^{-6}	0.891
4	0.0027	38.57	1.45×10^{-8}	2.30×10^{-8}	3.59×10^{-8}	-1700×10^{-6}	1.560

Table 2: Some parameters, which were calculated based on the data shown in table 1

Here: S - area under the Bragg peak (relative units);

C_{DNA} - concentration of DNA molecules unmodified by nano-Au (M);

C'_{DNA} - concentration of DNA molecules modified by nano-Au (M);

$C_{\text{Nano-Au}}$ - concentration of nano-Au (M);

ΔA_{270} - amplitude of abnormal band in the CD spectrum at $\lambda = 270$ nm (optical units);

R - the ratio of the number of nano-Au particles to the number of DNA molecules modified by nano-Au calculated according SAXS data.

The percentage of DNA molecules forming CLCD particles was calculated from the values of the area under the Bragg peak (S).

Specimens 1-4 - see Figure 4.

Table 2 demonstrates that for the case of 2 nm nano-Au the R-value is changed from ~ 0.5 (i.e. one nano-Au *per* two ds DNA molecules) to ~ 2 (i.e. two nano-Au *per* one ds DNA molecule).

High K_b - and small R-values speak in favor of a supposition that nano-Au may very strongly stick (fix) onto ds DNA molecules [33,34]. In the framework of the *chemical mechanism* of nano-Au fixation, a single nano-Au of any size can, in principle, form complexes with nitrogen base pairs (namely, N7 atoms of purine and N3 atoms of pyrimidine [35]) of ds DNA. Taking into account the possibility of deformation of spatial structure of nano-Au [36,37], chemical interaction between nitrogen bases and nano-Au appears to be a function of nano-Au size. In this case, an increase in the nano-Au size may lead to increase in the number of free electrons to share with nitrogen bases. The efficiency of the formation of complexes between nitrogen bases of ds DNA and nano-Au is limited due to the sterical location of nitrogen bases in the spatial structure of ds DNA, and thus cannot be considered as the main mechanism of ds DNA-nano-Au interaction.

So, one has to look for some other explanation for why nano-Au bind to ds DNA molecules. In our opinion, the “physical” mechanism of nano-Au-dsDNA interaction is a good candidate.

The «physical» mechanism of nano-Au - ds DNA interaction. (The theoretical description of this mechanism for the case of 2 nm nano-Au fixation nearby the ds DNA molecules is presented in **Addendum**, see below).

Taking into account the fact that CLCDs of ds DNA were obtained at high salt concentration leading to a decrease in electrostatic repulsion between nano-Au and ds DNA molecules, we advocate here the following mechanism of nano-Au-ds DNA interaction. The charges of the phosphate groups of ds DNA induce a dipole in the highly polarizable nano-Au. This mechanism is quite short ranged ($\sim 1/d^4$), where d is the distance between polarization center of nano-Au and DNA base pair local charge. One might think that the Coulomb repulsion keeps the species apart at longer distances. Under our conditions, the high ionic strength (~ 0.3) of the water-salt solution screens the long-range repulsion so the nano-Au are allowed an approach to the DNA base pair charges. At a certain distance, the ion-induced dipole interaction takes over, resulting in a net attractive force [29,30,37]. This is accompanied by fixation (immobilization) of nano-Au nearby the surface of ds DNA molecules. Besides, the polarizability of a conducting sphere is proportional to the cube of the sphere radius, resulting in a large drop in polarizability when changing from 15 nm to 2 nm nano-Au. This results in dropped efficiency of nano-Au-ds DNA interaction with decrease in the size of nano-Au.

If this is the case, it means that 15 nm nano-Au can be bound with ds DNA stronger in comparison to 2 nm. Indeed, Figure 3 shows that doping of ds DNA with 15 nm nano-Au is accompanied by much pronounced drop in an abnormal optical activity of the formed CLCD.

Taking into account that at formation of CLCD particle with an ordered arrangement of ds DNA molecules the “recognition” distance between them is close to 5.0–10.0 nm, one can expect that if nano-Au are fixed near the surfaces of these molecules, they must sterically prevent both their “recognition” and proper spatial orientation, which define mutual twist of ds DNA molecules.

Besides, the facet spatial structure of nano-Au [38–41] creates conditions, under which neighboring ds DNA molecules in water-salt solutions of high ionic strength, can be assembled on various facets of nano-Au [38,42]. This leads to disordered and uncorrelated fixation of ds DNA molecules on surface of nano-Au, i.e. to the formation of ds DNA aggregates. In the framework of this model, the greater the size of nano-Au, the effective the formation of ds DNA aggregates [4].

Comparison of Figure 3 to Figure 2 speaks in favor of this statement and shows that the greater the size of nano-Au, the smaller is the concentration of the nano-Au, which is necessary for disappearance of abnormal band in the CD spectrum typical for ds DNA CLCD.

Of course, although the elaboration of the physical model of the fixation of nano-Au between neighboring ds DNA molecules needs additional experimental work, it is not excluded that the combination of the “chemical” and the “physical” mechanisms can define peculiarities of negatively charged nano-Au fixation on the ds DNA molecules as well as high K_b - and small R - values.

Hence, negatively charged nano-Au are undoubtedly be bound to initial, free, linear ds DNA molecules in water-salt solutions of high ionic strength. Taking into account the possibility of uncorrelated positions of nano-Au nearby the neighboring ds DNA molecules, the phase exclusion of these complexes results in formation of disordered aggregates from ds DNA molecules linked via nano-Au. This allows one to suppose that the spatial topography of nano-Au may play a role of “random external field”, which suppresses both ds DNA molecules “recognition” and formation of cholesteric structure from (ds DNA-nano-Au) complexes. Analogous transformation of lamellar phases under influence of nanoparticles, including nano-Au (2 nm), was considered in [43,44].

Thus negatively charged nano-Au can be fixed nearby the surfaces of ds DNA molecules in the water-salt solution of high ionic strength. This process is accompanied by an appearance of ds DNA molecules, which are incapable to form the CLCD particles with specific optical properties. Hence, the change in the amplitude of the CD band is related to concentration of free, linear ds DNA molecules capable of forming the CLCD. This permits to construct the dependence between the amplitude of abnormal negative band in the CD spectra of the CLCDs and recalculated concentration of free, linear ds DNA still forming CLCDs (Figure 5). This dependence clearly shows that in contrast to the simplest explanation for the decrease in the amplitude of the abnormal band in the CD spectrum, doping of linear ds DNA molecules with nano-Au (~ 2 nm) results in the decrease in concentration of the these molecules capable of forming CLCD.

Hence, all results presented above show that negatively charged nano-Au can be fixed nearby the surfaces of ds DNA molecules in the water-salt solution of high ionic strength. This process is accompanied by an appearance of ds DNA molecules, which are incapable to form the CLCD particles with specific optical properties.

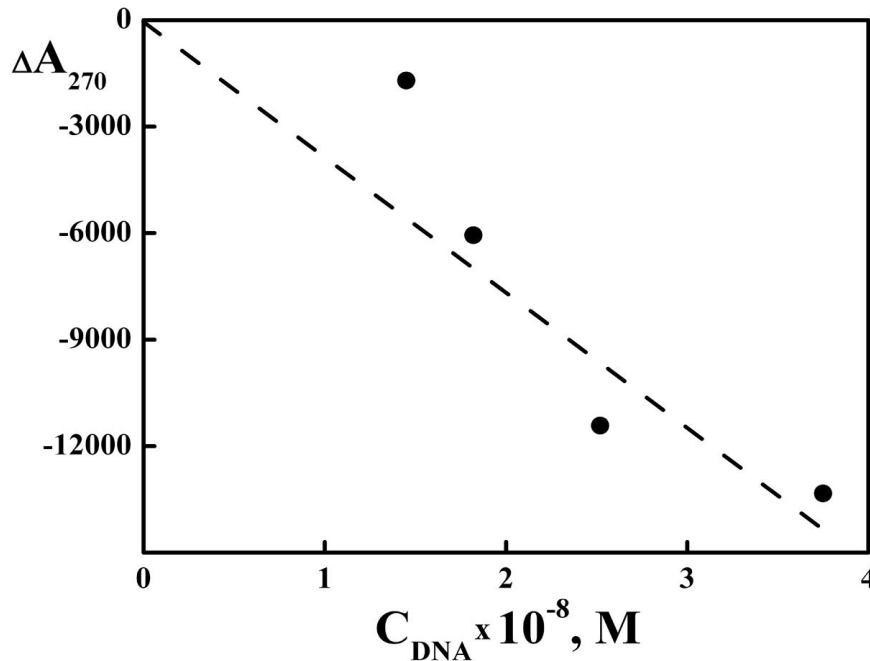


Figure 5: The dependence of the amplitude of negative band ($\lambda = 270$ nm) in the CD spectra of the DNA dispersions versus recalculated DNA concentration (in the case of nano-Au ~2 nm doping)

Addendum

Evaluation of electrostatic interaction between nano-Au and ds DNA molecule in water-salt solution

To confirm an ability of nano-Au to be fixed nearby ds DNA molecules, the theoretical calculation of strength of electrostatic interaction of these particles and ds DNA in water-salt solutions have been performed.

The interaction of DNA and a nano-Au is governed by two main contributions: the electrostatic interaction (due to the polarization of the nano-Au in the electrostatic field of fully dissociated DNA) [45,46], and the van der Waals interaction between the gold and DNA atoms. Van der Waals forces appear only at short distances and decay rapidly with increasing distance between the DNA and nano-Au (as a function of r^{-6}). One can say that the energy of interaction of DNA-nano-Au is proportional to the number of pairs of atoms involved in the interaction.

One can evaluate the electrostatic interaction of the polarized nano-Au and DNA in water-salt (0.3 M NaCl) solution. The DNA can be considered as a linear sequence of spherical units with identical diameter D , separated by a distance $0.34s$ with linear charge density $l = -2e/0.34s$, hereinafter s – a unit of length equal to 1 nm [45,46].

The electrostatic potential of a unit charge in the presence of low-molecular ions is described by the screened Coulomb potential:

$$\frac{U(r)}{k_B T} = \frac{\Gamma}{r} e^{-\kappa r}, \quad \kappa = \sqrt{4\pi(\rho_{Na} + \rho_{Cl})\Gamma\sigma^3}, \quad \Gamma = e^2 / 4\pi\epsilon_0\epsilon k_B T \sigma, \quad (1)$$

Here ρ_i is the average number density of Na^+ and Cl^- ions, k_B – Boltzmann's constant, T – absolute temperature (238 K), ϵ – the dielectric constant of the medium.

In the equation (1), and further, it is understood that all the distances r between interacting centers are calculated in σ units. For convenience, $U(r)$ is expressed in $k_B T$ units. For a vector of the electrostatic field $d\mathbf{E}(\mathbf{r})$, generated by DNA fragment dl (r – a vector from fragment from dl to a selected point in space), we can write the following equation:

$$d\mathbf{E}(\mathbf{r}) = -\lambda(\nabla_{\mathbf{r}}U(r))dl \quad (2)$$

A radial component $E_r(H)$ of the electrostatic field at a distance H from the axis of the DNA can be written as following equation:

$$E_r(H) = -\lambda \int_{-\pi/2}^{\pi/2} (\nabla_{\mathbf{r}}U(r)H / \cos(\psi))d\psi, \quad (3)$$

Here, we replace $dl = rd\psi/\cos(\psi)$ and $r = H/\cos(\psi)$ (ψ – angle between the vector $d\mathbf{E}(\mathbf{r})$ and the radial direction in nano-Au from its center to surface).

Evaluation of $E_r(H)$ shows that the electrostatic field of DNA in the case of water-salt solution (0.3 M NaCl, $\epsilon \approx 80$) decays very rapidly (Figure 6) and at distances greater than a diameter of the DNA is virtually absent.

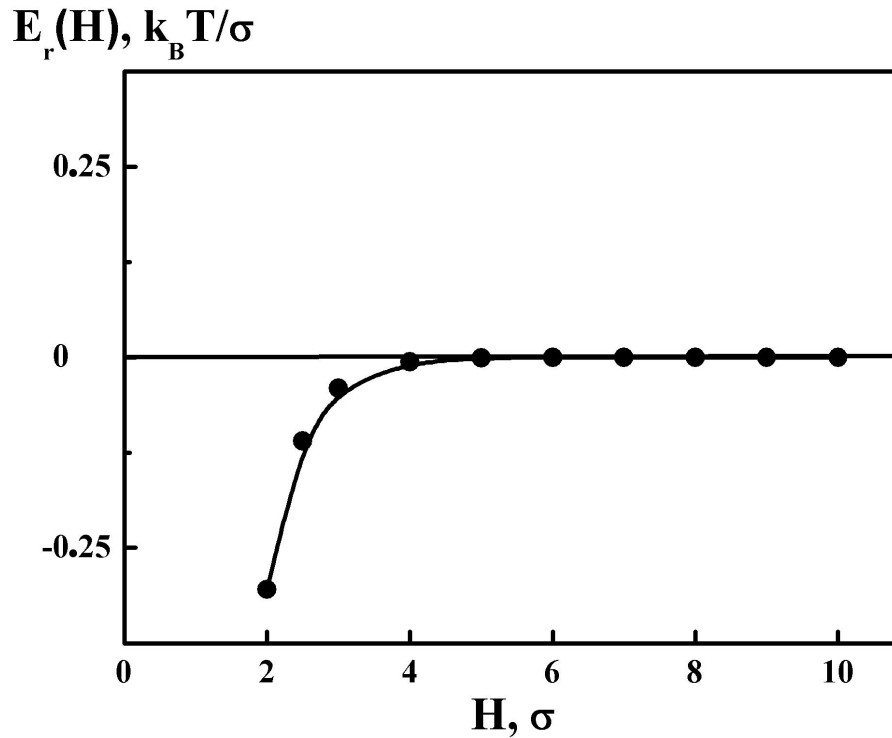


Figure 6: The radial component of the electrostatic field of DNA in aqueous solution in presence NaCl (0.3 M) ($T = 298$ K) as a function of distance (H , in σ units) from the center axis of the DNA

We consider that all nano-Au with $D \geq 2$ nm as conductors, what is confirmed by the presence of localized surface plasmon resonance [38].

The distance H is measured between the axis of the DNA and the surface area on nano-Au surface. Since the electrostatic field of DNA decays rapidly, we assume that the DNA-nano-Au interaction depends on the radial component of the Coulomb force $dF_r(D, \varphi, \theta, H)$ between the induced positive charges $d\Sigma_{ind}$ on the nano-Au surface $\Delta S(D, \varphi, \theta)$ and a DNA fragment dl (φ, θ – the polar angles in a reference frame associated with the nano-Au center).

The induced charge $d\Sigma_{ind}(D, \varphi, \theta, H)$ on the surface element $\Delta S(D, \varphi, \theta)$ facing towards to DNA can be easily found:

$$d\Sigma_{ind}(D, \varphi, \theta, H) = \frac{2\varepsilon_0 \varepsilon k_B T \sigma \Delta S(D, \varphi, \theta)}{e^2} \int_{-2D}^{2D} \Omega(D, \varphi, \theta, H) \cos(\mathbf{n}\mathbf{r}) d\mathbf{E}(\mathbf{r}), \quad (4)$$

Here we take into account contributions of all DNA elements dl which are “visible” from a point on nano-Au surface which has coordinates φ, θ . Vector \mathbf{n} is a normal to the nano-Au surface, and the function Ω is equal to 1 if the surface element dl “visible” from the point with coordinates (φ, θ) , or 0 otherwise. The limits of integration $\pm 2D$ chosen from the condition that $|\mathbf{dE}(\mathbf{r})| > 0.001 k_B T / \sigma$. Combining everything together we end with the following expression for the interaction force:

$$F_r(D, H) = \iint_{\varphi, \theta} \int_{-2D}^{2D} \cos(\mathbf{H}\mathbf{r}) d\mathbf{E}(\mathbf{r}) d\Sigma_{ind}(D, \varphi, \theta, H), \quad (5)$$

Here, the vector $H(D, \varphi, \theta, H)$ is external normal to the surface point of nano-Au to DNA and $H \perp dl$.

The calculated value of the force acting on the nano-Au as a function of the distance H between its surface and the axis of DNA is shown in Figure 7. As can be seen from the Figure 7, the radial component of the electrostatic force increases with increasing nano-Au diameter and rapidly decays with increasing H .

The above evaluations allow one to say (in the agreement with our experimental results) that the physical mechanism provides conditions for “fixation” of nano-Au nearby the surface of ds DNA molecules and efficiency of such “fixation” grows with the increase in the size of nano-Au.

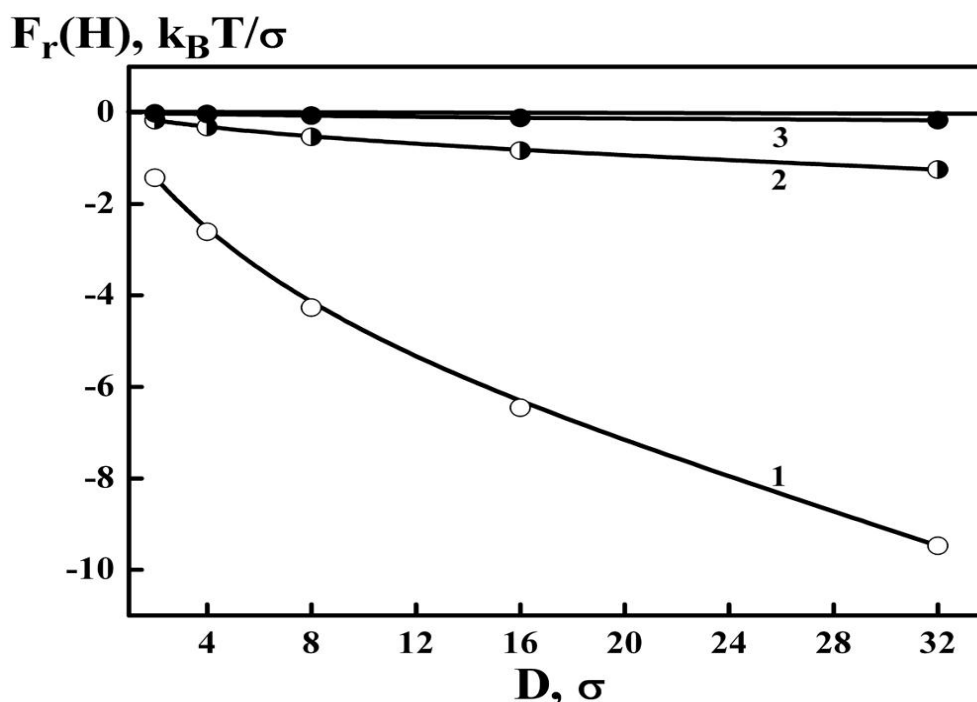


Figure 7: Evaluation of the radial component of the force acting on nano-Au from DNA in an aqueous solution of NaCl (0.3 M) ($T = 298$ K) as a function of the diameter D of the nano-Au in the case of different distances H (in σ units) between the DNA axis and the surface of nano-Au 1 - $H = 1.0$; 2 - $H = 1.5$; 3 - $H = 2.0$

Conclusion

Our experimental data, supplemented by a simple model numeric computations of screened (in the water-salt solution of high ionic strength) electrostatic energy for interacting ds DNA molecules and negatively charged but polarizable nano-Au, support the physical mechanism for fixation of nano-Au between neighboring linear ds DNA molecules. This is accompanied by so strong modification of these molecules that they are not able to align to form orientation ordering. Hence, the complexes of (ds DNA-nano-Au), in contrast to the free, linear ds DNA molecules, lose an ability to form spatially twisted structure at their condensation. Instead of ordered spatial structure of free, linear ds DNA CLCD only random disordered aggregates are formed by (ds DNA-nano-Au) complexes at their phase exclusion from water-salt polymer-containing solution.

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