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DNA SURFACE RECOGNITION NON-COVALENT DNA-AuNPS INTERACTIONS

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www.novapublishers.com

ISBN: 978-1-61942-470-8 2012

DNA AND RNA: PROPERTIES AND MODIFICATIONS, FUNCTIONS AND INTERACTIONS, RECOMBINATION AND APPLICATIONS

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Nova Science Publishers, Inc.
New York

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

ISBN: 978-1-61942-470-8

Published by Nova Science Publishers, Inc. † New York

CONTENTS

Preface		**vii**
Chapter 1	Non-Covalent Interactions: The Two State Model	**1**
Chapter 2	Gold Nanoparticles as Small Ligands	**11**
Chapter 3	Non-Covalent DNA-Gold Nanoparticles Interactions from a Thermodynamic and Kinetic Point of View	**17**
Chapter 4	Conclusion	**27**
Acknowledgments		**29**
References		**31**
Index		**37**

PREFACE

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 formation of these complexes. Therefore, it seemed interesting to address the thermodynamic and kinetic study of the interactions of DNA with nanoparticles acting as small ligands. Between nanoparticles, this review focuses on gold nanoparticles (AuNPs) due to an improvement in fabrication methods for both monolayer-stabilized clusters (MPCs) [1] and mixed monolayer protected gold clusters (MMPCs) [2]. The present contribution is divided into three sections. The first section provides a general overview of the two state model for the study of non-covalent interaction. Then, a brief introduction of gold nanoparticles as small ligands is described. Finally, in the third section, aspects of non-covalent DNA-gold nanoparticles interactions are covered through a thermodynamic and kinetic point of view.

Chapter 1

NON-COVALENT INTERACTIONS: THE TWO STATE MODEL

Nanoparticles covalently bound to DNA have a current interest as biosensors [3] and a potential use in vivo applications [4]. In this sense, the folding state of DNA aptamers on gold nanoparticle surfaces modulates their electrostatic interactions. Thus it also affects the aggregation of particles, which can be used to make colorimetric biosensors based on gold nanoparticles [5]. Most of the studies have been performed with DNA *covalently* bound to nanoparticles involving *small oligonucleotides*. However, there are relatively scarce studies in solution referred to *non covalent long chain* DNA/nanoparticles interactions [6]. Systematic studies of the conformational changes of the polyelectrolyte due to the binding of the nanosystems are also relatively infrequent.

Non-covalent interactions, INC, play a key role in different fields of chemistry and biology, such as antigen /antibody interaction, processes of solar energy conversion, environmental chemistry, phenomena of self-aggregation, etc. Interactions between ions, between them and molecules, hydrogen bonds, hydrophobic interactions and Π-stacking are, among others, INC. Non-covalent interactions are those that occur between chemical species (molecules, ions, etc.) always that these interactions do not involve the formation of a covalent bond between the interacting species. The energies at play in such interactions are generally lower than those of conventional chemical bonds, but in any case, its intensity is sufficient to produce redistribution of charge on the interacting species.

Such redistribution can affect - sometimes dramatically - their properties and, in particular, the chemical reactivity [7].

The kinetics of the processes that lead to formation of supramolecular entities involving interactions of the type considered is usually rapid, often controlled by diffusion of species that bind. Recently the study of such interactions has gained increasing interest (perhaps one might say, more properly, it has recovered) because they are essential in shaping the scene in many fields of scientific and technical interest. Thus, the antigen/antibody interactions are often not covalent interactions [8]. Within the field of biochemistry, it can be added that the interactions between sugars and metal cations condition important processes as the transport and storage of ions [9], the stabilization of membrane structures [10], the binding of glycoproteins to cells surfaces [11], the metabolism of toxic metals [12] and the joints proteins/sugars [13]. Noncovalent interactions are also relevant in relation to molecular machines [14], molecular electronic [15], the manufacture of sensors [16], catalysis (homogeneous or heterogeneous) [17], the capture and storage of solar energy [18], etc. It is clear that the situation in these fields is not uniform: some have just opened or are opening, others are well known. Among the former one may include the molecular machines (artificial) and DNA-AuNPs interactions; between the second, the field of catalysis.

The establishment of an interaction between two species always implies a change of their properties (thermodynamic and not thermodynamic). In fact, the binding of a substrate, S, and a receptor, R, both dispersed in a given environment, causes a change of the free energy of the substrate, ΔG_s. If the receptor is monodisperse, ΔG_s is given by [19]:

$$\Delta G_s = R\,T \ln \gamma_s \qquad \text{(a)}$$

$$\gamma_s = \frac{1}{1 + K\,[R]} \qquad \text{(b)} \qquad (1)$$

In the above equations, γs, is defined with respect to a reference state in which [R] = 0. On the other hand, K in Equation 1b, represents the equilibrium constant for the process:

$$S\ (\text{free}) + R \leftrightarrow S/R\ (\text{substrate linked to the receptor}) \qquad (2)$$

But not only the thermodynamic properties change. Other properties do the same. Thus, when the substrate and the receptor are charged their union results in a change in the electrical conductivity of the medium that contains both [20]. Changes in spectra of different types (NMR [21], absorption [22] or emission [23], transport coefficients [24], etc) often accompany the formation of supramolecular structures substrate/receptor. These properties changes are the foundation that fosters the development of techniques that allow the detection and quantification of non-covalent interactions. These techniques always start from the assumption common of the two state model [25]: the substrate is divided between the two states appearing in equation 2, free and bound. Under these conditions, fractions of one and another state are given by:

$$[S]_{libre} = \frac{[S]_{total}}{1 + K\,[R]} \qquad (a)$$

(3)

$$[S/R] = \frac{[S]_{total}\,K\,[R]}{1 + K\,[R]} \qquad (b)$$

Accordingly, if the properties of the free substrate and bound substrate found by the experimental technique are different, γ_S and $\gamma_{S/R}$, respectively, the magnitude of the observed property is given by [26]:

$$\lambda_{obs} = \frac{\lambda_S + \lambda_{S/R}\,K\,[R]}{1 + K\,[R]} \qquad (4)$$

Equation 4 is actually a simplification. Compliance requires certain conditions, depending on the type of property being handled. So if λ represents a rate constant, this constant must be smaller than the constants (diffusion) for the processes, forward and reverse, that appear in equation 2. Otherwise, there is coupling reaction/diffusion: "gated" processes [26].

Thus, measuring the property at various different concentrations of receptor, it is possible to obtain K and from this, the formal standard free energy for the substrate/ receptor binding. This property can be, for

example, the change in the rate constant of a ligand substitution process or of an electron transfer reaction in the presence and absence of the receptor [27].Usually, an electron transfer process has been used as a probe, taking into account its apparent simplicity: in these kinds of reactions an electron is transferred from a donor, D, to an acceptor, A, without breaking or forming new bonds, a fact which implies that the electron transfer reaction is one of the simplest of chemical processes [28]. It is important to note that the two state model consider a rapid distribution (in relation to the kinetic events) of the reactants between two states that are free and bound to some supporting *monodisperse* materials. On the other hand, the concentration of the partitioned substrate must be low enough to avoid the saturation of the receptor. Indeed, even in this case, it is implicit that the presence of a substrate molecule in the receptor neither encourages nor discourages the union of a second molecule of substrate: in other words, the binding of the substrate to the receptor is noncooperative in character.

If the measured property depends on the receptor binding to more than one substrate, as for example when a formal standard redox potential is measured, which depends on receptor binding to oxidized and reduced forms of the pair, the equation 4 must be modified in order to contain the binding constants of the two substrates. Specifically in the case of the potential the equation is [19]:

$$E^{o\prime} = E^{o} + \frac{RT}{nF} \ln \frac{1 + \mathrm{K_{red}}[R]}{1 + K_{\mathrm{ox}}\,[\mathrm{R}]} \tag{5}$$

K_{red} in this equation is the equilibrium constant for the binding of the reduced form of the redox couple with the receptor and K_{ox} has the same meaning for the oxidized form.

The use of this equation has allowed to confirm the interaction of a negatively charged ligand (particularly an anionic binuclear complex of iron and ruthenium) with DNA [29].

One can conclude that nearly always it will be possible to find an experimental technique that allows quantification (through K) of the substrate/receptor interactions. The only condition required is that the answers to the technique of free and bound substrate must be different. Two-state models are frequently used in order to rationalize the results of

different fields of chemical kinetics, such as heterogeneous catalysis [30], enzymatic reactions [31], salt effects [32], and, generally speaking, reactions carried out under restricted geometry conditions [33]. These are conditions in which one or both reactants are forced to remain totally or partially at the surface of a micelle, or a polyelectrolyte or in the cavity of a cyclodextrin, etc. In this sense, nanoparticles promote restricted geometry conditions considering that it is possible to control the surface charge on the nanoparticle and, therefore, to modulate the interaction between the NPs and charged ligands.

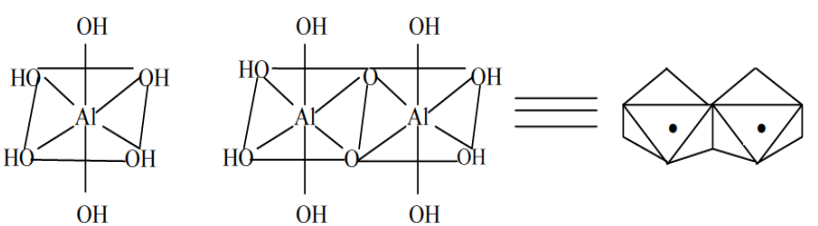
Thus, as has been described, measuring some properties at different receptor concentrations it is possible to obtain K and from this the standard free energy corresponding to the union substrate/receptor. *Changes of the rate constants of a given reaction in which S participates* can be used as a valid property in order to obtain K. As an example, following this approach, a systematic study of the interaction between a small anionic ligand, $S_2O_8^{2-}$, and nanosized boehmite particles (AlOOH-NPs positively charged at pH = 5.4), allows to obtain information about the energetic of the binding using spectrophotometric techniques [34]. The equilibrium binding constant K and, therefore, the free energy of binding of the persulphate ion (negatively charged) to the AlOOH-NPs can be obtained following the changes in the kinetics of the electron transfer reaction between a ruthenium complex, the $[Ru(NH_3)_5pz]^{2+}$, and $S_2O_8^{2-}$. From these kinetic data, *a two-state-model* allows the evaluation of, not only the *strength* of the binding, but also its *character* as a function of the AlOOH-NPs concentration and the ionic strength of the medium. On the other hand, working at different ionic strengths it is possible to separate the *electrostatic and non-electrostatic* contributions to the binding free energy. In this way, a complete picture of the binding can be obtained and, the values of the differences of electrostatic potential at the AlOOH/solution interface can be determined from the electrostatic component [34].

The effect of noncovalent interactions on the kinetics of chemical reactions has been studied for a long time: many catalytic processes (and in particular the enzymatic processes) are caused by such interactions between the catalyst (receptor) and the substrate. The interaction substrate/catalyst has to be less favorable (in a thermodynamic sense of the word) that the interaction transition state/catalyst to catalysis occurs [35-36]. These different interactions involve, of course, changes in the

free energy surface of the processes and, consequently, changes in the reaction rate. Thus, for unimolecular processes it can be shown that the rate constant in the presence of a receptor is given by [19a]:

$$k = k_o \frac{1 + K_{\neq}[R]}{1 + K[R]} \qquad (6)$$

In this equation ko is the rate constant in the absence of receptor (catalyst), that is, the reference state defined above, K has already been defined and K≠ has the same meaning as K, but referred to the transition state rather than to the substrate [37]. It is noteworthy that the reactivity, as the redox potential, depends on the relative strength of the joints of the receptor with two species (substrate and transition state for a unimolecular reaction). Hence k is a function of K and K≠, as in the case of redox potentials (K_{ox} and K_{red}). On the other hand, it can be easily shown that $k_oK_{\neq} = k_mK$, where k_m is the rate constant for the bound substrate [19a]. Note that making this substitution in equation 6, it takes the form of equation 4, with λ representing a rate constant, and it matches with the Pseudofase Model equation [25].



Scheme I. Boehmite structure. a) Structure of an octahedron. b) Functional group structure.

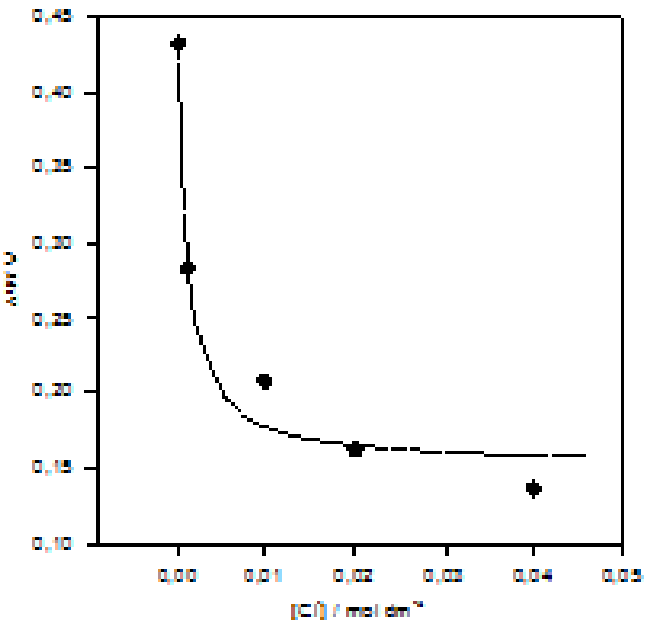


Figure 1. Plot of the the electrostatic potential at the AlOOH/solution interface, $\Delta\Psi$/Volts, vs. $[Cl^-]$/mol dm^{-3} (see reference 34).

In practice it is common to find systems in which things occur in more complex than suggested by the equation 6 (and earlier). The reason are the so called *cooperativity phenomen* [38]. These phenomena appear when the receptor has more than one point of attachment to the substrate (for example, if the receptor is a polymer). In such cases, the union is said to be cooperative if the interaction of a first substrate with the receptor do the union of a second substrate more favorable and this, in turn, that of a third, etc. Otherwise, if successive unions are becoming less favorable, it is called anticooperativity. Finally, if the successive unions substrate/receptor are neither more nor less favorable than before, the interaction is called non-cooperative. Cooperativity criteria are based on so-called Scatchard curves. In them it is represented $\nu/[S]_{free}$ vs ν (ν being the ratio [S/R]/[R]). The ratio $\nu/[S]_{free}$ is, of course, the association equilibrium constant receptor/substrate. Thus, a decrease of $\nu/[S]_{free}$ as ν increases indicates anticooperative character [39]. In any case there are similar representations to the Scatchard ones that have been proposed by other authors [40].

Formally, the cooperativity is manifested in a variation of the substrate/receptor equilibrium binding constants when the ratio of their concentrations changes [41]. From Equation 6 it follows that, except in the very unlikely event that K and $K_{\neq}$ change in the same magnitud [42], cooperativity phenomena allow to adjust the value of the rate constants merely changing the concentrations ratio substrate/receptor. By this procedure variations of k (equation 6) of several orders of magnitude using the appropriate receptors (eg, DNA in the case of reference [43]) can be obtained.

It should be noted that a change in the substrate/receptor binding constants by varying the concentrations ratio, although formally corresponds to a manifestation of cooperativity effects, not always it is due to these effects. Thus, the variation of the constants for the equilibrium 2 is reduced to a variation of these constants when the concentration of receptor is changed in kinetic studies in which concentrations of reagents usually remain constant. It is true that, in many cases, these changes in K correspond to manifestations of cooperativity. In other cases, however, it is not true: it was observed that the constants for the binding of charged substrates with receptors who are also charged (with opposite charges to those of the substrate) vary when changing the concentration of receptors. In these cases the origin of the phenomenon, at least in part, is not in cooperativity but in structural changes of the receptors, such as changes in the dissociation degree of charged groups of the receptor, aggregation number (when receptors are micelles , etc.), produced by varying the concentration of receptors or their precursors (monomers of surfactants in the case of micelles). Therefore, before granting a change in the intensity of interactions to substrate/receptor cooperativity phenomena other possibilities must be taken into account [41]. It follows that structural studies of receptors in different environmental conditions, are always recommended.

Cooperativity also depends on the nature of the dispersing medium containing the receptor and the substrate. For example, if both are charged, a variation of the ionic strength of the medium can change the intensity of non-covalent bonds and even its character (of non-cooperative to cooperative) [44]. This fact has been observed, for example, in the interaction of Ru $(NH_3)_5pz^{2+}$ with anionic dendrimers (see reference [44b]). This, of course, opens another channel for the modulation of reactivity, modulation that may be decisive when the

process in question is part of a series of coupled reactions. The modification of noncovalent interactions (in its intensity and character) as changing the medium has its origin in various causes. Thus, the medium may cause a greater or lesser stabilization of the substrate in the continuous phase (which could cause a decrease or increase of the substrate/receptor interaction). Another possible cause for the change of these interactions is that the molecules (or ions) of the dispersing medium compete with the substrate by the anchor positions in the receptor [45]. A third possibility is when the receptors are charged and, therefore, develope a surface potential. Certain additives (salt or cosolvent that varies the dielectric constant) can cause changes in the surface potential of the receptors. These changes, if the binding substrate/receptor is electrostatic, at least in part, cause changes in the free energy of the binding process [46]. A very interesting point on this third possibility is the determination of the fraction of the surface potential that truly affects the process of bringing the substrate to the receptor.

The non-covalent bonds (cooperative or non-cooperative) are very sensitive to temperature changes. They results in structural changes in receptors, such as changes in the aggregation number of micelles [47], in the association degree between these and counterions [48], in the conformational changes of receptors [49], etc . But these structural changes can also occur in the substrate or even in the dispersion medium. Thus, in the case of hydrophobic interactions substrate/receptor, the temperature marks its intensity through changes promoted in the solvent structure [50]. The same applies to electrostatic interactions solute/receptor: an increase in temperature usually causes a decrease in the dielectric constant of dispersion medium, and thus an increase in the intensity of the electrostatic interactions [51]. In any case, there are no many systematic studies on the effect that temperature does on non-covalent interactions, despite the interest of these estudies [52].

As a summary of the above it could be indicated that non-covalent interactions are present, and determine, a variety of important phenomena. In particular they are a key element in supramolecular chemistry, in catalysis (chemistry and biochemistry) and in processes of industrial interest (decontamination). It is important to note that under the name of non-covalent interactions are included very different types of them. Today, quantification can be done through a variety of techniques,

sometimes "static" as the spectroscopic techniques, and other "dynamic", as techniques for measuring transport properties or techniques based on their influence on the kinetics of chemical or biochemical processes. The type of technique employed should be selected specifically for each problem. Thus, it is clear that spectroscopic techniques can not be used in cases where there are no changes in the spectra due to the binding substrate/receptor. The study of noncovalent interactions between gold nanoparticles and a structured biopolymer as DNA implies knowledge of the free energy interaction from a thermodynamic point of view and the rate of formation of the aggregate from a kinetic point of view.

Chapter 2

GOLD NANOPARTICLES AS SMALL LIGANDS

According to the generally accepted Gouy-Chapman-Stern-Graham model, a certain number of counter ions are accumulated near to the charged surface of a solid material, attracted by an electrostatic Coulomb attraction. Considering that, at equilibrium, the electrical neutrality of the interphase region must be maintained, the distribution of charge can be described by the following equation:

$$\sigma_o + \sigma_B + \sigma_d = 0 \qquad (7)$$

where σ_o, σ_B and σ_d are the surface charge, the compact layer charge and the diffuse layer charge. Stability of different kinds of nanoparticles depends strongly on the valency of the counterions in the solution. For example, for negatively charged gold colloids, the flocculation value is about 20 mmol/L for monovalent cations, about 0.4 mmol/L for divalent cations, and in the range of 10^{-6} mol/L for trivalent cations [53]. Naturally, larger gold particles aggregate faster than the smaller particles due to stronger van der Waals forces between the larger particles. The fact that the stability of different kinds of nanoparticles depends strongly on the valency of the counterions in the solution is due to the electrokinetic or ζ-potential. This is the difference between the compact layer potential and the diffuse potential. Of course, the higher the ζ-potential the more stable the system because of a stronger Coulomb repulsion between the colloid particles, which prevents them from

coagulating. In this sense, the magnitude of the measured ζ-potential is an indication of the repulsive force that is present and can be used to predict the long-term stability of the nanoparticle.

Another parameter, related with the colloidal stability is the critical concentration of coagulation. The generalization that the critical coagulation concentration for a typical lyophobic sol is extremely sensitive to the valence of the counterions (high valence gives a low critical coagulation concentration) is called the Schulze-Hardy rule. If counter ions are added to the solution they tend to accumulate in the compact layer, thus decreasing the electrokinetical potential as well as the system stability. The point of zero charge (pzc) is also an important property of a colloid system. It must not be confused with the isoelectric point. The latter is defined as the pH value at which the ζ-potential equals zero; meanwhile the pzc is defined as the pH value at which $\sigma_o = 0$ (the number of positively charged surface centres equals that of negatively charge ones, so that the surface is electoneutral) [54]. When the pH is lower than the pzc value, the system is said to be "below the pzc". For example, for AlOOH nanoparticles, below the pzc, the acidic water donates more protons than the hydroxide groups, and so the adsorbent surface is positively charged (attracting anions). Conversely, above the pzc, the surface is negatively charged (attracting cations/repelling anions). Of course these two properties are identical only in the absence of specific ion adsorption. Several methods have been developed for determination of the point of zero charge. The Salt Titration (ST) method has been successfully and widely applied to determine the pzc of a colloid. Compared with the Potentiometric Titration (PT) method, operation of the ST method is simple, requiring a small quantity of sample and its result is accurate. The point of zero charge (pzc) and also the electrostatic potential at the NPs/solution interface can be determined through alternatives routes based on kinetic measurements [55] (see Figure 2). Nedeljkovic et al [55] studied the effect of pH on the reaction rate of iodide oxidation by persulphate in the pH range from 4 to 9.5 in the presence of fixed AlOOH nanospheres concentration. The authors chose the reaction of the oxidation of iodide by persulphate as a model system because the change of acidity of the medium does not appreciably affect the reaction rate [56]. Meanwhile the reaction rate in aqueous medium is not sensitive to pH changes they found a decrease of the reaction rate constant for almost 3 orders of magnitude in intermediate

acidity ranges (6.5 < pH < 8.5, see Figure 2). These results can be explained by transformation of the protonated hydroxyl surface groups into their deprotonated form around the pH_{pzc}. From the pH profile of the iodide-persulphate reaction rate constant, the point of zero charge can be determined. From the inflection point of the k = f(pH) curve, a value of pH_{pzp} = 7.1 was obtained, which is in good agreement with the value of pH_{pzp} = 7.2 obtained from stability measurements [54]. Results show how the sensitivity of the rate constant to the pH provides a convenient way for precise detection of the point of zero charge of dispersed material. Of course a pronounced change of the electron transfer rate constant in the narrow pH range around the point of zero charge is needed.

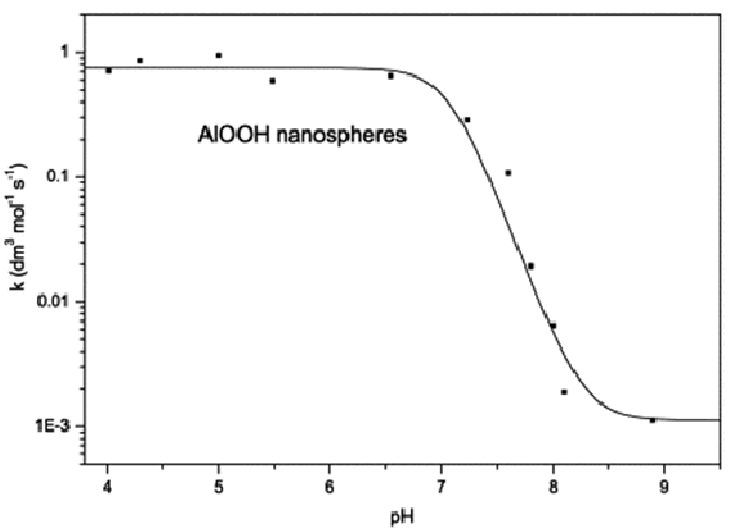


Figure 2. The pH profile of the iodide-persulphate reaction rate constant in the presence of the AlOOH nanospheres. Data are reproduced, with permission, from ref. [55]. Copyright (2003), with permission from Elsevier.

Among a wide variety of nano-sized materials, the optical probes currently used are mainly quantum dots such as differently coated CdS, CdSe and CdTe nanoparticles that have unique size-dependent fluorescent properties. However, their human toxicity and cytotoxicity disfavour in vivo applications [57]. As an alternative, Au nanoparticles are being taken into account because of potential non-cytotoxicity [58], even if recent publications have reported on conflicting data on their

toxicity [59]. Gold colloids are the most stable metal nanoparticles with promising applications because of their electrical, optical, magnetic and catalytic properties [60]. Nanoparticles are of course characterized not only by the properties of the metal cluster core but also by the organic molecules that constitute the monolayer, that is, on the capping agents. In this sense, alkanethiolate nanoparticles have received considerable attention due to their advantages of stability, suspendability in different solvents, and facile characterization by standard analytical techniques [61]. Gold nanoparticles can be functionalized with a wide variety of structural units by simple chemical transformations [62]. Murray's group has studied the chemical and reactive properties of nanoparticles protected by alcanethiolates self-assembled monolayers. This group found that alkanethiols of nanoparticles were able to exchange [63]. These reactions seem, at first, SN_2 nucleophilic substitution reactions, where choosing a more nucleophilic ligand it is possible to displace a thiol attached to the nanoparticle by this one. Although no one knows for sure what is the mechanism by which these thiols are exchanged, these authors suggest that it is not an SN2 reaction, but the surface of thnanoparticle core has small imperfections in vertices or corners where the S bond is weaker, providing that in these points some ligands can be exchanged by others [64]. These reactions require several days and the amount of ligand exchanged can be calculated from the resonance spectrum by the integration of signals corresponding to both ligands. However, this exchange reaction is limited to thiols while the disulfides are not able to be exchanged with thiols already anchored to gold.

A direct application of gold nanoparticles is their use as multivalent systems to study carbohydrate-carbohydrate interaction. Recently it has been designed and developed a new system called multivalent gold glyconanoparticles in order to study these carbohydrate-carbohydrate interactions. In the literature there are studies that describe the preparation and application of gold nanoparticles functionalized with proteins and nucleic acids [65]. However, these bionanoconjugates with important biologically carbohydrates allow to perform basic studies on receptor-ligand interactions or for intervention in biological systems, in vitro and in vivo. Glyconanoparticles can be described as a multipurpose system that presents a number of sugar molecules, alone or in combination with other molecules, arranged on a base or core metal (gold) of nanometric dimensions through a spacer, which length and

nature can be changed. The combination is done by reducing a gold salt, such as tetracloroauric acid ($HAuCl_4$) in the presence of neoglicoconjugates carrying a terminal thiol group. This methodology allows to prepare glyconanoparticles of different density if different ligands are used together in the reaction. The size of the nanoparticle will vary depending on the ratio thiol:gold. Normally, a large excess of thiol is added to prevent the growth of crystal gold and to force the gold nuclei formed to be stable. In any case the great interest for use in the study of noncovalent interactions with different substrates is the possibility of synthesizing gold nanoparticles loaded with functional groups, resulting in narrow size distribution curves.

Mixed monolayer-functionalized gold nanoparticles present a promising structure for the development of DNA-regulating molecules, and have already been shown to be highly effective transfection vectors. Quaternary ammonium-functionalized nanoparticle binds 37-mer DNA in a non-aggregated stoichiometric fashion (3-4 nanoparticles per 37-mer DNA strand) with high affinity [66]. The interaction of slightly negative gold nanoparticles capped with N-(2-mercaptopropionyl)glycine with double stranded DNA has also been proven. Groove binding of these hydrophilic nanoparticles occurs principally through the formation of stable hydrogen bonding between the tiopronin chain and the interior of the heterocyclic DNA bases and, as will be described, the kinetic of the interactions are biexponential and reveal the presence of three kinetic steps [67].

Despite all this, in applications of AuNPs as sensors of DNA are often used nanosystems not functionalized: nanoparticles specifically protected by citrate ions. Single and double-stranded DNA (ssDNA and dsDNA, respectively) have different propensities to adsorb onto unmodified gold nanoparticles, due to their dissimilar electrostatic interactions [68]. ssDNA is known to be able to adsorb on AuNPs and to stabilize colloidal suspensions in high salt concentration [69] meanwhile ssDNA can uncoil to expose its bases. Duplex DNA (dsDNA) caracterizes by its stable DNA-DNA double-helix geometry. It always isolates the nucleobases and presents the negatively charged phosphate backbone showing little affinity to negatively charged AuNPs. For this reason, dsDNA cannot protect AuNPs from salt-induced aggregation, as compared to ssDNA. This aggregation is detectable as color change of the colloidal solution and red shift of the surface plasmon peak. So,

colorimetric assay for ssDNA detection based on the aggregation of unmodified metallic nanoparticles have been developed [70].

Nanoclusters do not aggregate due to the negative citrate adsorbed on them [71]. It is the repulsion between these adsorbed citrate ions and the charged phosphate backbone of dsDNA the reason for that dsDNA will 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 and attractive van der Waals forces between the bases and the gold nanoparticle are sufficient to bring ssDNA to bond 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 allows the determination of whether or not ssDNA is present in a solution but not whether or not dsDNA is present. 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 effected by dsDNA oligonucleotides [72].

Chapter 3

NON-COVALENT DNA-GOLD NANOPARTICLES INTERACTIONS FROM A THERMODYNAMIC AND KINETIC POINT OF VIEW

The number of DNA-related studies, apart from purely related to genetics, has grown exponentially over the past two decades. That fact has to do with the interest of such studies in fields such as (among others):

1) The changes that DNA produces in the kinetics of the reactions that occur in its environment. These effects are due to two factors: the so-called concentration factors [36] and the so-called environmental factors, due to changes that DNA produces in the physical properties of environment [73].
2) Studies of the interaction of DNA with amphiphilic substances and similar substances are of interest for the ability to act as vectors for gene transport, much safer than the usual viral vectors and with the advantage of not causing immune response [74]. Recently, Gemini-lipids have been used as carriers. These vectors are reminiscent of the virus, but are artificially produced [75].
3) Construction of DNA-based molecular sensors or sensors for DNA, which are based on changes in properties of small molecules (ligands) when they bind to DNA (substrate).

> Recently it have appeared in the literature sensors that consist of metal nanoparticles functionalized with DNA. It has been shown that these particles show affinities to the ligands that are two orders of magnitude greater than the conventional sensors. This has been attributed to high packing density that can reach the DNA on the surface of the metal particle, which provides multiple binding sites for the ligand. However, affinity does not increase monotonically with packing, so that it seems to be a magical level of packaging which optimizes the detection of the ligand [76].

The three fields have, of course, specific characteristics. However, they all have a common root: the non-covalent interaction substrate/ligand. Many forms of cancer and infectious diseases are caused by attacks from external agents that can cause DNA mutations. Covalent attachment of small organic and inorganic molecules (alkylating agents, heavy metal ions, metal complexes, etc ...) to nucleic acids, can cause various types of damage to the macromolecule that, ultimately, could prevent the proper storage and transmission of the genetic code. Similar significant effects could be caused by small molecules that bind *non-covalently* to nucleic acids.

In the specific case of small ligands binding to DNA, there has been a close relationship between theoretical and experimental studies, so it is now possible to give a detailed description of how a DNA-ligand complex behaves at the molecular level.

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 give them a positive charge, its structure being quite similar in all organisms with a size of 7 nm [77]. The positive charge of histones, allows rapid bonding to DNA through the negatively charged phosphate groups. A key feature is that chromatin structure also allows an orderly packaging of DNA molecules, allowing in turn important processes to be carried out, such as the expression of genetic information and DNA replication. Of all the histones, the sequence of histone H4, in particular, seem remarkably well conserved throughout evolution. However, a given histone may undergo

numerous changes during the cell cycle. Processes such as phosphorylation or acetylation of histone H4 may play a regulatory role in transcription and DNA replication. For example, when the N-terminal residue is phosphorylated by a kinase, the net charge of the terminal residue can vary from +1 to -1. These changes may alter the affinity of N-terminal domain of histone H4 by DNA and also alter the affinity of histone to other proteins. For most of the cell cycle the chromatin is in the form of thin filaments of 30 nm thick. However, in humans the length of these figures range between 0.25 and 2 nm. That is why the structure of chromatin is very condensed. In this regard, the DNA-nanoparticle system is a model that simulates the interactions that occur between proteins and DNA on histones. 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 [78-80]. Some of these studies have clarified that the mode of interaction between semiflexible DNA molecules of long length chain and nanoparticles, is closely correlated with the conformation of DNA, the chain rigidity and the size of the nanosphere [78-79]. Specifically, structural studies of DNA/nanoparticle complex, 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") [78] (see Figure 3).

Figure 3.

The determination of the existence of a complex or another, can be carried out by direct observation of the number of nanoparticles per DNA chain using the technique of transmission electron microscopy. Anatoly

and collaborators determined the different types of DNA complexes (T4 (166,000 bp)) with gold nanoparticles under conditions of complete compaction of the biopolymer thorugh this technique [78]. 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 nanoparticle and a small number of particles per chain complexed DNA. Intermediate-sized nanoparticles in relation to the length of the DNA chain, type M, have a wrapping mechanism as 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 nano-spheres. 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 as the T4-type genomic DNA is extremely large. Whatever type of partnership arrangement in the various systems involved nanoparticles/DNA, 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 [78], TEM [77a, 77b, 78] or fluorescence microscopy [79]. In relation to the degree of compaction of DNA in the presence of nanoparticles, the saline effect is interesting. 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 [78].

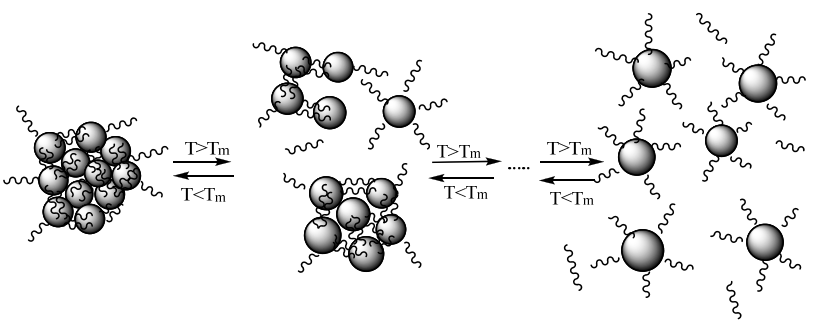
Bulking agents in the media induce DNA compaction by various causes, such as changes in electrostatic interactions between phosphate groups, changes in DNA-solvent interactions, excluding counter ions, and / or due to curvature or distortion in the structure of the helix. In

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, neutral, and more recently it has been found that it can be induced by the addition of 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. The compactness is a property that is of significant importance in gene therapy and efficiency in transfection [81]. In fact, compaction of DNA together with the reduction of its load, facilitates the transport of nucleic acids through celular membrane [82]. 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 formation of these complexes. Therefore, it is of interest to address not only thermodynamic but also kinetic study of the interactions of DNA with nanoparticles.

One of the basic reasons why it is important to perform a thermodynamic study of DNA-nanoparticle systems is the determination of the factors that govern the affinity and specificity of these nanosystems by the biopolymer. The equilibrium constants (association constant, K) and the corresponding Gibbs free energy can be determined following different procedures, both to obtain experimental data and to analysis. In connection with these systems, spectroscopic methods are generally effective procedures for obtaining the parameter K: interaction nanoparticle/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.

The processes of hybridization/deshibridation of DNA and the aggregation/dissociation of the systems DNA/nanoparticle, are influenced by many variables such as: the size of the nanoparticle, the surface density of oligonucleotides, the dielectric constant of the medium, the salt concentration and the concentration of DNA. Therefore, a complete thermodynamic model to explain these processes should treat, at least, the dependence of the equilibrium interaction constant of these systems according to these variables. During the last decades, several authors have directed their efforts towards the preparation of

nanoparticles covalently bonded to DNA [83]. The dissociation of the double strand, in systems containing aggregates of DNA molecules/nanoparticles is cooperative. A theoretical model 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 [84] (see Scheme II). They observed that the local concentration of salt in the aggregate gradually decreases as the dissociation of the DNA strands take place, and this decrease in salt concentration, implying a decrease in the local dielectric constant, causes a decrease in the melting temperarure 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. The thermodynamic model proposed by Jin Rongchao has been used in further theoretical studies of molecular dynamics at different concentrations of NaCl. The objective was to determine and compare the distribution of ions around the double helix of DNA with that existing in the presence of clusters or aggregates DNA [85].



Scheme II.

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 [84]. 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 increases with increasing the size of them.

Within the systems DNA/nanoparticle for the fabrication of biosensors is of interest to analyze and compare the thermodynamic properties of association of these systems with systems that use fluorescent probes. Mirkin et al [86] 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. Now the association/dissociation from nanoparticles (functionalized with oligonucleotides) with oligonucleotides (complementary) assuming a simple reaction scheme is considered by Mirkin et al, regardless of the possible aggregation of the system. A linear representation of 1/Tm against lnCT (CT is the total concentration of nanoparticle and fluorophore or quencher together with fluorophore), allows the determination of the equilibrium parameters of both systems (ΔH^0, ΔS^0, K) through the Breslauer equation [87].

The interaction between inorganic nanoparticles and DNA 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 (800 bp) has been investigated by spectrophotometric and spectrofluorimetric titrations [88]. 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 [88]. In this sense, Grueso et al observed that the CD technique provides an alternative path to evaluate the interaction of neutral tiopronin gold nanoparticles (Au@tiopronin) with long chains DNA when the most commonly employed spectroscopic methods are not appropriate [67]. The binding of 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, instead of Secco´s nanoparticles, neutral Au@tiopronin bind tightly to ctDNA.

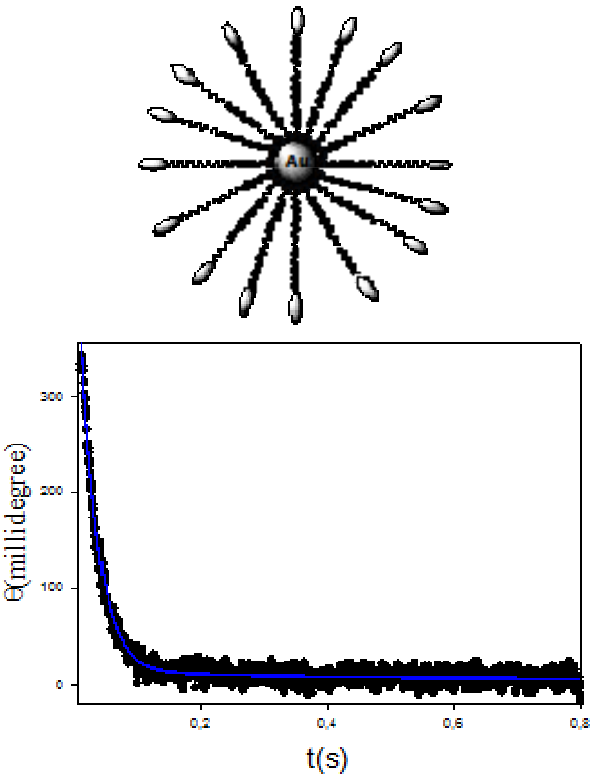


Figure 4. Au@tiopronin nanoparticle and plot of ellipticity, θ, vs time, t, in a typical kinetic experiment between DNA and Au@tiopronin . The curve is the best fit to the experimental data.

Kinetic studies of the hybridization/deshibridación processes of nanoparticles covalently bound to oligonucleotides are less frequent. Two processes are generally considered critical in the formation rate of aggregates: (i) the rate of union of the DNA strand to the complementary strand (annealing) characterized by a constant k_1 (ii) the rate of growth of aggregates, from DNA already hybridized in the nanoparticle, characterized by a constant k_2. Depending on the size of the oligo, if this one increases the speed of both processes would be reduced. 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 [89].

With respect to kinetic studies of noncovalent interactions between nanosystems and free DNA molecules they are extremely rare. Regardless of the complexation mode, the binding of NPs to DNA causes a reversible conformational change in the DNA structure to a more compact form, as demonstrated by circular dichroism [90], TEM microscopy [79], fluorescence [66] and fluorescence microscopy [79]. Even though the AuNPs–DNA system has been studied for the last decade [83], a comprehensive mechanism to control the kinetic behaviour of DNA-based nanosystems is required for the development of NP applications. As these systems allow for more sophisticated detection and increasing complex bottom-up construction, a protocol for the regulation of the nanoparticle–DNA assembly kinetics would be beneficial. Unfortunately, as saying before, nowadays kinetics and mechanistic studies of the non-covalent interaction of DNA with gold nanoparticles are scarce [78-79]. Specially interesting is a current kinetic study of the interaction of gold nanoparticles capped with N-(2-mercaptopropionyl) glycine with double stranded DNA [67]. This study has been carried out in water and in salt (NaCl) solutions. E. Grueso and one of the present authors found that the kinetic curves are biexponential and reveal the presence of three kinetic steps. The dependence of the reciprocal fast and slow relaxation time, on the DNA concentration, is a curve and tends to a plateau at high DNA concentrations. The simplest mechanism consistent with the kinetic results involves a simple three-step series mechanism reaction scheme. The first step corresponds to a very fast step that is related to a diffusion controlled formation of an external precursor complex (DNA, AuNPs); the second step involves the formation of a (DNA/AuNPs)I complex, as a result of the binding affinity between hydrophilic groups of the tiopronin and the DNA grooves. Finally, the third step has been interpreted as a consequence of a conformational change of the (DNA/AuNPs)I complex formed in the second step, to a more compacted form (DNA/AuNPs)II. Regarding the influence of the medium 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 [67].

Chapter 4

CONCLUSION

There are relatively scarce studies in solution referred to non covalent long chain DNA/nanoparticles interactions. Systematic studies of the conformational changes of the polyelectrolyte due to the binding of the nanosystems are also relatively infrequent. The study of noncovalent interactions between gold nanoparticles and a structured biopolymer as DNA implies knowledge of the free energy interaction from a thermodynamic point of view. This free energy interaction can be estimated thorugh a two state model that consider a rapid distribution (in relation to the kinetic events) of the reactants between two states that are free and bound to some supporting monodisperse materials. On the other hand, 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 formation of these complexes. Get information about the kinetic of compaction processes is fundamental because the mechanism of these processes is poorly understood. This information is particularly necessary in relation to gene transport, because the release of DNA from vectors, which obviously has kinetic implications, is one of the parameters that control this process.

ACKNOWLEDGMENTS

This work was financed by the D.I.G.Y.T. (CTQ2008-00008/BQU) and the Consejería de Educación y Ciencia de la Junta de Andalucía.

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INDEX

A

acetylation, 19
acid, 15
acidic, 12
acidity, 12
additives, 9
adsorption, 19, 20
AFM, 23
aggregation, 1, 8, 9, 15, 21, 23, 24
alcohols, 21
ammonium, 15
annealing, 24
antibody, 1, 2
antigen, 1, 2
arginine, 18
attachment, 7, 18
Au nanoparticles, 13, 23

B

base, 14
biochemical processes, 10
biochemistry, 2, 9
biological activity, vii, 21, 27
biological systems, 14
biopolymer, 10, 20, 21, 27
biosensors, 1, 23
bonding, 15, 18
bonds, 4

C

cancer, 18
carbohydrate, 14
carbohydrates, 14
catalysis, 2, 5, 9, 32
catalyst, 5, 6
catalytic properties, 14
cationic surfactants, 21
cell cycle, 19
chain rigidity, 19
chemical, 1, 4, 5, 10, 14
chemical bonds, 1
chemical kinetics, 5
chemical reactions, 5
chemical reactivity, 2
clusters, vii, 22
colloid particles, 11
color, 15, 16
compaction, 20, 27
construction, 25
covalent bond, 1, 8, 9
cytotoxicity, 13

D

decontamination, 9

detectable, 15
detection, 3, 13, 16, 18, 25
dielectric constant, 9, 21
diffusion, 2, 3, 25
direct observation, 19
diseases, 18
dispersion, 9
dissociation, 8, 21, 22, 23
distribution, 4, 11, 15, 22, 27
DNA, i, ii, iii, v, vii, 1, 2, 4, 8, 10, 15, 17, 18, 19, 20, 21, 23, 24, 25, 27
double helix, 22

E

electrical conductivity, 3
electron, 4, 5, 13
emission, 3
energy, 1, 2, 5, 6, 27
environment, 2, 17
environmental conditions, 8
environmental factors, 17
equilibrium, 2, 4, 5, 7, 8, 11, 21, 23
eukaryotic, 18
eukaryotic cell, 18
evolution, 18

F

fabrication, vii, 23
flexibility, 16
flocculation, 11
fluorescence, 20, 21, 23, 25
force, 12, 15
formation, vii, 1-3, 10, 15, 21, 24, 25, 27
free energy, 2, 3, 5, 6, 9, 10, 21, 27

G

gene therapy, 21
genetic code, 18
genetic information, 18
genetics, 17
geometry, 5, 15
glycine, 15, 23, 25
glycol, 23
glycoproteins, 2
gold nanoparticles, vii, 1, 10, 14, 15, 16, 20, 22, 23, 25, 27
growth, 15, 24

H

heterogeneous catalysis, 5
histone, 18
histones, 18
human, 13
hybridization, 21, 24
hydrogen, 1, 15
hydrogen bonds, 1
hydroxide, 12
hydroxyl, 13

I

immune response, 17
in vitro, 14
in vivo, 1, 13, 14
integration, 14
interface, 5, 7, 12
interphase, 11
intervention, 14
ion adsorption, 12
ions, 1, 2, 9, 11, 12, 15, 16, 20, 22
iron, 4

J

joints, 2, 6

K

kinetic curves, 25
kinetic studies, 8, 25
kinetics, vii, 2, 5, 10, 17, 21, 25, 27

L

lead, 2
ligand, vii, 4, 5, 14, 18, 21, 27
lipids, 17
liposomes, 21
lysine, 18

M

magnitude, 3, 8, 12, 18
materials, 4, 13, 27
measurements, 12, 23
media, 20
melting, 22, 23
melting temperature, 22
metabolism, 2
metal complexes, 18
metal ion, 18
metal ions, 18
metal nanoparticles, 14, 18
methodology, 15
microscopy, 20, 25
model system, 12
models, 4
molecular dynamics, 22
molecular sensors, 17
molecules, 1, 9, 14, 15, 17, 18, 22, 25
monolayer, vii, 14, 15
monomers, 8
mutations, 18

N

NaCl, 16, 22, 25
nanoparticles, vii, 1, 5, 11-15, 19, 21, 22, 23, 24, 25, 27
nanosystems, 1, 15, 21, 25, 27
neutral, 21, 23
NMR, 3, 21
nucleation, 24
nuclei, 15
nucleic acid, vii, 14, 18, 21, 27
nucleus, 18

O

oxidation, 12

P

permission, 13
permit, 16
permittivity, 21
pH, 5, 12, 13
phosphate, 15, 16, 18, 20
phosphorylation, 19
physical properties, 17
polyamines, 21
polymer, 7, 20
polymer chain, 20
polymers, 21
preparation, 14, 21
probe, 4
proteins, 2, 14, 18
protons, 12

Q

quantification, 3, 4, 9
quantum dot, 13
quantum dots, 13

R

reactant, 33
reactants, 4, 5, 27, 33
reaction rate, 6, 12, 13
reactions, 4, 5, 9, 14, 17
reactivity, 6, 8
reagents, 8, 23, 33
receptors, 8, 9
red shift, 15
redistribution, 1
relaxation, 25

replication, 18
repulsion, 11, 16
residues, 18
root, 18, 32
routes, 12
ruthenium, 4, 5

S

salt concentration, 15, 21
salts, 21
saturation, 4
sensitivity, 13
sensors, 2, 15, 17
showing, 15
signals, 14
solution, 1, 5, 7, 11, 12, 15, 16, 25, 27
solvation, 25
solvents, 14
species, 1, 2, 6, 25
spectroscopic techniques, 10
stability, 11, 12, 14
stabilization, 2, 9
state, vii, 1, 2, 3, 4, 5, 6, 23, 27
states, 3, 4, 27
storage, 2, 18
structural changes, 8, 9
structure, 6, 9, 15, 16, 18, 20, 25
substitution, 4, 6, 14
substitution reaction, 14
substrate, 2, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18
substrates, 4, 8, 15
surfactants, 8
suspensions, 15

T

techniques, 3, 5, 9, 14, 20
TEM, 20, 25
temperature, 9
thermodynamic properties, vii, 3, 21, 23, 27
thymus, 23
toxic metals, 2
toxicity, 13
transcription, 19
transfection, 15, 21
transformation, 13
transformations, 14
transmission, 18, 19
transmission electron microscopy, 19
transport, 2, 3, 10, 17, 21, 27
two-state model, 32

U

uniform, 2
unions, 7
UV, 21

V

valence, 12
variables, 21
variations, 8
viral vectors, 17
viscosity, 23, 25

W

water, 12, 25