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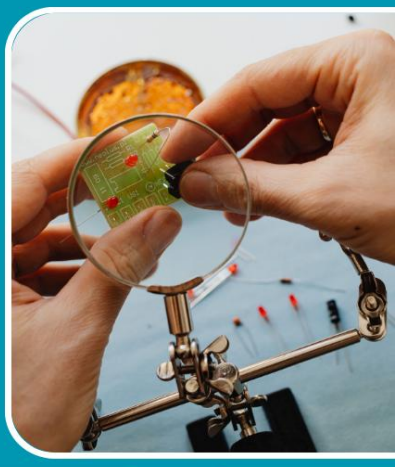
FRONTIERS IN MATERIAL SCIENCE AND NANOTECHNOLOGY

ISSN: (3065- 4114)



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Frontiers in Material Science and Nanotechnology
Volume1, Issue3, 2025

Spatial Packing of Double-Stranded DNAs "Controlled" by Negatively Charged Gold Nanoparticles

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Article Info

Received: 25-07-2025 ***Revised:02-09-2025*** ***Accepted:10-09-2025*** ***Published:22-09-2025***

Abstract

In this paper, we detail recent visual and small-angle X-ray scattering (SAXS) observations of the dispersion development in ds DNA molecules doped with nano-Au, which are negatively charged nanoparticles. The amplitude of the abnormal negative band in the circular dichroism spectra of the formed cholesteric liquid-crystalline dispersions (CLCD) is reduced when nano-Au is fixed near the surfaces of linear double-stranded DNA in a solution of high ionic strength (0.3 M NaCl) and then the (ds DNA-nano-Au) complexes are phase-extracted from a solution containing poly(ethylene glycol). The standard structural parameters derived from SAXS data, which represent the local ordering of ds DNA molecules, are unaffected by doping linear ds DNA with nano-Au and then phase excluding the resulting (ds DNA-nano-Au) complexes; however, the amplitude of the characteristic Bragg maximum is reduced. Doping double-stranded DNA with negatively charged nano-Au causes a population of "modified" double-stranded DNA molecules to emerge, according to our experimental data and a basic model numerical calculation of screened electrostatic energies for ds DNA molecules and negatively charged but polarizable nano-Au in a water-salt solution of high ionic strength. In contrast to free ds DNA molecules, these molecules are unable to form spatially twisted structures during phase exclusion, leading to the formation of chaotic aggregates rather than the ordered spatial structure often seen in ds DNA CLCD.

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

Introduction

Due to their potential applications and nontrivial biological consequences, double-stranded DNA spatially twisted liquid-crystalline dispersions (CLCD) doped with metallic nanoparticles, such as nano-Au or cobalt ferrite nanoparticles, have recently garnered a lot of experimental and theoretical attention [1-6]. It is well-established that dsDNA CLCD's physicochemical characteristics mirror those of these macromolecules found in biological things like chromosomes of early creatures (such the dinoflagellate's) and viruses that include DNA [7,8]. Researchers in the fields of nanotechnology and biology are therefore interested in the possibility of doping double-stranded DNA CLCDs with nano-Au. To determine why nano-Au is genotoxic, it could be helpful to examine how it affects DNA CLCD characteristics [9–11]. In the field of nanotechnology, DNA liquid-crystalline dispersions treated with nano-Au have the potential to give rise to novel materials with interesting characteristics [12]. In their seminal works, Mirkin [13] and Alivisatos [14] outlined the characteristics of linear single-stranded and double-stranded DNA molecules that had positively charged nano-Au attached to their surfaces.Discovering the effects of negatively charged nano-Au on linear double-stranded DNA molecules is the primary motivation for our research. Asking some broad questions and providing broad answers sounds like a good first step. In particular, we sought to address the following inquiries.

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

Creating nano-Au and finding their typical dimensions In this work, two different sizes of nano-Au were included into colloid solutions. Using the reduction of HAuCl4 as stated earlier, solutions (hydrosols) containing nano-Au with average diameters of 2 and 15 nm were produced. The Duff approach was used to

create 2 nm nano-Au with a numerical concentration of CN = 3.5×1015 particle ml-1 [15]. As per the Frens citrate technique [16], 15 nm nano-Au with a concentration of 3.5×1012 particle ml-1 were produced. Assuming full reduction of Au, the material balance was used to determine the numerical concentrations of all nano-Au samples. Every single nanoparticle had an opposite charge. They had ξ-potentials of about -40 mV at pH levels that were neutral.Transverse electron microscopy confirmed the typical dimensions of the nano-Au in the stock solutions. A Jeol-100CX electron microscope from Japan was used to acquire micrographs of the nanoparticles. Ingredients for dsDNA CLCDs and their preparation: We used depolymerized double-stranded DNA (dsDNA) from a calf thymus (Sigma, USA) that had a molecular mass of about (0.6-0.8)×106 Da after further purification. By using the established value of the molar extinction coefficient (εmax = 6,600 M-1 cm-1), the DNA content in the water-salt solutions was ascertained by spectrophotometry. No further purification was performed on the poly (ethylene glycol) (PEG; Serva, Germany; molecular mass of 4,000 Da) sample before its usage. Conception of double-stranded DNA circular DNA double-strand breaks: The technique of vigorously combining (shaking) a water-salt solution containing PEG with a water-salt ds DNA solution was used to create standard CLCDs of ds linear DNA molecules. Read [8] for all the information on this technique. Measurements The spectrophotometer used to capture the absorption spectra was a Cary 100 Scan, manufactured in Vari, USA. Spectra of circular dichroism (CD) were captured using a portable dichrometer called SCD-2, which was manufactured by the Institute of Spectroscopy of the Russian Academy of Sciences in Moscow-Troizk [17]. The dependency of the difference between the intensities of absorption of left- and right-handed polarized light (ΔA; YA = (AL - AR)) on the wavelength (λ) was used to illustrate the CD spectra. A small-angle X-ray scattering (SAXS) analysis was performed on pellets (~ 3 mg) of DNA CLCD particles and dispersion particles (~ 2 and 15 nm) formed by double-stranded DNA molecules doped with nano-Au and packed in a PEG-containing water-salt solution. The pellets were produced by slow speed centrifugation (5,000 rev. min-1, 40 min, 4 oC; centrifuge K-23, Germany). In the range of the moment transfer, which is defined as $4\pi\sin\theta/\Lambda$, where 2θ is the scattering angle and λ = 0.1542 nm is the X-ray wavelength, the measurements were performed on a laboratory diffractometer Amur-K with a Kratky-type (infinitely long slit) geometry. After initial processing according to established protocols, the experimental scattering profiles were adjusted to account for solvent background scattering [18]. In order to determine the peak location (smax) on the scattering patterns, the software PEAK was used to compute the repeating distances of the periodic motifs in the crystalline areas ($d= 2\pi/smax$) [18]. Using the usual equations provided in [19], the mean long-range order dimension, L (the size of crystallites), and the degree of disorder in the system, Δ/d, were determined.

Results

Figure 1 depicts the primary framework for ds DNA CLCD generation and the primary elements that impact this process. You can see the basic idea behind phase exclusion of double-stranded DNA molecules doped with negatively charged nano-Au in this figure. Nano-Au (~ 2 nm or 15 nm) was added to a linear dsDNA solution, and the mixture was let to sit at room temperature for one hour. The last step was to combine the finished product with a PEG solution while stirring. Consequently, any (ds DNA-nano-Au) complexes that do form undergo phase exclusion. The resultant mixes' CD spectra were measured after an extra hour of exposure. Since Lerman's investigations, the process of phase exclusion of linear double-stranded DNAs from solutions containing water and salt has been referred to as "ψ-condensation" (where psi stands for polymer-and-salt-induced) [20,21]. Phase exclusion from a PEG solution is followed by the production of CLCD in the instance of linear, rigid, low molecular mass ds DNA molecules (molecular mass of about (0.6-0.8)×106 Da) [8].The creation of double-stranded DNA CLCD is seen in Figure 2 along with the emergence of a strong negative band in the CD spectrum (curve 1), which is situated in the area where nitrogen bases are absorbed (λ ~ 270 nm). The presence of this band provides undeniable evidence of the spatial twisting of nearby "quasinematic layers" in DNA dispersion particles [22]. In CLCD particles, the left-handed twist of the right-handed DNA molecules (B-form) is confirmed by the negative sign of the aberrant band in the CD spectrum. The concentration of nano-Au in the solution determines the amplitude of the CD band (curves 2-4). An aberrant band in CD spectra of DNA dispersions is reduced to a larger extent with increasing nano-Au concentrations.

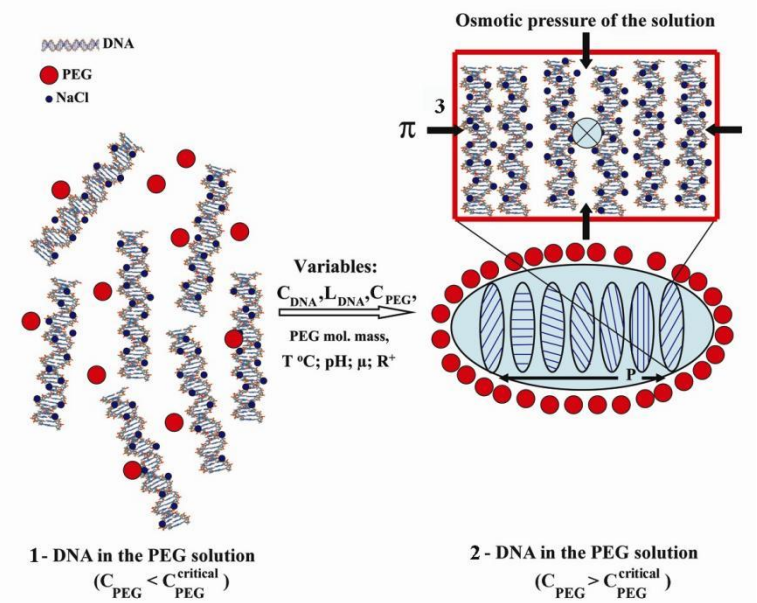

Figure 1: Schematic diagram of formation of CLCD of ds DNA molecules (1). Mobility of DNA molecules in quasinematic layers (3) attaches liquid properties to the formed particle (2), and the ordered location of DNA gives crystal properties, i.e. liquid-crystalline packing is typical of particles. Dispersion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

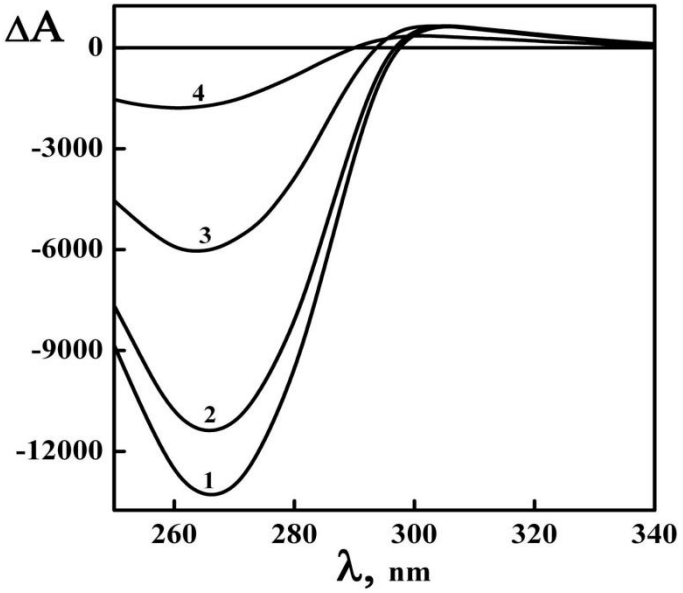

Figure 2: CD spectra of dispersions formed in water-salt PEG-containing solution by ds DNA molecules doped with nano-Au (the diameter of the nano-Au is ~ 2 nm)
1 – $C_{Nano\text{-}Au}$ = 0, 2 – $C_{Nano\text{-}Au}$ = 0.57×10^{-8} M,
3 – $C_{Nano\text{-}Au}$ = 1.72×10^{-8} M, 4 – $C_{Nano\text{-}Au}$ = 3.59×10^{-8} M.
C_{DNA} = 30 μg ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer.
$\Delta A = (A_L - A_R)\times10^{-6}$ optical units; l = 1 cm.

Also, the aberrant band in the CD spectra, which is responsible for DNA nitrogen base absorption, cannot be changed by the hypothetical aggregation of independent nano-Au outside of CLCD particles. A clear indication that the negatively charged nano-Au (~ 2 nm) must interact with free, linear ds DNA molecules in order to cause changes in the optical activity of the CLCD particles that are generated is the reduction in the amplitude of the CD band (Figure 2).Nano-Au doped linear ds DNA molecules measuring 15 nm also contributed to the formation of CLCDs. In Figure 3 (curves 2-5), you can see the CD spectra of various CLCDs. It is once again evident that the spectral impact is concentration dependent. The decrease in the intensity of the negative band in the CD spectra of DNA dispersions increases as the concentration of nano-Au in the solution increases. In addition, the captions of Figures 2 and 3 reveal that the spectrum alterations are about ten times more effectively induced by 15 nm nano-Au than by 2 nm nano-Au.

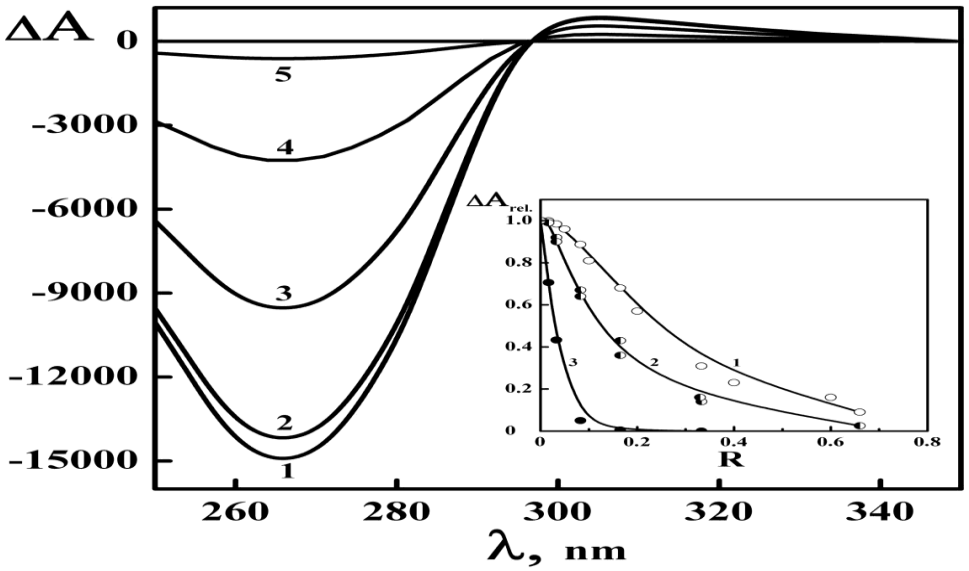

Figure 3: CD spectra of dispersions formed in water-salt PEG-containing solution by ds DNA molecules doped with nano-Au (the diameter of the nano-Au is ~ 15 nm)

1 – $C_{Nano\text{-}Au}$ = 0, 2 – $C_{Nano\text{-}Au}$ = 0.0125×10^{-8} M,
3 – $C_{Nano\text{-}Au}$ = 0.0614×10^{-8} M, 4 – $C_{Nano\text{-}Au}$ = 0.127×10^{-8} M,
5 – $C_{Nano\text{-}Au}$ = 0.309×10^{-8} M.
C_{DNA} = 30 μg ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^{+}-phosphate buffer.
$\Delta A = (A_L - A_R)\times10^{-6}$ optical units; l = 1 cm.
Inset: the dependence of the relative amplitude band (λ = 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.

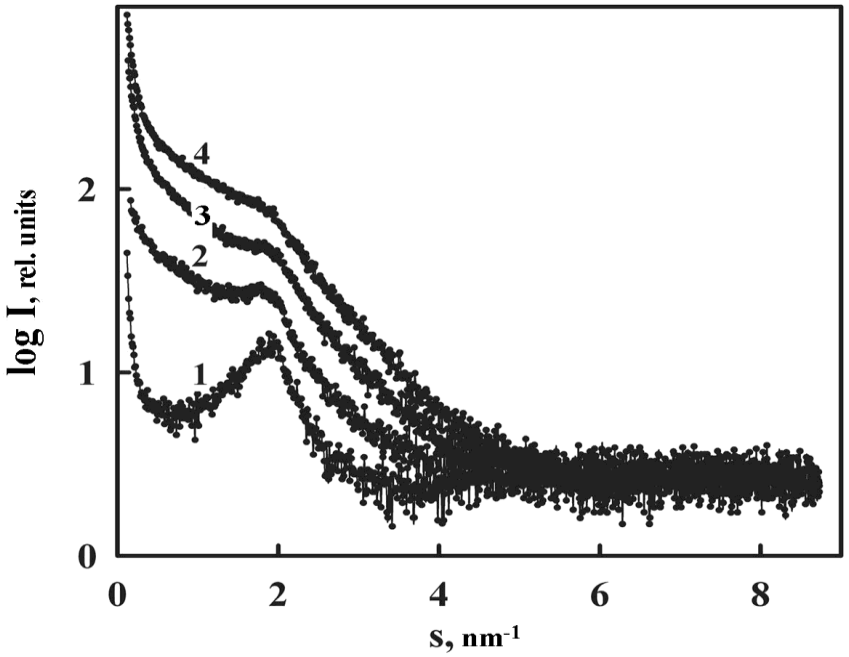

Figure 4: Experimental SAXS curves of phases obtained from the DNA CLCD (curve 1, control) and of phases formed in the PEG-containing water-salt solution by ds DNA molecules doped with nano-Au (curve 2-4)

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_{DNA} = 30 μg ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $C_{Nano\text{-}Au}$ = 0);
Specimen 2 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au (C_{DNA} = 30 μg ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $C_{Nano\text{-}Au} = 0.57\times10^{-8}$ M);
Specimen 3 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au (C_{DNA} = 30 g ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $C_{Nano\text{-}Au} = 1.72\times10^{-8}$ M);
Specimen 4 – phase obtained as a result of low-speed centrifugation of the particles formed by DNA molecules doped with nano-Au (C_{DNA} = 30 μg ml^{-1}, C_{PEG} = 170 mg ml^{-1}, 0.3 M NaCl + 0.002 M Na^+-phosphate buffer, $C_{Nano\text{-}Au} = 3.59\times10^{-8}$ M);
The diameter of the nano-Au is ~ 2 nm

Specimen of DNA phase	s_{max}, nm^{-1} (± 0.1 nm^{-1})	*d*, nm (± 0.1 nm)	L, nm (± 3.0 nm)	$\Delta/\bar{d}$	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 ~ 2 nm)

Here: s_{max} – wave vector ($s_{max} = 4\pi\sin\theta/l$; 2θ - scattering angle, λ - radiation wavelength equaling 0.1542 нм); *d*– interplane distance; *L* – size of crystallites; Δ/*d* – degree of disordering; **S** – area under the Bragg peak.
Specimens 1-4 - see Figure 4

Discussion

First, a few of things must be said. The efficacy of arranging ds DNA molecules in CLCD particles relies on the features of their secondary structure, which may be emphasized [8]. The CLCD particles are obtained by phase excluding everything except the dsDNA molecules with linear, stiff, or semi-rigid secondary structures. The capacity to generate CLCD particles is lost by flexible single-stranded DNA molecules and dsDNA molecules that have been changed by large chemical or biologically active substances [8]. But since they are phase excluded from solutions containing polymers, these molecules may nevertheless form unordered aggregates. The ds DNA liquid-crystalline dispersions, shown in Figure 1, are generated by phase exclusion from a water-salt (0.3 M NaCl) PEG solution. Each particle is around 500 nm in size and contains about 104 DNA molecules [8].Considering the technology behind their acquisition, it can be stated that the ideal way to compress a large number of linear, rigid DNA molecules into a small space is to minimize the excluded volume, which causes 104 ds DNA molecules to align

unidirectionally into particles with lateral hexagonal order. In spite of the hexagonal array's tight packing of linear high-molecular-mass ds DNA molecules, the resulting particle structure does not match that of a real crystal. Using this framework, three distinct sets of molecular or "quasinematic" [23] layers may be defined, according to aligning with the three primary axes of the hexagonal framework. Layers in this structure have a thickness that is very near to the interhelix spacing, and linear double-stranded DNA molecules lie flat on this plane. In addition to being able to spin about their longitudinal axis, double-stranded DNA molecules also exhibit some disorder around their locations, allowing them to slide and bend with regard to each other. There is a conflict between the propensity of helices to create spatially twisted structures and the unidirectional alignment and, by extension, spatial organization, of helical (chiral) molecules like double-stranded DNA. The primary consequence of chirality is that molecules of high molecular mass in DNA (or their "quasinematic layers" [24]) are less likely to pack parallel to their neighbors and more likely to pack at a small angle with regard to their neighbors. The orientation of double-stranded DNA molecules with a unique spatial pitch (P) is macroscopically twisted due to chirality. The high density of fixed surface charges (backbone phosphate groups and adsorbed counter ions) makes tight packing a key electrostatic interaction for double-stranded DNA molecules. It is quite probable that electrostatics will predominate in chiral interactions. Based on the results of the experiments, ds DNA liquid-crystalline particles have a spatially twisted structure with "quasin-ematic layers" that are spaced at 2.9 to 5.0 nm apart, depending on the osmotic pressure of the PEG solution. Each layer of ds DNA molecules in a CLCD particle is rotated at an angle of approximately 0.5o relative to the one before it [8]. The alignment of linear double-stranded DNA molecules has been elucidated by a number of interactions, including:

1. Attractive dispersion interactions (van der Waals) that extend over long distances between double-stranded DNA molecules; 2. Repulsive interactions that originate from the steric (chiral) structure of double-stranded DNA molecules and occur over short distances;

3. Dipole-quadrupole interactions, the most common kind of direct Coulomb contact, involving double-stranded DNA molecules that are electrically neutral [25]. No one has been able to agree on the specific microscopic interaction between ds DNA molecules is most important for making CLCD particles as of yet. In addition, the production technique, namely the boundary conditions at the CLCD particle surface, and the characteristics of the ds DNA molecules and the polymer (PEG) outside particles all have an impact on the spatial structure of the CLCD particles. It is possible for the aligning effect to spread into the particle as a result of surface boundary circumstances influencing the orientation of ds DNA molecules close to the surface. Typically, DNA molecule orientation as a result of surface boundary conditions, ordering effects on the liquid crystal as a result of DNA molecules attempting to align parallel to each other, and disordering effects of temperature and spatial twist will all compete with one another [26].

A delicate equilibrium between the CLCD particles' surface free energy and their bulk free energy determines their size, shape, and existence. Generally speaking, CLCD particles are larger when considering the bulk free energy, but smaller when considering the surface free energy, which is highly dependent on the surface tension between the cholesteric and isotropic DNA phases [27]. Consequently, the spatial arrangement and method of packing of double-stranded DNA molecules in CLCD particles are affected by a vast array of physical variables [28]. The theoretical claims that are most relevant to our study are as follows: [29] "the macroscopic properties of the cholesteric ds DNA phase are affected by the pattern of counterion adsorption" and "counterions bound to the ds DNA influence the efficiency of condensation of these molecules" [30,31]. Considering these comments, it is possible to go back to Figures 2 and 3. Theoretically, according to [22], the concentration, size, and long-range order of the chromophores of ds DNA molecules (nitrogen bases) determine the amplitude of the aberrant band in the CD spectrum of CLCD particles. (The amplitude of the aberrant band in the CD spectrum is dependent on the long-range order of these molecules due to the stiff fixation of nitrogen bases in the secondary structure of linear ds DNA molecules.) When all ds DNA CLCDs are obtained using the same fixed (standard) technique, the amplitude of the aberrant band is correlated with the level of twist in the spatial organization of the ds DNA "quasinematic layers" [22]. The mode of ds DNA's "recognition," which is necessary for the formation of a spatially twisted structure at phase exclusion, is defined by the peculiarities of the secondary structure of linear ds DNA molecules and the pattern of fixation of various compounds nearby the surfaces of neighboring molecules. It is reasonable to assume that the properties of the resulting (ds DNA-nano-Au) complexes are distinct from those of linear ds DNA molecules if negatively charged nano-Au are attached to the surfaces of surrounding ds DNA molecules in some manner [5]. The characteristics of the spatial structure that forms during phase exclusion and the value of an aberrant band amplitude in the CD spectrum may be significantly affected by the mechanism of "recognition" of the (ds DNA-nano-Au) complexes. It is reasonable to assume that the band's amplitude in the CD spectra will decrease as the degree of nano-Au fixation increases. Figures 2 and 3 show the CD spectra, and the most basic explanation for the reduction in the amplitude of the aberrant band is as follows [22]: The reduction in the strength of aberrant bands in CD spectra is associated with alterations in the degree of helical twist of double-stranded DNA "quasinematic layers" inside the CLCD particle structure. The unraveling of the helical structure of double-stranded DNA CLCD particles is caused by changes in the structural characteristics of linear double-stranded DNA

molecules caused by the fixation of nano-Au at double-stranded DNA surfaces prior to phase exclusion. Table 1's findings are interesting from this perspective. The findings show that the typical structural parameters (smax-, d- and L- values), which define the short-range order of nearby ds DNA molecules, remain nearly constant for all produced phases, regardless of the rise in concentration of nano-Au added to linear ds DNA. The structural characteristics of the ds DNA molecules that produce all of the phases that scatter X-rays are unaltered. This runs counter to the previously proposed most basic reason for the shift in the CD band. Table 1 demonstrates that the regions beneath the Bragg peaks are decreased for all phases that were produced. Assuming that we have utilized a constant concentration of double-stranded DNA molecules (CDNA ~ 3.75×10-8 M), followed standard protocols to collect samples for both the CD and SAXS studies, and conducted the SAXS experiments under standard conditions, it can be concluded (after [30]) that the area under the Bragg peak is correlated with the concentration of free, linear double-stranded DNA molecules in the solution, whose secondary structure is "suitable" for forming the CLCD at the phase exclusion [7,8]. According to this theory, all of the DNA molecules have the potential to form a CLCD if they are free, linear, initial double-stranded ds DNA molecules (line 1 in Table 1). Table 1 (line 1) displays the structural characteristics that were acquired from SAXS data, and Figure 2 (curve 1) shows that the optical property is represented by the negative anomalous band in the CD spectrum. It is clear from Table 1 that the liquid-crystalline phases are formed by ds DNA molecules with a constant initial secondary structure. This is because the distance between these molecules in the formed condensed phases and the characteristic structural parameters (smax, d- and L-values) that reflect the peculiarities of this structure remain unchanged. It can be inferred that doping linear ds DNA molecules with nano-Au causes a "population" of molecules incapable of forming the CLCD, since the structural parameters and phase exclusion of all phases formed after doping with nano-Au do not change (with the exception of the areas under the Bragg peak, or S). They instead agglomerate into what SAXS calls "invisible" substances. It is possible that the secondary structure of double-stranded DNA molecules is altered and that there is a particular method of distribution of nano-Au between them during the doping process, which is followed by the fixing of nano-Au adjacent the surfaces of the DNA molecules [32]. Thus, the concentration of free, linear ds DNA molecules that may form CLCD decreases as the nano-Au concentration increases. Since the area under the Bragg peak has decreased relative to the area under the peak for initial ds DNA molecules, the second (and subsequent) lines in Table 1 that show the same characteristic structural parameters but with a smaller value reflect the concentration of free ds DNA molecules, which has decreased in direct proportion to this value. Based on this theory, it is possible to determine the concentration of free, linear ds DNA molecules that form the CLCD (CDNA; Table 2, column 4), even when nano-Au is added to the original ds DNA solution. You may determine the concentration of linear double-stranded DNA molecules bonded with nano-Au (C*; Table 2, column 5), by comparing the starting concentration of ds DNA molecules (Table 2, line 1, column 4) with the obtained value. Because of this, one may make an approximation of the nano-Au "binding constant" (Kb) with respect to ds DNA molecules. The value of it is around 3.5×107 M-1. With the nano-Au concentrations in hand, we can utilize the SAXS data to determine the R-value, which is the ratio of the number of nano-Au particles to the number of DNA molecules changed by nano-Au (Table 2, column 8).

Specimen of DNA phase	S, (relative units)	% of DNA molecules forming CLCD particles	C_{DNA}, (M)	C^*_{DNA}, (M)	$C_{Nano-Au}$, (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);
$\mathbf{C}_{DNA}$ - concentration of DNA molecules unmodified by nano-Au (M);
$\mathbf{C}^*_{DNA}$ - concentration of DNA molecules modified by nano-Au M);
$\mathbf{C}_{Nano-Au}$ - concentration of nano-Au (M);
$\Delta\mathbf{A}_{270}$ – amplitude of abnomal 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.

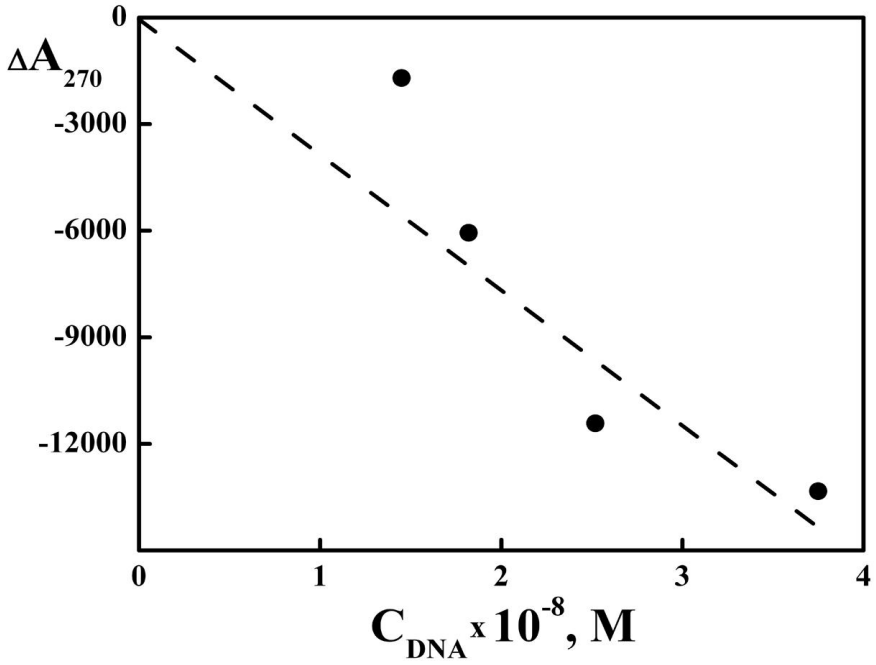

Figure 5: The dependence of the amplitude of negative band (λ = 270 nm) in the CD spectra of the DNA dispersions versus recalculated DNA concentration (in the case of nano-Au ~2 nm doping)

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:

$$\frac{U(r)}{k_B T} = \frac{\Gamma}{r} e^{-\kappa r}, \quad \kappa = \sqrt{4\pi(\rho_{Na} + \rho_{Cl})\Gamma\sigma^3}, \quad \Gamma = e^2 / 4\pi\varepsilon_0 \varepsilon k_B T\sigma, \qquad (1)$$

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_BT units. For a vector of the electrostatic field $d\mathbf{E}$ **(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:

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

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:

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

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

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

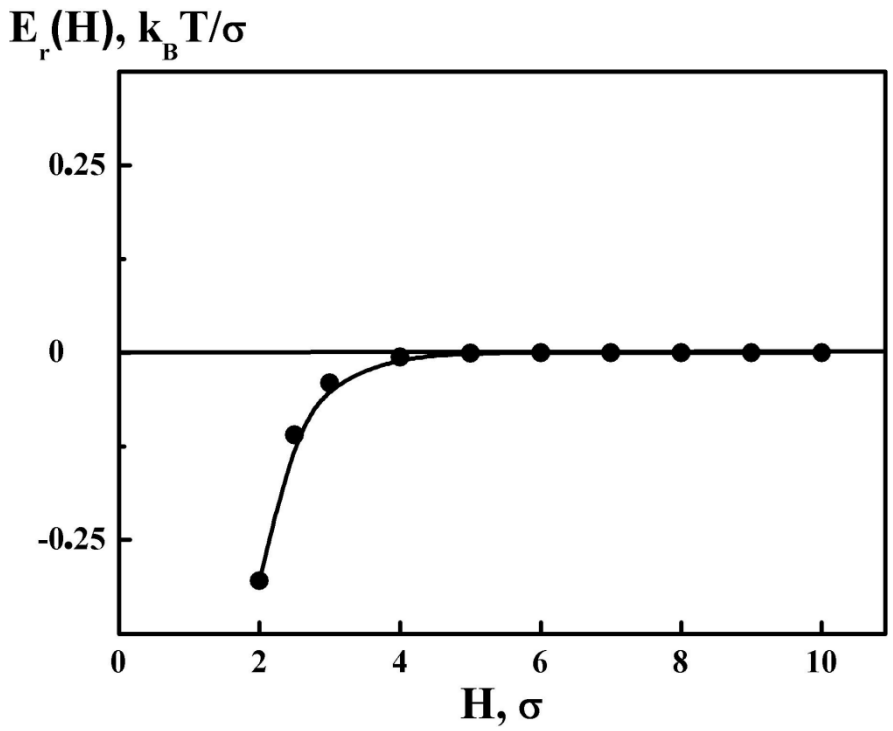

Figure 6: The radial component of the electrostatic field of DNA in aqueous solution in presence NaCl (0.3 M) (T = 298 K) as a function of distance (H, in σ units) from the center axis of the DNA

We consider that all nano-Au with D ≥ 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 ΔS(D,φ,θ) 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 ΔS(D,φ,θ) facing towards to DNA can be easily found:

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

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 n is a normal to the nano-Au surface, and the function 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 $|\mathbf{dE}(r)| > 0.001\ k_BT/\sigma$. Combining everything together we end with the following expression for the interaction force:

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

Here, the vector *H(D, φ,θ,H)* is external normal to the surface point of nano-Au to DNA and H□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.

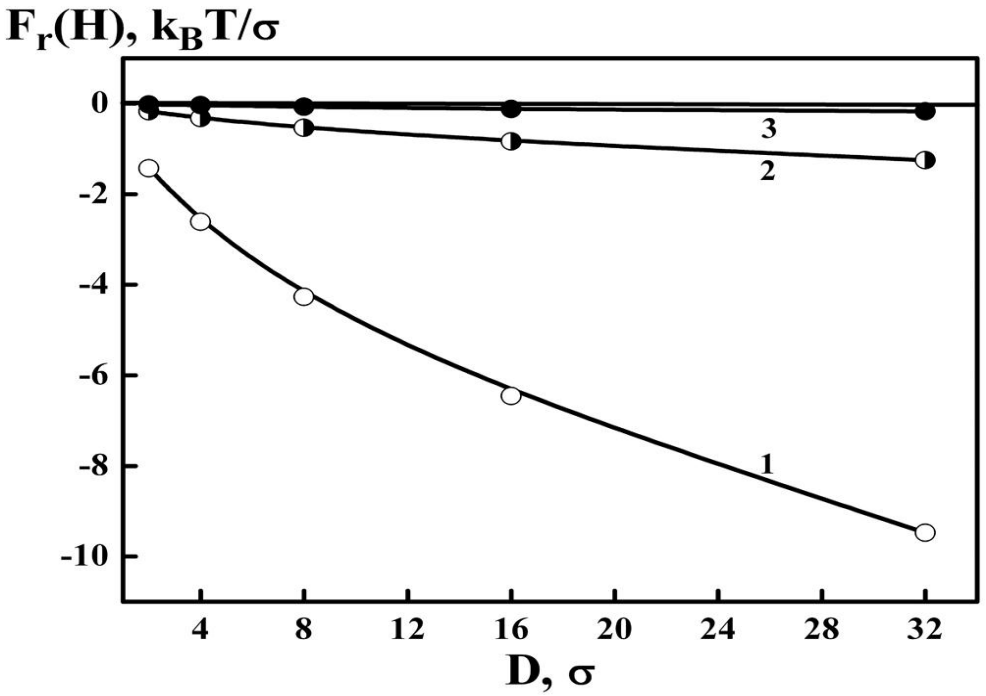

Figure 7: Evaluation of the radial component of the force acting on nano-Au from DNA in an aqueous solution of NaCl (0.3 M) (T = 298 K) as a function of the diameter D of the nano-Au in the case of different distances H (in σ units) between the DNA axis and the surface of nano-Au 1 – H = 1.0; 2 - H =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

Conclusion

The physical mechanism for fixation of nano-Au between neighboring linear ds DNA molecules is supported by our experimental data and a simple model numerical computation of screened electrostatic energy for interacting ds DNA molecules and negatively charged but polarizable nano-Au in a water-salt solution of high ionic strength. Along with this comes a level of molecular alteration so high that the molecules can no longer align to establish orientation ordering. Therefore, unlike free, linear ds DNA molecules, complexes of (ds DNA-nano-Au) are unable to construct a spatially twisted structure during condensation. When (ds DNA-nano-Au) complexes are phase expelled from a solution containing water and salt, they produce chaotic aggregates rather than the ordered spatial structure of free, linear ds DNA CLCD.

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