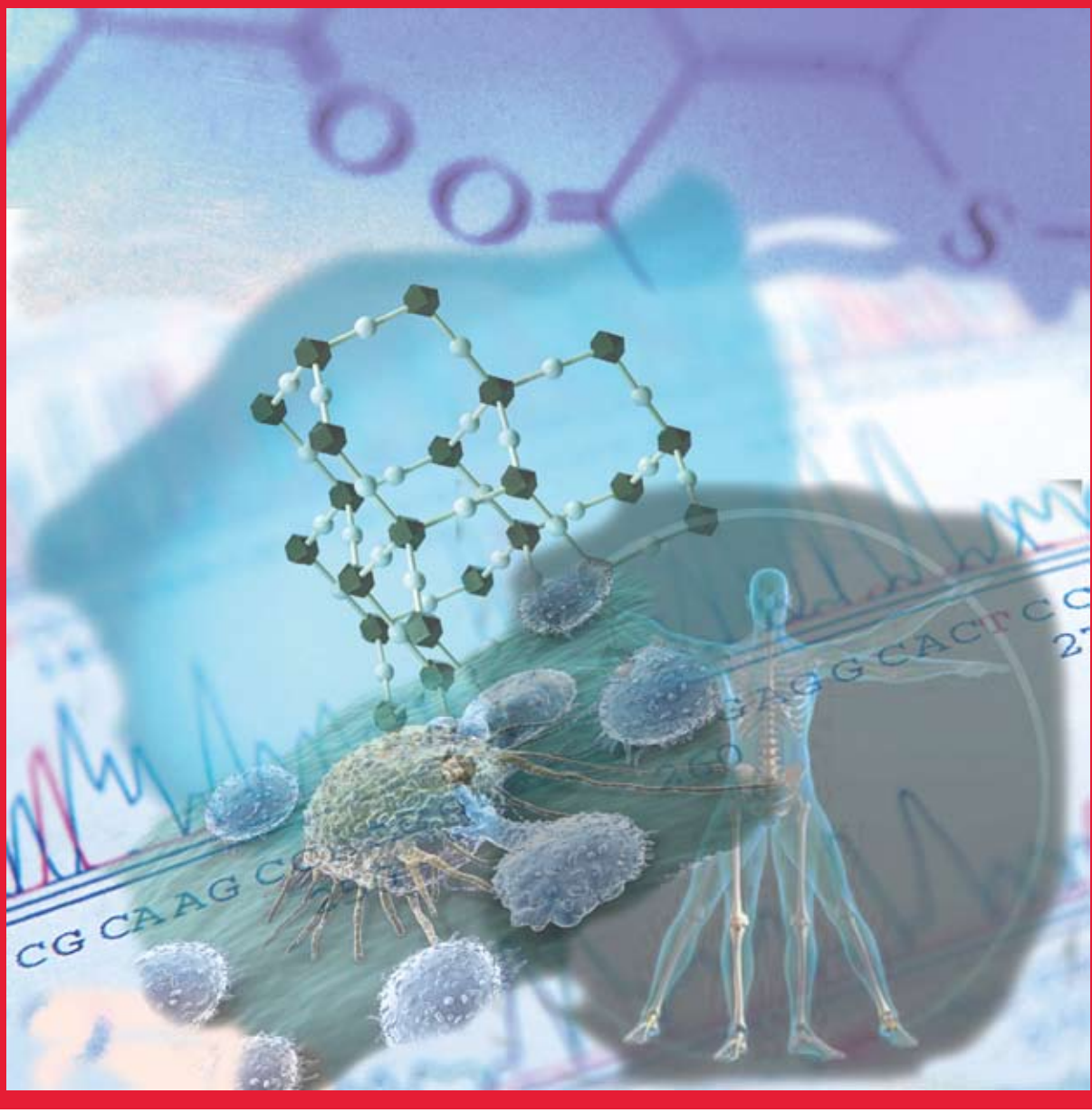
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Physicochemical and nanotechnological approaches to the design of 'rigid' spatial structures of DNA

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This review focuses on physicochemical and nanotechnological approaches to the design of 'rigid' particles based on double-stranded DNA molecules. The physicochemical methods imply cross-linking of adjacent DNA molecules ordered in quasinematic layers of liquid-crystalline dispersion particles by synthetic nanobridges consisting of alternating molecules of an antibiotic (daunomycin) and divalent copper ions, as well as cross-linking of these molecules as a result of their salting-out in quasinematic layers of liquid-crystalline dispersion particles under the action of lanthanide cations. The nanotechnological approach is based on the insertion of gold nanoparticles into the free space between double-stranded DNA molecules that form quasinematic layers of liquid-crystalline dispersion particles. This gives rise to extended clusters of gold nanoparticles and is accompanied by an enhancement of the interaction between the DNA molecules through gold nanoparticles and by a decrease in the solubility of dispersion particles. These approaches produce integrated 'rigid' DNA-containing spatial structures, which are incompatible with the initial aqueous polymeric solutions and have unique properties.
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I. Introduction

In recent years, many studies have focused on the design of new spatial structures based on linear double-stranded (ds) DNA molecules considered as a functional polymer (see, *e.g.*,[1,2]). However, DNA hydrogels rather than spatial structures are often used in practice.[3]

Double-stranded DNA hydrogels can be obtained, in particular, through creation of artificial cross-links between ds-DNA molecules,[4] formation of complexes with polyvalent cations (Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Al^{3+}),[5,6] cryopolymerization [7] or exposure to UV light. The ds-DNA hydrogels are macroscopic assemblies of irregularly cross-linked and disorderly arranged ds-DNA molecules. In hydrogels, ds-DNA molecules retain many of their inherent

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properties so that hydrogels are used as substrates or sorbents for various compounds.[8, 9]

However, from the physicochemical and practical standpoints, transformation of liquid particles of the DNA liquid-crystalline dispersion (LCD) into 'rigid' (gel-like) particles seems to be more promising as compared to uncontrollable cross-linking of neighbouring ds-DNA molecules.[10] This is due to the fact that the resulting 'rigid' particles are characterized not only by an ordered structure with a regular arrangement of neighbouring ds-DNA molecules but also by the high reactivity toward chemical, including biologically active, compounds. Such particles are used in bioengineering, biosensorics and medicine.[11]

The present review addresses the principles of approaches to the design of 'rigid' (gel-like) DNA particles (DNA nanoconstructions) from LCDs, with the focus on a new nanotechnological approach based on doping DNA cholesteric liquid-crystalline dispersion (DNA CLCD) particles with gold nanoparticles (nano-Au).

II. Properties of DNA liquid-crystalline dispersion particles

As known,[12] phase exclusion (condensation) of ds-DNA molecules (an average molecular weight of $\sim(6-8)\times10^5$ Da, which corresponds to an average molecule length of ~ 400 nm) from aqueous salt solutions of polymers [for example, poly(ethylene glycol), PEG] leads to the formation of a dispersion of these molecules (Fig. 1). Such a condensation is referred to as ψ-condensation (*psi* is the acronym for Polymer Salt Induced). Each of the dispersion particles (at the given molecular mass) has an average diameter of 400 – 500 nm and contains $\sim10^4$ ds-DNA molecules. The particle size is determined by the DNA molecular mass and the osmotic pressure of a PEG solution. These estimates are approximate since they have been calculated assuming that the particles, which are formed by ds-DNA molecules with the phosphate groups neutralized by counterions, are spherical objects.

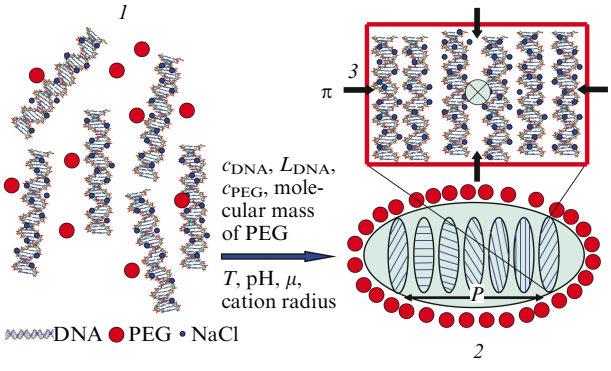


Fig. 1. Scheme of formation of a liquid-crystalline dispersion as a result of phase exclusion of linear ds-DNA molecules with a molecular mass of $(6-8)\times10^5$ Da from a PEG-containing aqueous salt solution.[12]
(*1*) ds-DNA molecules at a PEG concentration in a solution below the 'critical' value; (*2*) a particle of the DNA liquid-crystalline dispersion formed at a PEG concentration above the 'critical' value (*P* is the pitch of the helical twist of the spatial structure of a dispersion particle); (*3*) a quasinematic layer of ds-DNA molecules (the cross at the center denotes the rotation axis of the helical structure); the rectangular box and arrows correspond to the osmotic pressure (π) of a PEG-containing solution.

The DNA LCD particles have the following specific properties:

1. These particles do not involve water-soluble polymer molecules (PEG).

2. Dispersion particles are characterized by a high local concentration of DNA (in the range from 160 to 600 mg ml^{-1}), neighbouring DNA molecules being packed in layers. In the resulting lamellar structure, the distance between adjacent DNA molecules ranges from 2.5 to 5.0 nm (depending on the osmotic pressure of a PEG solution); in this respect, dispersion particles resemble one-dimensional crystals. The DNA molecules in dispersion particles retain almost all diffusion degrees of freedom; *i.e.*, the particles retain the properties of a liquid. Such a combination of properties enables the term liquid-crystalline dispersion to be applied to the resulting structure (see, *e.g.*, monograph [12] and references therein).

3. The ds-DNA molecules contain chromophores (UV-absorbing nitrogen bases), which are rather rigidly fixed (the tilting angle of the bases to the long axis of DNA molecules is $\sim90^\circ$). If phase exclusion leads to the formation of dispersion particles for which a layered (lamellar) packing of DNA molecules is typical, this means that the nitrogen bases are also oriented in a definite manner in the layer of DNA molecules.

4. In the physics of liquid crystals, each layer of a lamellar structure is referred to as quasinematic.[13] Since ds-DNA molecules are chiral, the interaction of adjacent molecules leads to that each next quasinematic layer is turned at a certain angle ($\sim0.5^\circ$) with respect to the preceding layer.[12] As a result, a spatially twisted structure of LCD particles is formed.

5. For the helically twisted packing of adjacent quasinematic layers of DNA molecules, calculations predict the appearance of a very strong (abnormal) band in the circular dichroism (CD) spectrum in the DNA chromophore absorption range.[14] Depending on the fact whether nucleic acid molecules belong to the B or A family, this band can have both the negative and positive sign of optical rotation. Calculations have enabled the refinement of conditions (the pitch of a helical structure, dispersion particle size, *etc*). under which the abnormal band in the CD spectra can be experimentally observed. This result indicates that rigid anisotropic DNA molecules in dispersion particles realize their propensity to be arranged into a spatially twisted (cholesteric) packing. For DNA LCD particles that give rise to the abnormal band in the CD spectrum, the term 'cholesteric liquid-crystalline dispersion' is used.[15, 16] Hence, the appearance of a chiral LCD (CLCD) of DNA is easily detected by the abnormal optical activity, which manifests itself, in particular, as a characteristic strong band in the CD spectrum in the absorption range of DNA nitrogen bases. This band can be a criterion for judgment of both the state of the secondary structure of ds-DNA molecules and the degree of integrity of the helical twist of the spatial structure of DNA CLCD particles.

It should be noted that, unlike the usual aggregation of DNA molecules, which can occur at any length of these molecules and is not accompanied by the appearance of optical activity, the CLCD is formed in the course of phase exclusion of ds-DNA molecules only if the length of the latter is >150 Å (~50 nucleotide pairs).

6. Noteworthy is the fact that there is free space between the ds-DNA molecules in quasinematic layers of the CLCD. The molecules of chemical (including biologically active)

compounds (but not the PEG molecules) can readily diffuse into this space and interact with DNA molecules. The high local DNA concentration and orderly arrangement of macromolecules in a quasinematic layer create conditions for efficient interaction of low-molecular-mass compounds with nitrogen base pairs of DNA (intercalation between base pairs, fixation in the grooves on the molecular surface, *etc*.). Modification of the secondary structure of ds-DNA driven by physical or chemical factors can have an effect not only on the properties of quasinematic layers but also on the character of interaction between them and, hence, on the structural features of CLCD particles and their properties, in particular, on the magnitude (and even the sign) of optical activity.[15] However, complete separation of strands of ds-DNA molecules in the quasinematic layer and their rolling up into single-stranded random coils (DNA denaturation) are impossible for steric reasons.

Each LCD particle can be conventionally treated as a drop of a concentrated DNA solution; the structure and properties of the particle are determined, in addition to the ionic strength, pH and temperature of the solution, by its critical osmotic pressure.[12, 17, 18] An LCD (or CLCD) particle cannot be taken in hand, seen or placed on the surface of a membrane filter since, in the absence of the critical osmotic pressure of the solution, the spatial DNA molecule packing must change so that the DNA molecules will transform from the condensed (liquid-crystalline) state into the initial isotropic one.

In studying the physicochemical properties of quasinematic layers in the DNA CLCD, the following two factors are of special importance:

— molecular mass of ds-DNA: the larger this mass, the lower the compatibility of ds-DNA with a PEG solution and the higher the efficiency of phase exclusion;

— solubility of DNA molecules: the lower the solubility, the higher the immiscibility of DNA molecules with a PEG solution and the efficiency of their phase exclusion.[12]

It can be stated that, in the case of LCD particles, the properties of DNA molecules and an aqueous polymer solution control the properties of these particles; *i.e*., the DNA packing in LCD particles is easily regulated. In this respect, the behaviour of dispersion particles is similar to that of biological objects. Biologically, LCD and CLCD particles are of interest since they enable a more accurate description of the properties of DNA-containing viruses or protozoan chromosomes, which are microscopic dispersed systems characterized not only by the ordered but also by the flexible DNA packing. Practical interest in CLCDs is due to a unique combination of physicochemical properties of DNA molecules and DNA liquid crystals that opens up possibilities for using dispersion particles as sensing elements (biosensors) of analytical devices intended to detect genotoxic agents targeted at the genetic material of cells.

Thus, studying the physicochemical properties of ds-DNA LCD particles has provided information on the conditions of their formation and properties. This information has been used for developing various scenarios of transformation of liquid DNA CLCD particles into rigid (gel-like) particles.

III. Physicochemical approach to the formation of rigid DNA particles

Two variants of the physicochemical (liquid-crystalline) approach are used to transform a 'liquid' DNA particle into the 'rigid' (gel-like) state:[19]

— through cross-linking of neighbouring ds-DNA molecules in CLCD particles;

— through salting-out of DNA molecules into CLCD particles.

III.1. First variant

The basic idea of the first variant of the liquid-crystalline approach to the design of rigid DNA particles has been formulated as follows:[20] 'The ds-DNA molecules in quasinematic layers of CLCD particles are in the 'soluble' state and there is a free space between them. Molecules of chemical or biologically active compounds (guests) that occur in this free space owing to diffusion can cross-link neighbouring DNA molecules. The cross-linking of all DNA molecules ordered in the quasinematic layers of one CLCD particle leads to the formation of an integrated structure, *i.e*., to a structure having a very high molecular mass. Such a spatial structure will be incompatible with a PEG solution. This means that cross-linking of the DNA molecules forming a CLCD particle initiates the transformation of the CLCD particle from the 'liquid' to the 'rigid' (gel-like) state.'

Physicochemically, this variant is nothing else than gelation caused by chemical cross-linking of isotropic linear ds-DNA molecules. However, it should be taken into account that the cross-links between polymer molecules in classical gelation can have different lengths and can be randomly arranged in space.[19]

In the case under consideration, gelation should occur inside a nanometre-sized CLCD particle. During this process, the fixed distance between neighbouring ds-DNA molecules in quasinematic layers of the same particle should remain constant, and the spatial helical structure of DNA CLCD particles should persist.

Hence, this variant implies the formation of cross-links with rather specific characteristics between ds-DNA molecules. Since the distance between DNA molecules is 2.5–5.0 nm depending on the osmotic pressure of a solution, these cross-links can be referred to as nanobridges and the resulting integrated structure can be regarded, as a nanoconstruction based on ds-DNA molecules or as a bionanoobject. The approach itself can be called DNA-based nanodesign.

To implement this approach, our research team has initiated experimental studies (see, *e.g*., Refs 21 and 22) and has performed theoretical analysis of the efficiency of nanobridge formation between ds-DNA molecules;[23, 24] results of these studies have been generalized and reported.[25]

It has been demonstrated that nanobridges consisting of alternating daunomycin (DAU, anthracycline antibiotic) molecules and divalent copper ions cross-link DNA molecules located in the same layer, as well as in adjacent quasinematic layers. This cross-linking dramatically changes the properties of liquid DNA CLCD particles. In particular, the amplitude of the abnormal band in the DNA chromophore absorption range ($\lambda \approx 270$ nm) of the CD spectrum increases several-fold; in addition, a new abnormal band is induced in the absorption range of the DAU chromophores ($\lambda \approx 510$nm) incorporated in the nanobridges (Fig. 2). Such an optical effect is evidence of 'freezing' of the spatial structure of CLCD particles. This result indicates that cross-linking of adjacent DNA molecules inside a CLCD particle leads to the formation of a structure with the properties sharply different from the properties of the initial liquid particle.

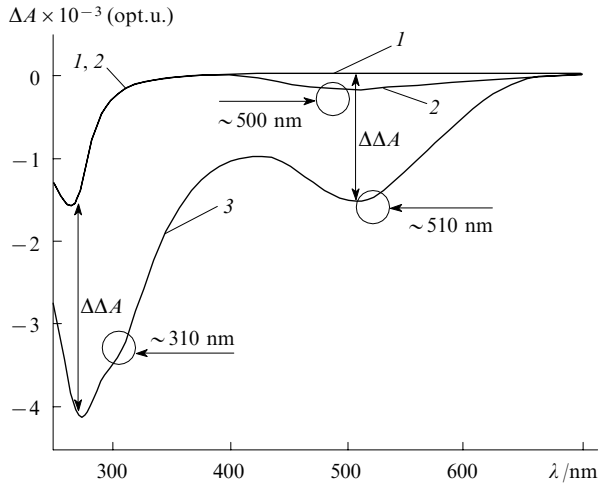


Fig. 2. Circular dichroism spectra of the initial DNA CLCD (*1*) and the same dispersion treated with daunomycin (*2*) and $CuCl_2$ (*3*).
Solution: c_{DNA} = 5.5 μg ml^{-1}; c_{PEG} = 170 mg ml^{-1}; 0.3 mol litre^{-1} NaCl + 0.002 mol litre^{-1} of a phosphate buffer; $c_{DAU} = 27.3 \times 10^{-6}$ mol litre^{-1}; $c_{Cu^{2+}} = 9.9 \times 10^{-6}$ mol litre^{-1}; here and in Figs 4, 8, 10, $\Delta A = (A_L - A_R)$, A_L and A_R are the absorbances for the left and right circularly polarized light, respectively; $\Delta\Delta A$ is the increase in band intensity caused by the formation of nanobridges.

The stability of cross-linked DNA CLCD particles depends on the number and properties of nanobridges rather than on the osmotic pressure as previously. This is evidence that gelation inside the initial liquid DNA CLCD particle promotes its transition to the rigid (gel-like) state. A rigid DNA particle can exist in the absence of the high osmotic pressure of a solution. Formation of such a structure opens up the possibility for immobilization of the resulting rigid DNA particles on the membrane filter surface, which makes atomic force microscopy (AFM) applicable to studying the particle structure (let us underline once more that the initial liquid DNA CLCD particles do not exist in the absence of the osmotic pressure of a solution).

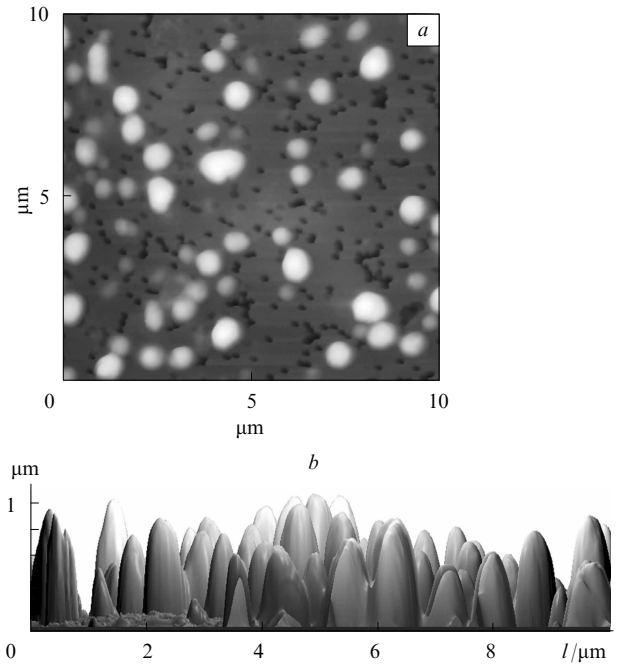


Fig. 3. AFM images of rigid particles formed by cross-linking through nanobridges of neighbouring ds-DNA molecules in quasinematic layers of CLCD particles and immobilized onto a nuclear membrane filter.[11]
(*a*) Top view, (*b*) side view. The dark spots in Fig. 3 *a* are holes in the nuclear membrane filter made of poly(ethylene terephthalate) (PETP).

The shape of rigid DNA particles is close to spherocylindrical (Fig. 3), and their average diameter is within the range of 400–500 nm, *i.e.*, coincides with the size of the initial DNA CLCD particles estimated previously for PEG-containing solutions (see above). The evaluation of the size of rigid DNA particles has enabled the refinement of some of their parameters.[26] In particular, it has been shown that a single rigid DNA particle contains $\sim 1.6 \times 10^4$ molecules, and its molecular mass is $(1.0-1.2) \times 10^{10}$ Da, which is rather close to the parameters of the initial liquid DNA CLCD particles obtained by phase exclusion.

Thus, the first variant of the liquid-crystalline approach makes it possible to design rigid DNA particles suitable for manipulation. Many specific details of this variant have been reviewed.[27]

III.2. Second variant

The principle of the second variant of the physicochemical approach to the design of rigid DNA particles has been formulated as follows:[28] 'The ds-DNA molecules in quasinematic layers of CLCD particles are in the dissolved state, and there is free space between them. Multiply charged cations that diffuse into dispersion particles can displace sodium ions from the hydration shell of DNA and efficiently neutralize the negative charges of the DNA phosphate groups, thus sharply decreasing the solubility of these molecules [down to DNA precipitation (DNA salting-out)].[12] The interaction even between separate moieties of poorly soluble neighbouring ds-DNA – multicharged cation complexes can lead to the formation of an integrated gel-like structure involving all DNA molecules ordered in the quasinematic layers of a CLCD particle. Such a gel-like (rigid) structure is incompatible with a PEG solution and can exist in the absence of the osmotic pressure of the PEG-containing solution'.

From the physicochemical point of view, this variant differs from the first one since it is based on initiation of gelation inside a CLCD particle as a result of the decrease in solubility of ds-DNA molecules (DNA salting-out) rather than on chemical cross-linking of ds-DNA molecules. It should be noted that binding of multicharged cations to ds-DNA molecules can be accompanied not only by a disturbance of the secondary structure of such molecules but also by a change in the character of mutual ordering of DNA molecules in the quasinematic layers of CLCD particles. This means that the properties of multicharged cations can modify the parameters of the spatial structure of DNA CLCD particles.

For salting-out ds-DNA molecules in the quasinematic layers of CLCD particles, multiply charged lanthanide cations are of special interest.[29] Such cations efficiently neutralize the negative charges of the DNA phosphate groups, the complexes of the cations with the phosphate groups being nearly insoluble (for example, the solubility constant of gadolinium phosphate is $\sim 10^{-12}$ mol litre^{-1}). In addition, binding of these cations to the base pairs of

linear ds-DNA causes changes in its secondary structure analogous to the B → Z transition.[30]

With taking into account the possible practical use of the resulting structures, gadolinium salts (in particular, $GdCl_3$) have been selected as the source of a basic multicharged cation. It has been shown that treatment of DNA CLCD with gadolinium salts results in the multifold enhancement of the negative abnormal band in the range of chromophore (nitrogen bases) absorption range of the CD spectrum (Fig. 4).[29] Such an optical effect is analogous to the effect caused by chemical cross-linking between DNA molecules. This is accompanied by a local disturbance of the secondary structure of ds-DNA molecules in quasinematic layers, which is manifested in the decrease (down to complete disappearance) of the characteristic peak on the small-angle X-ray scattering curves for the phases formed of DNA CLCD particles treated with a $GdCl_3$ solution.[31]

The amplitude of the negative band in the CD spectrum increases when the gadolinium concentration in the PEG-containing solution is sufficient not only for the displacement of Na^+ by Gd^{3+} in the nearest environment of ds-DNA molecules but also for the loss of solubility by ds-DNA molecules in which phosphate groups form complexes with Gd^{3+} (so that the DNA molecules are salted-out into CLCD particles) (Fig. 4 *b*). For steric reasons, the ds-DNA molecules cannot leave the physical volume of a CLCD particle; therefore, we can state the following: the disappearance of the 'fluidity' of DNA molecules, in combination with the enhancement of interaction between neighbouring DNA molecules (moieties) of different conformation in quasinematic layers, leads to the formation of an integrated structure. The high molecular mass of such a structure and the insolubility of the ds-DNA – Gd^{3+} system are responsible for its incompatibility with a PEG-containing solution. Liquid DNA CLCD particles transform into rigid (gel-like) particles.[32] As in the above case, rigid DNA particles can exist in the absence of the osmotic pressure of the PEG-containing solution, which supports their immobilization on the nuclear membrane filter surface (Fig.5 *a*,*b*). The particles are independent objects with uncompensated surface charges, which prevent their aggregation. The average

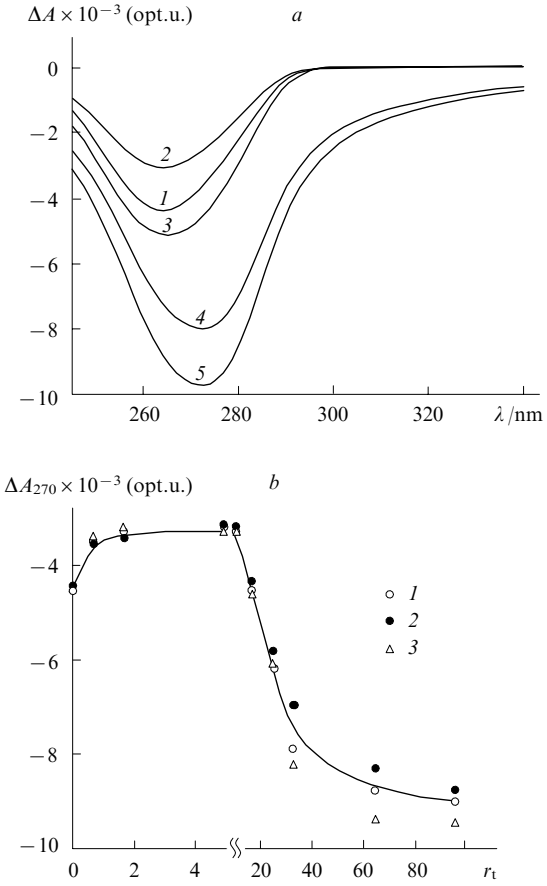


Fig. 4. CD spectra of DNA CLCD in the absence (*1*) and in the presence of $GdCl_3$ (*2*–*5*) in a solution (*a*) and the amplitude of the band in the spectrum (λ = 270 nm) as a function of the ratio of the $GdCl_3$ concentration to the concentration of DNA base pairs in solution (r_t) (*b*).[11]
(*b*) Data points *1*–*3* correspond to different series of experiments; (r_t) (*1*–0) initial DNA CLCD; (*2*) 1.65; (*3*) 16.5; (*4*) 32.7; (*5*) 95. Solution: c_{DNA} = 10 μg ml^{-1}; c_{PEG} = 170 mg ml^{-1}; 0.3 mol litre^{-1} NaCl.

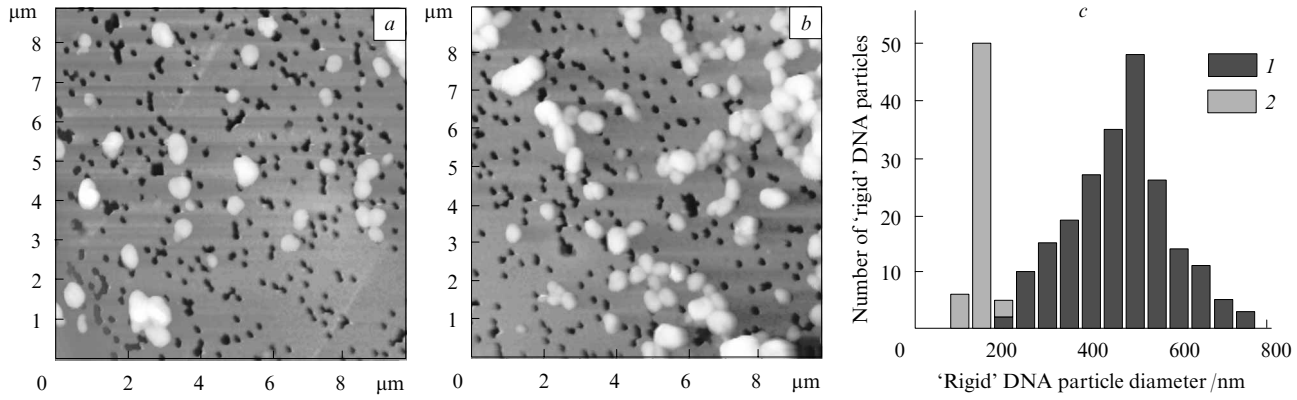


Fig. 5. AFM images of 'rigid' particles formed by treatment of DNA CLCD particles with a $GdCl_3$ solution and immobilized on a nuclear membrane filter (*a*, *b*) and the size distribution of 'rigid' DNA particles and holes in the membrane filter (*c*).[11]
(*a*, *b*) The images have been obtained at different concentrations of 'rigid' DNA particles [dark spots in the figures are the holes in the nuclear membrane filter (PETP)]; (*c*) (*1*) rigid particles, (*2*) holes.

size of rigid DNA particles is within the range 400–500 nm (Fig. 5 *c*) (*i.e.*, this size coincides with the size of initial liquid DNA CLCD particles). This is evidence that the packing of base pairs (chromophores) of DNA molecules in rigid particles formed by ds-DNA–Gd^{3+} complexes retains the high density.

Thus, the second variant enables the design of rigid DNA particles also suitable for manipulation. It should be noted that the above variants represent almost all approaches to the design of rigid DNA particles possible from the standpoint of the physical chemistry of polymers.

IV. Nanotechnological approach to the formation of rigid DNA particles

The advent of nanotechnology made possible yet another strategy — nanotechnological — for design of rigid DNA particles. The fact is that a novel branch of nanotechnology, which still has no name, is rapidly developing. This branch of nanotechnology is concerned with the creation of novel materials in nanoparticles–low-molecular-mass liquid crystals systems.[33–36]

The first nanoparticles used in similar systems were carbon nanotubes, by which liquid crystals were treated;[37–42] gold nanoparticles or nanoparticles with the gold core have been rather often used for this purpose.[43–46] Such a treatment can be responsible not only for the change in the physicochemical properties of low-molecular-mass liquid crystals[47] but also for specific ordering of nanoparticles in the liquid-crystalline phase.[48,49] These studies formulated the area of nanotechnology that opens up the possibility of tailoring optical and electrooptical characteristics of low-molecular-mass liquid crystals by means of nanoparticles, used as 'control' additives, and designing materials with new properties based on them.

As for liquid crystals of biopolymers, in particular, DNA, such systems have been treated not only with carbon nanotubes,[50,51] but also with nano-Au. The last series of studies was started in 2010.[52–54]

With taking into account the earlier results obtained for CLCD particles formed by high-molecular-mass ds-DNA molecules, the idea of the nanotechnological approach to the design of rigid DNA particles can be formulated as follows: The ds-DNA molecules in quasinematic layers of CLCD particles are in the dissolved state and there is a free space between them. Nanoparticles that have a size comparable to the distance between adjacent ds-DNA molecules in the quasinematic layers of CLCD particles and are able to diffuse into the free space can be located between neighbouring DNA molecules, acting as a mediator of efficient interaction between these molecules. Under these conditions, formation of an integrated structure involving all DNA molecules ordered in the quasinematic layers of a CLCD particle is probable. The nanoparticles (or their aggregates) located between ds-DNA molecules will decrease the solubility of the integrated structure. The resulting rigid (gel-like) structure will be incompatible with a PEG solution.

Certainly, it cannot be ruled out that the interaction between ds-DNA molecules in quasinematic layers through the nanoparticles can modify the parameters of the integrated structure of CLCD particles, as occurs in the case of low-molecular-mass liquid crystals.[47–49]

For this approach, noble metal nanoparticles, in particular, nano-Au, are of greatest interest since their size is comparable to the distance between the DNA molecules in quasinematic layers. Studies of the properties of the DNA CLCD–nano-Au system are stimulated by several factors: first, the chemical and physical properties of nano-Au depend on their size, shape, structure and dielectric environment;[55,56] second, information is accumulated on the genotoxic effect of gold nanoparticles on living organisms.[57–60]

IV.1. Linear DNA–positively charged gold nanoparticles system

There are two lines of investigation of the DNA–nano-Au system:

— interaction of nano-Au with linear single-stranded DNA molecules;

— interaction of nano-Au with linear double-stranded DNA molecules.

Studies of interaction between nano-Au particles capped with molecules containing sulfanyl and amino groups at the opposite ends (for imparting a positive charge to nano-Au) with linear single-stranded DNA molecules were pioneered by Mirkin and Alivisatos in 1996 (see, *e.g.*, Refs 61–73). It has been shown that, after the interaction with positively charged gold nanoparticles, single-stranded DNA molecules are hybridized and are even able to form spatial planar structures, despite the fact that the pristine DNA molecules exhibit anisotropic properties.

Formation of aggregates of adjacent gold nanoparticles near the surface of DNA molecules leads to the enhancement of the so-called localized surface plasmon resonance band (SPR band) in the visible range of the absorption spectrum and to the appearance of interaction between neighbouring plasmons (plasmon overlap) accompanied by a bathochromic or hypsochromic shift of the SPR band, depending on a number of parameters (distance between particles, size and shape of the resulting aggregates, dielectric constant of a medium, the presence of 'interlayers' between neighbouring nano-Au particles, *etc.*).[74,75]

These studies left almost unanswered the important question of whether unmodified gold nanoparticles coated with a shell of negatively charged ions (citrate, borate, *etc.*) can interact with DNA molecules. The answer to this question is very important since there has been reported that ds-DNA molecules cannot be sorbed onto gold nanoparticles.[76] The mode of binding of gold nanoparticles to ds-DNA and its conformation at the binding site(s) have been addressed in numerous works; however, the key conclusion is still reduced to the statement that this binding is of complicated character.[77]

IV.2. Properties of negatively charged gold nanoparticles

To adjust the nanotechnological strategy for designing rigid DNA particles, it is necessary to answer the above question and study the possibility of interaction of negatively charged gold nanoparticles of small size ($\sim$2–10 nm) with ds-DNA molecules by different physicochemical methods. Of special interest are the properties of such nanoparticles.

The citrate-stabilized anionic form of gold nanoparticles $\sim$10 nm in diameter is, as a rule, obtained by reduction of $HAuCl_4$, using, for example, the classical Turkevich method.[78] According to this method, a 1% citric acid solution is added to a boiling solution of $HAuCl_4$ under vigorous stirring. Citrate ions are both the reducing agents

for tetrachloroaurate ions and the stabilizers of gold nanoparticles. Smaller gold nanoparticles (~2 nm) are synthesized by the Duff method in which $HAuCl_4$ is reduced to gold by hydroxymethylphosphonium chloride under alkaline conditions.

The citrate form of gold nanoparticles is schematically shown in Fig. 6.[79–82] The anions on the nanoparticle surface are responsible for the high stability of the gold hydrosol, and repulsion between the anions prevents the aggregation of nanoparticles.[83,84–86] An analogous scheme is also applicable for other stabilizing ions (for example, phosphine ions)[81,82] on the nano-Au surface.

Following the authurs of Refs 81 and 87, we assume that a gold nanoparticle ~2 nm in diameter consists of 150 atoms. (Sometimes, it is stated[88] that a nano-Au particle 2.3 nm in diameter is an octahedron and consists of 300 atoms). A rough estimate has shown that, in a 2-nm particle, about 60% of all atoms are surface gold atoms with uncompensated valence.[80]

Nanoparticles (small quasi-metallic particles) exhibit properties responsible for their high propensity to react with various compounds. For gold nanoparticles ~2 nm in diameter, the d level approaches the Fermi level, which is accompanied by a decrease in the average Au–Au distance in nanoparticles as compared to the distance in gold metal.[89] As a result, the valence electrons become more accessible for binding to ligands.[90,91] It has been suggested[92] that the ligand sorption energy is determined by the difference between the Fermi level and the d band. The surface of nanoparticles contains different crystallographic faces [Au(111), Au(100), Au(110) *etc.*]. Their proportion dictates the particle polarity and shape and the efficiency of dipole–dipole interaction and ligand binding.[56,93,94]

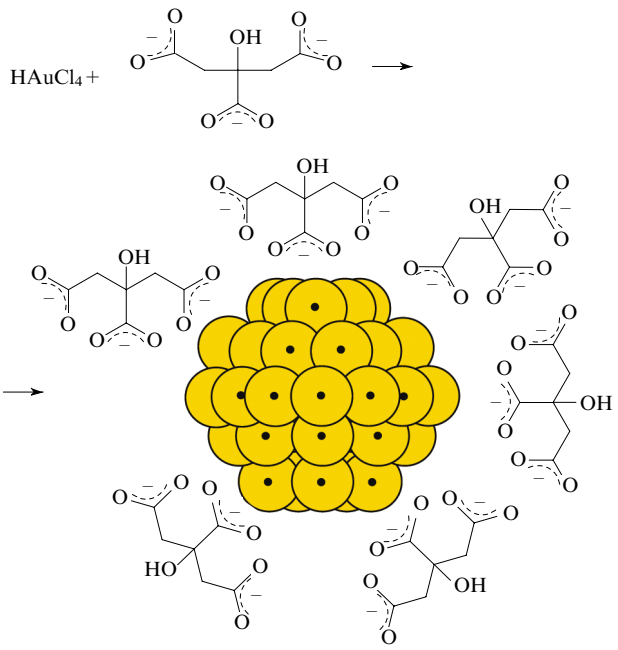


Fig. 6. Scheme of synthesis of gold nanoparticles from $HAuCl_4$ and the gold nanoparticle stabilized by citrate ions.

A very important result is that monodentate ligands weakly bound to the gold nanoparticle surface can be replaced by other ligands, including polydentate ones.[80] Indeed, it has been demonstrated that the capping citrate ions on the nano-Au surface can be replaced by triphenylphosphine,[82] triphenylphosphine sulfonate,[95] phenylmethanethiol[96] or pyridine.[83,97] Analogous substitution is possible for other ligands, in particular, phosphine ligands.[56] In this case, gold nanoparticles can change shape, like 'jelly', and undergo distortions depending on the type of substrate on the surface.[80]

IV.3. Possible mechanisms of binding of negatively charged gold nanoparticles to ds-DNA molecules in high salt solutions

Based on the above literature data, we can suggest two hypotheses concerning the mechanism of interaction of negatively charged gold nanoparticles with ds-DNA molecules.

It is evident that long-range electrostatic repulsion prevents ds-DNA molecules, which contain negatively charged phosphate groups, from coming closer together, as well as from binding to negatively charged nano-Au particles. The repulsion is determined by the dependence of the Debye screening length (Debye screening radius) on the ionic strength of a solution.[98]

A situation changes if the charges of the DNA phosphate groups are neutralized by counterions, which occurs in high salt solutions. In this case, the repulsion between gold nanoparticles and DNA molecules is minimized, they come closer together, and this gives rise to factors that trigger some processes.

First, when ds-DNA molecules approach nano-Au particles, the negatively charged phosphate groups of DNA act as substituents[81–83] for capping ions (citrate, phosphine, *etc.*) on the gold nanoparticle surface, and this process initiates polydentate interaction between ds-DNA and nano-Au.[98,99]

Second, chemical interaction between the nitrogen-containing groups (the adenine and guanine N(3) and N(7) atoms) of DNA molecule bases and an adjacent nano-Au particle starts playing a role.[79,98,100] Nitrogen bases differ in efficiency of binding to nano-Au, and this underscores the additional contribution of specific non-ionic interactions between nitrogen bases and nano-Au to the overall stabilization energy of DNA–nano-Au complexes in high salt solutions.[98] This is supported by the fact that the interaction between oligonucleotides and nano-Au gives (after the substitution of citrate ions) rather strong complexes.[101,102]

Third, it is not ruled out that the spatial structure of nano-Au is distorted[80] 'to match' the spatial parameters of ds-DNA molecules, which ensures maximal interaction. This assumption is quite consistent with the concept of the polyfunctional structural model of nano-Au.[83]

Fourth, it is not ruled out that the formation of strong DNA complexes with negatively charged gold nanoparticles is accompanied by the disturbance of the secondary DNA structure[103] and/or displacement of water molecules from the hydration shell of DNA, which is necessary for the maximal contact of the DNA molecule with the nano-Au surface.

Hence, it can be supposed that ds-DNA molecules in high salt solutions can chemically interact with negatively charged gold nanoparticles.

The physical mechanism of interaction of negatively charged nano-Au particles with ds-DNA molecules in high salt solutions can be described as follows.

If the negative charge on the phosphate group of DNA is Z_1e (where Z_1 is the number of charges on the phosphate group, and e is the electron charge) and if the gold nanoparticle is considered ignoring its polarization as a point charge, the Coulomb repulsion (V_C) between these species is described by the equation

$$V_C = \frac{Z_1 Z_2 e^2 \exp(-kr)}{4\pi\varepsilon r} \quad (1)$$

where Z_2e is the nano-Au charge, ε is the dielectric constant of a solution, k is the Debye screening radius, and r is the distance between the nano-Au particle and the DNA molecule.

However, a real gold nanoparticle is not a point charge; it has a finite size and can be polarized. The DNA molecule is a strongly charged polyelectrolyte and creates an inhomogeneous electric field around itself. It can be assumed that when, in a high salt solution, nano-Au and a ds-DNA molecule approach each other in the field of negative charges of the DNA phosphate groups, dipole moment is induced on gold nanoparticles. In this case, attraction appears between negatively charged phosphate groups and nano-Au [Keesom interaction (V_K)] caused by interaction of the charge–induced dipole type. The attraction between point charge Z_1e and a nano-Au particle is described by the equation

$$V_K = -\frac{Z_1^2 e^2 \alpha_2}{(4\pi\varepsilon)^2 r^4} \quad (2)$$

where α_2 is the polarizability of the gold nanoparticle proportional to its volume. Since the Keesom interaction is realized between charge Z_1e and a polarized particle, Z_2 is absent in Eqn (2).

Equations (1) and (2) demonstrate that the Keesom interaction energy is proportional to $1/r^4$; hence, is acts at shorter distances than the Coulomb repulsion proportional to $1/r$. Such a mechanism can act if dipole polarization is rather high and the ds-DNA charges are strongly screened (the Debye charge screening 'works' against the Coulomb repulsion but less efficiently than in the case of the Keesom attraction). The Debye screening of ds-DNA charges depends on the presence of counterions; therefore, it is rather efficient in high salt solutions. In his context, it can be assumed that, in high salt solutions in which the negative charges of the ds-DNA phosphate groups are screened by counterions ($kr > 1$) and the Coulomb attraction is small (the reduction of this barrier is necessary for a gold nanoparticle to approach a ds-DNA molecule), the Keesom interaction energy is responsible for the major contribution to the attraction between ds-DNA molecules and nano-Au particles. It is expected in this case that the larger the nano-Au size, *i.e.*, the larger its volume

$$V \approx a^3,$$

where a is the characteristic size (radius) of a nanoparticle, the higher the Keesom interaction energy, and thus the efficiency of nano-Au binding to ds-DNA will be proportional to the product $a^3c^{1/2}$, where c is the counterion concentration.

It should be noted that the electrostatic mechanism of binding (concentration) of negatively charged gold nanoparticles to the ds-DNA molecule in an aqueous solution where these nanoparticles are polarized has been studied and the high probability of this mechanism has been stated.[104]

Many details of specific mechanism of interaction of gold and other metal nanoparticles, in particular, magnetic nanoparticles,[105] with ds-DNA molecules remain unclear.

Thus, we believe that ds-DNA molecules in high salt solutions can interact with negatively charged nano-Au particles. Any of the above mechanisms implies that binding of negatively charged nano-Au particles to ds-DNA molecules is dynamic; *i.e.*, gold nanoparticles can move along the DNA molecule and be fixed at the strongest binding sites.[106]

It can be suggested that the nano-Au–ds-DNA interaction efficiency depends on a number of factors, in particular, on the base sequence in DNA molecules and features of the DNA secondary structure, as well as on the nano-Au size (diameter) or shape.[79]

On the basis of the above hypotheses concerning the possibility of nano-Au binding to ds-DNA, possible sites of fixation of gold nanoparticles (guests) near ds-DNA molecules in a quasinematic layer can be considered (Fig. 7).

First, nano-Au species of any size can interact with both the surface DNA molecules and the nitrogen base pairs of the terminal groups of DNA molecules in quasinematic layers, thus forming complexes (assemblies) with them.[79, 100] Binding of even a small number of negatively charged nano-Au species to DNA molecules (especially to terminal groups of these molecules) leads to the formation of dipoles (see Fig. 7, nanoparticles *1*, *2* and *3*, *4*). It should

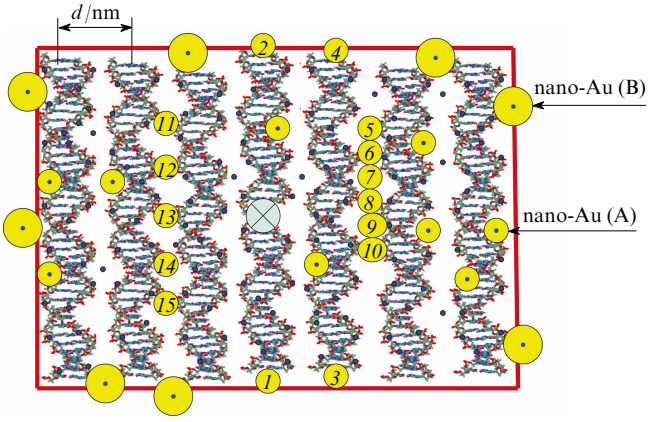


Fig. 7. Possible variants of arrangement of gold nanoparticles of different size (A and B) near ds-DNA molecules forming a quasinematic layer (symbol ⊗ at the centre denotes the rotation axis of the helical structure of the cholesteric DNA).[10]
(*1*–*15*) See text for details. The average distance between DNA molecules (d) depends on the solution osmotic pressure denoted by the red box.

be noted that this process does not require diffusion of gold nanoparticles into the quasinematic layer. The dipole formation through nano-Au binding to terminal DNA groups can play an important role. The DNA–nano-Au dipoles in adjacent quasinematic layers will tend to align parallel to each other.[67–69] Binding of negatively charged nanoparticles has no significant effect on the forces (steric, *etc.*) responsible for the propensity of adjacent DNA molecules to align parallel to each other but can change contributions (in particular, the anisotropic contribution to the van der Waals interaction) determining the helical twist of adjacent quasinematic layers. In this case, the helical twist will be disturbed, and the twist angle (~ 0.5) between the layers can become zero.[12] This is equivalent to the untwisting of the cholesteric helical structure of an LCD particle, which is accompanied by the disappearance of the abnormal band in the CD spectrum of LCD particles.[14]

Second, it can be assumed that gold nanoparticles with a size comparable to the size of the free space between ds-DNA molecules in quasinematic layers [Fig. 7, nano-Au (A)] can move among these molecules. Due to the interaction between reactive groups of DNA and nano-Au species, the latter can be fixed at different locations near the DNA molecule surface.[104] In this case, neighbouring nano-Au species can effectively interact with each other through DNA molecules. This enables not only the spatial fixation of separate nano-Au species but also the formation of various linear assemblies of adjacent nanoparticles (Fig. 7, nanoparticles *5–10* and *11–15*).

Hence, it is expected that the interaction (if any) between negatively charged nano-Au species and ds-DNA molecules in CLCD particles through the first or second mechanism will be, first of all, accompanied by a change in the abnormal optical activity characteristic for this dispersion.

It cannot be ruled out that the definite arrangement of neighbouring gold nanoparticles and formation of assemblies between DNA molecules can lead to the emergence of the SPR band in the absorption spectrum.[55, 56]

IV.4. Circular dichroism spectra of CLCDs formed from linear ds-DNA molecules preliminarily treated with gold nanoparticles

We have used a hydrosol containing quasi-metal gold nanoparticles ~ 2 nm in diameter obtained by the Duff method.[85] The zeta potential of nanoparticles is within -30 mV; *i.e.*, the nanoparticles are negatively charged. The capping ions on the nano-Au surface stabilize the properties of these nanoparticles, while the electrostatic interaction (repulsion) prevents the aggregation of nanoparticles and ensure their stable existence in solution.

As shown above, the phase exclusion of rigid linear ds-DNA molecules from an aqueous salt solution of PEG is accompanied by the formation of a CLCD that gives rise to the abnormal band in the CD spectrum.

Taking into account the possible interaction between negatively charged gold nanoparticles and linear ds-DNA molecules, as well as the change in the abnormal optical properties of the CLCD caused by this interaction, we have prepared CLCDs from DNA molecules preliminarily treated with gold nanoparticles.

Figure 8 *a* shows the CD spectra of a CLCD formed from pristine linear ds-DNA molecules (curve *1*) in an aqueous salt (0.3 M NaCl) solution of PEG and CLCDs formed under the same conditions from linear ds-DNA molecules treated with nano-Au (curves *2–7*). As seen, the higher the nano-Au concentration in a solution, the lower the amplitude of the abnormal negative band in the CD spectrum. The addition of one gold nanoparticle per 10 ds-DNA molecules in the CLCD is sufficient for the abnormal band amplitude in the CD spectrum to start decreasing (Fig. 8 *b*). The saturation of the effect is observed if there are 4–6 gold nanoparticles per 10 ds-DNA molecules.

The abnormal band amplitude in the CD spectrum of the CLCD is associated with the properties of DNA molecules forming quasinematic layers; therefore, the decrease in this amplitude is, first of all, evidence of the interaction of gold nanoparticles with DNA molecules in a high salt solution. The ΔA magnitude is related to the twist

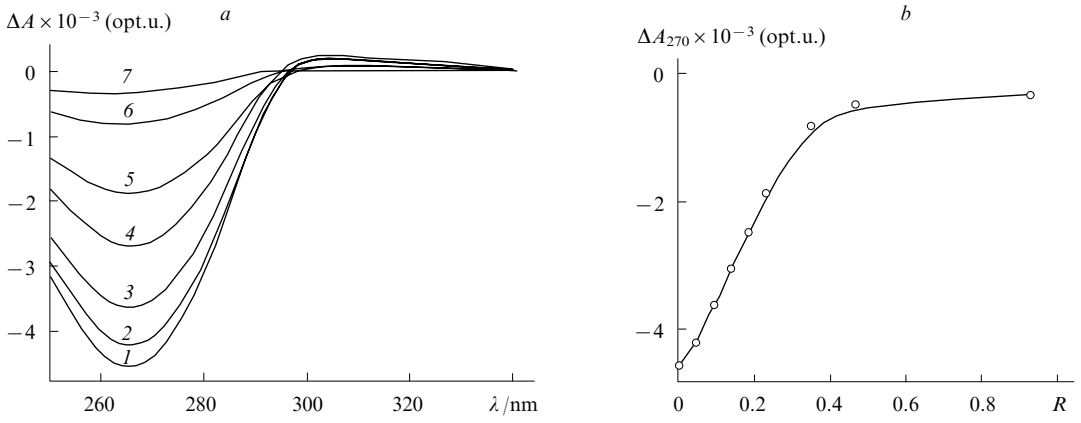


Fig. 8. CD spectra of CLCDs formed from linear ds-DNA molecules preliminarily treated with gold nanoparticles taken in different concentrations (*a*) and the plot of the ΔA_{270} *vs.* R (*b*).

Position *1*	*2*	*3*	*4*	*5*	*6*	*7*	
$c_{\text{nano-Au}}/10^{12}$ ml^{-1}	0	0.35	0.70	1.05	1.75	2.625	7.00
R	0	0.046	0.093	0.139	0.232	0.349	0.930

R is the ratio of the number of nano-Au particles to the number of DNA molecules in the solution. Solution: $c_{\text{DNA}} = 10$ μg ml^{-1}; $c_{\text{PEG}} = 190$ mg ml^{-1}; 0.3 mol litre^{-1} NaCl + 0.002 mol litre^{-1} of the phosphate buffer solution.

angle of the spatial structure (*i.e.*, to the structural chirality) of the DNA CLCD. Such a twist is determined at the moment of recognition of ds-DNA molecules when they come closer together. The energies required for the twist make a rather small contribution to the interaction of neighbouring DNA molecules. Such an interaction — of electrostatic or van der Waals nature — is extremely sensitive to the presence of gold nanoparticles. The fact that the decrease in the abnormal band amplitude in the CD spectrum is achieved at an extremely low concentration of gold nanoparticles is evidence that the latter should be bound to ds-DNA molecules to affect the recognition of these molecules [11–15] when they approach each other upon phase exclusion.

Hence, binding of negatively charged nano-Au particles to linear ds-DNA molecules in high salt solutions prevents the formation of CLCD particles exhibiting an abnormally high optical activity. The decrease in the abnormal band amplitude in the CD spectrum of CLCDs formed from linear ds-DNA molecules and treated with negatively charged nano-Au species can be, in principle, of general character and extended to CLCDs formed by molecules of synthetic double-stranded polynucleotides. However, the magnitude of the optical effect can depend on the secondary structure of double-stranded biopolymers.

IV.5. Effect of gold nanoparticles on the preliminarily formed ds-DNA CLCD

Figure 9 *a* shows the CD spectra of the CLCD formed in an aqueous salt (0.3 mol litre^{-1} NaCl) solution of PEG from pristine ds-DNA molecules (curve *1*) and of the same dispersion treated with different concentrations of nano-Au species (curves *2*–*7*), and Fig. 9 *b* shows the plot of the abnormal negative band amplitude (λ = 270 nm) in the CD spectrum *versus* R. It is seen that the treatment of the CLCD formed from linear ds-DNA molecules with gold nanoparticles leads to the decrease in the abnormal band amplitude. The optical effect is saturated if there are 5–10 gold nanoparticles per DNA molecule. This concentration is roughly one order of magnitude higher than the nano-Au concentration that 'prevents' the formation of a cholesteric from pristine linear DNA molecules (Fig. 8 *b*). This distinction is justified because it reflects the collective behaviour of neighbouring ds-DNA molecules in the CLCD particles. For the spatial structure of these particles to be disturbed, large energy inputs are required; therefore, this structure is disturbed when a noticeably larger number of nano-Au particles bind to ds-DNA molecules as compared to the number of nano-Au particles that modify the properties of single ds-DNA molecules in their recognition.

Since the abnormal band amplitude in the CD spectrum of ds-DNA CLCD correlates with the helical twist of a cholesteric structure, the observed decrease in the amplitude of this band is, in this case, evidence that nano-Au species bind to ds-DNA molecules forming quasinematic layers in the CLCD structure (Fig. 7). As underlined above, when even a small number of nano-Au species binds to DNA molecules in quasinematic layers, these layers tend to the parallel arrangement. In effect, this will lead to the decrease in the helical twist angle of neighbouring DNA molecules to zero. Indeed, according to theoretical studies,[14] unwinding of the spatial helical structure of DNA CLCD particles (nematization of this structure) reduces the abnormal optical activity.

The decrease in the abnormal optical activity of CLCD particles is a unique effect since none of the chemical or biologically active compounds interacting with ds-DNA molecules ordered in the structure of CLCD particles leads to nematization of the spatial structure of these particles at such a low concentration and room temperature.

Thus, the above results, reflecting different variants of action of gold nanoparticles on linear ds-DNA molecules, demonstrate that, in a high salt PEG-containing solution (0.3 mol litre^{-1} NaCl), small nanoparticles (2 nm) bearing a negative charge interact with (bind to) ds-DNA. Therefore, we agree with the authors of works [79, 98, 99] that the general statement about the lack of such an interaction made in Ref. 76, was erroneous.

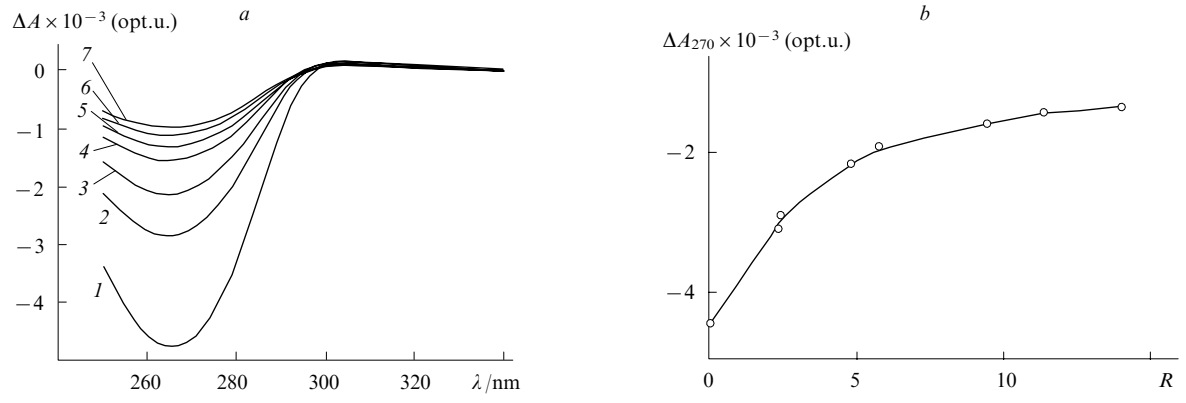


Fig. 9. CD spectra of DNA CLCD treated with gold nanoparticles taken in different concentrations (*a*) and the plot of the ΔA_{270} *vs.* R (*b*).

Position	*1*	*2*	*3*	*4*	*5*	*6*	*7*
$c_{\text{nano-Au}}/10^{12}$ ml^{-1}	0	1.74	3.465	6.863	10.194	13.462	16.667
R	0	2.39	4.77	9.42	14.00	18.49	22.90

Solution: c_{DNA} = 9.7 μg ml^{-1}; c_{PEG} = 190 mg ml^{-1}; 0.3 mol litre^{-1} NaCl + 0.002 mol litre^{-1} of the phosphate buffer solution.

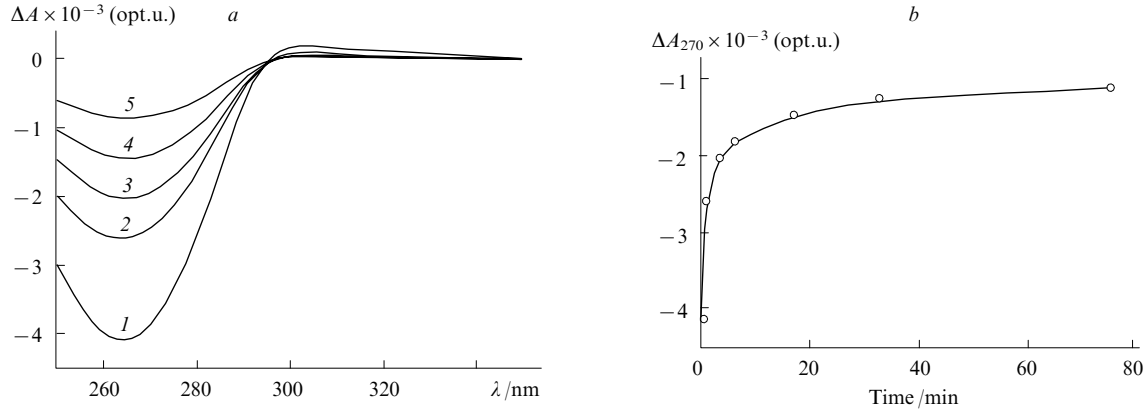


Fig. 10. CD spectra of the DNA CLCD recorded at different time intervals (t) after its treatment with gold nanoparticles (a) and the plot of the ΔA_{270} *vs.* time (b).
t /min: (*1*) 0, (*2*) 0.58, (*3*) 3.5, (*4*) 17, (*5*) 120. Solution: c_{DNA} = 10 μg ml^{-1}; c_{PEG} = 190 mg ml^{-1}; 0.3 mol litre^{-1} NaCl + 0.002 mol litre^{-1} of phosphate buffer; $c_{nano\text{-}Au} = 1.67 \times 10^{14}$ ml^{-1}, R = 22.19.

IV.6. Kinetics of the action of gold nanoparticles on the preliminarily formed ds-DNA CLCD

As an example, Fig. 10 *a* shows the CD spectra recorded at different intervals after the treatment of the ds-DNA CLCD with a hydrosol containing gold nanoparticles in high concentration, and Fig. 10 *b* shows the kinetic curve reflecting the change in time of the abnormal band amplitude (λ = 270 nm) in the CD spectrum. It is seen that the decrease in the abnormal band amplitude caused by the action of small nano-Au species (~2 nm) is completed in ~20 min. This means that nematization of the spatial structure of LCD particles driven by binding of a small number of nano-Au species to DNA molecules is a fast process.

It should also be noted that the form of the kinetic curve implies the occurrence of one more process related to the action of nano-Au on the ds-DNA CLCD, which makes only a small contribution to the change in the abnormal optical activity of dispersion particles.

IV.7. Change in absorption spectrum caused by the effect of gold nanoparticles on the preliminarily formed ds-DNA CLCD

The treatment of the ds-DNA CLCD with small gold nanoparticles (~2 nm) leads to changes in the UV and visible ranges of the absorption spectrum (Fig. 11).

First, the poorly pronounced band (*I*) at 505 nm (the localized SPR band)[56] starts changing. The amplitude of this band increases with time, and its maximum is shifted from ~505 to ~550 nm.

Second, the amplitude of the band in the UV range (*II*) decreases. (According to theoretical calculations,[66] analogous changes of two bands in the absorption spectrum correspond to the increase in the volume fraction of assemblies formed by gold nanoparticles).

The synchronous change of the SPR band (*I*) and the band in the DNA absorption range (*II*) indicates that the spectral changes are caused by changes in the properties of both the ds-DNA molecules and nano-Au species. It can be assumed that the observed evolution of the SPR band and its bathochromic shift (Fig. 11) are caused by the formation of nano-Au assemblies[55, 56] located, in this case, between ds-DNA molecules fixed in the quasinematic layers of CLCD particles.

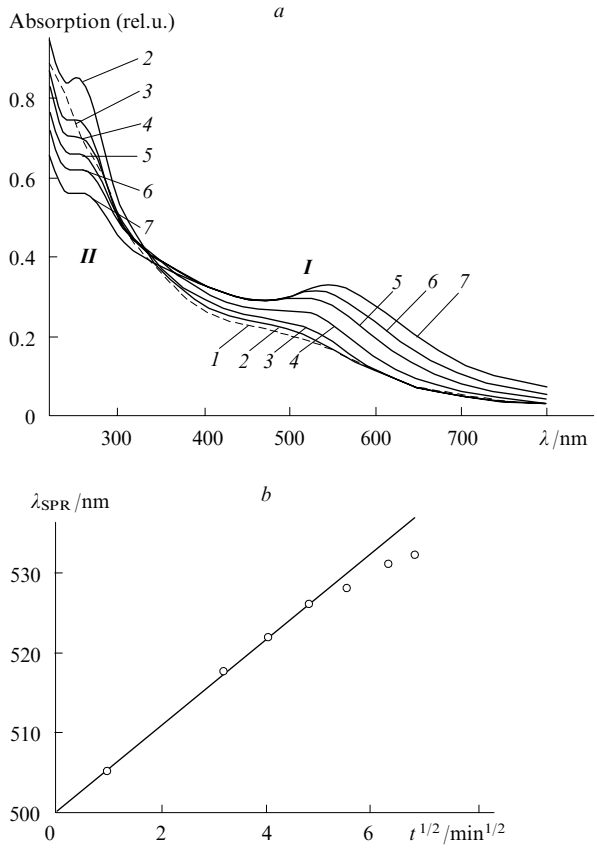


Fig. 11. Absorption spectra of the ds-DNA CLCD treated with gold nanoparticles for different time (a) and the SPR band maximum position as a function of time (b).
(*1*) (Dashed line) absorption spectrum of an aqueous salt solution of PEG containing nano-Au; t /min: (*2*) 1, (*3*) 10, (*4*) 23, (*5*) 46, (*6*) 148, (*7*) 1320. Solution: c_{DNA} = 10 μg ml^{-1}; c_{PEG} = 190 ng ml^{-1}; 0.3 mol litre^{-1} NaCl + 0.002 mol litre^{-1} of the phosphate buffer solution; $c_{nano\text{-}Au} = 1.67 \times 10^{14}$ ml^{-1}; (*I*, *II*) see text.

For illustration of the above statement, absorption spectra of a chaotically oriented ensemble of linear clusters of nano-Au particles (2 nm) in water have been simulated.[107] The distance between nano-Au particles was taken to be 0.2 nm, which corresponds to a rather weak interaction between them. The calculation was performed by the cluster T-matrix method.[108] Necessary computational details have been described elsewhere.[109, 110]

The calculated spectra qualitatively reproduce three key features observed in the experiment (Fig. 11):

— the increase in the SPR band amplitude;

— the bathochromic shift of the SPR band;

— the saturation of the plot of the plasmon shift *versus* the number of gold nanoparticles in a cluster.

Comparison of experimental data with the calculation results has shown that even the simplest monodisperse model of formation of nano-Au assemblies (linear clusters) with the adjusted interparticle distance reproduces rather accurately both the entire range of the change in the SPR band wavelength and the two-stage character of this change.

The formation of nano-Au assemblies is also supported by the dependence of the SPR band position on the time (t) of treatment of the ds-DNA CLCD with small gold nanoparticles. This dependence demonstrates that the SPR band position changes more slowly than the abnormal band amplitude in the CD spectrum. The change in the SPR band position (up to the moment of its saturation) is directly proportional to $t^{1/2}$ (Fig. 11 *b*). If we assume that the SPR band shift reflects the growth of the nano-Au assembly, the theoretical evaluation confirms the hypothesis of the possibility of formation of linear nano-Au clusters in DNA CLCD particles. The cluster growth is known to be determined by a competition between the bulk energy and the surface free energy, which includes the internal energy and entropy of all nanoparticles involved in cluster formation. The long-standing analysis of this process performed by Lifshitz and Slyozov[111] has demonstrated that the size (radius) of a spherical cluster increases proportionally to $t^{1/3}$. Using the approach by Lifshitz and Slyozov for describing the growth of a linear cluster of cylindrical shape has shown that, in this chase, the cylinder length increases proportionally to $t^{1/2}$ for any cylinder radius. This theoretical estimate is quite consistent with the experimental data (Fig. 11 *b*).

Thus, the change in the SPR band position as a function of time is associated with diffusion of nano-Au species into the ds-DNA CLCD particles and formation of linear nano-Au clusters in these particles.

IV.8. Structural analysis of phases formed from ds-DNA CLCD particles treated with gold nanoparticles

To verify the above assumption, structural analysis of the phases formed from ds-DNA CLCD particles treated with gold nanoparticles has been performed by the small-angle X-ray scattering method.[112] The assumption of the linear structure of nano-Au clusters and the calculation of their size with the GNOM programme[113] have enabled the construction of the model of nano-Au fixation between ds-DNA molecules in the quasinematic layers (Fig. 12).†

According to this model, nano-Au particles 2 nm in diameter form linear clusters with an average length of 13–15 nm and a maximal length of 40–50 nm.[54] The formation of nano-Au clusters has an effect on the spatial orientation of adjacent quasinematic layers of ds-DNA. Under these conditions, the helical twist of the layers in a ds-DNA CLCD particle changes in the same manner as it occurs in the case of low-molecular-mass liquid crystals.[47, 48]

The formation of linear gold clusters in the free space between ds-DNA molecules results in that the interaction between these molecules mediated by gold nanoparticles becomes stronger. This leads to the formation of the integrated structure

$$[...DNA...(nano\text{-}Au)_n ...DNA...(nano\text{-}Au)_m ...DNA...]$$

(where n and $m = 1, 2, etc.$), which involves nearly all DNA molecules of one particle. Neighbouring DNA molecules in quasinematic layers are physically cross-linked. In addition, the presence of separate gold nanoparticles and extended nano-Au clusters in the quasinematic layers of DNA molecules are responsible for the decrease in the solubility of the

† The figure was taken from our paper *Open Nanosci.* **8** 1 (2014) and is published with permission of the Editorial board of this journal.

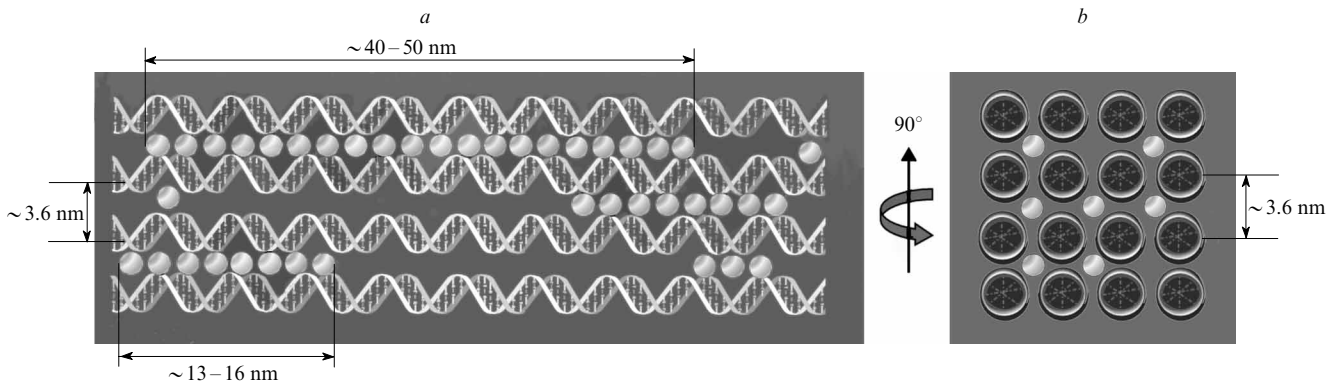


Fig. 12. Hypothetical model of the arrangement of linear clusters composed of gold nanoparticles between ds-DNA molecules forming the quasinematic layers in the CLCD (from small-angle X-ray scattering data).
(*a*) Side view; (*b*) end view.

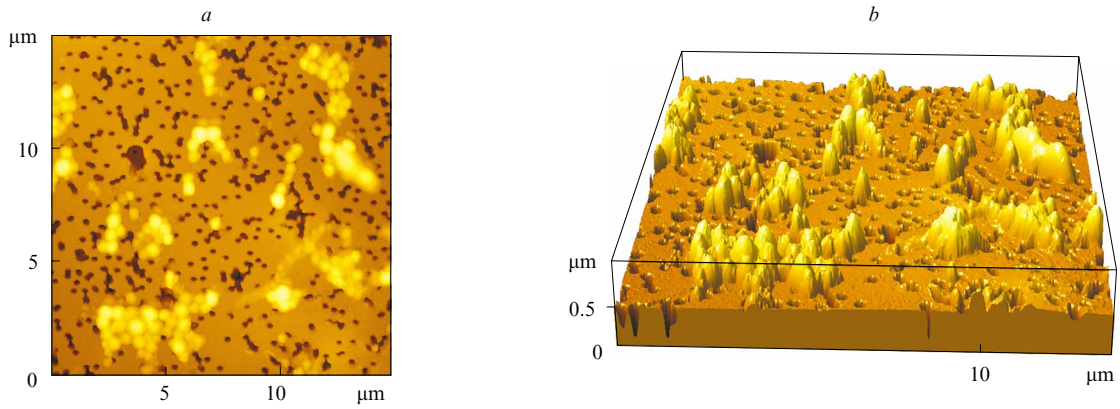


Fig. 13. 2D (*a*) and 3D (*b*) AFM images of rigid particles formed by means of treatment of the DNA CLCD with gold nanoparticles and immobilized on a nuclear membrane filter.[107]
Dark spots are the holes in the nuclear membrane filter (PETP).

resulting structure. The integrated structure with a very high molecular mass becomes incompatible with an aqueous salt solution of PEG. The stability of this structure is determined by the number and properties of separate gold nanoparticles and related nano-Au clusters rather than by the properties of the PEG solution. This means that ds-DNA CLCD particles are converted from the 'liquid' state to the 'rigid' state. The rigid (insoluble) structure can exist even in the absence of the high osmotic pressure of a solution.

IV.9. AFM images of rigid particles formed of ds-DNA CLCD particles treated with gold nanoparticles

Conversion of ds-DNA CLCD particles into the rigid state induced by formation of linear nano-Au clusters acting as physical cross-links between the ds-DNA molecules in quasinematic layers enables the immobilization of particles on the nuclear membrane filter surface and the study of their morphology by means of an atomic force microscope.

The 2D image (top view) of rigid particles formed upon the treatment of DNA CLCD particles with gold nanoparticles is shown in Fig. 13 *a* and their 3D image is in Fig. 13 *b*. The average size of rigid particles is within 450–500 nm. The height of the particles composed through cross-linking of neighbouring DNA molecules fixed in the quasinematic layers of CLCD particles by nano-Au clusters does not exceed 300 nm.

The data obtained are quite consistent with the results of theoretical calculations of the size of CLCD particles formed by ds-DNA molecules in solutions with constant osmotic pressure.

A distinction of rigid ds-DNA particles formed through gelation inside particles (see above) from the rigid particles formed by physical cross-linking is that the latter are weakly bound to the nuclear membrane filter surface. As a result, the metallized particles, *i.e.*, DNA CLCD particles treated with nano-Au, can be moved over the membrane filter surface by pushing them with the AFM cantilever and fixing at definite sites on the filter (Fig. 14).‡

‡ We have prepared a film demonstrating the spatial movement of a single DNA CLCD particle treated with nano-Au over the nuclear membrane filter surface.

Migration of metallized ds-DNA CLCD particles can be of interest for nanotechnology since it opens up the possibility to design matrices with specific properties.

Thus, the nanotechnological scenario enables the production of rigid DNA particles suitable for effective manipulation.

The above results, demonstrating that an extremely low nano-Au concentration (a few nanoparticles per ds-DNA molecule, which corresponds to an overall nano-Au concentration of $10^{11}-10^{12}$ ml^{-1}) disturbs not only the spatial order of linear ds-DNA molecules upon their phase exclusion but also the spatial structure of DNA LCD particles, are of great interest for biology. Here, attention should be paid to some circumstances.

First, it has been suggested that small nano-Au particles can interact with both the ds-B-DNA and ds-A-DNA molecules.[114]

Second, the experiments using ^{198}Au-labelled nano-Au have shown that 57.5% of radioactive gold is in the cytoplasm and 42.5% of ^{198}Au is in the cell nucleus.[99] After removal of nuclear proteins, it has been revealed that an extraordinary high concentration (21%–23%) of the radioactive material is bound to the condensed nuclear DNA. (It should be taken into account that DNA LCD particles model precisely the condensed ds-DNA state in protozoan cells).

Third, it has been shown for the first time that nano-Au particles exhibit genotoxicity and have an impact on spermatogenesis.[115]

Fourth, this work [115] has stimulated intensive studies of Russian scientists (see, *e.g.*, Ref. 59) not only confirming the nano-Au effect on spermatogenesis but also demonstrating a diversity of possible mechanisms of nano-Au action on animals. This is also supported by studies.[57, 58] Finally, Khlebtsov and Dykman in their review [116] addressing the toxic action of nano-Au have repeatedly stated that the toxic effect of nano-Au in different biological systems is manifested beginning precisely with a concentration of 10^{12} ml^{-1}. Such a coincidence between our values (see above) and the value reported in this review is hardly accidental.

The aforesaid allows us to assume that the interaction of nano-Au with ds-DNA and formation of nano-Au clusters between the ds-DNA molecules in condensed structures

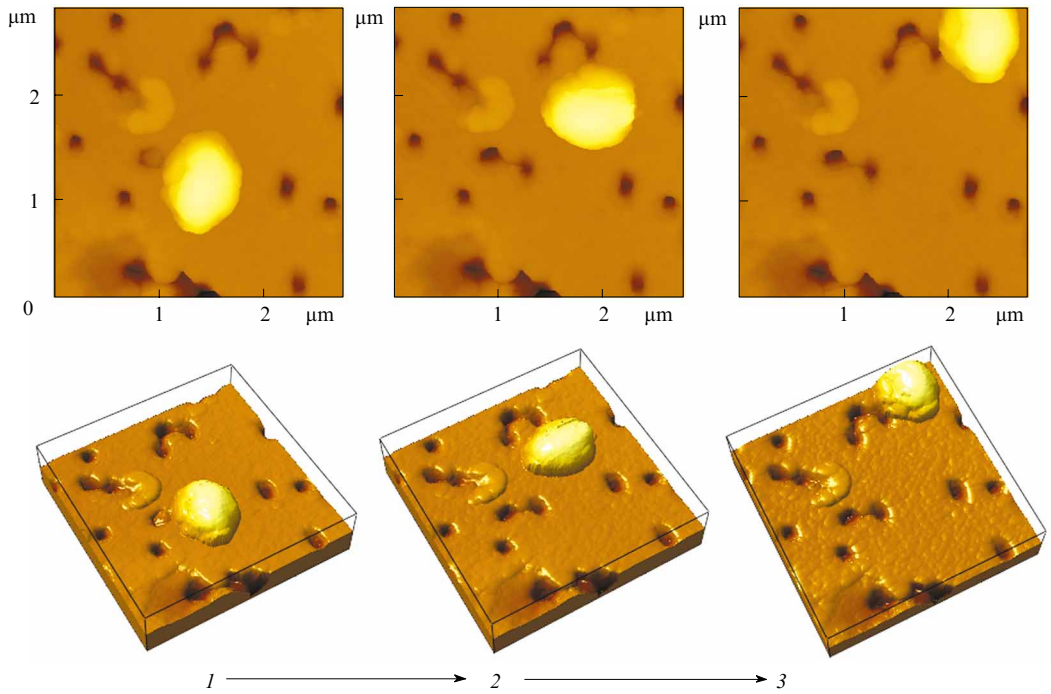


Fig. 14. Successive positions (*1–3*) of a rigid particle formed upon the treatment of the DNA CLCD with gold nanoparticles and moved over the membrane filter surface under the action of the AFM cantilever.[28]
Dark spots are the holes in the nuclear membrane filter (PETP).

formed by these molecules in biological objects (chromosomes, viruses and sperm cells), accompanied by the disturbance of the spatial structure of these objects, is one of the factors responsible for the genotoxic effect of small nano-Au particles.

V. Conclusions

In this review, different variants of the liquid-crystalline approach to formation of rigid ds-DNA particles have been compared.

It has been shown that physicochemical variants are reduced to the formation of different chemical 'gels' of linear ds-DNA molecules fixed in quasinematic layers of the spatial structure of the DNA CLCD.

A new possibility for formation of rigid DNA particles is offered by the nanotechnological approach. It has been shown that the treatment of CLCDs composed of high-molecular-mass ds-DNA molecules with negatively charged gold nanoparticles in a high salt solution leads to formation of a physical gel in which nano-Au particles are located between ds-DNA molecules ordered in the quasinematic layers of CLCD particles. Binding of nano-Au to ds-DNA molecules initiates two processes:

— disturbance of the spatial structure of the DNA cholesteric;

— formation of extended linear nano-Au clusters in DNA CLCD particles.

Under these conditions, the helical twist of the quasinematic layers of DNA molecules vanishes, as in the case in low-molecular-mass liquid crystals. The 'liquid' character of ds-DNA packing in CLCD particles disappears, and a DNA particle with a 'rigid' structure appears. A specific feature of rigid DNA particles formed using this approach is that such particles are weakly bound to the membrane filter surface. This enables their controlled movement over the filter surface and fixation at the required sites on the filter, which can be of nanotechnological importance.

The results obtained can be treated as evidence in favour of the concept that fixation of nano-Au in ds-DNA LCD particles modelling some properties of protozoa can be one of the factors responsible for the genotoxicity of these particles.

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