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Magnetic nanoparticles – DNA interactions: design and applications of nanobiohybrid systems

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Mechanisms of interaction between nucleic acid molecules and magnetic nanoparticles and methods of their conjugation in order to develop functional nanostructures are considered. The properties of nucleic acids and magnetic nanoparticles that are key to the design of nanobiocomposites are described. Prospects for applications of nanobiocomposites in nanoelectronics and medicine are analyzed. Possible harmful effects of magnetic nanoparticles on the genetic system are briefly outlined.
The bibliography includes 287 references.

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I. Introduction

Currently there is enormous interest in the design of nanobiocomposites comprising magnetic nanoparticles (MNPs) and nucleic acid molecules. Application of such systems is of great importance for progress in nanoelectronics (design of nanoscale electronic circuits, high-density data storage systems), biomedical diagnosis and therapy (design of highly sensitive biosensors and innovative drugs).[1]

The idea of development of memory devices based on templated MNPs dates back to the 1950s. There are two nanofabrication strategies called the 'bottom-up' and 'top-down' approaches.[2] Since the latter approach in the form of, *e.g.*, nanolithography is expensive and, moreover, miniaturization has its natural limits, the bottom-up approach is now on demand. It is based on utilization of small molecular building blocks to fabricate supramolecular complexes by self-assembly.[3]

Application of DNA molecules for the design of nanodevices is very attractive. In addition to being information carriers, DNA molecules are characterized by the mechanism of specific molecular recognition, and possess physicochemical stability and mechanical rigidity.[1] The possibility of high-precision manipulation of DNA-based building blocks is due to the ability of nucleic acids to hybridize by base pairing and to the existence of enzymes (restriction endonucleases, ligases and polymerases) that respectively allow one to cut, link and build the DNA-based structures.[4]

Rapid advancement of ideas on utilization of MNPs in biomedical research began in the 1960s.[5] Unique properties of magnetic materials in the nano state provides the possibility of detecting structures based on MNPs and to control them by external magnetic field.[6]

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The possibility of manipulation and targeting of MNPs by external magnetic field underlies magnetic separation of biological substances and drug delivery.[7] Magnetic resonance imaging (MRI), a widely used method of *in vivo* and *in vitro* diagnosis, as well as magnetosensitive biosensors based on nanoparticles owe their origin to the magnetic properties of nanoparticles.[8,9] Heating of MNPs by alternating magnetic field is basic to a method of treatment of pathological cells and (mainly) malignant tissues (hyperthermia).[10]

Conjugation of MNPs with biological molecules, in particular, nucleic acids, allows designing various nanobiohybrid systems that possess unique magnetic properties and biological selectivity to improve the efficiency of diagnosis and therapy of diseases.[5,8,9,11]

Various approaches to conjugation of nucleic acids with MNPs were proposed.[12] A nucleic acid molecule can directly bind to MNPs; otherwise, the formation of chemical bonds requires preliminary modification of the surface of MNPs and/or biomolecules.[13] Nevertheless, prevention of nonspecific interactions between nucleic acid molecules and nanoparticles, as well as determination of component ratios in complexes still remain topical for nanobiofabrication.[14] Not only do regularities of interaction between nucleic acids and MNPs underlie theoretical concepts and methodology of the design of nanobiocomposites, and also specify possible fields of application. Knowledge of relevant interaction mechanisms offers prospects for nanomaterial surface design in order to attain or suppress highly specific binding and to control bonds between components in the nucleic acid–MNP composites.

Assessment of biological and environmental risks is an important step in predicting the efficiency of utilization of nanotechnology products. Harmful effects of asbestos and uranium oxide nanoparticles, fullerenes,[15] carbon nanotubes[16,17] and products formed in internal combustion engines[18] on living organisms are well documented.

Potential risks of nanoparticles to human health are due to the following factors:[19]

— small particle size; nanoparticles can penetrate biological barriers including the blood–brain barrier;

— large specific surface area and high reactivity of nanoparticles; and

— poor knowledge of excretion and/or biodegradation mechanisms.

High risks of MNPs are associated with the activation of free radical generation due to release of metal ions from the surface and with the damage of biomolecules, cells and tissues by the oxidative stress mechanism.[20–22] Thereby, research on mechanisms of the DNA–MNP interaction is of paramount importance for assessment of the influence of MNPs on genetic material in biological systems, which is directly related to human safety.

In spite of the aforesaid, mechanisms of the interaction of MNPs with nucleic acid molecules are considered fragmentarily (see, *e.g.*, Refs 1, 8, 13, 23, 24).

In this review, we will briefly outline the properties of MNPs and nucleic acid molecules and discuss modern concepts concerning mechanisms of the interaction between them. Also, we will present a systematic description of methods for conjugation of MNPs with nucleic acid molecules (most of them were developed in the last two decades) and outline methods of investigation of the structure of nanobiocomposites. Fields of application and the functioning principles of nucleic acid-MNP nanobiohybrid systems will be considered, and genotoxic effects of MNPs will be discussed.

II. Properties of magnetic nanoparticles

Interest in magnetic nanomaterials is due to fundamental changes in magnetic properties of ferro- and ferrimagnets on going to the nano scale.[25,26] The nature of the phenomenon was physically substantiated by Stoner and Wohlfarth[27] in 1948 and thoroughly considered in a number of recent reviews.[9,11,28,29]

Understanding laws that govern the behaviour of MNPs is of paramount importance for optimization of magnetic properties and for the design of nanostructures based on MNPs. The magnetic properties of a bulk material mainly depend on its chemical composition, crystal structure, magnetic anisotropy and on the presence of vacancy defects. In contrast, the properties of nanomaterials depend primarily on their size and shape.[11] The size dependence of the properties of MNPs can be used for modulation of their magnetic parameters.[30,31]

1. Size-dependent effects in magnetic nanoparticles

Superparamagnetic behaviour of MNPs is one of the most interesting size-dependent effects (Fig. 1). The energy barrier for the magnetization vector is proportional to the product of the magnetic anisotropy by the volume of the magnet. For a bulk material, the energy required to change the direction of magnetization much exceeds the energy of thermal fluctuation, whereas for MNPs, the energy of thermal fluctuation is sufficient to induce a fast spin flip. This magnetic fluctuation leads to zero net magnetization, *e.g.* MNPs exhibit the superparamagnetic properties.[11] For instance, the temperature of the transition from superparamagnetic to ferromagnetic state (blocking temperature) for 2-nm and 13-nm cobalt nanoparticles is 10 and 370 K, respectively.

The size effect also manifests itself in changes in the coercivity. Compared to a bulk magnet with a multidomain

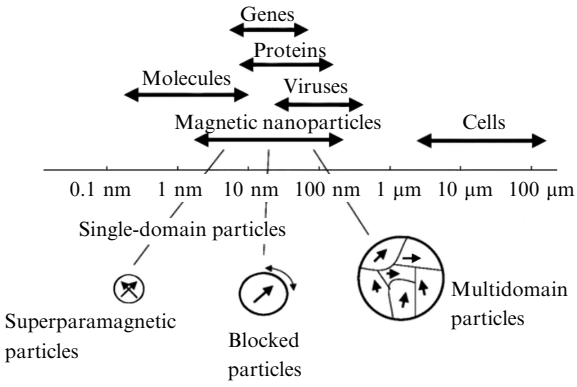


Figure 1. The size of magnetic nanoparticles and some biological entities: a comparison.†

† L Trahms, in *Colloidal Magnetic Fluids: Basics, Development and Application of Ferrofluids (Lecture Notes in Physics)* Vol. 763 (Ed. S Odenbach) (Berlin, Heidelberg: Springer, 2009 p. 327.

structure, MNPs whose size is smaller than a critical value have a single-domain structure with spin disorder.[9] In the single-domain state, the coercivity increases with the size of the MNP. Above some critical size, a transition to the multidomain state occurs. In particular, the coercivity of single-domain cobalt MNPs increases more than 4.5-fold as the nanoparticle size increases from 4 to 8 nm.[32]

The saturation magnetization of MNPs also depends on their size. In essence, all magnetic materials have a magnetically disordered (spin-glass-like) surface layer that is formed due to reduced spin–spin exchange coupling energy at the surface. For a bulk material, the volume of the disordered surface layer is small compared to the total volume of the magnet, and the surface spin canting effect can be neglected. However, for a nanoparticle the thickness of the disordered surface layer is comparable with its radius; therefore, the contribution of the surface spin canting effect becomes significant and affects the saturation magnetization.[33] For instance, an increase in the size of a Fe_3O_4 MNP from 4 to 12 nm causes its magnetization to increase from 25 to 102 emu g^{-1} at 1.5 T.[34]

By and large, the size and shape of MNPs have strong effect on the efficiency of magnetic separation, heating in alternating magnetic field and MRI contrast.[35]

2. Effect of the structure and composition of nanoparticles on their magnetic properties

The behaviour of MNPs is also influenced by their crystal structure. This can be observed in, *e.g.*, magnetic alloys with anisotropic crystal structure. Platinum-coated metallic cobalt nanoparticles have cores with fcc structure and exhibit the superparamagnetic properties (coercivity is zero) at room temperature. On heating, they form a CoPt alloy that has a a face centered tetragonal lattice (fct) and possesses ferromagnetic behaviour (coercivity is 5300 Œ) at room temperature due to a high magnetocrystalline anisotropy.[36]

Changes in the chemical composition of MNPs may significantly alter their magnetic properties. This influences the MRI-contrast properties of MNPs, namely, the spin–spin relaxation rate of iron oxide and manganese, cobalt and nickel ferrites is 218, 358, 172, 152 litre $mmol^{-1}$ s^{-1}, respectively.[37] The magnetic properties can also be affected by surface defects.‡

A wide variety of methods for preparation of metal and metal oxide MNPs from 4 to 100 nm in size were proposed in the 1960s. Presently, the range of available MNPs includes metallic (Co, Fe, Ni), iron oxide (Fe_3O_4 and γ-Fe_2O_3) and ferrite ($MgFe_2O_4$, $CoFe_2O_4$, $MnFe_2O_4$, $LiFe_5O_8$), as well as alloys (CoPt, FePt, MnAl, $SmCo_5$, $Fe_{14}Nd_2B$)[28,39–41] and core-shell MNPs comprising metal particles coated with graphene-like carbon,[42,43] silica,[44,45] natural and synthetic polymers.[46]

Oxide MNPs exhibit weaker magnetic properties compared to metallic MNPs. Nevertheless, iron(III) oxide nanoparticles are widely used for biomedicine due to low toxicity, stable magnetic properties, physicochemical stability and ease of preparation.[40,47]

‡ See, *e.g.*, Ref. 38 and E P Naiden, V I Itin, A A Magaeva, O G Terechova, V A Kostikova, L V Zagrebin, S S Shestov *Izv. Vyssh. Uchebn. Zaved. Poroshkovaya Metallurgiya i Funktsional'nye Pokrytiya* [*Powder Metallurgy and Functional Coatings*] (1) 3 (2011) (in Russian).

Table 1. The saturation magnetization (M_s) of MNPs and their acid/base stabilities.[42]

MNPs	Diameter /nm	M_s /A m^2 kg^{-1}	Acid/base stability
Fe_3O_4	10–100	30–50	low
Fe_3O_4/Fe	9	200	"
	200	172	high
C/Fe	20–30	104	"
C/Fe_3C	30	140	"
C/Co	30	162	"
C/Ni	5–100	30	–
C/CoFe	10–30	77	high
	7	215	"
C/FeNi	10–30	110	–
Au/Fe	–	80	medium
Au/Co	–	100	–
	40	135	low
SiO_2/Co	50	146	"
SiO_2/Fe	11	160	"
SiO_2/Ni	50–100	51	"

The saturation magnetization values and the acid/base stabilities of some widely used MNPs are collected in Table 1. All C/M and SiO_2/M (M is metal) nanocomposites possess magnetic properties. Compared to oxide MNPs, the C/M particles possess higher acid/base stabilities, thus being promising for biomedical applications.

One should keep in mind that MNPs are prone to form chain-like aggregates through dipole–dipole interactions.[48] Hydrodynamic interactions between MNP chains play the decisive role in magnetic separation, the effect of external magnetic fields being more pronounced and the separation time being shortened.

3. Specific features of the surface properties and modification of magnetic nanoparticles

Nanoparticles have large surfaces area. A trend towards minimization of the free energy and hydrophobic properties of the surface of MNPs predetermine their agglomeration in aqueous media.[28,38]

Biomolecules are usually studied in aqueous buffers. The pH condition, salt concentrations and temperatures of the buffers should not differ significantly from physiological values.[38,49] Modification of nanoparticles precludes their agglomeration and imparts hydrophilic properties to the surface of MNPs and protects them from oxidation, acid and basic corrosion and release of metal ions.[7,22] Coating of MNPs plays a key role for further conjugation with biomolecules.[50]

Methods of chemical modification of the surface of MNPs can be conditionally divided into two groups, *viz.*, methods of noncovalent (nonspecific) and covalent modification.[28,50–53] In the former case, there are no covalent bonds between organic molecules and the surface of MNPs (adsorption, electrostatic, van der Waals, hydrophobic interactions or hydrogen bonding occurs).[54] In the latter case, strong covalent bonds are formed.[42,55]

So-called core–shell structures representing MNPs coated with a layer of a polymer[46] or inorganic material[56,57] are most widely used. Nanoparticles can be coated during the synthesis or subsequently.[58]

Hydroxyl groups present on the surface of oxide nanoparticles react with molecules bearing hydroxyl, carboxyl, phosphate, phosphonate, sulfate and sulfonate groups through formation of hydrogen bonds or direct coordination as a result of ligand exchange.[49, 50]

The surface of MNPs can be modified with both organic (high-molecular-mass compounds and surfactants)[46, 49, 59, 60] and inorganic compounds.[61, 62] Possible surface modifiers that are used to prepare nucleic acid–MNP composites include synthetic and natural polymers, namely, polyethyleneimine (PEI),[59, 63, 64] poly(ethylene glycol) (PEG),[65] cationic lipids,[66] dendrimers,[67] dextrans (carboxymethyl dextran, aldehyde-aspartic dextran, epichlorohydrin cross-linked dextran),[68, 69] streptavidin[70–72] and proteins.[47, 73] These polymer molecules can directly interact with unmodified surface of MNPs or with organic or inorganic coatings that were applied to the surface of MNPs in advance. Polymeric coatings can be formed directly on the surface of MNPs by copolymerization of preliminarily adsorbed monomers.[49, 67, 74]

Modification of nanoparticle surface with low-molecular-mass organic compounds is also possible.[50] In particular, tetramethylammonium,[75] pyrrolidone,[76] β-mercaptoethylamine,[77] hemin,[78] *etc.*, are appropriate for further conjugation of MNPs with nucleic acids.

Currently, MNPs bearing various functional groups (azide,[74] amino,[79] carboxyl,[80] sulfhydryl[81]) on the surface are available. These groups ensure covalent immobilization of nucleic acid molecules in the next steps. Often, the surface of MNPs is coated with gold[62, 82] or silica[83] for efficient immobilization of nucleic acid molecules.

Silanization is widely used for modification of MNPs.[84, 85] In this case, the coating being formed is highly stable owing to formation of a covalent bond with the MNP surface.[54] This approach is used to impart hydrophilic properties to the surface and to prepare MNPs bearing various functional groups (COOH,[83] SH,[86] NH_2,[45] aldehyde groups[87]) that are convenient for subsequent bioconjugation.

Aromatic diazonium salts are promising and convenient reactants for the formation of reactive functional groups (FG) on the surface of MNPs and subsequent binding to nucleic acids. These salts readily interact with the surfaces of many metals, oxides and especially nanostructured carbon with evolution of nitrogen (Fig. 2).

To this end, aryldiazonium chlorides and tetrafluoroborates are routinely used.[52] In contrast to the traditionally utilized salts, recently synthesized aryldiazonium *p*-tosylates $ArN_2^+TsO^-$ are more stable and show good solubility in water and many organic solvents.[88] These salts exhibit enhanced reactivity towards many surfaces; in particular, they spontaneously and rapidly react with carbon layers on the surface of metal nanoparticles, in aqueous suspensions,[89] thus making the MNP surface coated with reactive functional groups.

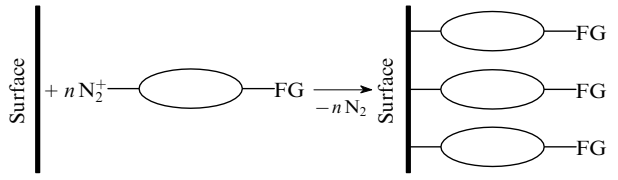


Figure 2. Covalent modification of the surface of nanomaterials with aromatic diazonium salts.

Surface modification may change the magnetic properties of MNPs.[75, 90–94] Electrostatic interactions between the coating molecules and the MNP surface can enhance the relaxation properties of the nanocomposite as compared to those of the initial MNPs.[95, 96] Coordination at the surface can lead to a considerable decrease in the coercivity of MNPs. This effect can be explained by crystal field theory. Binding of ligands to transition metal atoms on the MNP surface causes splitting of d-orbital energy levels and reduction of magnetic anisotropy that originates from spin–orbit coupling.[97]

It does not always happen that surface modification completely rules out subsequent nonspecific interactions of the nucleic acid molecule being conjugated with MNPs.[13] Mechanisms of the interaction of the coating with the surface of MNPs, the grafting density of the surface, stability in time and stability in different media often remain poorly studied.[13, 22] For instance, dextrans that are widely used for surface modification do not form strong coatings and can be desorbed form the surface.[54, 98]

Surface modification of MNPs has been a subject of some thematic reviews (see, *e.g.*, Refs 28, 40, 51, 99–101).

III. DNA molecules as components of nanobiocomposites

The concept of self-assembly of structures underlies the idea of using DNA as scaffold (template) for controlled arrangements of nanosized building blocks.[102]

The DNA macromolecule is almost ideal material for fabrication of structures with size from 5 nm to a few micrometres, and very attractive as component of nanosystems.[103] Timeliness of utilizing DNA is first of all due to the existence of the mechanism of highly specific molecular recognition based on the hybridization of two complementary strands.[1] For instance, an oligonucleotide 20 nucleotide base long is sufficient to recognize a target in a genome containing 3×10^9 base pairs.

There is a broad range of natural biomolecules (endonucleases, ligases, DNA-modifying enzymes) that can be used as tools for high-precision manipulations of DNA molecules.[4, 104, 105]

A DNA molecule to 40 nm long with any specified sequence can be synthesized and amplified by the polymerase chain reaction (PCR).[106] Double-stranded DNA molecules are convenient building blocks for constructing nanostructures because they can be linked 'tail-to-tail' to form extended linear molecules or structures with more complex architectures.[107] In 1982, Seeman[108] proposed to use nonlinear DNA molecules for assembling ordered networks with specified branchings and junctions, to form cubes,[109] triangles,[110] bilayer structures[111] and various DNA knots.[112] At present, diverse nonlinear DNA-structures are also assembled from modified nucleotides, proteins and RNAs.[113]

DNA molecules can be modified with various functional groups that specify sites for possible binding to other molecules.[114] The DNA molecule exhibits a relatively high physicochemical stability and mechanical rigidity of short double helices and can therefore behave as linker between two functional components attached to both ends.[1, 115]

In biomedical nanostructures, DNA/RNA molecules usually play the role of genetic information carriers or provide highly specific interaction with targets, *viz*., complementary DNA molecules, proteins and cell receptors.

1. Peculiar features of DNA molecules. How they govern DNA interaction with nanoparticles

Knowledge of DNA structure is a key to understanding of DNA interaction with other entities, such as molecules, molecular ensembles (complexes) and nanoparticles.[116, 117]

In solution, polymer molecules of single-stranded and double-stranded DNA have a characteristic secondary structure. Conformation of DNA depends on the DNA sequence and on parameters of the medium, *viz*., hydration level, ionic strength, pH, presence of metal ions, *etc*. A DNA strand can form loops, hairpins, cruciform structures as well as right- and left-hand helices of one up to four strands.[118]

A significant role in the interaction of DNA with other molecules and supramolecular complexes is played by protonation/deprotonation of particular atomic groups, which occurs in the range of pH values from 3.5 to 10.[119]

The idea of DNA molecule as a regular and rigid structure with fixed geometry is too simplified because DNA molecules in solution are characterized by internal mobility, fluctuation and local deformations.[118]

Persistent *in vivo* interaction of proteins, such as histones, transcription factors, polymerases, *etc*., with DNA molecules plays a significant role in their functioning and maintenance of particular conformations.[120, 121] The structure and functions of DNA depend strongly on metal ions.[122] Most DNA-binding proteins and enzymes contain metal ions as cofactors;[123] these ions play a significant role in for activation of enzymatic reactions involving DNA. Optimal rates of these reactions correlate with average concentrations of particular ions.

Magnetic nanoparticles are about 10 nm in size. This is comparable with the size of protein molecules that interact with DNA *in vivo* (see Fig. 1). The surface of MNPs is charged and bears particular functional groups;[124] this specifies the character of its interaction with the biomolecule as well as possible conformational transitions and properties of DNA upon binding.

2. Participation of DNA atoms in interaction: the rules

Usually, peculiar features of DNA interactions with various substances can be explained by the character of the charge density distribution in the biomolecule and by steric accessibility of particular atoms.

The largest negative charge is localized on phosphate groups present on the surface of DNA molecule and in the minor groove.[125, 126] Electron-donating properties of oxygen atoms of the phosphate groups involved in the phosphodiester bond are less pronounced, being suppressed by the free oxygen atoms bearing a partial negative charge.[125]

Amino groups of DNA bases exhibit weak acidic properties and act as proton donors in the formation of hydrogen bonds, whereas oxygen atoms of keto groups and nitrogen atoms of heterocyclic bases are proton acceptors.[116]

According to calculations,[127] the largest negative charge is localized on the N(3) atoms of purine bases and O(2) atoms of pyrimidine bases in the minor groove, as well as on the N(7) atoms of guanine and adenine in the major groove of B-DNA. In GC-tracts, the potential energy minimum responsible for the most favourable conditions for electrostatic interactions is localized in the major groove of DNA molecule; in AT-tracts, it is localized in the minor groove.[126, 127]

Steric accessibility of atoms in the B-DNA molecule characterizes the ability of particular atomic groups to interact with other molecules and ions and decreases in the following order:

$$\mathrm{PO(2) > N(7)(G) > O(6)(G) > N(7)(A) > O(4)(T) > > O(2)(T) > N(3)(A), O(2)(C)}$$

Endocyclic nitrogen atoms and first of all, the N(7) atoms of purines and the N(3) atom of cytosine, have lone electron pairs and are good ligands. However, interaction with the N(3) atom of cytosine is sterically hindered due to the flanking keto and amino groups.[116]

Oxygen atoms of deoxyribose rarely exhibit electron-withdrawing properties owing to steric hindrances.[125]

3. Interaction of DNA molecules with metal ions

DNA molecules have a number of metal-ion binding sites. There are oxygen atoms of phosphate groups, hydroxyl groups of sugars, endocyclic nitrogen atoms and exocyclic keto groups of bases. Interactions of metal ions with different sites of DNA molecules are specific and depend strongly on the nature of the ions.[128]

Usually, DNA is present in living cells in complexes with, *e.g*., Na^+, K^+, Mg^{2+} and Ca^{2+} ions. Metal ion binding to phosphate groups stabilizes the double helix.[129] This type of interaction is characterized by anti-cooperative effect because of strong Coulomb repulsion between cations.

Unlike alkali-metal and alkaline-earth metal ions, transition metal ions bind to both phosphate groups and DNA bases.[130–132] Interaction of transition metal ions with oxygen atoms of phosphate groups is accompanied by the formation of ionic bonds between them. When these cations interact with nitrogen atoms and keto groups of bases, coordination bonds are formed.§ Amino groups of bases are not involved in direct binding to metal ions.

Coordination to transition metal ions may destabilize DNA molecules, reduce the helix–coil transition temperature and change the molecular conformation. Complexation with transition metal ions is favoured by conformational fluctuation of DNA molecules.[122]

X-Ray diffraction studies showed that Fe^{2+} cation is directly coordinated to the N(7) atom of guanine and indirectly, through water molecules, to the O(6) atom of guanine and phosphate groups, thus forming an octahedral coordination complex of iron.[134–136] Also, simulation of the interaction of iron cation with oligonucleotide duplex and relevant NMR data showed that Fe^{2+} forms coordination bonds with the N(7) and O(6) atoms of guanine, an oxygen atom of phosphate group and N(7) atoms of two neighbouring adenine residues.[134] According to crystallographic data, a Co^{2+} ion interacting with double-stranded DNA is directly coordinated to the N(7) atom of guanine. In this case, filling of the coordination sphere will lead to

§ See Ref. 133 and J K Barton, in *Bioorganic Chemistry* (Mill Valley, CA: University Press, 1994) p. 455.

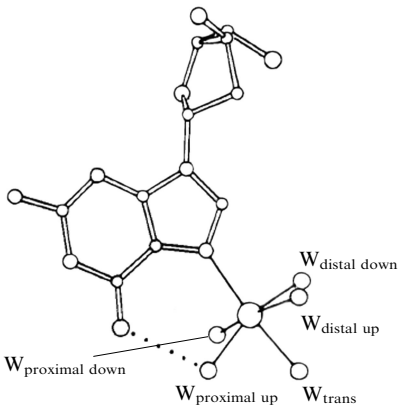


Figure 3. Octahedral coordination geometry of Co ion at the N7 position of guanine.[137]
Shown are five water molecules of the hydration shell.

formation of indirect coordination bond (through water bridge) with the O(6) atom of guanine, whereas water molecules coordinated in axial positions will induce conformational change in the adjacent base pair (Fig. 3). This unavoidably will cause significant conformational rearrangements in the DNA molecule.

Interaction of Mn^{2+} with DNA bases also induces structural transition.[130] Initiation of structural rearrangement of the entire molecule favours positive co-operativity of complexation with transition metal ions.[129]

Water molecules play a significant role in DNA complexation with metal ions because they form the hydration shell of the ion and its complex.[129, 138] Hydrated ions interact with hydrophilic atoms in the nucleic acid molecule through the hydration shell owing to formation of a weak bond (outer-sphere complex). If one or more water molecules in the hydration shell ion are replaced by oxygen and/or nitrogen atoms of the nucleic acid molecule, a direct bond is formed (inner-sphere complex).[125] The formation of outer-sphere complexes with DNA molecules is more characteristic of Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} ions compared to Ag^{+}, Cu^{+} and Cu^{2+} ions (Cu^{2+} mainly forms inner-sphere complexes with DNA molecules).[122]

Significant differences are observed for binding ions to double- and single-stranded DNA. Double-stranded DNA molecules are characterized by much higher density of negative charges on the surface. In addition, in this case some active sites of DNA bases are 'hidden' in the interior of the molecule. Therefore, for double-stranded DNA the affinity of metal ions to phosphates is higher than to bases.[129] Preferred binding of metal cations to phosphate groups of DNA rather than to bases as follows:¶

$$Mg^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+}$$

The ability of metal ions to bind to DNA underlies some original methods of formation of MNPs from solution on the surface of the biomolecule.[93]

¶ See J K Barton, in *Bioorganic Chemistry* (Mill Valley, CA: University Press, 1994) p. 461.

IV. Methods of fabrication of DNA – magnetic nanoparticle composites

There are two types of nanocomposites, namely, DNA on MNPs and MNPs on DNA. Realization of a specified geometry of the composite being formed is of great importance and is determined by the mechanism of the interaction between its components. The structure of the nucleic acid – MNPs systems can be studied using methods of investigation of the interaction of organic molecules with the surface of MNPs.

1. Methods of investigation of the interaction of organic molecules with magnetic nanoparticles

Usually, the formation of a coating on MNPs is confirmed by transmission electron microscopy (TEM)[75, 93, 139, 140] and atomic force microscopy (AFM).[141 – 143] These methods are also used to determine the size and morphology of nanocomposites. Changes in the size of MNPs upon coating can be detected by dynamic light scattering.[54, 58] The distance between neighbouring MNPs formed on a DNA molecule can be found from small-angle X-ray scattering data.[75]

By analyzing the adsorption isotherms, including those measured upon variation of parameters of the solution (pH, ionic strength, chemical composition), one can estimate the strength of a chemical bond and make assumptions of its nature. Data of adsorption experiments used together with the specific surface areas of MNPs can be basic to calculations of the component ratio in the nanocomposite; they also allow one to decide between mono- or polymolecular adsorption layer formed on the surface.

Measurements of electrophoretic mobility and potentiometric titration make it possible to determine the charge of MNPs and to calculate the number of proton-binding sites on the surface of MNPs.[50, 58, 144] These data are used in simulation of mechanisms of the interaction between components in nanocomposites.

Thermogravimetric analysis (TGA) makes it possible to calculate the enthalpy of adsorption of organic molecules on the surface of MNPs. The strength of the bond between molecules and the surface can be estimated from desorption temperatures (from mass loss).[94] TGA data are also used to calculate the grafting density and to determine the number of adsorption layers.[90, 144, 145]

IR spectroscopy is widely used to confirm the formation of nanocomposites and to determine group of atoms involved in the formation of bonds with the surface. Attenuated total reflectance Fourier transform IR spectroscopy can be used to examine samples in solution.[94, 139, 141, 144 – 147]

Investigations of nanocomposites by circular dichroism help to assess conformational changes in polymer molecules (including DNA) upon binding to nanoparticles.[147]

X-Ray photoelectron spectroscopy (XPS) provides valuable information on the character of bonding between the molecule and the surface and on the nature of interacting ligands.[139, 141] By analyzing the photoelectron spectra one can determine the thickness of the adsorption layer and conformations of polymer molecules on the surface.[54] XPS data are used to calculate the atomic ratios in the nanocomposite being formed and the number of ligands bound to a single nanoparticle.[90]

Solid-state magic-angle spinning NMR spectroscopy enables observation of high-resolution ^{1}H NMR signals of

organic molecules (unsaturated acids, codeine phosphate, sulfonated heterocyclic polyfunctional ligand with imidazole and quinoline units) covalently bonded to iron oxide MNPs.[148] Combining this technique with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is suitable to determine local conformations of organic molecules on the surface of MNPs.

Static secondary ion mass spectrometry (SSIMS) was used to analyze the surface structure and the elemental composition of the organic coating.[54] Modification of iron oxide nanoparticles with [3-(2-aminoethylamino)propyl]-trimethoxysilane led to formation of Fe–SiO mixed clusters, which confirmed that the coating is covalently bound to the surface. The presence of an iron peak in the positive ion SSIMS spectrum of dextran-modified iron oxide nanoparticles suggested an incomplete coverage of the surface.

Coordination of organic molecules to the surface causes structural changes in MNP that can be detected by X-ray adsorption spectroscopy. Bidentate coordination of organic ligands of coating molecules with MNPs is convenient for filling oxygen vacancies on the surface of MNPs and for formation of octahedral configurations. Using spectroscopic methods, namely, X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS), one can determine the local geometry and electronic structure of atoms, as well as the coordination geometry and the oxidation state at the interaction with adsorbents.[149, 150]

Information on changes in magnetic properties of MNPs upon binding to organic molecules can also be used for understanding of the mechanisms of interaction between components of nanocomposites. For instance, a decrease in the magnetization of Fe_2O_3 nanoparticles bound to octadecylphosphonic acid was explained by changes in the spin state of iron atoms upon coordination.[151]

Electron paramagnetic resonance studies of Fe_2O_3 nanoparticles coated with octyl phosphonate revealed a transition from superparamagnetic to antiferromagnetic behaviour.[94] It was concluded that this transition is a result bridging bidentate coordination of phosphate group between two neighbouring Fe^{3+} ions on the nanoparticle surface.

In a study on the magnetic properties of γ-Fe_2O_3–DNA composite the observed increase in the coercivity compared to the coercivity of initial nanoparticles was explained by reduction in interparticle interaction upon DNA coating.[140]

Differential pulse voltammetry is used to study the electrochemical behaviour of DNA in order to prove structural changes in DNA upon binding to MNPs.[142]

2. Mechanisms of interaction of DNA molecules with the surface of unmodified magnetic nanoparticles

High affinity of ferrite MNPs to DNA was reported.[142, 152, 153] However, studies on the mechanisms of interaction between unmodified MNPs and DNA are scarce.

Taylor *et al.*[153] investigated binding of nucleic acids to MNPs and showed that regularities of adsorption/desorption on magnetite nanoparticles differ from those established for classical sorbents (*e.g.*, silica).

Byrne *et al.*[95] studied the formation of Fe_3O_4 nanoparticles by co-precipitation in a solution containing single-stranded denatured and double-stranded native herring DNA and concluded that nanoparticles bind to DNA molecules through formation of Fe–O–P bonds. Also, the interaction of nanoparticles with the double-stranded DNA was accompanied by cross-linking of phosphate groups, which led to DNA compaction. Binding of nanoparticles to the single-stranded DNA was more efficient than to the double-stranded DNA.

To understand the nature of the chemical bond formed between MNPs and DNA, it is appropriate to analyze original publications concerning the interaction of nanoparticles with organic molecules containing similar group of atoms. For example, detailed investigations of the interaction between iron oxide nanoparticles and phosphorus-containing organic molecules were carried out. Phosphonic acids and phosphonates adsorbed on iron oxide MNPs are strongly attached to their surface through mono- or bidentate coordination of phosphonate group to Fe^{3+} ions located in octahedral sites of spinel structure.[53, 94, 151] It was concluded[90] that binding of alkyl phosphonate to the surface of an iron oxide nanoparticle is followed by the formation of a quasi-bilayer coating, the primary layer being covalently bound to the surface while the other layer being adsorbed through hydrogen bonds and van der Waals interactions.

Studies of adsorption of phosphate anion on the surface of magnetite MNPs showed that the process depends on the pH value of the medium and occurs through either electrostatic interactions of phosphate with $Fe-OH_2^+$ or ligand exchange (oxygen atoms of phosphate are more electronegative than hydroxyl groups and displace them from the surface).[144] There are also ionized surface sites capable of coordinating phosphates. The phosphate anion is adsorbed on the surface of a magnetite MNP as a result of the interaction with Fe^{3+} in octahedral sites. The process mainly results in binuclear complexes that can be protonated.[144, 154] Binding of polycyclic codeine phosphate was also accompanied by chemisorption owing to symmetrical coordination of phosphate group to the magnetite surface.[148]

At physiological pH values (pH 7.2–7.4), phosphate groups of DNA molecule and the surface of iron oxide MNPs are mainly deprotonated.[155–158] Reduction of the pH value of the medium may lead to an increase in the grafting density of phosphate groups because of protonation of both phosphate groups of DNA and the surface of MNPs; this creates prerequisites to the formation of additional hydrogen bonds.[154, 159, 160]

Lengthening of the DNA molecule and, correspondingly, an increase in the number of phosphate groups causes the number of potential binding sites to increase; this should make bonds with the surface of MNPs stronger.[50, 161] However, oligonucleotides attach to the surface stronger than polynucleotides. This is due to the fact that polymer molecules bind to the surface through particular segments, whereas nonadsorbed segments form loops.[54, 162] Theoretical schemes of adsorption of polynucleotide and oligonucleotide molecules on the surface of MNPs are shown in Fig. 4.

The conformation of polynucleotide DNA molecule on the surface of MNPs is still to be clarified. It was proposed[93] that DNA molecule can wrap around MNPs similarly to the wrapping of the DNA around the histone core in the nucleosome through electrostatic interactions of phosphate groups with the surface of MNPs.

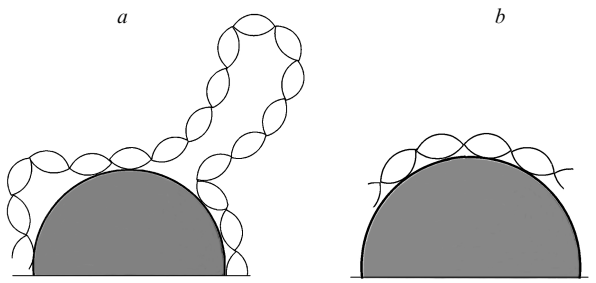


Figure 4. Adsorption of polynucleotide (*a*) and oligonucleotide (*b*) DNA molecules on the MNP surface.

In spite of the leading role of phosphate groups in the bonding with MNPs, DNA bases can also be involved. For instance, binding $Ni_{0.5}Zn_{0.5}Fe_2O_4$ nanoparticles to double-stranded DNA molecules leads to disruption of some hydrogen bonds between complementary strands.[142] This enhances the accessibility of the base atoms to the interaction with MNPs or is a direct consequence of participation of the nitrogenous bases in the bonding.

ATR-FTIR studies of nanocomposites of cobalt ferrite nanoparticles with single-stranded and double-stranded DNA showed that both oxygen atoms of carbonyl groups of heterocyclic bases and phosphate group are involved in the bonding.[163, 164] Nitrogen-containing heterocyclic compounds can strongly attach to the surface of Fe_3O_4 nanoparticles through coordination of carbonyl group to iron ion (C=O···Fe).[76]

Preparation of nanoparticles by co-precipitation onto DNA molecule was accompanied by the formation of small (1–2 nm in diameter) and large (5–10 nm in diameter) nanoparticles. It was concluded that small particles are formed upon binding of iron ions to the N(7) atom of guanine in the major groove, whereas large particles are formed as a result of binding of iron ions to phosphate groups of DNA.[93]

Taking into account the affinity of amino groups to the surface of iron oxide MNPs, one can assume that NH_2 groups of DNA bases can participate in the hydrogen bonds formation.[49, 54]

3. Nanobiocomposites: methods of preparation

Methods of conjugation of MNPs with nucleic acids can be divided into two groups with respect to specifity of interactions:

— molecular recognition where nanoparticles bind to certain DNA/RNA bases;

— electrostatic interactions, chemical reduction, *etc.*; these methods are less specific.[165]

The starting point in the design of a nanobiocomposite is the desired field of application. The key parameters are as follows:

— orientation of DNA molecule on the nanoparticles surface or mutual position of nanoparticles on the aligned molecule; *e.g.*, the arrangement of chemically modified bases on the DNA molecule allows placing the nanoparticles on the controlled distance;

— nature of the bond between MNPs and DNA molecule; dissociation of this bond as a result of changes in pH value, ionic strength or upon addition of compounds that possess higher affinity to the binding site on the surface can either have dramatic consequences or form the basis of functioning of the nanobiocomposite.†

Methods of conjugation of MNPs with nucleic acid molecules are characterized in Table 2.

Using modified nucleic acid molecules, one can achieve strong, oriented and highly specific binding of biomolecules to nanoparticles. Covalent immobilization is often attained with thiolated and aminated molecules. To this end, amino, sulfhydryl, carboxyl and azido groups are formed on the surface of nanoparticles. Covalent bonds are formed using traditional methods of bioconjugation and 'click'-chemistry approaches including carbodiimide activation, thiol–disulfide exchange, aldehyde–amine condensation, azide–alkyne cycloaddition, *etc.* Bioconjugation of DNA with nanoparticles is very often attained using cross-linkers.[196–199]

To avoid nonspecific interactions during immobilization of nucleic acids on the surface of MNPs, unreacted functional groups are blocked in different manner.[74, 87, 178]

DNA molecules can also be bound to nanoparticles without formation of covalent bonds, namely, through electrostatic interactions with the surface. For instance, positively charged ferrite nanoparticles modified with pyrrolidine during the synthesis form ionic bonds with λ phage DNA.[200]

To prepare nanobiocomposites, DNA molecules are often immobilized on the surface of MNPs modified with cationic lipids[66] and polymers, *e.g.*, cationic proteins, amino acids (polylysines, polyarginines),[201] chitosan,[202] PEI,[172] *etc.*[203] Yet another approach involves formation of poly(amidoamine) dendrimers on pre-silanized surface of MNPs.[67, 204] Poly(amidoamine) molecules can spontaneously form complexes with negatively charged DNA molecules through electrostatic interactions.[205] It should be noted that the efficiency of DNA binding to PEI-coated nanoparticles depends on the pH value of the medium.[63]

The sequences and secondary structure motifs of the biopolymer can favour binding of MNPs to DNA molecules. The experimentally established dependence of the efficiency of binding of single-stranded homooligonucleotides to cobalt ferrite MNPs on the nucleotide composition as well as preferential binding of nanoparticles to GC pairs of double-stranded DNA suggest the ability of these nanoparticles to selectively interact with particular motifs in the DNA structure.[190, 206]

One more strategy of preparation of nanobiocomposites is to form nanoparticles directly on the DNA molecule.[207, 208] The essence of the method is that metal cations M^{n+} from the salt solution bind to DNA [$-PO_2^-$, N(7)] and subsequent increase in the pH value of the solution causes the formation of MNPs (co-precipitation method).[77, 93]

Often, specific binding of MNPs to DNA is attained using high-affinity noncovalent interactions biotin–streptavidin[71, 72, 209, 210] or protein–aptamer.[78] This requires preliminary modification of the surface of MNPs and nucleic acids with appropriate molecules.

† The first steps in fabrication of nanobiocomposites are associated with publication of two key studies[166, 167] on covalent bonding of alkanethiol-modified DNA molecules with gold nanoparticles. At present, thiolated DNA is used for immobilization on gold-coated MNPs.[62]

Table 2. Methods for conjugation of magnetic nanoparticles with nucleic acid molecules.

Nanoparticle	Surface modifier	Functional group	Nucleic acid	Possible applications	Ref.
Electrostatic interactions between positively charged nanoparticle and negatively charged DNA molecule (PO_2^-)					
FePt	tetramethylammonium	–	double-stranded DNA (37 bp)	biomedicine	75
γ-Fe_2O_3	–	–	calf thymus DNA	the same	140
Co	β-mercaptoethylamine	–	poly(dC) and poly(dG)	data storage	77
$CoFe_2O_4$	2-pyrrolidone	–	λ phage DNA	the same	168
Fe_3O_4	2-pyrrolidone	–	λ phage DNA	"	169
Fe_2O_3	2-pyrrolidone	–	λ phage DNA	"	105
Fe_2O_3, $CoFe_2O_4$	2-pyrrolidone	–	λ phage DNA	MRI contrast agent	96
Electrostatic interactions between cationic polymer coating a nanoparticle and negatively charged DNA molecule					
Fe_3O_4	APTES and PAMAM	–	asODN	gene therapy	67
Fe_3O_4	oleic acid and cationic lipid	–	siRNA	"	66
Fe_3O_4	polyacrylic acid and PEI	–	plasmid DNA	"	170
Mesoporous SiO_2 impregnated with Fe_2O_3	PEI	–	plasmid DNA	"	59
Fe_3O_4	Si – OH-copolymer and PEI	–	plasmid DNA	"	171
Fe_3O_4	PEI	–	plasmid DNA	DNA vaccine	63
Fe_3O_4	PEI	–	plasmid DNA	transfection vector	172, 173
Bond between thiol group and gold atom					
Fe_3O_4	Au	–	$HS-(CH_2)_6-ODN$	biomedicine	62
Co	Au	–	$HS-(CH_2)_6-ODN$	the same	62
$CoFe_2O_4$	APTES, MPTMS and Au	–	$N'-Cys-\hat{O}-\hat{O}-ODN$	*in vitro* diagnosis	82
Disulfide bond in thiol-disulfide exchange reaction					
γ-Fe_2O_3	TEOS and MPTMS	SH	$5'$-$HO-(CH_2)_6-S-S-(CH_2)_6-ODN$	the same	81
Fe_3O_4 and γ-Fe_2O_3	TEOS and MPTMS	SH	N′-Lys – ODN – Ala – Ala – Cys(4-mercapto-pyridine)NH_2	"	86
Fe_3O_4 and γ-Fe_2O_3	TEOS and MPTMS	SH	$5'$-S – S – ODN	"	see [a]
Phosphoramidate bond between phosphate group and amino group (carbodiimide activation)					
Fe_3O_4	APTES	NH_2	$5'$-PO_4 – ODN	"	174
Amide bond between carboxyl group and amino group (carbodiimide activation)					
Fe_3O_4	TEOS	COOH	$ODN-NH_2$-$3'$	genotoxicity studies	83
Fe_3O_4	SiO_2	COOH	$5'$-NH_2-ODN	*in vitro* diagnostics	175
MFe_2O_4 (M = Co, Mn, Fe)	alkylphosphonate and oleate	COOH	$5'$-$NH_2-(CH)_6-dT_5-ODN$	the same	176
Fe_3O_4	carboxymethyl dextran	COOH	$5'$-$NH_2-(CH_2)_6-ODN$	tumour diagnosis and therapy	80
$Fe_3O_4-SiO_2$[Ru(bpy)$_3$]	APTES and succinic anhydride	COOH	$5'$-$NH_2-dT_{10}-ODN$	tumour therapy	177

Table 2 (continued).

Nanoparticle	Surface modifier	Functional group	Nucleic acid	Possible applications	Ref.
Amide bond — aldehyde – amine condensation with Schiff base formation and reduction of imine bond					
MNPs [b]	Aldehyde – aspartic dextran	CHO	ODN – NH_2 – C3-3′	*in vitro* diagnosis	178
Cross-linking using homobifunctional cross-linker (glutaraldehyde)					
Fe_3O_4	TEOS and APTES	NH_2	5′-NH_2 – $(CH_2)_n$ – dA_7 – ODN	the same	179
Fe_3O_4	APTES	NH_2	5′-NH_2 – $(CH_2)_6$ – dC_6 – ODN	"	180
Fe_3O_4	APTES	NH_2	5′-NH_2 – $(CH_2)_6$ – dA_{12} – ODN	*in vitro* diagnosis	181
Fe_3O_4	APTES	NH_2	5′-NH_2 – $(CH_2)_6$ – dC_6dT_{25}	biomedicine	see [c]
Cross-linking using heterobifunctional cross-linker containing amino-(*N*-hydroxysuccinimide) group and sulfhydryl group					
SPDP cross-linker					
$(Fe_2O_3)_n(Fe_3O_4)_m$	dextran and epichlorohydrin	NH_2	5′-HS – $(CH_2)_6$ – ODN, 5′-ODN – $(CH_2)_3$ – SH	*in vitro* diagnosis	68
$(Fe_2O_3)_n(Fe_3O_4)_m$	dextran and epichlorohydrin	NH_2	5′-HS – ODN, 5′-ODN – dA_{12} – SH	the same	182
$(Fe_2O_3)_n(Fe_3O_4)_m$	dextran and epichlorohydrin	NH_2	5′-HS – dT_n – ODN	"	183
$Mn_x(Fe_2O_3)_n(Fe_3O_4)_m$	cationic bovine serum albumin	NH_2	5′-HS – siRNA – Cy5	*in vitro* diagnosis and therapy	184
SIA cross-linker					
$(Fe_2O_3)_n(Fe_3O_4)_m$	dextran and epichlorohydrin	NH_2	5′-HS – $(CH_2)_6$ – ODN	*in vitro* diagnosis	185
MBS cross-linker					
$(Fe_2O_3)_n(Fe_3O_4)_m$	dextran and epichlorohydrin	NH_2	5′-S – S – $(CH_2)_6$ – siRNA	diagnosis and therapy *in vivo*	186
Sulfo-SMCC cross-linker					
MNPs [b]	–	NH_2	5′-HS – dT_{12} – ODN – FAM	*in vitro* diagnosis	187
γ-Fe_2O_3	APTES	NH_2	5′-S – S – $(CH_2)_6$ – siRNA	gene therapy	188
Cu(I)-catalyzed azide – alkyne cycloaddition					
MNPs [b]	SiO_2 and copolymerization OEGMA – N3 and OEGMA – OH	N_3	Alk – ODN	*in vitro* diagnosis	74
Fe_3O_4	polyacrylic acid	C≡C – CH_2 – O –	N_3 – ODN	the same	189
Fe_3O_4 – NH_2	azidobutyrate *N*-hydroxysuccinimide ester	N_3	Alk – dA_{10} – ODN	biomedicine and date storage	see [d]
$M^{2+/3+}$ ion binding to DNA [$-PO_2^-$, N(7)] in solution, formation of nanoparticles on DNA molecules by co-precipitation upon increase in pH					
Fe_3O_4	–	–	plasmid DNA	biomedicine	93
Fe_3O_4	–	–	double-stranded herring testes DNA, double-stranded λ phage DNA	nanowires	141
α-Fe_2O_3	–	–	double-stranded herring testes DNA	hyperthermia	147
Fe_3O_4	–	–	double- and single-stranded herring testes DNA	*in vitro* diagnosis	95

Table 2 (continued).

Nanoparticle	Surface modifier	Functional group	Nucleic acid	Possible applications	Ref.
Coordination of functional groups and atoms of DNA molecules with $M^{2+/3+}$ ions on the nanoparticles surface					
Bonds with PO_2^-, C=O and N(7)					
$CoFe_2O_4$	–	–	double-stranded salmon testes DNA, poly(dT), ODN	*in vitro* diagnosis	164, 190
Bond with PO_4^- group					
Fe_3O_4	sodium silicate and Al_2O_3	–	ODN	"	179
Biotin – streptavidin affinity interaction					
Fe_3O_4	TEOS	avidin	5′-biotin – ODN	"	191
MNPs [b] (70% iron oxide)	–	streptavidin	5′-biotin – ODN	"	70
Fe_3O_4	L-aspartate	streptavidin	5′-biotin – ODN	"	192
Fe_3O_4	Au	streptavidin	5′-biotin – ODN	"	192
Fe_3O_4	APTES	streptavidin	5′-biotin – ODN	"	192
Protein – aptamer affinity interaction					
Fe_3O_4	hemin	–	ODN	"	78
Intercalation into double-stranded DNA					
Fe_3O_4	carboxymethyl dextran	TO-PRO1	double-stranded λ phage DNA	"	193
$Ni_{0.5}Zn_{0.5}Fe_2O_4$	adipoyl chloride	doxorubicin	calf thymus double-stranded DNA	tumour therapy	142
Fe_3O_4	phosphated starch	mitoxantrone	double-stranded DNA	the same	194
Hydrogen bonds (Watson – Crick complementary interaction)					
Fe_3O_4	hemin	ODN	polynucleotide	*in vitro* diagnosis	78
MNPs [b]	–	dT_n – ODN	ODN	nanofabrication	195

Note. APTES stands for (3-aminopropyl)triethoxysilane; PAMAM stands for poly(amidoamine), a dendrimer; asODN stands for antisense oligo(deoxy)nucleotides; siRNA stands for small interfering RNA; Si – OH-copolymer stands for thermally cross-linked poly[3-(trimethoxysilyl)propyl]methacrylate-*p*-PEG-methyl methacrylate-*p*-*N*-acryloxysuccinimide ester; MPTMS stands for (3-sulfanylpropyl)trimethoxysilane; Cys stands for cysteine; Ô stands for 8-amino-3,6-dioxaoctanoic acid; ODN stands for oligo(deoxy)nucleotide; Lys stands for lysine; dA stands for adenine; dC stands for cytosine; dG stands for guanine; dT stands for thymine; Ala stands for alanine; TEOS stands for triethoxysilane; $Ru(bpy)_3$ stands for tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate ($C_{30}H_{24}Cl_2N_6Ru \cdot 6\,H_2O$); SPDP stands for 3-(2-pyridyldithio)propionic acid *N*-succinimide ester; Cy5 stands for cyanine fluorescent dye; SIA stands for *N*-succinimidyl iodoacetate; MBS stands for *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester; Sulfo-SMCC stands for sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; FAM stands for fluorescein fluorescent dye; OEGMA – N3 stands for azido-modified oligo(ethylene glycol)methyl methacrylate; OEGMA – OH stands for hydroxyl-containing oligo(ethylene glycol)methyl methacrylate; and TO-PRO1 stands for fluorochrome intercalated into double-stranded DNA.

[a] See S Santra, R Yapec, N Theodoropoulou, J Dobson, A Hebard, W Tan *Langmuir* **17** 2900 (2001).

[b] Commercially available nanoparticles (no chemical composition specified).

[c] See A del Campo, T Sena, J-P Lellouchec, I J Brucea *J. Magn. Magn. Mater.* **293** 33 (2005).

[d] See J I Cutler, D Zheng, X Xu, D A Giljohann, C A Mirkin *Nano Lett.* **10** 1477 (2010).

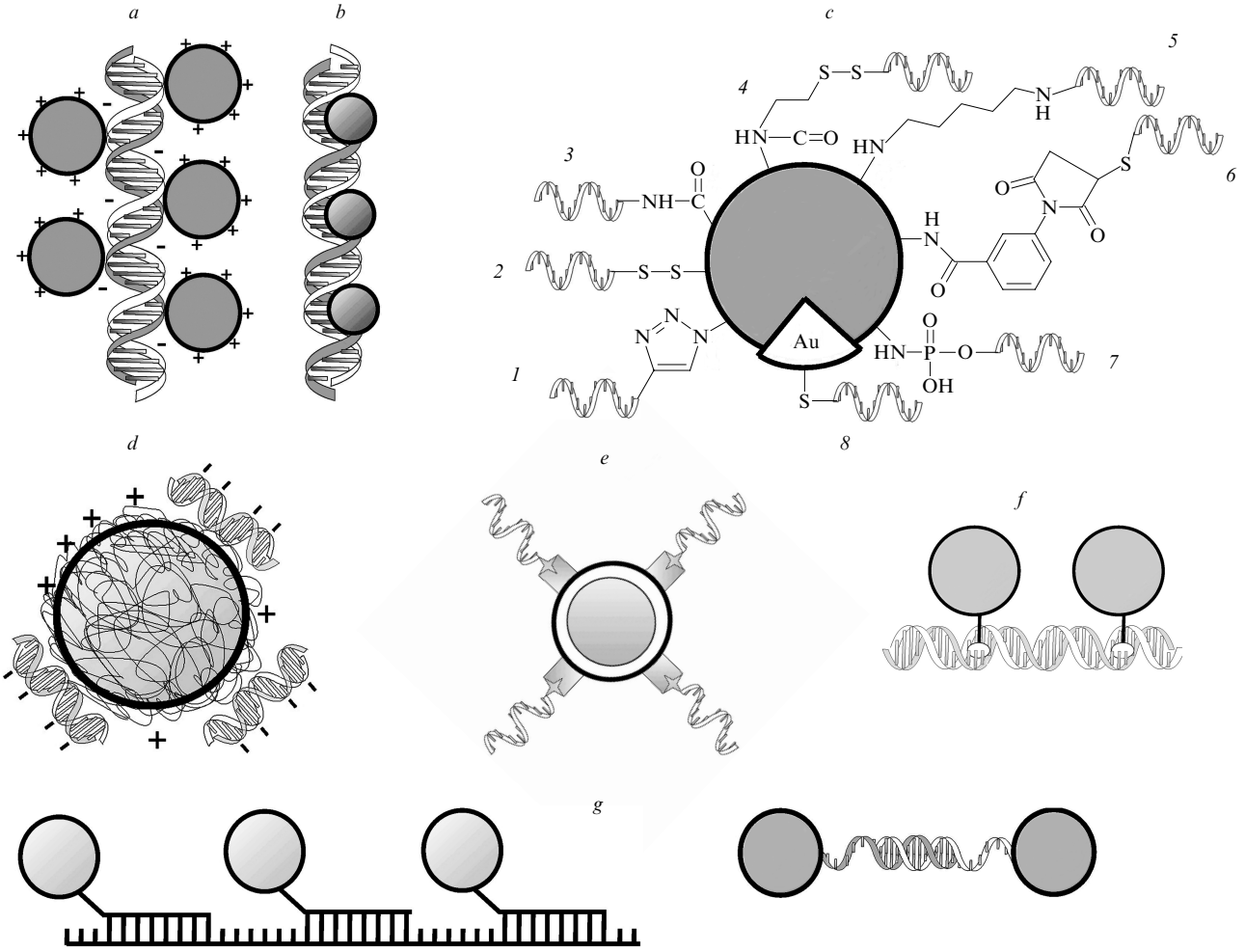


Figure 5. Structures of nucleic acid – MNP nanobiocomposites formed through various interactions.
(*a*) Electrostatic interactions;[105] (*b*) formation of MNPs on DNA molecule;[211] (*c*) covalent bonding of modified nucleic acid with functional groups on the modified surface of MNPs in azide – alkyne cycloaddition reaction (*1*),[74] through formation of a disulfide bond (*2*),[81] amide bond (*3*),[80] use of SPDP (*4*),[68] glutaraldehyde (*5*)[180] and MBS (*6*)[186] cross-linking, phosphoramidate bond (*7*),[174] interactions of sulfonyl group with gold coating (*8*);[82] (*d*) electrostatic interaction of nucleic acid with coating the MNPs surface;[67] (*e*) high-affinity noncovalent interactions between pre-functionalized surface of MNPs and nucleic acid;[70] (*f*) intercalation of specific molecules immobilized on MNPs into double-stranded DNA;[193] and (*g*) formation of complementary hydrogen bonds between oligonucleotides immobilized on MNPs and long single-stranded nucleic acid molecule (left)[78] or between the oligonucleotide molecules (right).[195]

Formation of hydrogen bonds between complementary DNA strands is basic to the fabrication of nucleic acid – MNP hybrid nanocomposites by self-assembly. Oligonucleotides immobilized onto MNPs act as cross-linkers between two (and more) MNPs or allow one to arrange MNPs on a longer single-stranded DNA molecule.[78, 195] Complementary binding of an oligonucleotide immobilized on MNPs and target nucleic acid molecule (hybridization) underlies the operating principles of some diagnostic assays.[74, 174, 178, 180]

Complexes of MNPs with DNA can be obtained by functionalization of the surface of nanoparticles with molecules capable to intercalate into double-stranded DNA.[142, 193, 194]

Possible types of nucleic acid – MNP nanobiocomposites formed through various interactions are shown in Fig. 5.

V. Applications of DNA – magnetic nanoparticle nanostructures

1. Nanoelectronics devices

Controlled arrangement of MNPs using DNA molecules as template offers great prospects for the design of high-density data storage devices, nanowires for interconnection of functional elements of nanoelectronics devices, *etc*.[212, 213] Using MNPs 5 – 10 nm in diameter arranged at a distance of 5 nm from one another, it is theoretically possible to reach an storage capacity of the order of a few Terabits per square inch.[97]

The design of such nanostructures begins with controlled positioning of DNA molecules on an appropriate substrate (*e.g.*, mica, silanized glass, *etc*.).[200] This can be done using various methods, namely, 'molecular combing' (DNA strand is stretched and oriented normal to the meniscus); hydrodynamic and electrophoretic methods

(DNA molecule is oriented under the action of mechanical or electric field); positioning through specific interactions with particular surface sites (biotin–avidin, gold–thiol).[165] Often, 'stretching' on the surface is preceded by modification of DNA molecule and/or surface.

To date, the greatest advances were achieved in DNA nanowire fabrication. Metallization of DNA occurs by coating through chemical reduction. A DNA nanowire thus obtained had variable diameter (from 25 to 250 nm) and exhibited the properties of a paramagnet. DNA was immobilized by deposition onto mica treated with Crystal Violet dye. Subsequent 'activation' with palladium chloride followed by treatment with boron hydride dimethylamine led to formation of nanoclusters on DNA. Reduction of nanoclusters in an electrolyzer in the presence of cobalt afforded a magnetosensitive nanowire.[143]

Magnetic nanowires can also be fabricated by binding MNPs to DNA.[214] For instance, coating of DNA molecule with iron oxide nanoparticles gave a magnetic nanowire 10 nm in diameter. The magnetization of single-domain nanoparticles in the nanowire could be changed using a magnetic tip.[213]

Solution methods of formation of MNPs on DNA are very attractive for fabrication of nanowires because it is possible to limit the particle size. For instance, regularly coated magnetic nanowires 5–6, 14–15 and 23–24 nm in diameter (Fig. 6) were prepared by co-precipitation of iron oxide nanoparticles onto DNA from a salt solution.

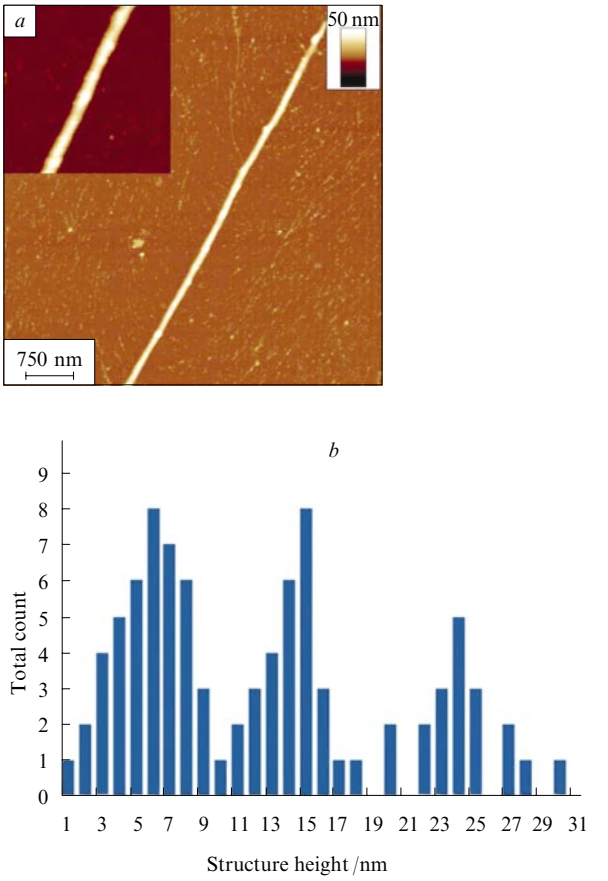


Figure 6. AFM image of a λ-phage DNA-templated Fe_3O_4 nanowire aligned upon a trimethylsilane-modified Si/SiO_2 substrate (inset: granular structure of the nanowire can be seen) (*a*); histogram of the size (diameter) distribution of the nanowires (*b*).[141]

Not only 1D, but also 2D and 3D structures can be fabricated by positioning nanoparticles on DNA molecules. Hybridization of poly(dC) and poly(dG) bound to 12-nm cobalt nanoparticles by ionic bonds led to formation of a network structure on mica surface. Individual nanoparticles had different directions of magnetization, similar to 'bits' in magnetic data storage devices.

2. *In vitro* biomedical studies

Magnetic nanoparticles are widely used in *in vitro* diagnosis. Nanosized structures based on MNPs can bind to particular biomolecules and thus significantly improve the sensitivity of analysis.[6,215] Unique magnetic properties of such systems ensure ready isolation and concentration of target biomolecules and offers prospects for efficient real-time detection.[5]

Magnetic nanoparticles are widely used for magnetic separation of biomolecules. This procedure is much less robust compared to filtration and centrifugation.[152,204] PEG-modified nanoparticles, either silanized or silica-coated, were successfully utilized for isolation of genomic[153] and plasmid DNA.[216] Application of MNPs allows one to enhance the efficiency of DNA isolation from biological material with low DNA content. For instance, genomic DNA present in urine sample at a concentration of 50×10^{-6} mol litre^{-1} was isolated using carboxylated MNPs as adsorbent.[217] DNA/RNA isolated using MNPs is used for carrying out polymerase chain reactions, restriction digestion and transfection.[44,218,219]

An interesting version of highly sensitive PCR with real-time MRI detection (an alternative to fluorescent detection) was proposed.[193] Carboxymethyl dextran coated iron oxide nanoparticles were covalently bound to TO-PRO 1 fluorochrome. The addition of nanocomposites to the PCR mixture was followed by binding nanoparticles to fragments of double-stranded DNA through intercalation of fluorochrome and then by aggregation that could be detected by light scattering and from changes in the spin–spin (transverse) magnetic relaxation time. High sensitivity of the technique led to significant reduction of the number of PCR cycles.

The ability of nucleic acids to hybridization of complementary stands is a prerequisite to the development of a convenient and highly sensitive method for detection of specific DNA/RNA in biological samples. Immobilization of a specific oligonucleotide sequence on the surface of MNPs enables highly sensitive pathogen detection,[220] as well as detection of specific nucleic acids,[181] DNA-binding molecules and development high-throughput genotyping systems.[221,222]

Isolation of specific nucleic acids from samples in order to improve the efficiency of subsequent PCR-analysis is attained using MNPs bearing an oligonucleotide sequence complementary to the matrix RNA (mRNA) or fragment of the DNA target (gene of interest).[178,180,209] Magnetic nanoparticles are also used in elaboration of methods for SNP-analysis in order to detect DNA/mRNA molecules with a single base mismatch for diagnosis of diseases.[82,223]

Attempts to improve diagnostic systems based on MNPs are aimed at enhancing the sensitivity and reducing the time of analysis by excluding, *e.g.*, the amplification step.[224]

A method of amplification-free herpes simplex virus DNA assay in picomolar concentration using MNPs and fluorescent detection was developed.[74] An original method for fluorescent detection of DNA with a detection limit of $<40\times10^{-12}$ mol litre^{-1} was reported.[179] Riboflavin-5′-monophosphate was bound to the surface of Al_2O_3-coated Fe_3O_4 nanoparticles *via* phosphate-Al chelation. Binding to iron oxide nanoparticles was followed by quenching of riboflavin fluorescence. Once DNA was added, riboflavin was displaced in solution by the PO_4 group of DNA, its fluorescence enhanced and could be measured.[179]

Methods for targeting of nucleic acids using MNPs with electrochemical,[174] chemiluminescent,[87, 175] surface plasmon resonance[225] and light scattering[226] detection were developed.

Conjugation of MNPs with aptamers offers prospects for the development of methods for detection of specific proteins[227] and pathological cells.[191] An approach for ultrasensitive fluorescent immunoassay based on conjugation of biotin-labelled oligonucleotide with streptavidin-coated MNPs was proposed.[210]

A DNA molecule immobilized on MNPs can serve as substrate for binding genotoxic substances for isolation and subsequent analysis.[83]

Exclusion of nonspecific interaction of nucleic acid molecules is an important step in the design of diagnostic systems. A possible method to prevent nonspecific adsorption of nucleic acids as *via* electrostatic interactions with the surface is to coat MNPs with negatively charged polymers.[178, 193] Potential binding sites for nucleic acids and proteins can also be blocked by polymer molecules.[74, 87, 175]

To avoid nonspecific interactions between the immobilized oligonucleotide (probe) and the surface of a nanoparticle and to eliminate steric hindrances to subsequent hybridization, the DNA probe is immobilized using linkers. These can be cross-linker molecules[178] or additional nucleotide[177, 182, 183] or hydrocarbon spacers.[68, 80, 186]

One should take into account the fact that certain parameters of free DNA and DNA conjugated with nanoparticles can be different.[14] For instance, the melting temperature of a double-stranded DNA formed by hybridization of single-stranded oligonucleotides attached to nanoparticles is higher and the temperature dependence of denaturation of the DNA helix (melting curve) is much steeper than for free DNA in solution. Also, an increase in the nanoparticle grafting density, as well as extension of the spacer between the MNP surface and the probe leads to an increase of the melting temperatures of the DNA-duplex being formed.

3. Magnetosensitive biosensors

Progress in applications of MNPs for *in vitro* diagnosis is aimed at fabricating magnetosensitive biosensors whose surfaces are functionalized for specific recognition of molecular targets. There are three types of magnetic sensors based on MNPs depending on the physical principle underlying the operation of the device and on the signal detection technique.[228]

1. Magnetic relaxation switch (MRS) sensors. Their operation is based on the ability of MNPs to change the relaxation rate of water protons of surrounding molecules. Binding of MNPs to the target molecule produces a local inhomogeneity of the magnetic field, the spin–spin relaxation time is shortened (or the relaxation rate increases) and the contrast of the MRI image changes. Aggregates formed by MNPs upon binding to the target analyte enhance dephasing of the spins of surrounding water protons compared to dephasing caused by MNPs in the dispersed state.[196] For instance, there is a method involving MNPs bearing two types of oligonucleotide sequences complementary to two ends of the oligonucleotide target. In the presence of the oligonucleotide target, MNPs form large aggregates, which can be detected by measuring the magnetic spin–spin relaxation time.[68] Note that aggregation/dissociation of MNPs upon binding to the target can be initiated by varying the temperature or pH value, as well as by increasing the concentration of the target molecules; in all cases, a signal is produced. The size of the MNP aggregates depends on the nature and concentration of the target nucleic acids.[229] This type of sensors is characterized by high sensitivity and can be used to study DNA–DNA interactions,[230] enzymatic activity[79, 185] and to target specific protein molecules (Fig. 7).[182, 183]

A mercury sensor based on Fe_3O_4 nanoparticles conjugated with oligonucleotides was reported.[231] In this case, aggregation is due to the ability of mercury ions to form cross-links between thymine bases.

Measurements are performed with NMR relaxometers (MRI scanners); benchtop models are widely used. Recent advances in the field include fabrication of small-size on-chip NMR analysers.

2. Magnetic particle relaxation (MPR) sensors. Here, signal detection is based on measurements of the relaxation of particle magnetic moments after switching off external magnetic field. Usually, measurements are carried out with SQUID-magnetometers. MPR sensors enable detection of trace amounts of specific DNA molecules present in concentration $(3-10)\times10^{-12}$ mol litre^{-1}.[232] A method for

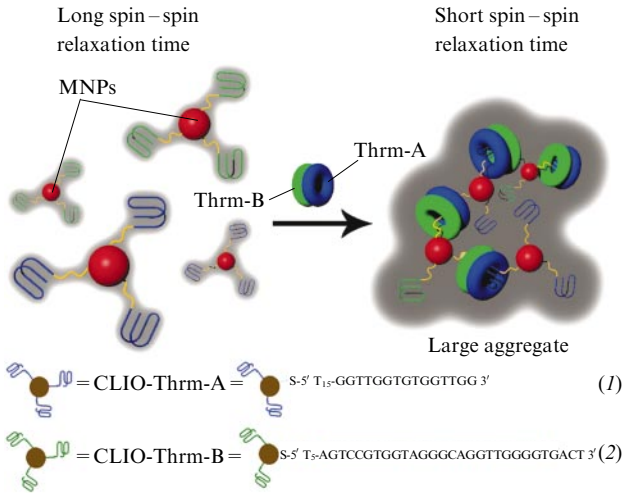


Figure 7. The operating principle of a MRS sensor for thrombin detection: a schematic illustration.[183]
Magnetic nanoparticles were modified with either Thrm-A, an antithrombin aptamer that binds to fibrinogen-recognition site (*1*), and Thrm-B that binds to the heparin-binding site (*2*). The addition of thrombin to the medium causes aggregation of the aptamer-functionalized nanoparticles; this can be detected from shortening of the spin–spin relaxation time.
CLIO is Cross-Linked Iron Oxide.

multiplex detection of specific DNA sequences in the sample using the MPR sensor was proposed.[233] If the sample contains the target DNA-sequences, probe ends are closed and their ligation occurs. Then, 'rolling circle' amplification of DNA proceeds, resulting in single-stranded concatemers that form a DNA coil about 1 mm in diameter. The addition of different-size MNPs functionalized with oligonucleotides complementary to a DNA coil to the amplification mixture is followed by their hybridization and formation of a large aggregate. Aggregation can be detected with a SQUID magnetometer.

Further improvement of the technique is aimed at developing less expensive detectors, for instance, sensors based on the Hall effect.[187]

3. Magnetoresistive sensors generate responses to the presence of MNPs at the surface of electrodes sensitive to changes in magnetic field.

Target molecules are detected using methods of direct (analyte conjugated to MNPs interacts with the modified surface of the electrode) or indirect (based on the 'sandwich' principle used in enzyme-linked immunosorbent assay) interaction. In the former case, the streptavidin–biotin system or recognition of complementary DNA is used.[234] There are two types of magnetoresistive sensors, namely, giant magnetoresistance (GMR) sensors and magnetic tunnel junction (MTJ) ones. In both cases, different-size MNPs are used. These sensors may exhibit a sensitivity of 10^{-12} mol litre^{-1} for DNA detection.[235] The operating principle of the GMR-sensor for detection of specific DNA sequences (amplified by PCR) sandwiched between an antibody at the sensor surface and a streptavidin coated superparamagnetic particle is illustrated in Fig. 8.

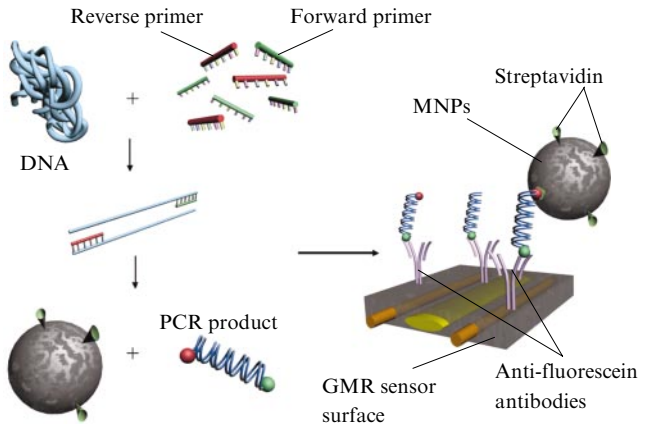


Figure 8. Detection of specific PCR product (DNA) using a GMR sensor and MNPs.‡
The polymerase chain reaction is carried out using specific primers, *viz*., a 5′-biotin forward primer and a 5′-fluorescein reverse primer. The PCR product is mixed with streptavidin-coated superparamagnetic particles. The surface of the GMR sensor is functionalized with anti-fluorescein antibodies; the formation of an antibody–PCR product–MNPs 'sandwich' at the sensor surface causes generation of a signal.

‡ M Koets, T van der Wijk, J T W M van Eemeren, A van Amerongen, M W J Prins *Biosens. Bioelectron.* **24** 1893 (2009).

In the presence of superparamagnetic iron oxide nanoparticles on the surface of a MgO-based MTJ sensor the device generates a signal that is proportional to the coverage of MNPs.[71] This effect underlies the design of a sensor for detection of DNA in concentration 2.5×10^{-6} mol litre^{-1}. To this end, the sensor surface was pre-passivated with SiO_2 and spin-coated with PEI. Then, a single-stranded DNA probe was immobilized on the sensor surface and hybridized with complementary target 3-′biotin-labelled DNA. Streptavidin-coated iron oxide nanoparticles bound to these DNA molecules at the sensor surface.

At present, MNPs are used in different types of biosensors in order to enhance their sensitivity and to increase the throughput. Improvement of composites based on MNPs, modulation of magnetic parameters during the synthesis of nanoparticles, as well as development of novel measuring facilities predetermine high potentialities and great prospects for future progress in this field.

4. *In vivo* applications of magnetic nanoparticles

Key limiting factors for *in vivo* applications of nanobiocomposites include the particle size and biocompatibility. In this case, the prime objective in the design of coatings for MNPs is to stay on nano scale in biological media.[236] The final diameter and shape of the nanobocomposites are of great importance for safe intravenously administration. These parameters correlate with the biodistribution of the nanoparticles, rate of their accumulation in organs, the blood circulation time, renal clearance, uptake and elimination by the reticuloendothelial system.[237, 238]

The optimal size of nanobiocomposites ranges from 10 to 150 nm. Penetration of large aggregates through capillary walls is hampered; moreover, they can cause embolism.[237] However, nanoparticles 5 nm in diameter are excreted renally within a few minutes while required blood circulation times for MRI-contrast agents is sufficient longer (hours). An increase in the diameter or length of a nanobiocomposite often leads to extension of its circulation time.[238]

The cellular uptake of MNPs depends on cells vary significantly depending on the cell type, incubation conditions, surface properties, type and purity of nanomaterials. Usually, MNPs penetrate into cells by endocytosis.[239] However, some types of nanoparticles can penetrate the cell membrane and nuclear membrane by passive transport and even active transport.[240, 241] Penetration into cells depends on the nature of ligands on the surface of MNPs.[236, 242] Coating of nanoparticles and attachment of specific ligands to the surface may favour efficient penetration into cells.[243]

Positively charged nanoparticles nonspecifically bind to cells and penetrate into them more readily than negatively charged nanoparticles.[244] The latter rapidly accumulate in liver while neutral nanoparticles show the longest circulation time.[238]

Nanoparticles or structures based on them can form aggregates in the presence of serum proteins. This reduces the expected efficiency of cellular uptake or even causes cytotoxic effect.[237] Improved biocompatibility is attained by coating nanoparticles with, *e.g*., dextran, PEG, albumin and silica. Coating with dextran precludes opsonization of MNPs introduced into the bloodstream by cells of reticuloendothelial system.[40, 245] Modification of nanoparticles with PEG reduces their uptake by macrophages; polar and

nonpolar groups attached to the surface of nanoparticles favour more efficient penetration through the cell membrane.[65] Biocompatibility of other polymers used to form nucleic acid–MNP composites can be improved by modification. For instance, complexes of polycationic polymers with DNA are characterized by high density of positive charges, which leads to adsorption of negatively charged blood proteins and may invoke cytotoxicity. Adverse effects can be significantly reduced using copolymers with PEG.[246] Alkylation of branched PEI improves its stability in physiologocal conditions and enhances the transfection efficiency.[247] An alternative route to biocompatibility is to confine nanoparticles or structures based on them within a phospholipid bilayer (formation of liposomes) that can fuse with cell membrane.[248]

More detailed information on biocompatibility of MNPs and nanostructures based on them can be found in the literature.[13, 23, 249]

5. *In vivo* studies using magnetic nanoparticles

Magnetic resonance imaging is widely used for detection of pathologies in the body.[250] Magnetic nanoparticles influence the resonance frequency and relaxation rate of nuclear spins of surrounding water protons, namely, signal broadening occurs and the relaxation rate increases.[13] The use of, *e.g.*, magnetite MNPs as MRI contrast agents enables significant enhancement of the signal and improves the sensitivity of the technique.[9]

Grafting of probe molecules onto the surface of MNPs offers prospects for MRI-detection of femtomolar concentrations of pathological (including tumour) cells or target molecules in organisms.[9] Renshaw *et al.*[251] were the first who proposed to use 'molecularly targeted' nanoparticles and prepared a composite based on dextran-coated iron oxide nanoparticles bearing antibodies on the surface.

Methods of application of oligonucleotide–MNPs conjugates for MRI detection of specific mRNAs in cells are developed intensively.[252] Conjugation of MNPs with DNA leads to abrupt increase in the relaxivity of proton signals in T1-weighted MRI sequences and corresponding signal enhancement and improved sensitivity of MRI analysis even at low magnetic field.[95]

Conjugates of DNA with cobalt ferrite and Fe_2O_3 nanoparticles are promising contrast agents. To improve their biocompatibility, the system DNA–MNPs was encapsulated by coating with a number of alternating layers of cationic and anionic polyelectrolytes [polyallylamine hydrochloride and poly(styrene sulfonate)].[238] A comparison of the MRI phantoms obtained in water and in matrigel showed that encapsulation led to a decrease in relaxivity of the nanocomposites. However, tests in cell culture revealed no differences between the MRI signals of encapsulated and non-encapsulated nanocomposites. This is due to the fact that cell uptake of encapsulated nanocomposites was three times more efficient compared to non-encapsulated nanocomposites.

6. Therapy using nucleic acid–magnetic nanoparticle composites

The idea of targeted drug delivery using MNPs was for the first time proposed in 1978.[253] Taking into account permeability of biological tissues to magnetic fields, local accumulation of MNPs and composites based on them in a particular region of body (organ) and control of the behaviour of MNPs by of alternating magnetic field seem to be possible.

Small size of nanoparticles ensures safe parenteral administration of nanocomposites based on them without risk of embolism.[19] An additional advantage of most drug delivery systems based on MNPs consists in the possibility to be detected by MRI. Targeted delivery to a certain organ or into particular cells is possible.

Gene therapy is a promising method of treatment of various human diseases including monogenic, multifactorial, oncological, infections, degenerative and neurological ones.[254] Targeted delivery of therapeutic nucleic acids to cells can change gene expression as well as initiate or suppress the synthesis of particular proteins. Practically, gene therapy progresses towards the design of highly specific systems for delivery and stabilization of therapeutic molecules (nucleic acids) and improvement of transfection efficiency.[255, 256] Gene therapy using nucleic acid–MNP nanocomposites to deliver DNA/RNA therapeutical molecules into target cells seems to be a good alternative to viral vectors because there is no risk of immune response and development of infections diseases.[40] Functionally active DNA [257] and RNA molecules immobilized on MNPs were successfully transfected into pro- and eucaryotic cells.[258]

Delivery of plasmid DNA to human respiratory epithelial cells using superparamagnetic particles was reported.[259] Experiments on transfection of small interfering RNAs (siRNAs) conjugated with MNPs in HeLa cells were carried out and changes in gene expression were analyzed.[203] High level of transfection of antisense RNAs (asRNAs) immobilized on MNPs was attained in the *in vitro* experiments with human cancer cell line, namely, MCF-7 breast cancer (Fig. 9), melanome MDA-MB-435 and liver carcinoma HepG2.[67] Superparamagnetic iron oxide nanoparticles conjugated with plasmid encoding interleukin-12 was successfully used for immuno-gene therapy of murine adenocarcinome.[170] Composites based on PEI-modified MNPs were utilized to improve the efficiency of antimalarial DNA-vaccine.[63]

It should be noted that conjugation of nucleic acids with MNPs often enhances resistance of biomolecule to nuclease degradation.[260] This favours a solution to the problem of abrupt reduction of the efficiency of transfected genes owing to fast cleavage with endo- and exonucleases.

Composites based on MNPs used as transfecting agents improve the efficiency of transfection under the action of alternating magnetic field (magnetofection).[203, 261–264]

It should be noted that the expression efficiency of a gene being transfected in the complex with nanoparticles can vary over a wide range depending on the MNPs/DNA molar ratio and on the cell type.[171]

Nucleic acid–MNPs nanocomposites are developed as components of drug delivery systems with controlled drug release. A possible design for the preparation of such a composite is as follows.[188] Iron oxide nanoparticles are encapsulated in mesoporous silica matrix; single-stranded DNA molecules are immobilized on the silica surface and then hybridized with complementary molecules attached to the MNPs. Heating of nanoparticles by alternating magnetic field causes denaturation of DNA-duplex. As a result, pores on the silica surface are opened and fluorescein (used as model drug in that study) is released. On cooling to 20 °C complementary oligonucleotides are hybridized with each other and pores are blocked by nanoparticles (Fig. 10).

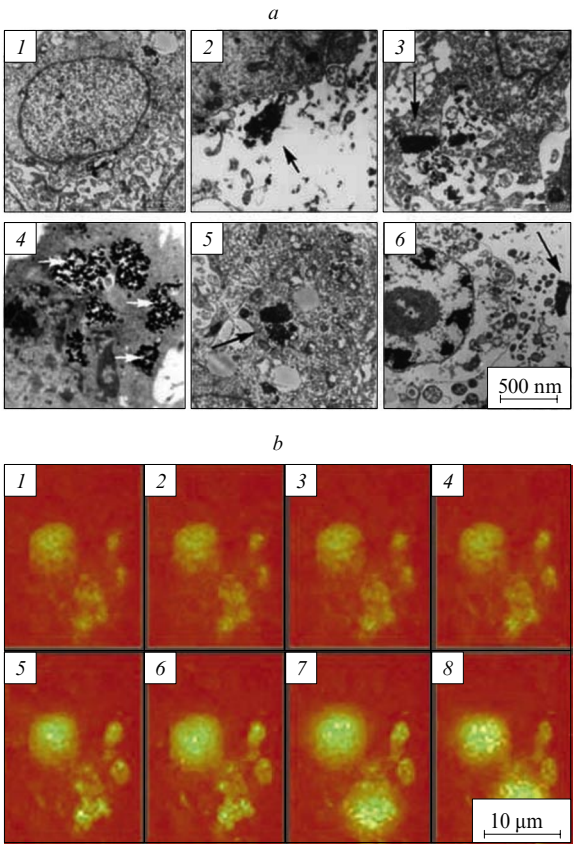


Figure 9. Ultrastructure visualization of MCF-7 cells using a high-resolution transmission electron microscope (*a*) and a confocal laser microscope (*b*).[67]
(*a*) No MNPs–asODN added (*1*); after incubation with MNPs–asODN for 0–4 (*2*), 4–12 (*3*), 12–24 (*4*), 24–48 (*5*) and > 96 h (*6*); *b*: after incubation with MNPs–asODN for 1 (*1*), 3 (*2*), 5 (*3*), 7 (*4*), 10 (*5*), 15 (*6*), 20 (*7*) and 30 min (*8*).

In spite of the advances achieved, there are some problems that preclude wide clinical applications of drug delivery systems based on MNPs. They include risk of embolism due to accumulation of nanoparticles in the target region, as well as possible toxic effects. At present, it is still impossible to suppress activation of cells of reticuloendothelial system upon introduction of nanocomposites. High efficiency of magnetic targeting using therapeutic complexes based on MNPs was demonstrated on cell culture and small laboratory animals, but it is not always possible for humans because very strong magnetic fields are required.[237] In this connection, the search for and design of nanocarriers are being in progress.

7. Multifunctional magnetic-fluorescent nanocomposites

Fabrication of multifunctional magnetic-fluorescent nanocomposites is a promising area of nanobiotechnology. These systems are attractive because they can be simultaneously controlled by two independent methods based on different physical principles. Magnetic-fluorescent nanocomposites are used for solving a number of topical biomedical problems, such as *in vitro* and *in vivo* fluorescent and MRI visualization of, *e.g.*, targeted drug delivery, improvement of the efficiency of tumour therapy (by combining photodynamic therapy and hyperthermia), cytometry and magnetic separation of biomolecules, as well as fabrication of highly sensitive chemo- and biosensors.[184, 265–267]

A magnetic-fluorescent nanocomposite was prepared [186] using MNPs coated with aminated dextran and successively conjugated with cyanine fluorescent dye (Cy5.5), peptide [Myr-Ala-$(Arg)_7$-Cys] for efficient cell uptake of the nanocomposite and siRNA that suppresses gene expression of green fluorescent protein (Fig. 11). In the second series of experiments, survinin siRNA was used. The nanobiocomposite was used for the fluorescent and MRI detection of siRNA delivery to the tumour.

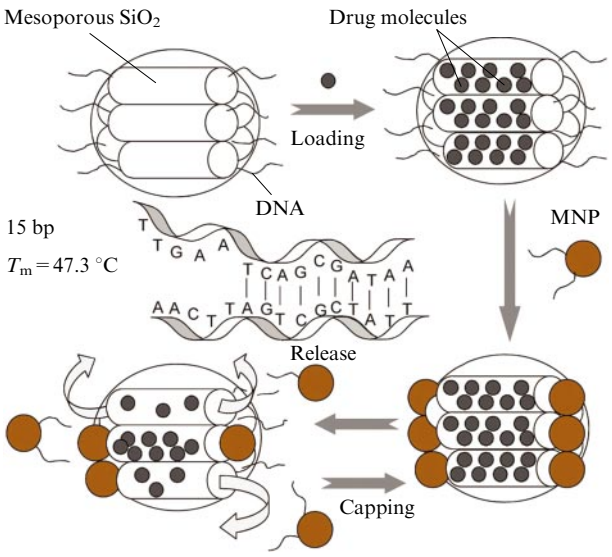


Figure 10. Nanogate based on MNP-modified mesoporous silica particles for controlled drug release through DNA hybridization/dehybridization.[188]

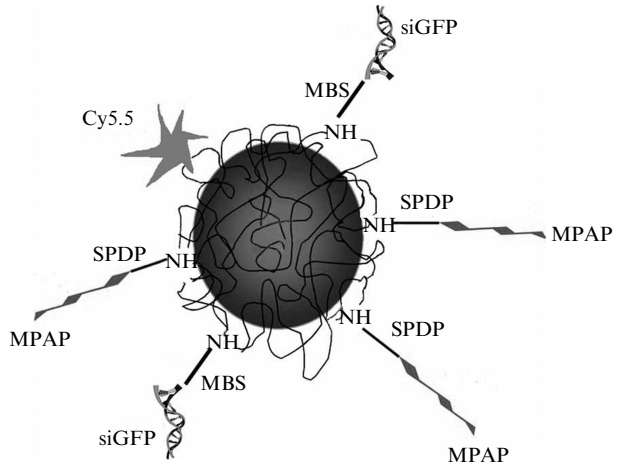


Figure 11. Multifunctional magnetic-fluorescent nanocomposite for MRI and gene silencing in tumours.[186]
Cy5.5 is a fluorescent cyanine label, MBS and SPDP are heterobifunctional cross-linkers, siGFP is siRNA against green fluorescent protein gene expression and MPAP is a peptide serving as a membrane translocation module.

Another interesting example is provided by a magnetic-fluorescent nanocomposite based on magnetite nanoparticles coated with silica modified with tris(2,2′-bipyridyl)ruthenium fluorophore and functionalized with AS1411 aptamer that contains 26 guanine residues and can bind to nucleolin overexpressed on the surface of tumour cells. The nanocomposite was successfully used for fluorescent and MRI visualization of tumours. The nanocomposite also acted as photosensitizer for photodynamic therapy of tumour. In addition, the G-quadruplex was intercalated with 5,10,15,20-tetrakis(1-methylpyridin-4-yl)porphyrin whose macromolecule could penetrate into tumour cells upon binding the nanocomposite to nucleolin.[177]

Magnetic-fluorescent nanocomposites are also on demand for nanoelectronics because positions occupied by MNPs on templates can be visualized using a confocal microscope.[266]

VI. Genotoxic effects of magnetic nanoparticles

Genotoxic effects of MNPs can occur through direct interaction with DNA (DNA binding or DNA breaks) or indirectly (oxidative stress mediators, enzyme activators/inhibitors).[22] As a consequence, DNA damage may lead to mutations, cell malignization and apoptosis. Nanoparticle-binding may cause a negative impact on the structure, functioning and stability of the biomolecule.[22, 268, 269]

Experiments in cell culture showed that superparamagnetic iron oxide nanoparticles coated with carboxy dextran can have an impact on cytoskeleton and cell proliferation and to alter the expression of various genes involved in cell cycle regulation, cell signalling, genes of growth hormones and regulation of iron homeostasis.[22] Cobalt ferrite MNPs can influence gene expression both in cell culture and *in vivo*, namely, DNA damage and repair, apoptosis, carcinogenesis, inflammation, oxidative stress and growth arrest.[270] It is believed that changes in gene expression are primarily due to oxidative stress and to the response to damage caused by reactive oxygen species. Local changes in the concentration of iron can also contribute to the process.[22]

Many researchers emphasize that toxic effect of nanoparticles is concentration dependent.[271, 272] The character of the impact of nanoparticles on the genetic system to a great extent depends on their physical parameters including the size, shape, specific surface area, charge, composition and the type of the crystal lattice.[273]

A study in fibroblast cell culture revealed that the toxic effect of superparamagnetic iron oxide nanoparticles on DNA depends not only on the concentration of MNPs, but also on the nature of functional groups attached to surface [O^- (uncoated nanoparticles), OH, COOH, NH_2] and on the charge of the MNP surface.[236] It was emphasized [23, 270] that coating of MNPs significantly reduces toxic effects. Nevertheless, genotoxic effects observed in fibroblasts after incubation with uncoated iron oxide nanoparticles were much less pronounced than, *e.g.*, those observed using iron oxide nanoparticles modified with (3-aminopropyl)trimethoxysilane. This was explained by the fact that the modified MNPs bear positive charge, which favours direct interaction with negatively charged DNA molecule. Citrate-modified MNPs bearing a negative charge also had a stronger genotoxic effect compared to unmodified particles; this was associated with activation of the generation of reactive oxygen species.[236]

DNA damage induced by MNPs mainly is a consequence of generation of reactive oxygen species involving transition metal ions. Experiments [274] revealed increased mutation frequency of *E. coli* incubated with γ-Fe_2O_3 nanoparticles due to increased concentration of Fe^{3+} cations in the medium. Other transition metal ions (manganese, cobalt, nickel) can also participate in Fenton-like reactions that proceed as follows:[275, 276]

$$M^{n+} + H_2O_2 \longrightarrow M^{n+1} + OH^- + HO^{\bullet}$$

$$M^{n+1} + H_2O_2 \longrightarrow M^{n+} + H^+ + HOO^{\bullet}$$

Damage of plasmid DNA after incubation with nickel and cobalt MNPs 20 nm in diameter also originates from activation of free-radical processes.[277]

Activation of inflammatory processes as a result of oxidative stress upon inhalation of technogenic nanoparticles containing transition metals (Fe, Mn) is considered among key pathways of cell malignization.[18, 278–280] On the molecular level, one deals with the formation of DNA adducts, DNA breaks and oxidative damage of DNA associated with the formation of 8-hydroxydeoxyguanosine.

Oxidative damage of DNA depends strongly on the MNP size and on the release rate of transition metal ions.[19, 281, 282] For instance, 6-nm and 10-nm-diameter cobalt ferrite particles produced a genotoxic effect, although at different concentrations (5×10^{-6} mol litre^{-1} and 5×10^{-5} mol litre^{-1} or more, respectively), whereas 120-nm particles appeared to be safe. This was explained by negative effect of the cobalt ions. The proportion of cobalt ions transferred in solution was 30% after 48 h for the 6-nm particles (*cf.* 0.01% for the 10-nm-diameter particles). It is important that modification of the 6-nm particles with functional groups reduced the genotoxic effect because the proportion of cobalt ions transferred in solution decreased to 2.45%.[283]

The authors of a study [284] of the influence of cobalt MNPs on T-lymphocytes explained the observed DNA damage by the release of cobalt ions. When applied *in vivo*, these ions can induce both direct and indirect DNA damage, namely, DNA break, covalent cross-linking of protein to DNA owing to production of free radicals, as well as replacement of metal ions in 'zinc-finger' proteins and metal-containing enzymes leading to their inactivation.

The experimentally found ability of Co^{2+} and Mn^{2+} ions to activate DNA cleavage by topoisomerase II can also be a reason for DNA damage in the presence of Co- and Mn-containing MNPs.[123, 285] Transition metal ions can disturb the functioning of DNA repair systems.[286, 287] Possible pathways of genotoxic effects of MNPs are presented in Fig. 12.

It should be noted that toxic effects of MNPs on DNA molecules and genetic system are first of all due to specific features of nanoparticles and nanosized structures. An important role is also played by the chemical composition, MNP size and dose. Design of nanocomposites based on particular MNPs in order to solve specific problems requires a balanced approach including optimization and equilibrated between possible benefits and adverse effects.

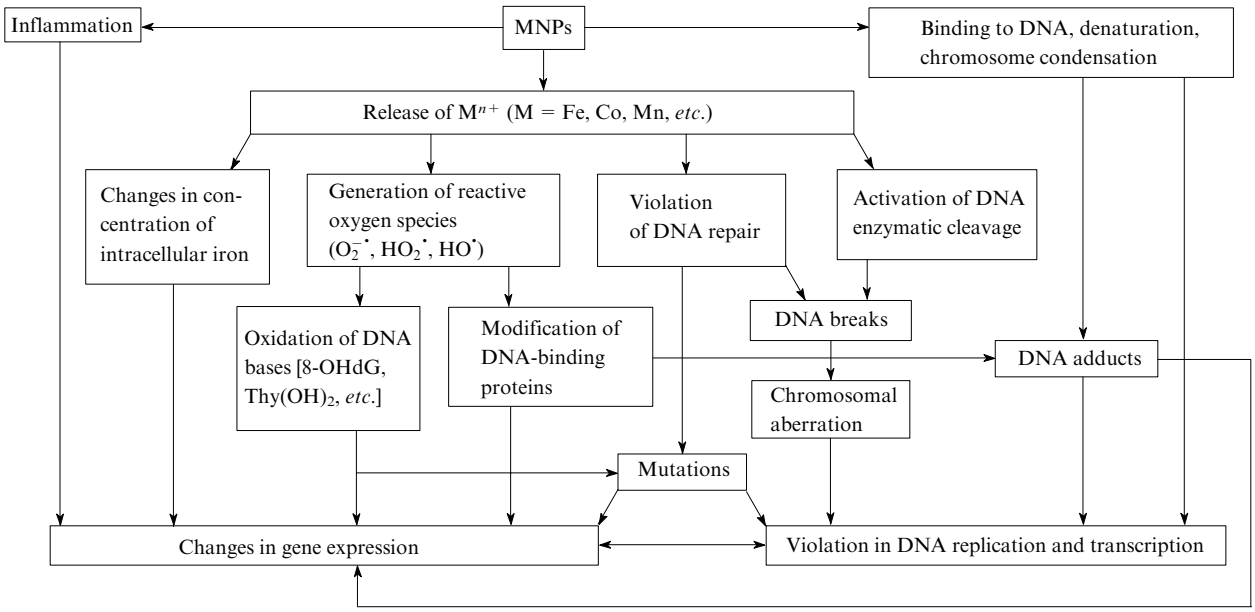


Figure 12. Harmful effects of MNPs on the genetic system: possible routes to cell malignization and/or cell death.

VII. Conclusion

Nucleic acid – MNP nanobiocomposites hold great potential for nanoelectronics and nanomedicine. In spite of impressive advances in nanobiofabrication achieved in the last two decades, many problems are still to be solved. The primary goal of nanobiotechnology is fabrication of smart hybrid nanodevices biomolecule – nanoparticle *de novo* — nanorobots, nanotools and nanomanipulators.

In this connection, research in the fields of nanoelectronics, fabrication of relatively cheap, high-throughput nanosized sensors with high noise immunity, sensitivity and specifity, as well as the design of programmed nanodevices for repair of different-level biological systems become of crucial importance.

Progress in nanobiotechnology is aimed at deeper interpenetration of nanoelectronics and biotechnology, consolidation of the results obtained, where nanodevices are based on biomolecules, while biological systems are studied and controlled by nanoelectronics devices and sensors. Generation of knowledge on the regularities of the interaction between nanoparticles and biomacromolecules underlies progress of future technologies.

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