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Principles of the design of nanostructures with nucleic acid molecules as building blocks

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Abstract. The review covers two known strategies for the design of nanostructures based on liquid-crystalline dispersions of double-stranded nucleic acids: the 'entropy' condensation of nucleic acids and the 'enthalpy' condensation of nucleic acid – polycation complexes. Examples of the design of nanostructures based on DNA molecules and DNA – chitosan complexes are given. The unique properties of nanostructures dictating the scope of their practical application are described. The bibliography includes 60 references.

I. Introduction

The rapidly evolving field of research, which was originated at the crossroad between physics, chemistry and electronic and computer engineering and is known under the name of nanotechnology,[1, 2] is focused on the so-called nanosized structures ranging from subnanometres to hundreds of nanometres. These structures have attracted interest because, as was found at the turn of the 21st century, individual atoms (or individual molecules) can be used as building blocks for the atom-by-atom design of desired three-dimensional structures. Nanosized systems include one-, two- and three-dimensional structures (individual nanoparticles, nanofilms, rods and tubes), nanostructured and nanoporous materials and nanodevices. The upper limit of the size range for these structures is rather arbitrary, and the lower limit is determined by the sizes of atoms and molecules.

In spite of diversity of nanosystems, all these materials, apparently, have common features. First, it is the so-called size effect.[3, 4] Actually, an atom on the surface differs from atoms of the same element in the bulk of usual materials. If a large number of atoms are located on the surface, their average coordination number is smaller. Weaker biding of atoms on the surface results in lower melting points of the surface layers. The notion of melting, which is a cooperative process, becomes rather ambiguous for a small number of atoms in an ensemble (cluster), and the phase transition (melting) becomes 'blurred' as the number of atoms in the cluster decreases.[4] (This is, apparently, associated with the fact that clusters behave as small molecules rather than as a massive material. In this case, it is more correct to speak of different structural isomers, which exist in a particular temperature range, rather than of phases.) Moreover, the properties of an atom involved in a small cluster and located on the surface depend on the type of the substrate or on whether the cluster contains an 'impurity' of one or several atoms of other elements. To elucidate the relationship between the properties and sizes of the clusters, one should bear in mind that not only the average size of the clusters, but also their size distribution are of importance.[3] As a result, the properties change non-monotonically with a decrease in the particle size, and the corresponding plots have specific points.

In addition, nanomaterials are characterised by the following specific features:[3]

— the size effects can have a quantum nature,

— there are numerous factors masking the size effect (as opposed to usual materials),

— the catalytic properties of transition metal atoms on the surface, including the selectivity, strongly depend on the cluster size.

It can be said that structures consisting of a limited number of building blocks manifest fundamentally new dominant physical phenomena, such as quantum effects, the scaling of the properties depending on the sizes of the structures, the predominant effect of surface properties, and the absence of bulk defects. In addition, the presence of free valences in atoms of the surface layer is responsible for considerable 'energy saturation' and, correspondingly, high reactivity of the resulting structures. All the aforesaid shows that structures acquire unique, often unpredictable properties: mechanical, electrical, magnetic, optical, chemical, *etc*. Thus, nanostructured materials are 'more than the size', because the appearance of unique properties, which are a function of the particle size of nanomaterials, rather than the size as such is essential.

Finally, the problem of stability of nanomaterials is of great importance because thermal, chemical and radiation fields often lead to the complete or partial loss of the unique properties of nanomaterials.[3, 4]

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Hence, it is evident that interdisciplinary studies are required to investigate the properties of nanostructured materials.

Taking into account all the aforesaid, nanotechnology can be described as an interdisciplinary science concerned with the problems of the design and application of structured materials, devices and systems the properties of which are determined by the geometric sizes or specific physicochemical features of nanostructures.[5]

Nanomedicine is one of the relatively new fields in nanotechnology (to be more precise, it is more correct to speak of nanotechnology for the solution of medical problems). An appealing idea is that nanotechnology will help in making the treatment of many diseases more effective. Indeed, the drug distribution in organisms of patients can be changed so as to deliver the drug to the desired target. This problem can be solved with the use of nanostructured carriers, which are called also nanoparticles (nanosystems) for the targeted drug delivery and which are designed taking into account the biochemical features of the organism of a particular patient. Since the unique feature of nanoparticles is that they have a very developed (compared to traditional materials) surface, the use of nanosystems for the drug delivery makes it possible to overcome the low solubility and low absorption properties (absorbability) of newer generation drugs. It should be noted that nanoparticles are used not only for the treatment of particular pathologies, but also for the disease diagnostics. In this connection, the design of new nanomaterials having better characteristics for the therapeutic and diagnostic applications is of great importance.

It should be noted that the biological role of nanomaterials remains an open question, and detailed investigations of their biological properties are required. The results of these studies would also be of considerable importance for understanding of the nature of toxicity of nanomaterials and elucidation of the potential hazard associated with nanotechnology. First discussions on this problem were held in USA [6] in December 2005 and at the Institute of Crystallography of the Russian Academy of Sciences (Moscow) in October 2006. The International experts who participated in these discussions noted that, because of the specific properties of nanoparticles of any origin (the developed surface, high chemical reactivity, high catalytic activity, the ease of transport in a flow of air or liquid, the absence of metabolism in many cases, *etc.*), their appearance in the environment or animal or human organisms can have unpredictable impacts. This has initiated the discussion of the rules of working with nanomaterials and the rules of their controlled applications in different fields of medicine, engineering and science.[7]

Materials of recent seminars and symposia (see, for example, materials of the International Conference *NanoTech 2005*, May 8–12, Anaheim, California, USA, or materials of the *NATO Workshop 'Nanomaterials for Application in Medicine and Biology'*, 2006, October 4–6, Bonn, Germany) show that the number of investigations in nanotechnology where biological macromolecules are employed rapidly increases. Nanotechnology based on double-stranded nucleic acids (dsNA) has attracted particular attention due to the specific properties characteristic of only these molecules.[8] In the present review, the strategies for the nanodesign are considered, the features of these strategies are discussed and the possible scope of application of nanostructures is outlined.

II. Strategy for the design of nanostructures based on double-stranded nucleic acids

Two fundamentally different strategies for the nanodesign were developed. These strategies are based on the use of different physicochemical properties of dsNA molecules; the structures of nanomaterials can be controlled at a molecular level regardless of the strategy.[9–11]

1. Step-by-step strategy

The technology for the nanoscale design pioneered by Seeman [9] in 1982 is based on the formation of three-dimensional structures by successive modifications of the starting DNA molecule giving rise first to a planar nanolattice and then to cube-like structures.

The first step involves the preparation of NA molecules containing complementary single-stranded terminal fragments (called sticky ends; **A** in Fig. 1 *a*) by biochemical methods or direct chemical synthesis. Interactions between the sticky ends of different NA molecules give rise to structures having two breaks in the sugar-phosphate backbone (**B** in Fig. 1 *a*). The enzyme ligase joins these breaks to form a base-paired dsNA structure (**C**). The second step gives rise to a branching point (cruciform structure **D**) by using NA molecules with a specific sequence of nitrogen bases or by introducing single-strand breaks into one of the NA chains. The cruciform structures can consist of three or four different branches, *i.e.*, can have different spatial shapes. Sticky ends are formed in cruciform dsNA molecules with the use of enzymes. The hybridisation followed by cross-linking of the sticky ends of the adjacent NA molecules gives rise to the primary nanostructure, *viz.*, a planar nanolattice composed of dsNA molecules (**E** in Fig. 1 *b*).

Since these structures consisting of DNA molecules are rather mobile at branching points, the resulting planar nanolattice is not very rigid. To form more rigid structures, several approaches were used. These approaches can be considered as supplementary to the approach suggested by Seeman. In particular, the technique suggested by Niemeyer [12] is based on the use of bis-biotinylated DNA molecules and the biotin-binding protein streptavidin. This technique allows the construction of nanocircles. Bergstrom [13] performed the nanodesign with the use of synthetic molecules derived from 1,1-bis[4-(2-hydroxyethyl)phenylethynylphenyl]ethane, in which both hydroxy groups are covalently linked to self-complementary single-stranded oligonucleotides. The complementary hybridisation of oligonucleotide fragments affords cyclic structures containing from two to seven building blocks. The rigidity of these structures (they are called multiarm stars) is provided by an alternation of hydrocarbon units with fixed spatial structures and double-stranded oligonucleotides.

Seeman prepared a cube-shaped nanoconstruction, in which dsDNA molecules served as the stiffening ribs, with the use of DNA molecules with a specified nucleotide sequence and an appropriate cruciform structure (Fig. 2). The aspects of the step-by-step strategy and different nanostructures (octahedra, dodecahedra, *etc.*) were considered by Seeman in his publication.[10]

It should be emphasised that the use of the above-described approach requires large expenditures. Actually, it is necessary to obtain NA molecules with known sequences of nitrogen bases, use a wide range of enzymes (restrictases and ligases) for

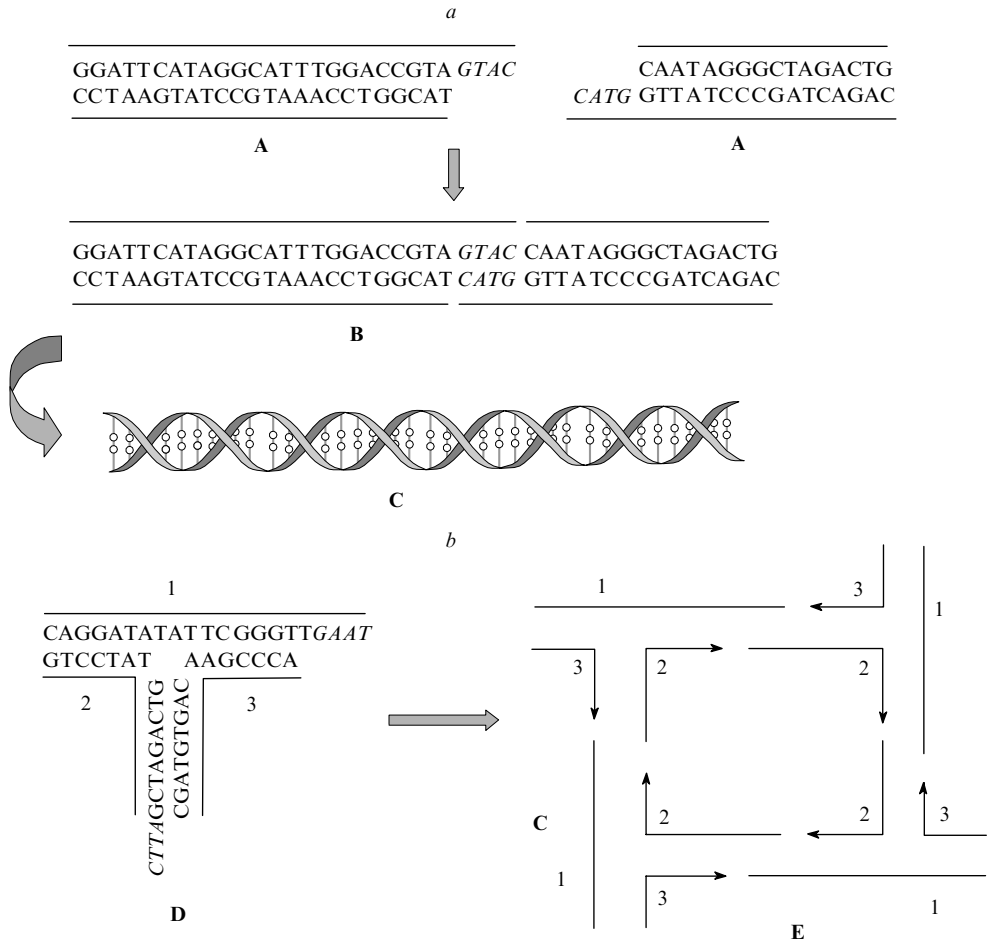


Figure 1. Scheme of the formation of a planar nanolattice by double-stranded DNA molecules.
(*a*) The formation of double-stranded DNA with a specified sequence; (*b*) the design of a planar nanostructure (see the text).

the cleavage followed by cross-linking of NA fragments between the required base pairs, isolate the product from the reaction mixture and analyse its properties by modern methods (such as atomic force microscopy) in all steps of the synthesis. Hence, this technique is labour-consuming and requires the control over the 'quality' of each step of the process.

Recall that the important aim of the nanodesign is to form three-dimensional nanoconstructions with controllable properties. The NA molecules involved in nanoconstructions retain physical and chemical characteristics and parameters typical of the B-form secondary structure. This opens a way of introducing various molecules (guests) into nanoconstructions, which should impart new useful properties to materials (see Fig. 2).

To design three-dimensional nanoconstructions containing guest molecules, Mirkin *et al.*,[14] Letsinger *et al.*[15] and Alivisatos *et al.*[16] virtually simultaneously (in 1996) employed an approach based on the use of synthetic single-stranded NA fragments linked to colloidal gold nanoparticles as building blocks. If a foreing NA with a nitrogen-base sequence complementary to the starting NA, is added to this system, the hybridisation affords a rigid dsNA molecule. In this case, gold nanoparticles are located at a fixed distance, which can be controlled with a step of 0.34 nm (the distance between the base pairs). The use of gold nanoparticles capped with more than one single-stranded NA fragments allows the formation of 3D nanostructures consisting of gold atoms and dsNA molecules alternating at regular intervals.

The formation of a nanoconstruction containing carbon nanotubes (CNT) and peptide nucleic acid (PNA) molecules, a synthetic compound in which (as opposed to natural NA molecules) the sugar-phosphate backbone is replaced by a peptide chain, was documented.[17–20] In the PNA structure, the NA nitrogen bases are retained and the distance between these bases is equal to that in natural NA. Peptide nucleic acids have some advantages over NA. They are more stable in commonly used solvents, are protected from degradation with some hydrolytic enzymes and, finally, contain the uncharged peptide backbone, resulting in an increase in stability of hybrid PNA–NA complexes. The attachment of a PNA molecule to a chemically modified CNT affords the CNT–PNA complex. This complex is hybridised with the complementary single-stranded fragment of the NA molecule covalently bound to the surface of a solid support to form a rigid double-stranded structure (PNA–NA) containing carbon nanotubes arranged in a particular fashion. Hence, carbon nanotubes can be oriented in desired directions with the use of NA molecules arranged in a specific fashion on the surface of an electronic unit.

In addition, the use of planar lattices consisting of DNA molecules as masks followed by the deposition of current-conducting compounds, such as gallium arsenide or indium phosphite, into these lattices opens, in opinion of Di Mauro and Hollenberg,[21] a way for designing elements (chips) for

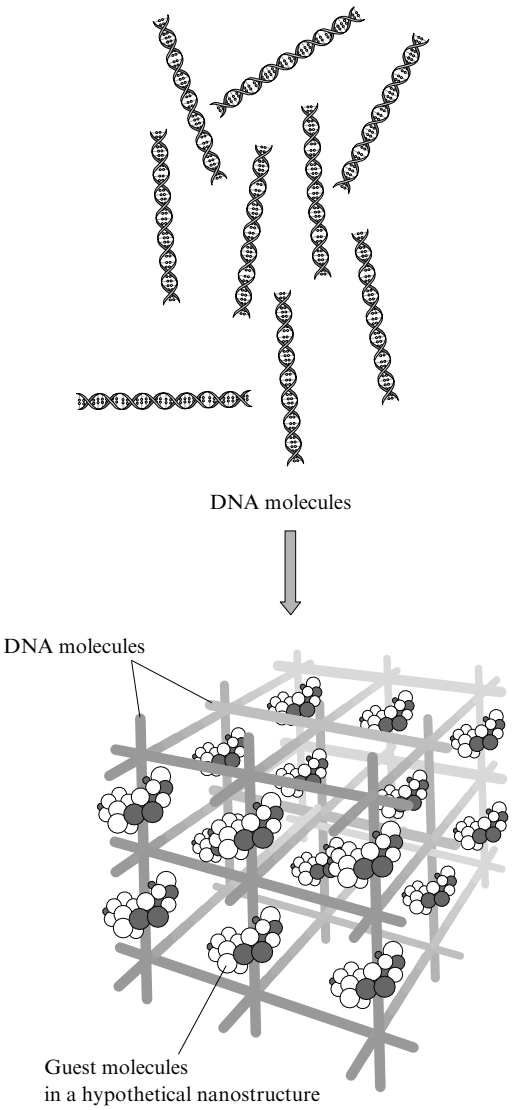


Figure 2. A scheme illustrating the idea of the step-by-step strategy: the formation of a 3D nanoconstruction that can bind guest molecules from dsDNA molecules by their successive modifications.

microelectronics with an accuracy greatly exceeding the accuracy of conventional techniques.

A comprehensive review [22] covered different aspects of the formation and possible practical applications of systems consisting of metal nanoparticles and oligonucleotide (nucleic acid) molecules.

Guest molecules in nanoconstructions can serve as targets for other biologically active compounds. *i.e.*, nanoconstructions can be used for the design of biosensors for the detection of compounds recognising guest molecules.

The three-dimensional nanostructure presented in Fig. 2 provides an explanation of the basics of different strategies for the accumulation of molecules of chemical, including biologically active, compounds (guest molecules) in nanoconstructions. According to one approach, guest molecules in nanoconstructions can be located within NA molecules. In fact, the linear dsDNA molecule can bind intercalators (*i.e.*, planar molecules inserted between nitrogen base pairs) or compounds filling one of the grooves on the surface of the macromolecule (polypeptides, proteins, *etc.*). The equilibrium concentration of such compounds depends on the characteristic features of the secondary structure of dsDNA (the distances between base pairs, the base pair tilt, the charge density of phosphate groups, *etc.*) and follows the known laws of adsorption. The limiting number of guest molecules can be equal, for example, to one molecule per 5 base pairs, and this parameter, all other factors being the same, depends only on the structure of the guest molecule.

Obviously, two ways of accumulating guest molecule in DNA-based nanoconstructions are, in principle, possible. For example, guest molecules can be attached to the starting DNA molecule, and the desired 3D nanoconstruction can be formed from the DNA – guest complex using the step-by-step strategy. Evidently, the concentration of guest molecules after several biochemical and physicochemical steps of formation of nanostructures, which are performed under different conditions, will most likely be low. According to another approach, the required 3D nanoconstruction can be formed from the starting dsDNA molecules using the step-by-step technique, and then guest molecules can be bound to dsDNA molecules serving as stiffening ribs. Since the physicochemical characteristics of DNA molecules in nanoconstructions remain virtually unchanged, the number of guest molecules bound to dsDNA molecules in nanoconstructions cannot be substantially different from the number of guest molecules bound to the starting dsDNA molecule. Therefore, the possibility of attaining superhigh concentration of guest molecules with the use of the step-by-step strategy is unlikely.

However, there is another possible approach to the problem of accumulation of guest molecules in dsDNA-based nanoconstructions. There is a free space between DNA molecules ordered in nanoconstructions (see Fig. 2), and there are *a priori* no restrictions on the filling of this space with guest molecules. Since the distance between the adjacent dsDNA molecules in the ordered structures of these macromolecules can be controlled within 2.5 – 5.0 nm, attempts can be made to attain a high concentration of guests in the free space between DNA molecules. Hence, there is a possibility of a fundamentally different way of immobilising (accumulating) molecules of various chemical compounds in 3D nanostructures.

2. All-at-once strategy

As mentioned above, procedures developed for the ordering of adjacent NA molecules allow the efficient use of the space between these molecules for the positioning of guest molecules. The distance between adjacent dsDNA molecules in ordered structures equals to a few nanometres. It can be attempted to attain high concentration of guests in the free space between DNA molecules by intercalating the guest molecules into the cross-links between adjacent DNA molecules. Taking into account the distances between the DNA molecules, these cross-links can be called nanobridges. In this approach, for the accumulation of guest molecules in nanoconstructions the concentration of guests is determined by the geometric parameters of the free space and the number of guest molecules in nanobridges and is not, apparently, directly associated with the characteristic features of the secondary structure of dsDNA molecules. Evidently, this approach to the accumulation of guest molecules will work if adjacent dsDNA molecules are spatially ordered and linked by bridges. Hence, a fundamentally different approach to the accumulation of molecules of various chemical compounds in 3D nanoconstructions is possible.

However, the following questions should be answered:

— how can adjacent dsDNA molecules be ordered;

— how can adjacent DNA molecules be linked by nanobridges containing guest molecules;

— what properties will the resulting nanoconstructions show.

The technique for the formation of nanoconstructions developed at the Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences [23, 24] is based on the idea that NA molecules (or their complexes) can be ordered to give various three-dimensional structures. The strategy for the nanodesign is based on the use of structures that are self-generated upon phase exclusion of these molecules from water-salt solutions rather than individual NA molecules.[25]

It is known that when concentrating water-salt solutions containing rigid helical dsNA molecules with a low molecular mass ($\sim 1 \times 10^6$ Da), the spontaneous ordering of these molecules proceeds to give different types of liquid-crystalline phases (from cholesteric to orthorhombic).[26, 27] It should be noted that the formation of cholesteric structures is a specific feature of dsNA molecules (DNA or RNA) [28] exhibiting the geometric and optical anisotropy.

The procedures for the formation and the physicochemical properties of liquid-crystalline dispersions (LCD) of dsNA by the condensation of these molecules in water-salt solutions were documented.[25]

a. Methods for the preparation of liquid-crystalline dispersions of nucleic acids

There are two fundamentally different procedures for the preparation of such dispersions (Fig. 3), which determine the formation of different types of nanoconstructions.[29]

The first procedure for the condensation is based on the so-called 'entropy' condensation, *i.e.*, the process in which the change in the entropy of the system is the driving force.[30] This condensation is achieved in the case of the phase exclusion of NA molecules from water-salt solutions upon the addition of certain polymers, in particular, of poly(ethylene glycol) (PEG). This method is known also as the ψ(psi) condensation (psi, in an acronym for polymer-salt-induced). Under certain critical conditions, rigid low-molecular-mass dsNA molecules are ordered and form LCD. (The violation of the critical conditions, in particular, a decrease in the PEG concentration below the critical value, leads to decomposition of LCD particles and the transition of DNA molecules into the isotropic state. It should be emphasised that, due to the 'liquid' character of the packing of NA molecules in LCD particles, these molecules cannot be, for example, immobilised on the surface of membrane filters.) The theoretically estimated particle size of LCD is close to 500 nm; the particles contain approximately 10^4 NA molecules each. As demonstrated earlier,[25] LCD particles generated by the phase exclusion of linear low-molecular-mass dsNA molecules, have several characteristic features, which can be used for the formation of nanoconstructions.

First, polymers are not involved in dispersion particles (see Fig. 3); LCD particles of dsNA are characterised by the retention of reactivity of the structural elements (nitrogen bases, *etc.*) of NA, as well as by a high (ranging from 160 to 400 mg ml^{-1}) local NA concentration and the ordered arrangement of adjacent NA molecules in LCD particles.

Second, the distance (d) between adjacent NA molecules in LCD particles can be controlled within 2.5 – 5.0 nm by changing the osmotic pressure of the solution. The free space between adjacent DNA molecules is accessible for molecules of many compounds. A considerable distance between DNA molecules

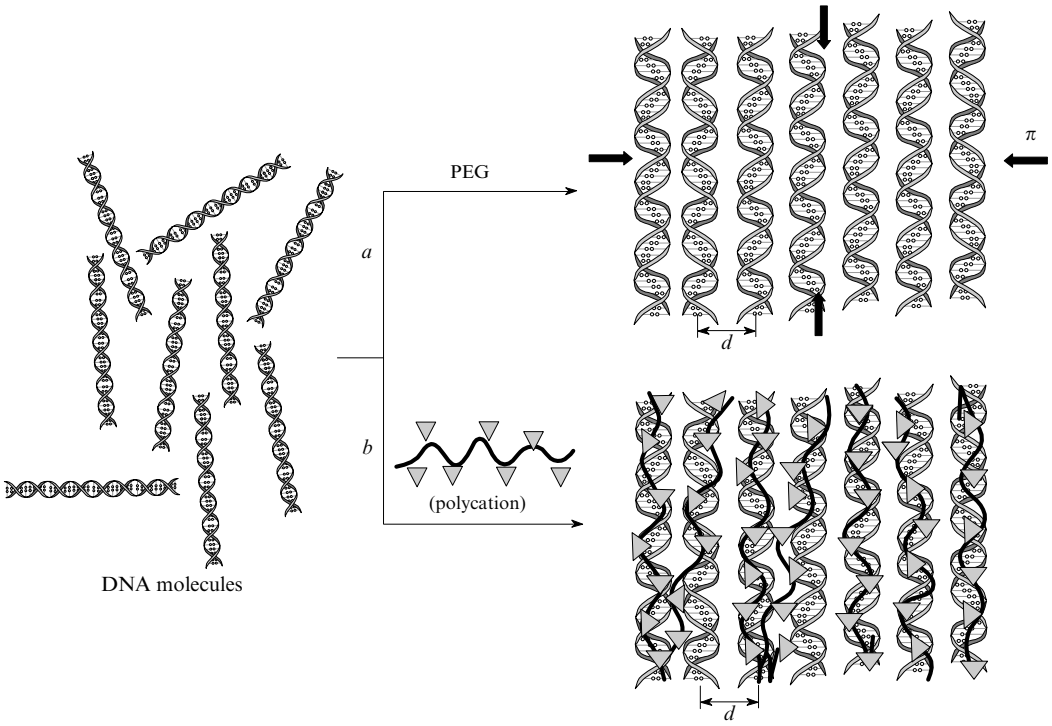


Figure 3. A scheme illustrating two possible ways of the spontaneous ordering of dsDNA molecules in particles of a liquid-crystalline dispersion (layers perpendicular to the axis of the particle are shown).
(*a*) The 'entropy' condensation induced by the addition of poly(ethylene glycol); the osmotic pressure of the solution (π), which is necessary for the existence of dispersion particles, is indicated by arrows; (*b*) the 'enthalpy' condensation induced by interactions between DNA molecules and polycations.

and the 'liquid' character of the packing of these molecules provide conditions for fast diffusion of guest molecules between both DNA molecules in a single layer and DNA molecules in adjacent layers in the structure of LCD particles. In addition, since it is impossible to 'take in hand' LCD particles, all estimates of their parameters are based on the results of indirect experimental methods and different theoretical approximations.

Third, as mentioned above, NA molecules form cholesteric LCD (CLCD) due to their geometric and optical anisotropy. These cholesteric phases are 'coloured' because they absorb UV light (due to the presence of nitrogen bases serving as chromophores). The theory developed specially for dispersion particles of coloured cholesteric phases (which has received considerable experimental support)[31] predicts that the UV region of the circular dichroism (CD) spectrum should show an abnormal band. The latter can have different signs depending on the parameters of the secondary structure of NA molecules. The theory does not impose restrictions on the number of molecules of coloured compounds (chromophores), which can be introduced into the NA structure. Hence, the CD spectra would be expected to show abnormal bands in the absorption regions of any coloured compounds, which are not only intercalated between base pairs of NA molecules but are also anisotropically located in the space between adjacent NA molecules that form CLCD particles. Consequently, abnormal bands in CD spectra serve as an analytical criterion allowing the monitoring of minor violations of the structures of the starting NA molecules and the resulting LCD particles.

Finally, reactive groups (sites) are present on the surface of adjacent NA molecules in CLCD particles or, alternatively, these groups can be chemically inserted into NA molecules, the ability to form CLCD particles being retained. In particular, nitrogen bases forming complexes with metal ions can serve as such sites; ligands additionally added to the system or active groups of polycations bound to NA molecules can also serve as such sites.

Thus, the retention of reactivity of the structural elements of NA (nitrogen bases, *etc*.), high NA concentration and the ordered arrangement of nucleic acid molecules in dispersion particles, which does not restrict the diffusion of guest molecules, can provide a high rate of penetration of these compounds into particles and a high rate of their reactions with NA molecules. The aforesaid shows that there is, in principle, a theoretical possibility of cross-linking of adjacent NA molecules in CLCD particles. In addition, the abnormal optical activity of the starting NA molecules and liquid-crystalline dispersions will allow the monitoring of minor changes not only in the secondary structure of NA molecules, but also in the three-dimensional structure of CLCD particles. Since the distance between NA molecules in CLCD particles is 2.5 – 5.0 nm, the cross-link can be called the nanobridge, and the resulting construction containing a large number of such bridges can be called the nanoconstruction based on dsNA molecules. This approach to the design of three-dimensional structures of NA can be referred to as the nanodesign, the principles of this approach being fundamentally different from the above-considered step-by-step strategy.

Another approach to the formation of CLCD particles from rigid linear low-molecular-mass NA molecules is based on their phase exclusion from water-salt solutions as a result of correlation interactions (dispersion force attractions)[32, 33] between segments of NA, in which the negative charges of the phosphate groups are neutralised by counterions (see Fig. 3). Since the change in the enthalpy of the system is the driving force for the process, this procedure for the phase exclusion is called the 'enthalpy' condensation. This approach requires the presence of polycations that neutralise most (80% – 90%) of the negative charges of the phosphate groups of NA. As the surface charge density of dsNA molecules decreases, the dispersion forces first equilibrate, and then become stronger than the electrostatic repulsions between adjacent molecules. This leads to the spontaneous exclusion of the resulting molecules of the NA – polycation complex from the solution. Evidently, the efficiency of interactions between the polycation and the NA molecule depends on both the positive charge of the polycation and the character of the charge distribution along its molecule.

The most important features of the dispersions of NA – polycation complexes are illustrated by Fig. 3. First, the phase exclusion of the complexes is achived when the critical concentration of polycations in solution comparable with the concentration of the starting NA molecules is attained. In this case, the molecular mass of the polycations is of considerable importance. The higher this mass the more efficient the binding of the polycations to the NA molecules and the lower the critical concentration of the polycations necessary for the condensation of NA. Second, polycations are always involved in the resulting dispersion particles.[34 – 36] Third, polycation molecules adopting a rigid conformation provide one fixed distance (d) between adjacent NA – polycation complexes in the resulting particles; as a rule, this distance is approximately equal to 2.5 nm.[37] Fourth, in some 'lucky' cases, depending on the structure of polycations in NA – polycation complexes, it is possible to form LCD the particles of which are characterised not only by a larger (but still fixed!) distance between the NA – polycation complexes, but also by an intense band in the CD spectrum,[37] *i.e*., it is possible to form CLCD. Finally, the formation of LCD particles of NA – polycation complexes can lead to a substantial decrease in the reactivity (due to the steric factors associated with the arrangement of the polycations on the surface of NA molecules), but other reaction sites in polycation molecules appear. These sites are also diffusion-accessible. A high local concentration of polycations in NA CLCD particles, the presence of chemically active groups in the polycations and the ordered arrangement of the polycations can provide conditions for the cross-linking of adjacent NA – polycation complexes in CLCD particles by bridges and the formation of nanoconstructions.

A comparison of two modes of the spontaneous arrangement of NA molecules or their complexes leads to the conclusion that there is at least the theoretical possibility of the use of NA molecules fixed in the three-dimensional structure of LCD particles as building blocks with controlled properties, *i.e*., nanoconstructions with desired properties can be designed.

Consequently, in both above-considered cases, the fundamental problem is to fix guest molecules and construct bridges between adjacent NA molecules (or polycation – NA complexes) to impart stability to the construction as a whole.

b. Nanodesign based on cholesteric liquid-crystalline dispersion of DNA

To form nanoconstructions according to the former technique of the phase exclusion of NA molecules (see Fig. 3), adjacent DNA molecules in the structure of CLCD particles should be linked so as to retain the character of the spatial ordering of dsDNA molecules. Obviously, this requires that sites that can serve as the beginning and end of such bridges were present on the surface of NA molecules. Metal ions bound to base pairs or molecules of an additional ligand can, in principle, serve as

such sites. Evidently, two adjacent DNA molecules can be linked only if they are arranged in a particular way with respect to each other (*i.e.*, the spatial orientation of the molecules should be consistent). Hence, the formation of nanobridges is a finely balanced process, which can proceed only if certain conditions are met.

Nanobridges should link DNA molecules located both in a single layer and in the adjacent layers of the cholesteric structure of CLCD molecules resulting in the loss of the diffusion degrees of freedom of DNA molecules. This can lead to dramatic changes in the properties of CLCD particles (Fig. 4). In particular, the 'liquid' character of the packing of adjacent DNA molecules and the diffusion mobility should disappear, and the structure can acquire certain features of a solid material (above all, the stability). Finally, if the cross-linking of adjacent DNA molecules could be performed without distortion of the three-dimensional structure of CLCD particles, the abnormal optical activity will allow the control of changes in the secondary structure of the starting DNA molecules and the appearance of bridge-forming components.

A detailed analysis of the properties of compounds that can be used as constituents of nanobridges led to anthracycline antibiotics as compounds of choice.[11] These compounds can form planar, rather rigid chelate complexes with metal ions, those with Cu^{II} ions being particularly stable. Under certain conditions, these complexes can contain up to 10 repeating subunits, *i.e.*, anthracyclines can form extended planar structures with Cu^{II} ions (polymeric chelate complexes[38]). Molecules of this class of antibiotics, in particular, daunomycin (DAU), can be intercalated between nucleic acid base pairs and synthetic polynucleotides only if they exist in the B-form. Upon the intercalation, the reactive groups of the DAU molecule (C=O and OH groups) are inaccessible for chemical reactions. However, DAU and its analogues are known to form the so-called external complexes with nucleic acids in the B- or A-forms. In these complexes, the reactive groups of DAU remain accessible for chemical reactions, in particular, for the chelation. The presence of 'active' oxygen atoms at positions 5, 6, 11 and 12 of the aglycon of the anthracycline was demonstrated[39,40] to be a prerequisite for the formation of extended chelate complexes.

The technique for the preparation of dsDNA-based nanoconstructions was documented in detail.[39,41] Cholesteric LCD was formed from dsDNA molecules followed by the successive

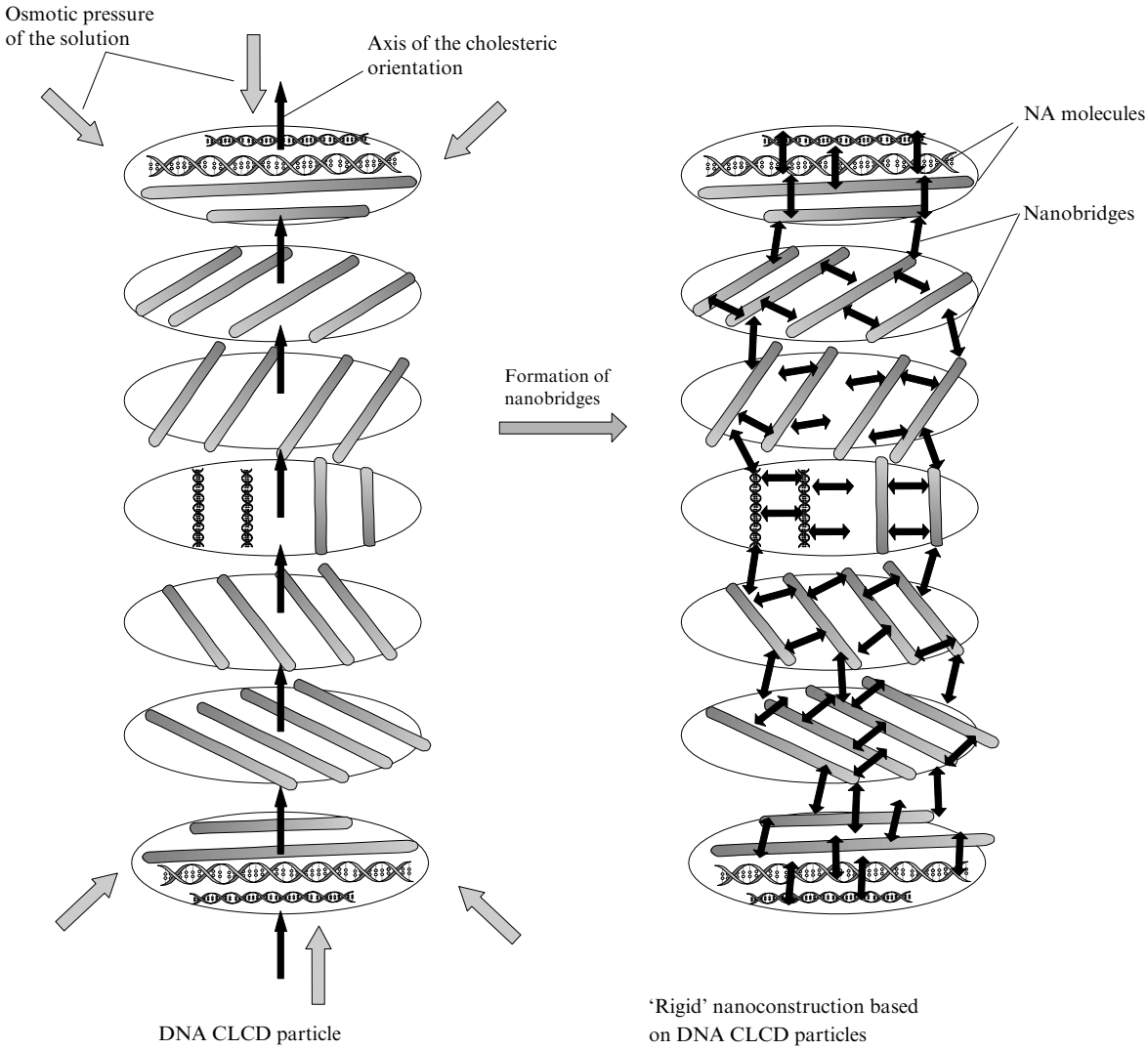


Figure 4. A scheme illustrating the all-at-once strategy: CLCD particles in which dsNA molecules are ordered exist only under a particular osmotic pressure of the solution; it is necessary to cross-link NA molecules by bridges without distortion of the three-dimensional structure of CLCD particles.

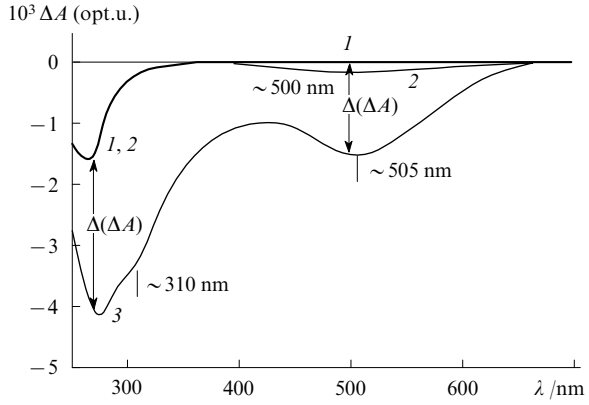


Figure 5. Circular dichroism spectra of the starting DNA CLCD (*1*) and the same dispersion after the treatment with daunomycin (*2*) and $CuCl_2$ (*3*).
The DNA concentration was ~5.5 μg ml^{-1}; phosphate buffer, pH 6.7. Here and in Figs 7 and 8, $\Delta A = A_L - A_R$, where A_L and A_R are the absorbance for the left and right circularly polarised light, respectively. $\Delta(\Delta A)$ is an increase in the intensity of the bands associated with the formation of a nanoconstruction.

addition of daunomycin and a $CuCl_2$ solution. In the circular dichroism (CD) spectrum measured after this treatment, the amplitude of the band at $\lambda \approx 500$ nm corresponding to the chromophore of the [DAU – Cu^{II}] complex sharply increases (Fig. 5). Under these conditions (the molecular mass of DNA is $\sim 8 \times 10^5$ Da, the DNA concentration is ~5 μg ml^{-1}), the maximum amplitude of this band can be as high as ~2500 units of ΔA (1 unit of ΔA is 10^{-6} optical units). Such a considerable increase in the amplitude of the band at $\lambda \approx 500$ nm indicates that the chromophore of the [DAU – Cu^{II}] complex is anisotropically fixed with respect to the axis of the DNA molecules in CLCD particles.[31]

Although there are two different models of the fixation of the [DAU – Cu^{II}] complexes near the surface of DNA molecules, experimental data [39, 41] provide unambiguous proof for the model of a chelate complex serving as a bridge between adjacent DNA molecules. In particular, this model was supported by the fact that the violation of the conditions for the formation of CLCD particles (for example, the dilution of a water-polymer solution to a PEG concentration below the critical value) is accompanied by dissociation of these particles, the transition of DNA molecules into the isotropic state and complete disappearance of the abnormal CD band in the absorption region of the DNA chromophores. However, this was not observed upon dilution of a water-polymer solution of CLCD particles pretreated with daunomycin and a copper chloride solution. The abnormal CD bands corresponding to chromophores of both DNA and DAU persist in all cases; their amplitude only decreases proportionally to the degree of dilution. Other evidences confirming the formation of bridges between the adjacent DNA molecules fixed in the structure of CLCD molecules were also documented.[42]

The efficiency of the formation of nanobridges depends on the concentrations of both DAU molecules and copper ions.[41] Both plots are S-shaped with high consistency. A close correspondence between the plots reflecting the increase in the intensity of the bands located in different regions of the CD spectrum suggests that the formation of bridges initiates a 'phase transition' between different cholesteric structures; in particular, the cholesteric structure that is formed by cross-linking of DNA molecules by bridges is apparently more twisted than the starting structure.

The experimental data obtained by different physical methods, including low-temperature magnetometry,[43] as well as the results of recent calculations,[44] showed that six divalent copper ions are involved in the [$\cdots - Cu^{II} - DAU - Cu^{II} - \cdots$] bridge (Fig. 6). These nanobridges are planar chelate complexes providing the cross-linking of spatially ordered DNA molecules located both in a single layer and in the adjacent layers.

Thus, the formation of bridges between adjacent DNA molecules leads to changes in the properties of the starting cholesteric phase [43] and the formation of a nanoconstruction (see Fig. 4).[45]

Let us mention selected properties of the resulting construction (nanomaterial). First, unlike the starting 'liquid' DNA CLCD particles, the stability of the nanoconstruction is independent of the osmotic pressure of the solution; instead, it is determined by the number of nanobridges. Second, this DNA construction is characterised by enhancement of the abnormal optical activity manifested in an increase in the amplitude of CD bands in the absorption regions of DNA and the antibiotic. Third, the nanoconstruction is characterised by two thermal structural transitions. One of these transitions can be called the 'CD melting' of nanobridges: the bridges between DNA molecules break as the temperature is raised, which is accompanied by the disappearance of the abnormal CD band of DAU characteristic of the nanoconstruction. Another transition can be called the 'CD melting' of the DNA cholesteric phase; the destruction of the cholesteric structure is also manifested in CD spectra. The 'melting point' of nanobridges increases as their concentration increases (Fig. 7); nevertheless, it is lower than the 'melting point' of the starting dsDNA cholesteric phase.[46] Finally, the nanoconstruction is characterised by a high local concentration of not only DNA molecules, but of the antitumour antibiotic daunomycin as well.

The stability of the nanoconstruction, which, in the absence of the osmotic pressure of the solution, is determined only by the number of nanobridges, allowed immobilisation of the particles on the surface of a membrane filter, which, in turn, opened a possibility to visualise particles with an atomic force microscope**.** (Let us emphasise once again that the starting DNA CLCD particles do not exist in the absence of the osmotic pressure of the solution.) Particles of the nanoconstruction resemble elongated cylindres in shape. The estimation of the size of 400 particles showed that the particle size ranges from 0.4 to 0.8 μm, and the average size is ~0.5 μm. Hence, the size of the resulting rigid particles of the nanoconstruction is equal to the size of the starting 'liquid' CLCD particles of dsDNA molecules obseved in solutions with the fixed osmotic pressure (this size has been estimated earlier by theoretical calculations).

Thus, not only a rigid nanoconstruction based on DNA CLCD particles was formed, but also the data characterising the macroscopic parameters of this material were obtained. Consequently, the hypothetical three-dimensional structure suggested for a rigid nanoconstruction (see Fig. 4) exists in reality.

Presumably, there are also other conditions under which dsDNA molecules that initially form a 'liquid' cholesteric phase can loose solubility and, consequently, pass into the 'rigid' cholesteric phase. The properties of the new type of materials can be controlled. In this case, the abnormal optical activity serves as a criterion reflecting the state of the three-

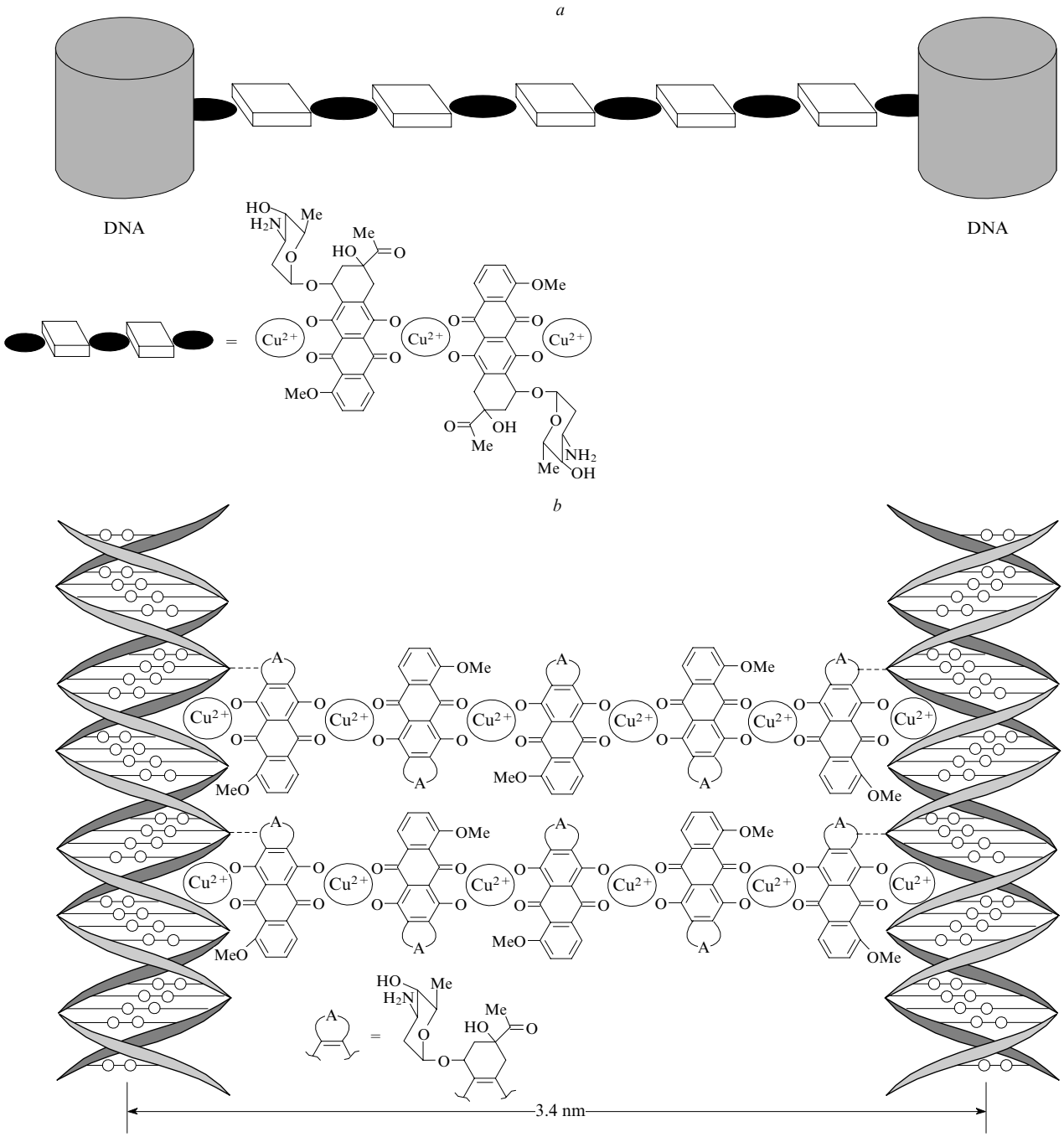


Figure 6. Schematic representation of nanobridges between two adjacent DNA molecules.
(*a*) The top-view projection of the nanobridge along the axis of the DNA molecule; (*b*) the more detailed structure of the nanobridges between DNA molecules (for clarity, the bridges are rotated by 90° with respect to their real position). It should be noted that one DNA molecule is rotated by 180° about the vertical axis compared to another DNA molecule.

dimensional structure of the nanoconstruction. This opens a possibility of the practical use of nanoconstructions of DNA as biosensors, *i.e.*, sensitive elements of sensor devices for the detection of chemical, including biologically active, compounds that violate the structural integrity of elements of nanobridges [47, 48] and, hence, the abnormal optical activity.

Thus, DNA CLCD particles can be used for the design of materials exhibiting unique properties. As regarding the scientific significance of nanoconstructions, the previously unknown structures were prepared based on DNA CLCD particles, which is of great theoretical interest.

It should be noted that these materials are called differently in different publications (nanoconstructions, nanomaterials, liquid-crystalline elastomers, rigid cholesteric materials, *etc.*). The correct name of these materials remains an open question.

c. Nanodesign based on a cholesteric liquid-crystalline dispersion of DNA – polycation complexes

The second approach to the phase exclusion of DNA (see Fig. 3) for the design of nanoconstructions is based on the formation of bridges between adjacent polycation molecules rigidly fixed on the surface of DNA molecules so as to retain

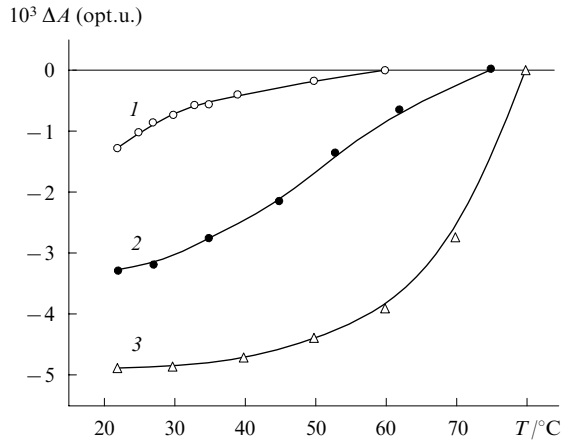


Figure 7. Temperature dependence of the amplitude of the band (λ = 310 nm) in the CD spectra of liquid-crystalline dispersions of DNA containing nanobridges at different concentrations of daunomycin (c_{DAU}) and $CuCl_2$ (c_{Cu}) (in mol litre^{-1}).
The poly(ethylene glycol) concentration was ~170 mg ml^{-1}; 0.3 M NaCl; 2×10^{-3} M phosphate buffer, pH 6.7.
(*1*) $c_{DAU} = 1.5\times10^{-5}$, $c_{Cu} = 5\times10^{-6}$; (*2*) $c_{DAU} = 2.73\times10^{-5}$, $c_{Cu} = 8.99\times10^{-6}$; (*3*) $c_{DAU} = 6.4\times10^{-5}$, $c_{Cu} = 2.0\times10^{-5}$.

the character of spatial ordering of DNA – polycation complexes in CLCD particles. A high local concentration of polycation molecules in NA CLCD particles, the presence of reactive groups in the polycation and the ordered arrangement of polycation molecules can provide conditions for the cross-linking of adjacent DNA – polycation complexes in LCD particles. The solution of this problem is associated primarily with a search for polycations that would ensure not only the neutralisation of the negative charges of the phosphate groups of DNA, but also the appearance of new reactive groups in the system.

From this point of view, the biodegradable biocompatible polyaminosaccharide chitosan [poly(1→4)-2-amino-2-deoxy-β-D-glucopyranose] prepared by deacetylation of natural chitin has attracted attention.[49,50] Due to the characteristic features of the three-dimensional structure of chitosan (Chi) molecules, the positive amino groups of Chi can interact with the negatively charged phosphate groups of DNA in an alternating manner. Chitosan not only neutralises the negative charges of the phosphate groups of DNA but also causes the specific distribution of the positively charged amino groups at the surface of DNA.[51]

An analysis of the absorption spectra observed upon titration of a water-salt solution of DNA with a chitosan solution showed that when the critical concentration of the latter is achieved, the spectrum shows absorption in the region ($\lambda > 320$ nm) where neither DNA nor chitosan have absorption (the so-called apparent absorbance) and the absorption at $\lambda \approx 260$ nm increases. These changes in the absorption spectra are associated with the formation of a disperse phase of DNA – Chi complexes, the particles of which bring about UV scattering.[51]

A comparison of the CD spectra of the starting B-form of DNA and the spectra of dispersions of DNA – Chi complexes showed that when the critical concentration of chitosan is attained, the abnormal positive CD band appears in the absorption region of DNA ($\lambda \approx 270$ nm). According to the theory,[31] this band reflects the cholesteric packing of DNA – Chi complexes in dispersion particles. However, unlike classical DNA cholesteric phases, which generally have abnormal negative CD bands and, consequently, the left-handed twist of the three-dimensional cholesteric structure, DNA – Chi cholesteric phases are characterised by the positive CD band and, consequently, the right-handed twist of the three-dimensional structure.

The X-ray scattering patterns from a phase formed by CLCD particles of the DNA – Chi complex exhibiting the abnormal positive optical activity have one Bragg reflection $d_{Bragg} \approx 2.6$ nm).[52] The absence of high-order small-angle reflections in the X-ray diffraction patterns is indicative of the absence of the regular three-dimensional order in the arrangement of the molecules of the DNA – Chi complex. The Bragg reflection characterising the distance between the adjacent DNA – Chi complexes in the resulting phase is substantially weaker than d_{Bragg} characteristic of DNA CLCD particles and corresponds to the transition region between the cholesteric and hexagonal liquid-crystalline phases. The fact that the Bragg reflection changes only slightly with increasing temperature shows that the packing of DNA – Chi complexes is not as 'liquid' as that in the cholesteric phase of the starting DNA.

Since the adjacent free amino and hydroxy groups in chitosan molecules form planar chelate complexes with copper ions with high efficiency,[53–56] the adjacent DNA – Chi complexes fixed in the structure of CLCD particles were cross-linked according to the technique used in the case of DNA CLCD particles. The interaction of dsDNA molecules with chitosan afforded CLCD, then a solution of DAU (or $CuCl_2$) was added and the mixture was treated with a $CuCl_2$ (or DAU) solution.

It was found that after the addition of a $CuCl_2$ solution to CLCD of the DNA – Chi complex having the positive sign of the CD band followed by the treatment with DAU, the spectrum shows an intense positive band at $\lambda \approx 500$ nm corresponding to the optical properties of the chromophore of the [DAU – Cu^{II}] complex.

An analogous increase in the amplitude of the band is observed also in the case of CLCD formed by chitosan molecules characterised by a different amino group content, CLCD of the DNA – Chi complex being characterised by the negative CD band. An increase in the amplitude of the band in the absorption region of the [DAU – Cu^{II}] chromophore indicates that this chromophore becomes spatially fixed with respect to chitosan molecules in any CLCD formed by different DNA – Chi complexes.

An important difference between CLCD of the DNA – Chi complexes and CLCD of DNA is that the order of the addition of DAU and $CuCl_2$ solutions has no effect on the increase in the abnormal optical activity. The efficiency of the increase in the intensity of the abnormal CD band corresponding to the liquid-crystalline dispersion of DNA – Chi complexes depends, as in the case of a DNA dispersion, on the concentration of DAU molecules and copper ions. However, the absence of the critical concentration for copper ions is evidence that, unlike DNA, the chitosan molecules in the DNA – Chi complex always contain groups sterically accessible for the chelation with copper ions. Amino groups and hydroxy groups of the sugar residues of chitosan serve as such groups. Chelate complexes of these groups with copper ions are characterised by the high binding constant [$\sim(1-4)\times10^{14}$].[57,58]

It should be noted that the data for the DNA – Chi complexes are in qualitative agreement with the results reflecting the formation of bridges in the case of DNA. Hence, it can be stated that an increase in the amplitude of the band at $\lambda \approx 500$ nm in the CD spectra of CLCD of various DNA – Chi

complexes is attributed to the formation of [$\cdots-Cu^{II}-DAU-Cu^{II}-\cdots$]-type nanobridges between the adjacent chitosan molecules involved in complexes with DNA molecules of CLCD particles.

As in the case of DNA, the bridges can be formed between adjacent chitosan molecules located both within a single layer and in the adjacent layers, *i.e.*, the increase in the intensity of the band at $\lambda \approx 500$ nm is indicative of the formation of the three-dimensional structure. This is evidence that the formation of [$\cdots-Cu^{II}-DAU-Cu^{II}-\cdots$] bridges (and, consequently, NA-based nanoconstructions) has the same optical manifestation in all cases. However, the maximum amplitudes of the bands observed upon the formation of nanoconstructions based on DNA – Chi complexes are approximately three times lower than the amplitude of the band characteristic of nanoconstructions formed by dsDNA CLCD. This difference can be attributed to several factors. First, this can be associated with the smaller number of nanobridges. Second, the nanobridges can be shorter because the distance between the DNA – Chi complexes is shorter than the distance between the DNA molecules. Finally, this may be due to the fact that the tilting angle (with respect to the macromolecule) of the bridges between the chitosan molecules in DNA – Chi complexes in CLCD differs from the angle in the case of bridges between DNA molecules, which can be associated with the conformation of chitosan molecules bound to adjacent DNA molecules. Further investigations should be performed to draw the unambiguous conclusion about the factor responsible for the differences.

The facts that the size of nanobridges in CLCD of the DNA – Chi complexes can be small and that the copper ions can form very stable chelate complexes with adjacent amino and hydroxy groups of the sugar residues of chitosan [59, 60] suggest that the properties of these nanoconstructions can differ substantially from the properties of DNA-based nanoconstructions. In particular, as can be seen from Fig. 8, the amplitudes of the abnormal bands ($\lambda = 505$ nm) in the CD spectra of dispersions of nanoconstructions based on different DNA – Chi complexes are virtually unchanged with temperature. This indicates that the three-dimensional structure of CLCD of DNA – Chi complexes remains unchanged in this temperature range. On the whole, the thermal stability of nanoconstructions based on DNA – Chi complexes is substantially higher than the thermal stability not only of dispersions of the starting DNA – Chi complexes (curves *1* and *2*), but also of DNA-based nanoconstructions (see Fig. 7).

The conformation of chitosan molecules is of great importance for the nanodesign, as evidenced by the dependence of the amplitude of the band at $\lambda = 505$ nm in the CD spectrum of CLCD of DNA – Chi complexes on the amino group content in the chitosan molecules.[29] This dependence shows that there is the minimum (possible shortest) distance between amino groups (~ 13 Å, which is approximately equal to the distance between amino acids spaced one residue apart), at which adjacent nanobridges from the [DAU – Cu^{II}] complexes can be located. The small abnormal optical activity, in spite of a high concentration of active groups in the sugar residues of chitosan, *i.e.*, in spite of a high concentration of potential bridge-forming sites, is evidence that the spatial orientation of the bridges is not optimal. Only as the distance between the amino groups of chitosan increases, the three-dimensional structure of this molecule ensures the orientation of active groups favourable for the formation of optimal nanobridges in the structure.

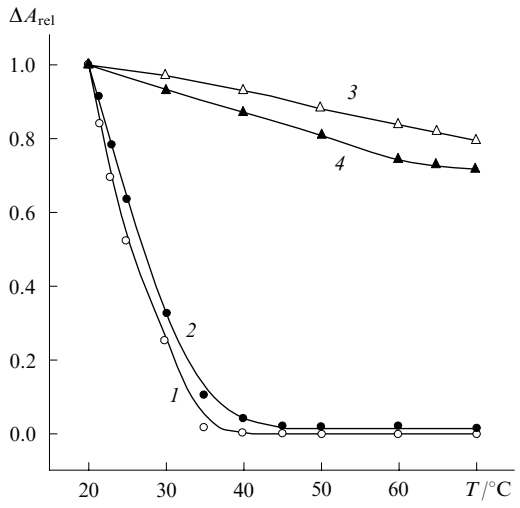


Figure 8. Temperature dependence of the amplitude of the band ($\lambda = 270$ nm) in the CD spectra of liquid-crystalline dispersions of the DNA – Chi complexes with different chitosans (*1*, *2*) and bands ($\lambda = 505$ nm) in the CD spectra of nanoconstructions based on these dispersions (*3*, *4*). $\Delta A_{rel} = \Delta A/\Delta A_0$, where ΔA_0 refers to the initial temperature.
The content of free amino groups in chitosan was 65% (*1*, *3*) and 55% (*2*, *4*). The DNA concentration was ~ 15.5 μg ml^{-1}, the chitosan concentration was 28.7 μg ml^{-1}; the molecular mass of chitosan was 5 kDa; $c_{DAU} = 5 \times 10^{-5}$ mol litre^{-1}, $c_{Cu} = 1.5 \times 10^{-5}$ mol litre^{-1}; 0.05 M NaCl, 0.02 M phosphate buffer, pH 6.7.

Thus, a comparison of the above-considered data with the results of the nanodesign based on DNA shows that bridges can be formed between adjacent chitosan molecules in the DNA – Chi complexes fixed in the three-dimensional structure of CLCD particles.

Evidently, the creation of a particular three-dimensional model of nanobridges between adjacent molecules of DNA – Chi complexes and, consequently, the design of the three-dimensional model of nanoconstructions as a whole require additional theoretical and experimental investigations.

The existence of rigid nanoconstructions based on CLCD particles of DNA molecules or DNA complexes with polycations, which retain the abnormal optical properties, opens a way for the design of other analogous materials. In particular, other polycations fixed on the surface of dsDNA molecules can be used. It is not inconceivable that analogous 'solid' cholesteric phases can be represented by DNA-containing bacteriophages or chromosomes of animals and plants.

III. Conclusion

The data summarised in the present review show that dsNA molecules are important polyfunctional objects of nanotechnology. The directed and controlled changes in the properties of these molecules provide considerable scope for the formation of nanostructures that can find practical applications in a number of fields of science and engineering.

Nanoconstructions in which the DNA concentration is higher than 200 mg ml^{-1} can be used in medicine and bioengineering as carriers of genetic information or various biologically active compounds, including drugs.

Nanoconstructions based on dsDNA (or PNA) CLCD particles can serve as sensitive elements of optical biosensors for the detection of biologically active compounds, in partic-

ular, of genotoxicants, in physiological liquids. These sensors have found use in medicine, bioengineering and environmental studies.

Nanoconstructions with controlled physicochemical properties and incorporated in polymeric films (hydrogels) can find application in modern optical and electronic devices (in particular, as optical filters).

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