CHAPTER I

INTRODUCTION

1.1 Research rationale

Currently, DNA mediated charge transport and electron transfer attracts considerable interest because of its relevance for the oxidative damage and/or mutations of DNA. [1,2] DNA is an important element molecule to function in living cells storing the information needed to maintain life. Errors introduced into those instructions generally have harmful consequences, so there is great pressure to correct them. Concerning the molecular level, the reaction that changes the structure of DNA damages the instructions and introduces errors. Several reactions cause structural changes in DNA that among the most important is one-electron oxidation.[3] Oxidation of DNA can result from normal cellular metabolism, from exposure to ionizing radiation, or from interaction with light. [4,5] When DNA is oxidized, it loses an electron and a radical cation ("hole") is generated.

Oxidation of DNA (loss of an electron) generates a radical cation that can migrate long distances to remote guanines in G_n steps which may occur as the charges are tunneling from guanine base pair to another. The unique material properties of DNA also hold great promise for molecular electronics [6,7] and electrochemical sequencing.[8] In the context of materials applications, the DNA oligomers can be used as ligands and spacers to control shape and size of semiconductor nanoparticle assemblies [9] or to construct silver nanowires. [10]

The double-stranded DNA molecule is presented as wire-like molecule. Its electrical resistance at room temperature was measured, after it had been placed between contacts; a surprisingly large electrical conductivity was found. Though the conduction mechanism is not understood yet, the prospects are great, because one is now able to use the full machinery of biochemistry for assembling electronic networks. This discovery will most likely become the starting point for exciting new interdisciplinary research.

Experimental and theoretical studies of charge migration in DNA have demonstrated its strong sensitivity to the structural flexibility of DNA and to modifications of the nucleotides. Several recent reviews describe the experimental investigations on the charge transport in DNA. [11–14] An important problem in this context is the distance dependence of the electron transfer rate in DNA. [15]

Most experimental data on the electron transfer in DNA have been obtained for DNA complexes with chromophores as probes because they provide a clearly defined point of charge injection. The chromophore-DNA interactions affect the local structure of DNA. Yet, hardy anything is known about the consequences of these structural (and electronic) effects on the measured charge migration behavior. This casts some uncertainty on the interpretation of experiments with standard models. [16,17]

1.2 DNA-intercalated aromatic chromophore complexes

Chromophores are the fluorescence molecules. In recent years, many efforts have been made to investigate aggregation processes and structure-property relationships of diverse molecular aggregates due to their unique physicochemical properties. An important reason for the strongly increased interest in molecular aggregates was their potential technological application in various fields. Based on biological assays are of ever increasing importance in the development of homogeneous and non-isotopic techniques used in drug discovery. Several techniques are already used widely.

In addition, fluorescent dyes have become the preferred method of detection for nucleic acids in Molecular Biology. Some common examples include automated fluorescent DNA sequencing, DNA detection, and quantitative target detection. Most applications involve covalent attachment of the chromophore to DNA. [18] Fluorescent changes in physical properties, which occur after conjugation to DNA were studied in detail by Sjoback. [19] When attached to a single-stranded oligo, the pKa of fluorescein shifts from 6.4 to 6.9 and the quantum yield decreases from 0.93 to 0.72. Further, the absorption efficiency of fluorescein decreases by 1/3 after conjugation to DNA. [20] On the other hand, rhodamine does not undergo a similar change in absorption efficiency following conjugation.

Moreover, the scientists are interested in the charge conduction properties of DNA and try to see whether DNA can be used as a molecular wire for connecting molecular components to form bigger circuits. Much experimental information on charge transfer in DNA pertains to migration of positive charge (electron holes) and chromophores have been used successfully as hole donors after excitation. The broad variety of organic or inorganic intercalators has been used as charge donor and it is attached with DNA duplexes via a flexible linker. These systems differ significantly in their structural properties, their redox potentials, and their absorbing wavelengths. It has been observed in the systems that the positive charge can be transported with high efficiency over very long distance (up to 200 Å). [21,22]

The introduction of charge is typically prepared by using of intercalators which oxidize the bases in the DNA or direct photo-induced radical ion formation on one of the bases of the DNA strand. Many organic chromophores interact strongly with DNA forming intercalated and other types of complexes. [23,24] In addition, intercalated transition metal complexes in electronically excited state were shown to initiate the electron transfer process in DNA duplexes. [25,26] For the advantages of this method, the most obvious is the capability to examine the final chemical results initiated by charge transfer: true chemistry at a distance. Others include being able to analyze charge transport over large distances. There is no limited of a fast timescale analysis.

Rhodamine is a fluorophore widely utility in biological research. [27] Dietrich A. and co-worker [28] studied the fluorescence resonance energy transfer (FRET) efficiency of different donor-acceptor labeled model DNA systems in aqueous solution from ensemble measurements and at the single molecule level. The donor dyes: tetramethylrhodamine (TMR); rhodamine 6G (R6G); and a carbocyanine dye (Cy3) were covalently attached to the 5'-end of a 40-mer model oligonucleotide. The acceptor dyes, a carbocyanine dye (Cy5), and a rhodamine derivative (JA133) were attached at modified thymidine bases in the complementary DNA strand with donor-acceptor distances of 5, 15, 25 and 35 DNA-bases, respectively.

The dyes change their conformation with respect to the oligonucleotide on a slower time scale in the millisecond range. In addition, the measured acceptor fluorescence intensities and lifetimes also partly show fluorescence quenching effects independent of the excitation wavelength, *i.e.* either directly excited or via FRET.

They suggest that the π-stack of the DNA double helix mediates electron transfer from the donor to the acceptor, even over distances as long as 35 base pairs.

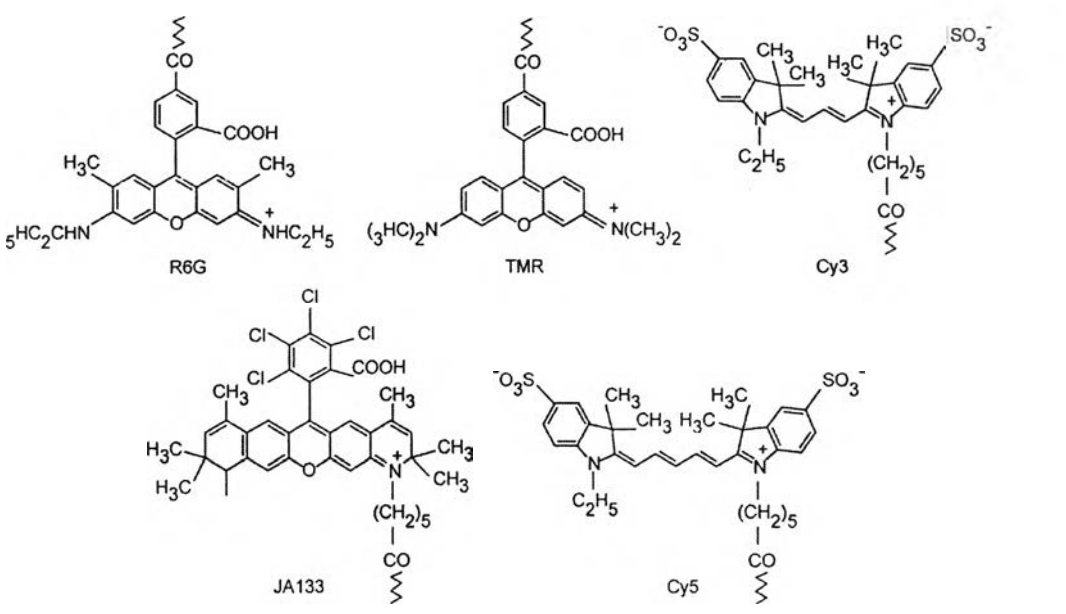

Figure 1.1 Molecular structures of the used dyes.

1.3 Charge transfer in DNA double helix

Research groups have contributed significantly to this research topic, which made the charge transfer in DNA molecule a subject of intensive investigation. DNA with its highly specific recognition between complementary nucleotides and the ability to self-assemble may have a new technological potential in constructing complex nano-wire networks. Moreover, the study of charge transfer in DNA could come through understanding the mechanism of oxidative DNA damage which the damage of DNA can result in cancer.

In principle, DNA-mediated charge transfer processes can be categorized as either oxidative hole transfer or reductive electron transfer processes.[29] The first remark was published over 40 years ago [30] that the π-stacked of base pairs in B-form DNA may represent a unique medium for electron transfer. Efficient, long-range charge transfer mediated by the DNA base stack has been observed in many different

systems. [12,26] Utilizing intercalating reactants which are well coupled to the DNA π stack reveals shallow distance dependence for charge transfer between donors and acceptors *through* the DNA double helix. [31] DNA was considered to be a molecular wire, a semiconductor, or an insulator. [32-33]

Barton and coworkers pioneered this research through remarkable contributions about photoactivated charge transfer chemistry in DNA. [34] They announced surprising results about using DNA as a "conductive" bridge between an organometallic donor and acceptor complexes positioned far from each other. They claimed that the photoinduced electron transfer through DNA could occur over distances far as much as 40 Å.

There are several biochemical techniques developed to investigate long-range, DNA-mediated charge transport. The most widespread one is a probe for oxidative damage in DNA. Such damage is observed primarily at guanine (G), as predicted by theoretical and experimental studies which have determined that G is the most easily oxidized base. The specific residues of damage, usually the 5' G in a 5'-GG-3' or 5'-GGG-3' sequence, are correlated with the oxidation potential of G in different sequence contexts.[35,36]

As an example we mention 9-amino-6-chloro-2-methoxyacridine (ACMA; see Figure 1.2). [37] Because of the ordering of the oxidation potentials of single nucleobases in solution, [38,39] it was inferred that hole hopping occurs between guanine (G) bases.[40–42] Furthermore, it was shown experimentally that G+ can be generated in DNA far away from an oxidant due to long-range hole transport. [21]

Consider, for another example, charge transfer was studied on the double stranded 63-mer DNA by Núñez and coworker. [21] The charge is introduced into the DNA by a metallointercallator, which is either Rh(phi)2bpy'3+ or Ru(phen)(bpy')(dppz)3+. Guanine in DNA is oxidized preferentially because it has a lower oxidation potential among the four bases. The oxidation potential of dG nucleoside is 1.29 V as compared to 1.42 V for adenine and 1.6 V and 1.7 V for cytosine and thymine, respectively. It is well known that the 5'-G of the guanine doublet site has a lower oxidation potential and thereby becomes the signature for recognition of oxidative damage in DNA.

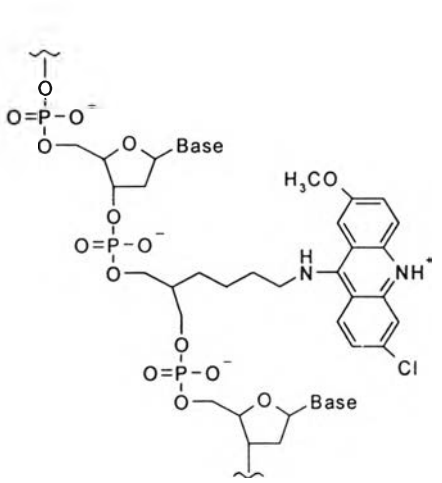

Figure 1.2 Schematic representation of the acridine derivative ACMA, covalently .phosphate backbone of DNA-linked to the sugar

Moreover, there are experiments in many laboratories using a variety of photooxidants aimed at exploring the mechanism of DNA-mediated charge transport. [21,43-47] Schuster and coworkers used various anthraquinone photooxidants attached to the end of the duplex. [48,49] They have been studied guanine oxidation at a distance through the double helix in assemblies. Saito *et al.* have looked at guanine oxidation triggered by derivatives of excited state benzophenone. [50,51]

Lewis and coworkers have extensively examined photoinduced charge separation between guanine and photo-excited stilbene in a series of synthetic DNA hairpins. [16] In these structures, the double helix was capped by the stilbene but remains stacked so that relatively efficient ET behavior is observed. Finally, direct base-base electron transfer was probed by examining the quenching of the fluorescent base 2-aminopurine by both guanine and 7-deazaguanine using ultrafast spectroscopies. [40] The characteristic of these experiments demonstrating efficient DNA mediated ET is the coupling of the reactants to the DNA base stack.

The mechanism of charge transfer is an issue of widespread controversy both in the experiment and theoretical scientists. The superexchange mechanism and the coherent and incoherent hopping mechanisms were thought to be the options till about

a few years ago. [52,53] Schematic representations of possible mechanisms for charge transport through DNA were proposed and displayed in Figure 1.3.

i) Superexchange: the charge tunnels from the donor (D) to the acceptor (A) through the bridge in a nonadiabatic process. The mechanism involves tunneling through an energy barrier (in this case the A:T base pair sequences), from the donor to the acceptor (the single guanine base and the triplet guanine base pair group, respectively). [52,54] A pictorial representation is provided in Figure 1.3a. An exponential decrease in the rate of charge transport with increasing length of bridge is predicted.

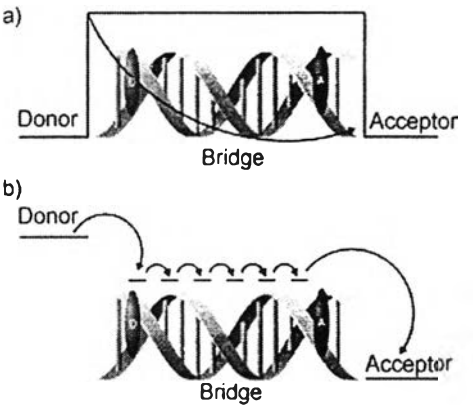

Figure 1.3 Schematic representations of possible mechanisms for charge transport through DNA. (a) Superexchange: the charge tunnels from the donor (D) to the acceptor (A) through the bridge in a nonadiabatic process. (b) Hopping: charge occupies the bridge in travelling from donor to acceptor by hopping between discrete molecular orbitals on the bridge.

ii) Hopping: charge occupies the bridge in traveling from donor to acceptor by hopping between discrete molecular orbitals on the bridge (See Figure 1.3b). This inspired the hypothesis that the charge transfer efficiency depends on the maximum run (length of A:T bridges) of A:T sequences between the G:C base pairs. [52,54] This may occur as the charges are tunneling from one G:C base pair to another, at a time through the A:T bridges, instead of directly from the donor to the acceptor. If the rate of charge migration is faster than trapping, the charge should be able to migrate over long distances before getting trapped.

1.4 DNA structure

Since the discovery of the double helical structure of DNA by Watson and Crick, scientists have been wondering whether the DNA duplex is capable of charge transport. The polymeric nature of DNA, consisting of a negatively charged sugar-phosphate backbone outside the duplex and aromatic nucleobases stacked on top of each other inside the duplex, represents a well-characterized system containing an extended π stack within its interior (Figure 1.4).

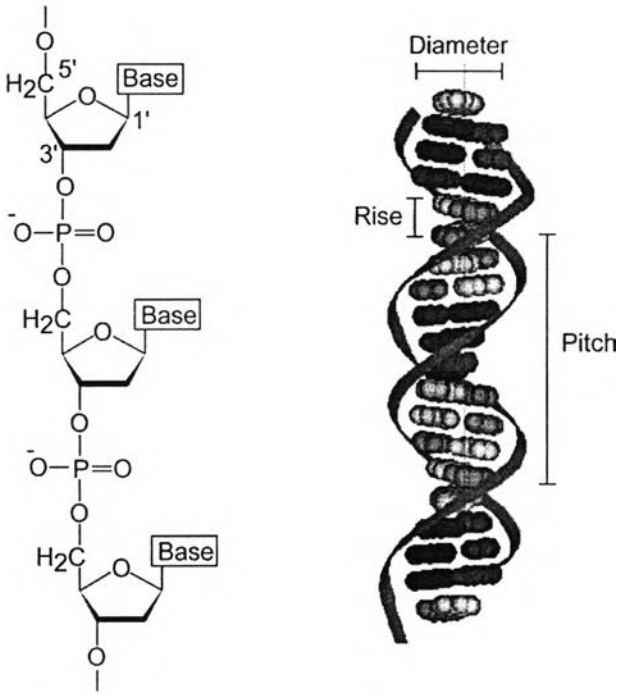

Figure 1.4 Structure of part of a DNA strand (left), and DNA double helix (right).

A single DNA strand is composed of a polyanionic sugar-phosphate backbone linking together combinations of the DNA bases that belong to adenine (A), guanine (G), purine family, and pyrimidines, cytosine (C) and thymine (T) (see Figure 1.4 and Figure 1.5). Each sugar contains an aromatic base bound to C1' of the sugar. The two strands are normally complementary so that when they combine to form the duplex, each base on one strand forms Watson-Crick hydrogen bonds with its counterpart (G

with C and A with T) on the opposite strand. At normal physiological pH (ca. 7.4), the phosphates of the backbone polymer are fully ionized, so there must be a counterion (Na+) for each phosphate.

Base pairs are approximately perpendicular to the helical axis. The stacking is held by van der Waals interaction. Because of, the unique and appealing properties of DNA, the structurally well-defined may provide an effective path for electron transfer. [30·55]

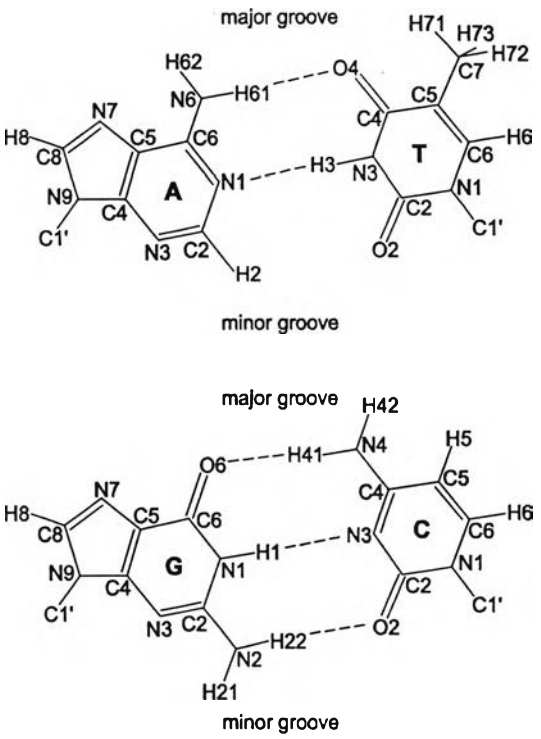

Figure 1.5 Watson-Crick base pais found in double stranded DNA; adenine (A), guanine (G), purine family, and pyrimidines, cytosine (C) and thymine (T).

Table 1.1 demonstrates the geometric features described DNA structure. The average values are given for the features found in A-DNA, B-DNA and Z-DNA structures in the Nucleic Acid Database. The parameters can be used to classify the DNA form, such as orientation of the double helix, inter-strand phosphate distance,

diameter, distance between two neighboring base pair (rise), rotation angel per base pair (twist), length of the helix per turn, a number of base pair per turn (pitch).

Table 1.1 Average structural parameters for A-DNA, B-DNA and Z-DNA.

Parameter	A-DNA	B-DNA	Z-DNA
Orientation	Right-handed	Right-handed	Left-handed
Helix diameter (Å)	26	20	18
Rise (Å)	2.56	3.38	3.70
Pitch(Å)	28.2	33.8	44.5
Base pair/turn	11	10	12
Helix twist (°)	32.7	36.0	-30.0
Major groove width (Å)	2.7	11.7	2.0
Minor groove width (Å)	11.0	5.7	8.8

1.5 Basic electron transfer theory

Charge transport through DNA, however, has been observed over distances as great as 200 Å, meaning that the decay with distance is exceptionally shallow. [21] Understanding the unique characteristics of DNA that allow such long-range events remains a major challenge of theory and experiment alike.

Most research groups interpreted their results according to the Marcus theory of non-adiabatic electron transfer:

$$k_{ct} = \frac{2\pi}{\hbar} H_{DA}^2 \frac{1}{\sqrt{4\pi\lambda k_b T}} \exp\left[-\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_b T}\right]. \qquad (1.1)$$

The model presumes that the overlap between relevant electronic orbitals of the donor (D) and acceptor (A) is small. The initial and final states of the system can be represented as two harmonic free energy curves, corresponding to two states before (D/A) and after (D^+/A^-) the electron transfer, as shown in Figure 1.6. The expression for the rate constant for such system is given by the following equation. When, ΔG^0 is

the free-energy of the reaction and λ is the reorganization energy, as shown in Figure 1.6. This λ is the energy required to transfer the electron from the bottom of the energy profile of the acceptor (product) state up to the energy profile of the acceptor state in the same nuclear configuration as the energy minimum of the donor state.

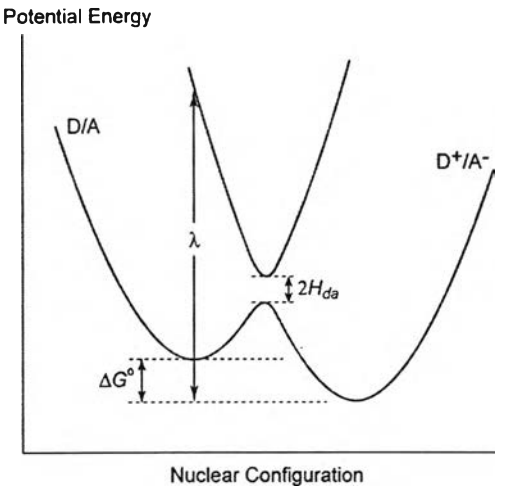

Figure 1.6 Energy diagram for the reactants (D/A) and products (D+/A-) as a function of nuclear configuration.

The magnitude of the electronic coupling matrix element, H_{DA}, depends on the overlap of donor and acceptor wave functions and determines. In case donor is equivalent as acceptor by symmetry, the magnitude of the splitting can be easily approximated as one-half of the energy difference between the two adiabatic states, E_1 and E_2. This approach is referred to as minimum splitting method.

$$H_{DA} = \frac{1}{2}(E_1 - E_2) \tag{1.2}$$

The distance dependence of electron transfer rate originates from the distance dependence of H_{DA}. The electronic matrix element can not be measured directly but is usually recovered from the charge transfer rate constant. For weak coupling, the distance dependence of the charge transfer rate constant is often fitted with the following equation:

$$k_{ct} = k_0 \exp(-\beta r), \qquad (1.3)$$

where the distance dependence of charge transfer is reflected by the beta decay parameter. This process provides a concise way of comparing charge transfer rates for different D-A bridges and intervening media. So far, experimentally measured beta values in DNA were reported to vary in a range between 0.1 and 1.4 $Å^{-1}$. Low beta values reported by Barton were surprising because they appeared to be substantially lower than those reported for proteins, which typically range within 0.9-1.2 $Å^{-1}$. [56]

Recently, the Munich group designed the semiempirical method NDDO-G for studying structural and spectroscopic properties of organic and biological molecules. [57] This method predicts bond lengths and bond angles with accuracy similar to that of AM1 and PM3. Several applications corroborate the success of the method in simulating spectroscopic properties of large organic compounds and biomolecules, in particularly, of the chromophore of the green fluorescent protein. [58] The NDDO-G method will be used to describe the electronic structure of chromophores and their complexes with DNA. Here, we mention recent quantum chemical calculations to support experimental investigations on the phothophysics of DNA complexes with ACMA (Figure 1.2); [37] also, spectroscopic experiments are carried out for such complexes in the Munich institute. [37]

Furthermore, the Munich group considered the energetics of hole transfer in DNA assuming idealized geometries. [59] A systematic computational study of triads 5'-XBY-3' (X, B, Y = A, G, C, T) revealed that the stabilization of B+ within duplexes 5'-XBY-3' is considerably affected by the subsequent base Y while the effect of the preceding base X is rather small. [59] Coupling matrix elements for the hole transfer between neighboring nucleotides and Watson-Crick pairs were determined at the SCF-Hartree-Fock (HF) level, [59,60] resulting in a detailed elucidation of the bridge specificity and directional asymmetry of the electron transfer.

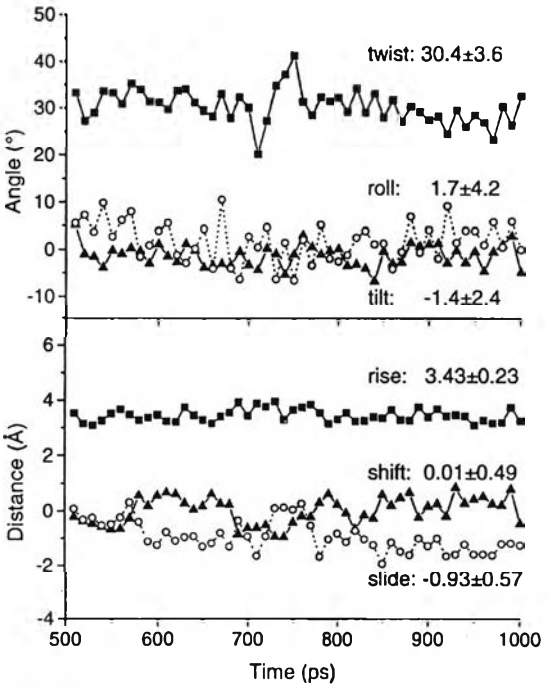

Figure 1.7 Structure fluctuations in an oligomer of 10 Watson-Crick pairs extracted from a MD trajectory of 1 ns.

The Munich group was the first to point out the importance of conformational fluctuations of DNA on the electron transfer coupling in π-stacks. [61] With a QM/MD approach that uses MD simulations of DNA fragments to generate structures, the electronic couplings were shown to be very sensitive to variations of the mutual positions of the Watson-Crick pairs; electronic couplings considerably change with time (Figure 1.7). [61] Furthermore, the intra-strand adenine-adenine interaction was found to be more sensitive to conformational changes than the inter-strand interaction. [61]

Based on quantum-chemical results for the energetic of hole transfer and the electronic coupling of π-stacks, the Munich group estimated the effective electronic couplings of hole donors and acceptors connected via short bridges. [62] The resulting distance decay parameters, β_{el} = 0.79 $Å^{-1}$ for $(T)_n$ and $(A)_n$ bridges, β_{el} = 0.68 $Å^{-1}$ for $(AT)_{n/2}$ π-stacks, satisfactorily agrees with experiment.[12–14]

1.6 Research objectives

In view of the fact that the majority of experiments on charge migration in DNA relies on charge injection via chromophore-DNA complexes and the demonstrated sensitivity of electron transfer to details of the geometric and electronic structure, the project pursues the following goals.

(i) The properties of free and solvated chromophores were investigated using quantum chemical methods: structure, relative stability, spectroscopic parameters (excitation energies, oscillator strengths, dipole moment in the excited state) of chromophores in solution.

(ii) The geometries for chromophores were optimized at the DFT level with Gaussian98 program. After that, excitation energies and osciilator strengths were calculated using configuration interaction of selected singly excited states with the semiempirical method. The calculations were carried out with the program SIBIQ.[57] Moreover, the absorption spectra are investigated using TDDFT calculations at B3LYP/6-31G* level with Gaussian98.

(iii) Molecular dynamic simulations of Rhodamine 6G (R6G) and Pyronine 6G (P6G) in aqueous solution were performed using AMBER package program. This program is very useful for studying the structure and conformational change of the biological and organic molecules. Unfortunately, P6G and R6G molecules are not the standard molecule in AMBER library. Therefore, new parameter set representing electrostatic interaction of the chromophore has to be generated. The atomic partial charges on the dye molecules were derived according to standard procedures consistent with the force field used, namely HF/6-31G* on the geometry optimized at the B3LYP/6-31G* level.

(iv) Before we were able to begin MD simulations on the structure and relative mobility of rhodamine-DNA complexes, a more detailed computational study to find an appropriate protocol for MD simulations of dimers of P6G and R6G in aqueous solution was undertaken. In the following, the results were presented, discussed and compared them to those of previous investigations.

(v) Molecular dynamics simulations of rhodamine 6G derivative (Rho) and DNA complexes were carried out. The derivative rhodamine 6G (Rho) studied in this work is a neutral dipolar form (zwitter-ion). It uses to be intercalators as charge donor and it is attached with DNA duplexes via a flexible linker. Simulations are for the three systems; the first one is Rho connected to 5'-Cytocine of the duplex (Rho-5C) whereas the second and the third systems, the dye is bound to 5'-Gaunine (Rho-5G and Rho-5G'). This aims to study structure and dynamic stability of Rho-DNA complexes to understand how structural characteristics of the chromophore affect the π-stacking interaction between the DNA oligomer.

1.7 Scope of the work

The intended computational investigations were focused on chromophore-DNA complexes to assist in the interpretation of related spectroscopic measurements that aim at an improved understanding of the interdependence of conformational and structural characteristics on the one hand, and electronic and optical properties complexes on the other hand.

For this purpose we *(i)* apply QM methods for a detailed study of the electronic structure of chromophores, *(ii)* MD simulation of the R6G and P6G in aqueous solution and *(iii)* MD modeling of DNA duplexes with rhodamine 6G derivative.