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CHEM
THE ALL RESULTS JOURNALS

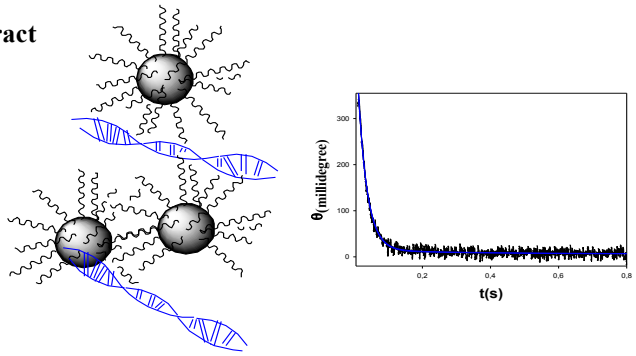
Issue 1, Vol 4, 2013, 1-9

AuNPs-DNA non-covalent interactions: improve of some studies and new avenues of research from negative results.

David Alcantara[1*], Elia Grueso[2], P. M. Castillo[2], Rafael Prado-Gotor[2*]

1) Andalusian Center for Nanomedicine and Biotechnology (Bionand), Severo Ochoa, 35, 29590 Campanillas. Malaga, Spain; 2) Department of Physical-Chemistry, University of Seville, Profesor Garcia Gonzalez, S/N, 41012, Seville, Spain. E-mail: dalcantara@bionand.es; pradogotor@us.es. * To whom all correspondence should be addressed.

Grafical Abstract



Abstract: Due to their chemical stability, high biocompatibility, excellent structural, optical, magnetic and catalytic properties, gold nanoparticles (AuNPs) have been widely used as therapeutics, delivery agents and transfection vectors. Since successful therapy for curing cancer, and other genetic diseases, requires the transport of DNA into the cell by delivery vehicles, the effective complexation of the DNA is a subject of great interest. In this sense, increasing concerns have been raised in regards to the thermodynamics and kinetics of non-covalent interactions of AuNPs with DNA. Although insights have been gained into the effects of AuNPs on DNA systems, there is still much ground to be covered, particularly in respect to our knowledge of the binding modes, conformational changes, the salt effects and the aggregation properties of these systems. This review highlights recent progress in the study of the interactive effects of AuNPs with DNA and the factors that influence the kinetics and thermodynamic of this interaction.

Keywords: *gold nanoparticles, DNA, biopolymers, thermodynamic, kinetics.*

Introduction

Damaging the replication machinery of DNA, either by covalent or non-covalent binding, is how normal anticancer drugs exhibit their antitumoral properties. Intercalation and groove fitting are the major modes of non-covalent interaction (NCI).[1,2] NCI, those that occur between chemical species (molecules, ions, etc.), do not involve the formation of a covalent bond between the interacting species and play a key role in different fields of chemistry and biology, such as antigen/antibody interaction, the processes of solar energy conversion, environmental chemistry, self-aggregation phenomena, etc. Specifically, the recognition of proteins' surfaces provides an attractive tool to regulate protein-protein interactions and enzymatic activities. Recently, research has been directed to the use of nanoparticles (NPs) as receptors

that bind to the protein's surface through multivalent interactions. The binding affinity and stability of NPs can be adjusted by 1) the surface's composition of the nanoparticle and functionality, and 2) varying buffer ionic strength. On the other hand, the size of nanoparticles is comparable to that of biomacromolecules, providing an efficient scaffold for binding biomacromolecules. Positively charged nanoparticles can be associated with DNA molecules to give very stable complexes, resulting in the disruption of DNA transcription. Furthermore, complex formation can also be exploited to protect DNA from enzymatic digestion, and positively charged nanoparticles can act as vectors to transfer DNA into cells.[3] Studies concerning non-covalent interactions between nanoparticles and biopolymers are very few and have only been conducted recently. Published studies concerning nanoparticle-biopolymer interactions involving DNA and lysozyme [4], cytochrome C[5] or albumin[6] as model proteins, are very recent compared with studies concerning other types of substrate/receptor complexes. The importance of studying NCI is completely understood considering the so-called conformational diseases, like Alzheimer's disease, where β-amyloid peptide undergoes a conformational change that leads to self-aggregation, giving rise to toxic species. Rotello et al. have described how highly charged nanoparticle-based hosts could serve as refolding agents by interacting with charged residues on denatured proteins, facilitating refolding and preventing aggregation.[7] This review focuses on different aspects of NCI nanoparticles/biopolymers and how negative results could be a challenge to new avenues of insight.

2. Nanomaterials and biomolecule interactions

A large number of applications in the field of nanotechnology research are directly related to the binding of metal nanoparticles to sugars[8], proteins[9], dendrimers[10], surfactants[11], small ligands[12] or DNA.[13] Nanoparticles have been used for the construction of new materials, development of bioassays and as multivalent systems to study the interactions of different substrates/ligands. Especially interesting is the development of so-called glyconanoparticles for the study of interactions between carbohydrates. Of these, gold nanoparticles are the most stable metal nanoparticles with excellent biocompatibility and with promising applications, particularly due to their structural, electronic, optical, magnetic and catalytic properties.[14] These interesting properties are having a significant impact in many areas of science, particularly in materials science and molecular biotechnology.[15-17] Faced with metal or semiconductor particles, the properties of nanoparticles, in general, are characterized not only by those related to the metal cluster which constitutes the core of the structure, but also by the organic molecules on their surface, whose principal function is to confer stability to the structure and prevent the formation of metallic clusters. More specifically, NPs with alkanethiolates as protective agents, have received considerable attention because they confer certain advantages over other agents system: high stability, solubility in water, suspensibility in different solvents, simple characterization by standard analytical techniques such as NMR, UV-Vis and TEM[18] and a flexible and versatile functionalization. Moreover, alkanethiolate gold nanoparticles are stable in physiological conditions; they are bioinert and provide non-toxic carriers for drug and gene delivery applications.[18] During the last few decades, researchers have focused on the development of systems of nanoparticles covalently bonded to DNA.[19] Some applications are related to the diagnosis and treatment of diseases[20], detection of pathogens[21], DNA biosensing[22], probes and drug transport[23] and fabrication of biosensors.[24-26] Most of these studies have been done with small DNA molecules and oligonucleotides. However, studies of non-covalent interaction DNA/nanoparticle[27], and more particularly with long chains of DNA, are relatively scarce in scientific literature.[27a,28]

Nanomaterials with high levels of porosity are suitable to achieve a more controlled application of drugs. In gene therapy, the success depends on developing safe and effective gene vectors. Non-viral vectors, nanoparticles and lipid-DNA polymer complexes have been proposed as alternatives to viruses for introducing specific genes to cells.[29] Nanostructures are often unstable because of the small size of their constituents and their high chemical activity. Therefore, a major challenge is to increase the thermal stability and structural chemistry of these materials.

Recently, sensors consisting of metal nanoparticles functionalized with DNA have appeared in the literature. It has been shown that these particles show affinities to the ligands that are, at least, two orders of magnitude greater than other conventional sensors.[30] This has been attributed to the high density (packing) of the DNA on the nanoparticle's surface, which provides multiple binding sites for the ligand (multivalency effect). However, affinity does not increase proportionally to packing, as it is necessary that a particular level of packaging is needed for optimizing ligand detection.[31] The optimization of these interactions play an important role because of the possibility of synthesizing nanoparticles coated with amphiphilic or similar substances, which are able to act as vectors for gene transport and are much safer than the usual viral vectors, with the advantage of not causing immunological responses.[32] It is known that the complex DNA vector must fulfill two main requirements: a) be relatively small and b) remain stable in the bloodstream.

Inhibition of DNA hybridization by metal nanoparticles has been studied.[33] In these studies, small oligonucleotides are used and the non-specificity of the interaction is discussed

using melting techniques, UV-visible and transmission electron microscopy. DNA melting point analysis showed that the oligonucleotides adsorb strongly and nonspecifically on small metal nanoparticles, inhibiting the hybridization of complementary DNA sequences in common buffered solutions.

Indeed, studies related to the nanoparticle-nucleic acid interactions focus mostly on the primary objective of a) the construction of biosensors, b) the synthesis of efficient vectors for the so-called photodynamic therapy (PDT)[34] and c) obtaining inorganic nanoparticles as carriers of nucleic acids to cells.[35] In this regard, there are studies related to the use of gold nanoparticles functionalized with short oligonucleotide DNA sequences "linkers"-O-(CH2)-SH in the extreme 5 'end of DNA (authors used only 24 nucleotides).[36] Other studies are concerned with the adsorption of single–stranded oligonucleotides on gold nanoparticles stabilized with sodium citrate. It appears that the displacement of citrate ions that stabilize the gold nanoparticles depends on the DNA sequence.[37]

Gold nanoparticles “mix and read” sensors, together with sequence-specific oligonucleotides, have been used for the detection and recognition of mercury ions through changes from red to blue experienced by the electronic absorption band characteristic of gold nanoparticles (surface plasmon resonance).[38] The synthesis of highly fluorescent gold nanoparticles to sense Hg have also been reported. These nanoparticles consist of alkanethiols and can be modified depending on the alkanethiol chain.[39] The method is based on aggregation-induced quenching of the fluorescence of 11-mercaptoundecanoic acid (11-MUA) protected AuNPs (11-MUA-AuNPs; (2.0 ±_0.1) nm) in the presence of 2,6-pyridinedicarboxylic acid (PDCA). This work provided the first example of a system for sensing Hg (II) ions based on fluorescence quenching through Hg (II)-induced aggregation of AuNPs.

3. Thermodynamic and kinetic DNA-nanoparticle interaction

It is well known that within the nucleus of eukaryotic cells, and more specifically within the chromatin, DNA is packaged and tightly bound to proteins. Chromatin is a complex of DNA and proteins, whose protein component is designated along with the name of histones (H1, H2A, H2B, H3 and H4). They contain many residues of arginine and lysine, which gives them a positive charge, its structure being quite similar in all organisms with a size of 7 nm.[40] The positive charge of the histones, allows rapid bonding to DNA through the negatively charged phosphate groups. A key feature is that the chromatin structure also allows the orderly packaging of DNA molecules, therefore also allowing important processes to be carried out, such as the expression of genetic information and DNA replication. This is why the study of these structures is of such high importance. However, the precise structure of the chromatin fibre in the cell is not known in detail, and there is still some debate over this. In this regard, the DNA-nanoparticle system may be a good model to simulate the interactions occurring between proteins and DNA on histones. Complexes of DNA with oppositely charged particles in-vitro seem to be promising as model systems to reveal fundamental mechanisms of the natural packing of DNA by histone octamers. Accordingly, in recent decades research on the microscopic structure of different complexes composed of DNA molecules of great length and different complexing agents has been of particular interest. [27a, 28, 41] Some of these studies have clarified that the mode of interaction between semi-flexible long DNA molecules (ssDNA or dsDNA) and nanoparticles, is closely correlated with the conformation of DNA, the chain rigidity and the size of the nanosphere.[27a, 28] Specifically, structural studies of systems consisting of well-defined monodisperse cationic nanoparticles (NP) (with various sizes ranging from 10 to 100 nm) and single-chain bacteriophage T4 DNA (57 μm contour length, 166 000 base pairs) have suggested the existence of different types of complexes and binding modes, depending on the size of the nanoparticle and the length of the DNA chain. These include: adsorption of DNA on the surface of the nanoparticle ("adsorption"), wrapping of DNA around the nanoparticle ("wrapping") and association of nanoparticles on the DNA strand ("collection")[27a] (Figure 1).

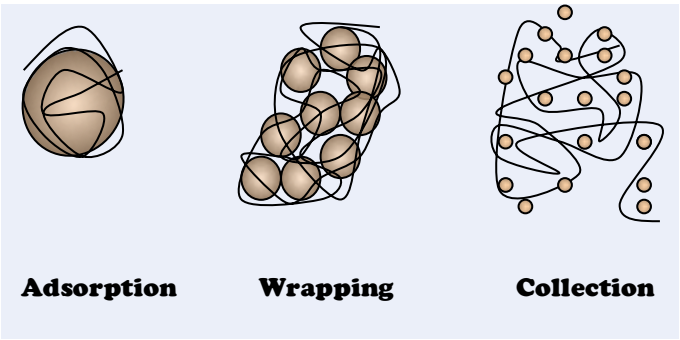


Figure 1. Different types of complexes and binding modes. Adapted from original picture of ref (27a).

TEM observation allows the determination and characterization of the type of complex by direct observation of the number of nanoparticles per DNA chain. Zinchenko et al. determined the different types of DNA complexes [T4 (166,000 bp)] with gold nanoparticles under conditions of complete compaction of the biopolymer through this technique.[27] They studied compaction of single-stranded DNA by histone-inspired nanoparticles and found that the DNA compaction by nanoparticles is stepwise and progressive at the single-chain level. Thus, they distinguished between XL nanoparticles (from 5 to 8 nanoparticles per

DNA chain), L nanoparticles (between 40 and 50 NPs for DNA strand), M nanoparticles (between 600 and 1200 NPs per chain) and S nanoparticles, containing more than 5000 nanoparticles per chain. Nanoparticles of large size, XL and L, were found to interact with DNA by the so-called adsorption mechanism. This type of complexation is characterized by the existence of a large amount of DNA adsorbed on the nanoparticle and a small number of particles per chain of complexed DNA. Intermediate-sized nanoparticles in relation to the length of the DNA chain, type M, have a wrapping mechanism as their characteristic mode of interaction, in which the rigidity of the polymer chain becomes significantly important and the complexation is achieved by one or more turns of the DNA strand around the nanospheres. Indeed, the ability of DNA to wrap nanoparticles is more efficient for larger particles. Finally, in the extreme case of small nanoparticles, type S, the mechanism of interaction is a simple partnership in which small nanoparticles are adsorbed on the surface of DNA. In this case, the number of nanoparticles required to saturate a long chain of the biopolymer, such as the T4-type genomic DNA, is extremely large. Whatever type of partnership arrangement in the various systems involved DNA/nanoparticles, the binding of this particular type of ligands to DNA causes compaction of the biomolecule. The association of nanoparticles to DNA causes a reversible conformational change in the structure of DNA to more compact and condensed forms, as has been demonstrated by using different structural techniques, such as circular dichroism [27a, 27b, 42], TEM [27a, 27b, 28], AFM[42-44] or fluorescence microscopy.[28] In relation to the degree of compaction of DNA in the presence of nanoparticles, the saline effect has also been studied. The ability of DNA to wrap nanoparticles, similar to DNA-histone interaction, is optimal at physiological salt concentration. In general, regardless of the size of the nanoparticles, the addition of salt to AuNPs-DNA system decreases the nanoparticle concentration necessary to produce the compaction of DNA chains. This effect has been proven to be more pronounced for the interaction of small nanoparticles (S and M) with DNA.[27a]

4. In-vivo DNA interactions: thermodynamic and kinetic aspects

All processes in-vivo happen in the condensed DNA phase and all binding events should be treated accordingly. An adequate description of DNA-binding processes in crowded macromolecular environments is a real challenge which will require merging existing biochemical, electrostatic, thermodynamic and bioinformatic approaches.

Macromolecular crowding is the basis for phase separation in the cytoplasm[45] and condensation of DNA into the nucleus of bacterial cells.[46,47] In-vitro, the compaction of the DNA molecule can be accomplished by adding agents, such as polyamines, multivalent metal cations, hydrophilic polymers, cationic polymers, cationic liposomes, cationic surfactants and, more recently, by nanoparticles. These changes can also be induced by varying the relative permittivity of the medium, the addition of cosolvents such as alcohols, and salts of highly charged ions. Alcohols have both electrostatic and structural effects on DNA, leading to three regimes of condensation. At the lowest alcohol concentrations, the B conformation is stable and condensation is relatively slow, allowing time for the packing adjustments necessary to form toroids. At intermediate alcohol concentrations, condensation is faster.

The combined effects of solvents and ions as $Co(NH_3)_6^{3+}$ locally destabilize the double helix permitting DNA foldbacks that lead to rod like condensates. As the dielectric constant decreases from 80 to 65, rods become shorter and at the lowest dielectric constants, alcohol and $Co(NH_3)_6^{3+}$ produce A-DNA.[48] The compactness is a property that is of significant importance in gene therapy and the efficiency of transfections.[49] As non viral vectors, polycations may work well for efficient cell uptake and endosomal escape, because they form compact and smaller complexes with plasmid DNA and carry amine groups, which give a positive charge and buffering ability that allows safe escape from the endosome/lysosome.[50] In fact, compaction of DNA, together with the reduction of its charge, facilitates the transport of nucleic acids through the cellular membrane.[51]

The biological activity of nucleic acids not only depends on the thermodynamic properties of DNA-ligand complexes. It can be conditioned by the kinetics of the formation of these complexes. Binding kinetics may represent another important discriminating factor in structure-activity relationships. Therefore, it is of interest to analyze not only thermodynamic but also kinetic study of the interactions of DNA with nanoparticles.

4.1 DNA-NP interaction thermodynamics

One of the basic reasons why it is important to perform a thermodynamic study of DNA-nanoparticle systems is to determine the factors that govern the affinity and specificity of these nanosystems by the biopolymer (DNA). The equilibrium constants (association constant, K) and the corresponding Gibbs free energy can be determined following different procedures for both, to obtain experimental data which can be analysed. Over the years, equilibrium binding constants K have been calculated using a variety of methods developed by Scatchard, Mc. Ghee and von Hippel or Nordén.[52] Spectroscopic methods are generally effective procedures for obtaining the parameter K. Interaction of nanoparticles with DNA induces changes in the spectroscopic properties of the nanoparticle (or DNA) and

these changes can be followed by the use of an adequate spectroscopic technique (UV-Vis, CD, NMR, or fluorescence). Given the multitude of ways by which a nanoparticle can bind to DNA, multifunctional nanoparticles are designed to determine the contribution of different kinds of interactions to the binding. Accordingly, for each DNA/ nanoparticle system, a global effect is exerted in conjunction with both the metal cluster and the capping agent of the nanocluster. Specifically, capping agents contribute to different abilities of the coordinated group, leading to intercalation, hydrogen bonding, and electrostatic interaction.[53, 54]

Indeed, the interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. Within this field, an area that has been largely unexplored is the interaction of metal nanoparticles with viruses.[55] Besides, each diagnostic/therapeutic technique requires a different chemical or physical property of the particle involved, which depends on the specific function played by the NPs in that therapy. An external interacting agent can be used to activate the particle function (for example, through magnetic field, light, radiation, etc). In this sense, the requirements for NPs as biomedical agents span a broad range of novel materials, synthesis strategies and research fields.[56]

Reversible hybridization of complementary DNA is fundamental to biological processes, such as replication and transcription. The hybridization kinetics experiment is executed with the use of a single-stranded DNA (ss-DNA) probe attached to a substrate to detect the target DNA in solution. The processes of hybridization/dehybridization of DNA and the aggregation/dissociation of the DNA/nanoparticle systems, are influenced by the size of the nanoparticle. However, other variables must be taken into account, such as the surface density of oligonucleotides, the dielectric constant of the medium, the salt concentration and the concentration of DNA. For this reason, in order to consider a complete thermodynamic model to explain these processes the dependence of the equilibrium interaction constant of these systems according to these variables must be studied. During the last few decades, several authors have directed their efforts towards the preparation of nanoparticles covalently bonded to DNA.[57] The dissociation of the double strand, in systems containing aggregates of DNA molecules/nanoparticles, is cooperative. One of the most important theoretical models for evaluating the relative importance of various factors in the processes of aggregation of nanoparticles covalently linked to oligonucleotides has been developed by Jin Rongchao et al[58] (Figure 2). They observed that as the dissociation of the DNA strands take place there is a decrease in the local concentration of salt in the aggregate. This gradual decrease in salt concentration implies that there is a decrease in local dielectric constant, causing a decrease in the melting temperature of the system. These facts were key to suppose a cooperative mechanism for dissociation/aggregation of gold nanoparticles, in which the first step of the mechanism is associated with a higher melting temperature, and hence a smaller equilibrium constant for the next step and so on. Recently, also through cooperative melting properties, J. S. Lee et al have found that salt concentration-induced dehybridization of DNA-gold nanoparticle conjugate assemblies for diagnostic applications. [59]

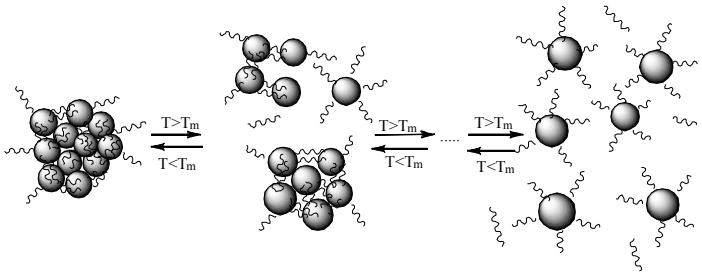


Figure 2. Cooperative mechanism for dissociation/ aggregation of gold nanoparticles. Adapted from reference (58).

From a quantitative point of view, Rongchao et al. found that the influence of the core size of gold nanoparticles functionalized with oligonucleotides (13, 31 and 50 nm) results in values of free enthalpy of 275.8, 473.8 and 706.8 kcal.mol-1 respectively.[58] Therefore, the melting curves are more pronounced or vertical as the size of the nanoparticle increases. This increase in enthalpy of dissociation with the nanoparticle size implies that the number of connections through the oligonucleotides between each pair of nanoparticles proportionally increases with size. In the field of nanoparticles as biosensors, fluorescent nanoparticles that can be attached to biological molecules are being developed for use in microscopic sensor devices. Within the DNA/nanoparticle systems for biosensors, fabrication is of interest to analyze and compare the thermodynamic properties of association of these systems with systems using conventional fluorescent probes. Mirkin et al[60] have compared the interaction between the oligonucleotide chains of both type systems through the study of the melting curves, depending on the concentration of reagents. In this work, only the association/dissociation of nanoparticles (functionalized with oligonucleotides) with complementary oligonucleotides is considered, regardless of the possible aggregation of the system. From a linear representation of 1/Tm against lnCT (CT is the total concentration of nanoparticle and fluorophore, or quencher together with fluorophore) they can determinate the equilibrium parameters of both systems (ΔH_0, ΔS_0, K) through the Breslauer equation.[61]

Although different factors determine the interaction between inorganic nanoparticles and DNA, this one is mostly driven by the average size of the first. Recently, the interaction of 9

nm monohydroxy-(1-mercaptoundec-11-yl)-tetraethylene-glycol-capped neutral Au nanoparticles of about 2.8 nm of core diameter with shortened calf thymus DNA (ct-DNA, 800 bp) has been investigated by spectrophotometric and spectrofluorimetric titrations.[62] Negative results were obtained by the authors. Due to the nature and length of the capping agent, no significant nanoparticle spectral variation upon DNA addition could be observed by Secco et al. in order to analyse the binding constant DNA/AuNPs quantitatively.[62] In this sense, and starting from these negative results, Grueso et al. observed that, although absorption spectra were not valid to measure the equilibrium binding constant DNA/AuNPs, the circular dichroism (CD) technique provides an alternative path to evaluate the interaction of neutral tiopronin gold nanoparticles (Au@tiopronin) with long DNA chains.[42] The binding of ct-DNA with gold nanoparticles capped with N-(2-mercaptopropionyl)glycine was investigated through AFM, absorption, intrinsic circular dichroism, viscosity measurements, melting analysis and steady state-fluorescence. The results indicate that neutral Au@tiopronin bind tightly to ct-DNA. This is a clear example were negative results provide a valuable resource for researchers.

4.2 DNA-NP interaction kinetics

Kinetic studies of hybridization/de-hybridization processes involving nanoparticles covalently bound to oligonucleotides are less frequent than thermodynamic studies. Two processes are critical in the cluster formation rate: a) the annealing, characterized by a constant k_1 and b) the aggregation growth rate from DNA already hybridized in the nanoparticle, characterized by a constant k_2. The size of the oligonucleotide controls the speed of both processes.[63] Recent studies have allowed a first estimation of the rate constants corresponding to the aggregation of these systems, using Avrami's law for the growth of molecular aggregates by nucleation.[64] Another interesting study reports on the kinetics of hybridization events. The scope of this work is to apply the plasmonic heating effect to the AuNPs/DNA system in an optical trap, so allowing the investigation of the kinetics of nanoparticle oxidation and growth.[65] Although insights have been gained into the kinetics of hybridization of AuNPs/DNA system, all of these studies correspond to AuNPs functionalized with DNA. Kinetic studies of non-covalent interactions (NCI) between nanosystems and free DNA molecules are extremely rare. Regardless of the complexation mode, the binding of NPs to DNA causes a reversible conformational compacted change in the DNA structure. However, a comprehensive mechanism to describe the kinetic behaviour of DNA-based nanosystems is still required.

As these systems allow for more sophisticated detection and increasingly complex bottom-up construction, a protocol for the regulation of the nanoparticle–DNA assembly kinetics would be beneficial. Unfortunately, nowadays kinetics and mechanistic studies of the non-covalent interaction of DNA with gold nanoparticles are scarce. Prado-Gotor et al. has recently studied the interaction of gold nanoparticles capped with N-(2-mercaptopropionyl) glycine (Au@tiopronin) with double stranded DNA.[42] This study was carried out in water and brine (NaCl) solutions and revealed the presence of three kinetic steps with biexponential kinetic curves (a typical kinetic experiment is given in Figure 3).

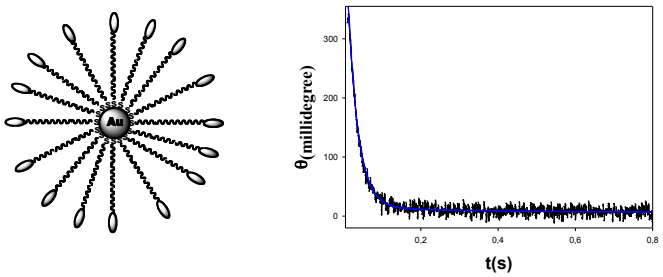


Figure 3. Au@tiopronin nanoparticle and plot of ellipticity, θ, vs time, t, in a typical kinetic experiment between DNA and Au@tiopronin. The curve is the best fit to the experimental data. Adapted from reference (42).

There is a dependence of the reciprocal fast and slow relaxation time curve on the DNA concentration. At high DNA concentrations both reciprocal relaxation times tend to plateau. Although different mechanisms are consistent with the kinetic results, the simplest ones involve a three-step series mechanism reaction scheme. The first step corresponds to a very fast process related to a diffusion controlled formation of an external precursor complex (DNA, AuNPs); the binding affinity between hydrophilic groups of the tiopronin and the DNA grooves involves a second step with the formation of a $(DNA/AuNPs)_I$ complex. Subsequently, a conformational change of the $(DNA/AuNPs)_I$ complex formed in the second step to a more compacted form $(DNA/AuNPs)_{II}$ corresponds to the third step. The values of the rate constants of each step decrease as NaCl concentration increases. The results were discussed in terms of solvation of the species and changes in the viscosity of the solution.[42] Information about the kinetics of compaction processes is fundamental because the mechanism of these processes is poorly understood. The dynamics of higher-order chromatin compaction play a crucial role in transcription and other biological processes inherent to DNA. Kinetic information is particularly necessary in relation to gene transport because the release rate of DNA from vectors is one of the many parameters that control this process.[66]

Indeed, DNA release into the tissue can occur rapidly, as in bolus delivery, or extend over days to months. [67-69]

5. Negative results of Nanoparticles as DNA sensors

Kyungnam el al[70] studied the effect of adding salt to DNA and gold nanoparticles coated with citrate (negatively charged). They found that the addition of DNA and salt to the system produced a change in the electrostatic properties of the system that favors the aggregation of nanoparticles. The aggregation of nanoparticles can be easily detected by UV-Vis techniques due to the change of colour that the dissolution suffers as the aggregation of nanoparticles occurs. This colour change results in a shift in the UV-vis absorption spectrum of the system to longer wavelengths (red shift). This property has allowed the use of these systems as biosensors for detecting DNA.[71] Until salt concentrations corresponding to 0.015 M in NaCl, negligible changes were observed in the UV-vis spectrum, ruling out the aggregation of these nanoparticles under these concentrations.[70] The addition of salt implies the destabilization of the gold nanoparticles due to shielding of electrostatic repulsion between them, suggesting that the control of salt concentration of the medium is somehow regulating the electrostatic repulsion between gold nanoparticles, inducing a selective aggregation of them.

To date, when using gold nanoparticles as colorimetric sensors, it is only possible to detect the presence of single-stranded DNA in a biological fluid. The results are negative when detecting double-stranded DNA (dsDNA) using anionic nanoparticles protected by citrate ions. The reason is that compared to dsDNA, single stranded DNA (ssDNA) has different propensities to adsorb onto unmodified gold nanoparticles due to their dissimilar electrostatic interactions.[72] In this sense, ssDNA is known to be able to adsorb on AuNPs and to stabilize colloidal suspensions in high salt concentration.[73] ssDNA can uncoil to expose its bases, but duplex DNA (dsDNA) is characterized by its stable DNA-DNA double-helix geometry. For this reason dsDNA always isolates the nucleotides presenting the negatively charged phosphate backbone, showing little affinity to negatively charged AuNPs. Thus, dsDNA cannot protect AuNPs from salt-induced aggregation, as compared to ssDNA. Normally, this process is detectable as a color change of the colloidal solution and red shift of the surface plasmon band. This characteristic behaviour allowed to develop colorimetric assay for ssDNA detection based on the aggregation of unmodified metallic nanoparticles.[74]

Citrate coated gold nanoclusters[75] present a negative charge of about –30 mV in water, enough to keep the particles dispersed and with a stable particle size. The repulsion between these two negative entities (citrate NPs and the charged phosphate backbone of dsDNA) is the reason why dsDNA do not adsorb. One of the characteristics of ssDNA is its flexibility. Thanks to that, the ssDNA can partially uncoil its bases, so that they can be exposed to the gold nanoparticles. Under these conditions, the negative charge on the backbone is sufficiently distant of the nanaoparticles. This fact together with the attractive van der Waals forces between the bases of the biopolymer and the gold nanoparticle (bare particles) is sufficient to bond ssDNA to the gold. This mechanism is not operative with dsDNA because the duplex structure does not permit the uncoiling needed to expose the bases. This system therefore allows the determination of ssDNA in a solution, but not dsDNA. Meanwhile color changes of the solution, triggered by the addition of additive such as NaCl, are retarded if the solution contains ssDNA, color alteration of colloidal gold solution is not affected by dsDNA oligonucleotides.[76] This negative result is a challenge in the current field for developing new biosensors based on non-functionalized nanoparticles.

Acknowledgments. This work was financed in part by the Otri (2010/00000762), by the D.I.G.Y.T. (CTQ2008-00008/BQU) and by the Consejería de Educación y Ciencia de la Junta de Andalucía. D. Alcantara is recipient of a Senior Marie Curie IEF (grant number PIEF-GA-2012-327151).

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