Nucleic Acids as a playground for the computational study of the photophysics and the photochemistry of multichromophore assemblies.

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Conspectus

The interaction between light and multichromophoric assemblies (MCA) is the primary event of many fundamental processes, from photosynthesis to organic photovoltaics and it triggers dynamical processes that share remarkable similarities at the molecular scale: light absorption, energy and charge transfer, internal conversions, emission, and so on. Those events often involve many chromophores and different excited electronic states that are coupled on an ultrafast time scale. This contribution aims to discuss some of the chemical physical effects ruling these processes, a fundamental step towards their control, based on our experience on nucleic acids.

In the last 15 years we have, indeed, studied the photophysics and photochemistry of DNA and its components. By combining different quantum mechanical methods, we investigated the molecular processes responsible for the damage of the genetic code or, on the contrary, those preventing it by dissipating the excess energy deposited in the system by UV absorption. Independently of its fundamental biological role, DNA, with its fluctuating closely-stacked bases stabilized by weak non-bonding interactions, can be considered a prototypical MCA. Therefore, it allows to tackle within a single system many of the conceptual and methodological challenges involved in the study of photoinduced processes in MCA.

In this contribution, by using the outcome of our studies on oligonucleotides as a guideline, we thus highlight the most critical modellistic issues to be faced when studying, either experimentally or computationally, the interaction between UV light and DNA, and, on the same time, bring out their general relevance for the study of MCA.

We first discuss the rich photoactivated dynamics of nucleobases (the chromophores), highlighting the main effects modulating the interplay between their excited states and how the latter can affect the photoactivated dynamics of the polynucleotides, either providing effective monomer-like non-radiative decay routes or triggering reactive processes (e.g. triplet generation).

We then tackle the reaction paths involving multiple bases, showing that in DNA duplex the most important ones involve two stacked bases, forming a neutral excimer or a charge transfer (CT) state, which exhibit different spectral signatures and photochemical reactivity. In particular, we analyze the factors affecting the dynamic equilibrium between excimer and CT, such as the fluctuations of the backbone or the rearrangement of the solvent.

Next, we highlight the importance of the effects not directly connected to the chromophores, such as the flexibility of the backbone or the solvent effect. The former, affecting the stacking geometry of the bases, can determine the preference between different deactivation paths. The latter is particularly influential for CT states, making very important an accurate treatment of dynamical solvation effects, involving both the solvent bulk and specific solute-solvent interactions.
In the last section, we describe the main methodological challenges related to the study of polynucleotides excited states and stress the benefits derived by the integration of complimentary approaches, both computational and experimental. Only exploiting different point of views, in our opinion, is possible to shed light on the complex phenomena triggered by light absorption in DNA, as in every MCA.

Key references (3)

- Improta, R.; Barone, V. Interplay between "Neutral" and "Charge-Transfer" Excimers Rules the Excited State Decay in Adenine-Rich Polynucleotides *Angew. Chemie Int. Ed.* **2011**, *50*, 12016-12019.

[1]A key study where, treating a tetranucleotide in water at a full Quantum Mechanical level, we discuss the main important photoactivated processes in a single strand, propose their extension to the duplex, and provide a unifying framework for the interpretation of the available experimental results, profitably used also in following years.

- Cerezo, J.; Liu, Y.; Lin, N.; Zhao, X .; Improta, R .; Santoro, F. Mixed Quantum/Classical Method for Nonadiabatic Quantum Dynamics in Explicit Solvent Models: The ππ*/nπ* Decay of Thymine in Water as a Test Case. *J. Chem. Theory Comp.* **2018**, *14*, 820-832.

[2]A study where we study the quantum dynamics of the ππ*/nπ* decay for Thymine in water addressing the effect of the dynamical response of the solvent, proposing a feasible protocol of general applicability for rigid systems and ultrafast processes.

- Martinez-Fernandez, L.; Changenet, P.; Banyasz, A.; Gustavsson, T.; Markovitsi, D.; Improta, R. Comprehensive study of guanine excited state relaxation and photoreactivity in G-quadruplexes. *J. Phys. Chem. Lett.* **2019**, *10*, 6873-6877.

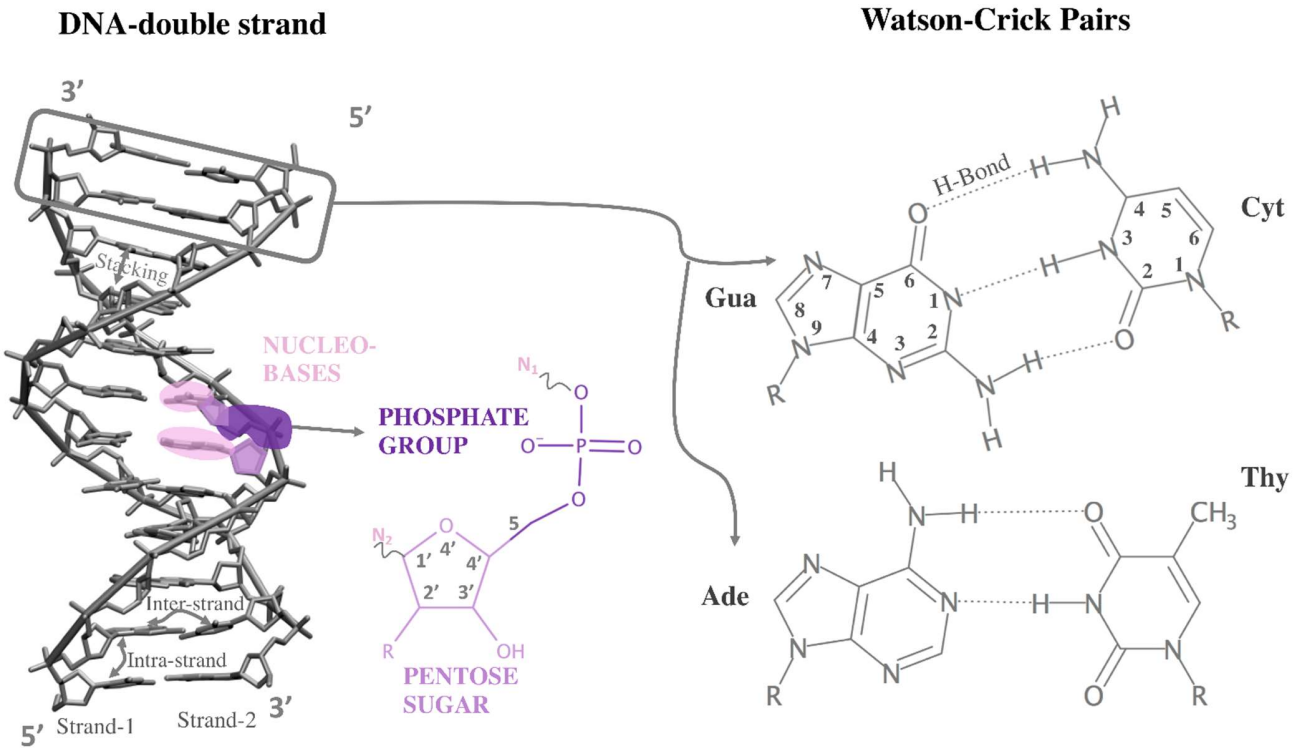
[3]A study addressing the photophysics and photochemistry of quadruple helices, structures which have a key biological role, treating systems containing a dozen of nucleobases by QM/MM calculations, and a nice example of integration between experimental and computational approaches.

Absorption of UV light by DNA triggers a cascade of photophysical events and photochemical reactions with possible pathological consequences (damage of the genetic code, apoptosis, carcinogenesis).[4] This observation has motivated, especially in the last two decades, many studies devoted to elucidate the processes elicited in polynucleotides and in their components (the nucleobases) by UV irradiation.[4] Thanks to the impressive development in Time Resolved (TR) spectroscopies and in computational methods, our understanding of the photoactivated dynamics of Nucleic Acids (NAs, either DNA or RNA), even on the high-resolution sub-picosecond (ps) time-scale, has made important advances, as witnessed by the many reviews and books in this field.[5-9] Though several issues are still matter of a very lively debate, there is general consensus on some basic points, such as the existence of very effective, sub-ps non radiative decay paths to the ground state (GS) for single

nucleobases or the predominance of longer living excited states, involving multiple bases, for oligonucleotides.[5-9]

Besides their biological role, NAs (Scheme 1) have intrinsically interesting features: they are self-assembling closely-stacked multichromophoric arrays (MCA), stabilized by different weak non-bonding interactions, like hydrogen bonds (HB) and van der Waals ones and, due to many charged moieties (phosphate, counter-ions), they generate a strongly anisotropic environment in solution. Oligonucleotides are also flexible and, at room temperature, they adopt different conformations, which can affect their spectroscopic features and excited state dynamics.[5-9] Finally, the photoactivated dynamics in NAs span several orders of magnitude of time, from femtosecond (fs) to several millisecond (ms), if not more.[5-10] We are thus in the presence of systems/processes with strong multiscale character, in size and time, so that their theoretical study necessitates the integration of several computational methods and approaches, from quantum mechanical (QM) to classical molecular mechanics (MM) ones. On this ground, NAs are an excellent playground to investigate the main photoactivated processes occurring in MCA, unveil the underlying chemical-physical effects, and, on the same time, tackle the related methodological challenges.

This is exactly the perspective of this contribution, aimed to highlight key features of UV-absorbing NAs that are potentially relevant for any irradiated MCA, and therefore for the biological processes or the technological applications they are involved in. We shall mainly discuss examples taken from our computational studies on the photoactivated dynamics of NAs in the last 15 years. Overall, our philosophy has been to focus on models close enough to the systems investigated by TR experiments to provide a direct help for their interpretation, meaning that in our models we pointed to account all the main chemical-physical effects into play. Consequently, most of our calculations has been performed in solution and on models containing more than one nucleobase, enabling us to map all the main decay paths, treated, preferentially, at the full QM level. Due to the size of the systems studied, our reference electronic methods have thus been Density Functional Theory (DFT) and Time Dependent-DFT (using long-range corrected functionals), resorting to continuum or mixed explicit/continuum models to include solvent effect. In several studies we complemented static calculations with dynamic ones, pursuing a fully quantum dynamical (QD) description of the nonadiabatic processes, even if, in most of the cases this has limited our analysis to the ultrafast regime (100 fs). We start focusing on effects strictly related to the nucleobases (the chromophores), before discussing those operative in the oligonucleotides and those related to the 'environment', like the (deoxy)ribose-phosphate backbone and the solvent. The last section discusses some of the key methodological features of our approach.



Scheme 1. Schematic description of a DNA double helix, showing also the phosphodeoxyribose backbone and the four bases in Watson-Crick pairs, Guanine(Gua)-Cytosine(Cyt) and Thymine(Thy)-Adenine(Ade).

The Monomers: small does not mean simple.

Nucleobases, are π-systems consisting of one six-member cycle (pyrimidines, Pyr) or fused six- plus-five-member cycles (purines, Pur) (Scheme 1), substituted at some positions with functional groups such as NH_2 or C=O. This peculiar chemical structure results in the coexistence of diverse types of frontier orbitals (Lone Pairs, LP and π), which in turn give rise to different low-lying excited states (nπ* or ππ*) (Figure 1) and possible decay mechanisms (Figure 2)**.** Indeed, in the Franck-Condon (FC) region there is, at least, one dark nπ* excited state quite close in energy to the spectroscopic bright ππ* state(s).[8] These nπ* states (Figure 1) involve the excitation from a LP of a Nitrogen atom of the ring (Ade, Cyt) or from an Oxygen atom of the carbonyl substituent (Ura, Thy, Cyt, Gua). In a series of papers, we mapped the main excited state decay routes for both nπ* and ππ* states for all the nucleobases in different solvents (Figure 2).[11-14] In agreement with the indications of most of the theoretical studies in the field,[8] our calculations show that for all the bright ππ* excited states an almost barrierless path connects the FC point with a crossing region with GS, giving account of the sub-ps decay of the fluorescence signal (Figure 2).[5,7] Although the dark states are not directly excited in FC, we suggested that an ultrafast partial population transfer can occur for Ura[12] and, to a lesser extent, for Thy, a prediction confirmed by experiments.[15] The population of a dark state, likely involving the carbonyl LP, has actually been recorded for Cyt.[11,16,17] We have investigated this population transfer also by QD simulations, for the five nucleobases (studying also its impact on the absorption spectrum),[18] dedicating specific studies to Thy[2], Ura[19], Cyt [20] and Ade.[21]

Our studies show that a subtle interplay between different effects governs the shape of the nπ* and ππ* Potential Energy Surface (PES) in nucleobases and rules their interaction. QD studies[18] on Ura, Thy, Cyt and Ade

in the gas-phase indicate that even small distortion of the ring planarity induces a large electronic coupling between ππ* and nπ* states. Moreover, nπ* states involving the carbonyl LP are characterized by a large reorganization energy. Consequently, also when the nπ* states are less stable than the spectroscopic ππ* states in the FC region (Cyt), a non-negligible part of the photoexcited population can be trapped in their minima. It is also noteworthy that small changes in the ring substituents can have significant effects on the decay mechanisms. This is the case of a key epigenetic base, 5methylcytosine (5mCyt), where the simple presence of a methyl substituent, causing an energy barrier along the decay path of the ππ∗ state, increases fluorescence QY (Figure 2) and, by 7 times, the excited lifetime in solution.[11] On the same time, the lowest energy nπ* states are relatively destabilized and their population is suppressed.[22]

Solvent strongly impacts on the population transfer between ππ* and nπ* states. Here we only quote two examples concerning the lowest energy nπ* associated to the LP of the C4 carbonyl group of Ura derivatives, postponing a more detailed discussion to the following pages. With full QD models we have proposed 1) that fluorescence lifetime of 5fluorouracil is shorter in acetonitrile than in water,[19] because only in the former the ππ* →nπ* decay channel is active, and 2) the nπ* population in Thy is drastically reduced moving from gas phase to a water solution.[2]

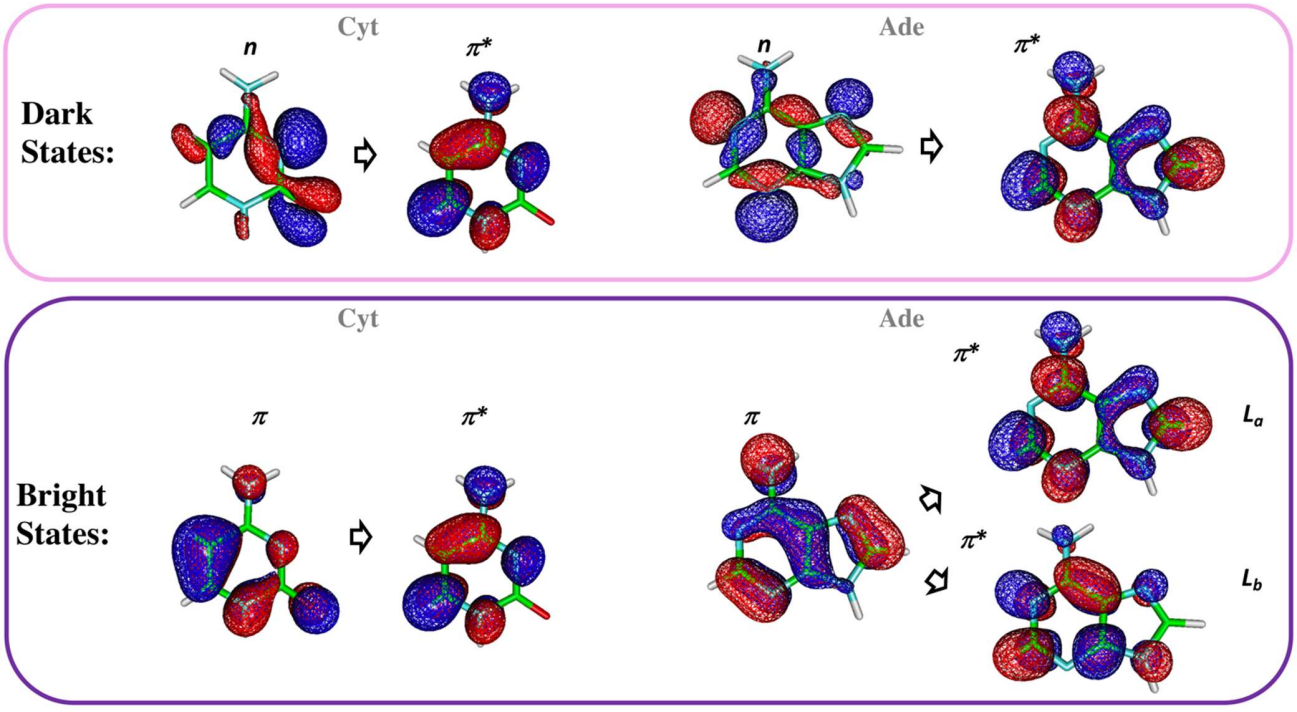


Figure 1. Schematic description of the frontier orbitals of the excited dark (top) and bright (bottom) states involved in the photoactivated dynamics of Cyt (left) and Ade (right).

These considerations are valid also for another kind of 'dark' state potentially affecting the nucleobase photophysics, i.e. triplets. The singlet-triplet inter-system crossing quantum yield (QY) of natural coded nucleobases is extremely small[23] (though not zero).[24] However, the formyl substituent in the epigenetic base 5formylCyt (5fCyt) leads to a triplet QY≥ 0.7, in a mechanism where other dark states (a nπ* and an

intramolecular CT state) act as a doorway for intersystem crossing (Figure 2).[25] In this respect, due to El Sayed rules, nπ* minimum is the most likely doorway state for populating the most stable ππ* triplets.

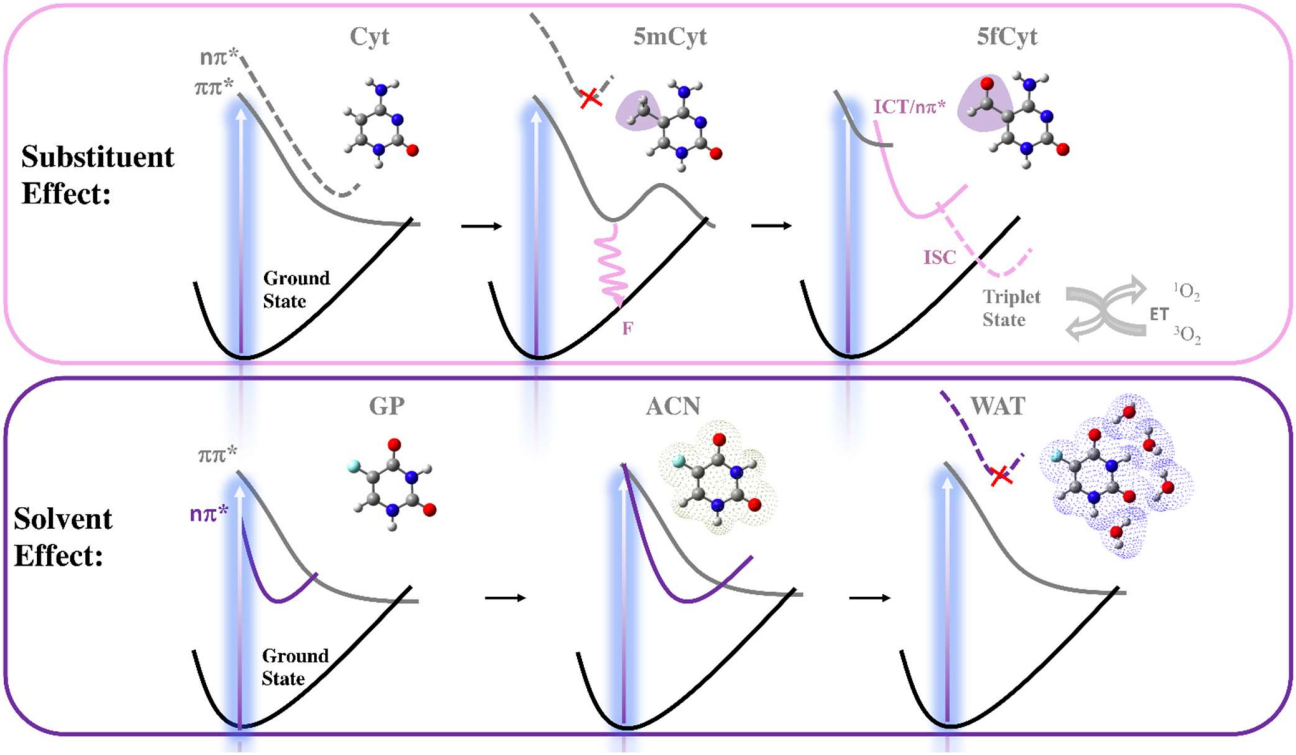


Figure 2. Schematic description of the major deactivation pathway in isolated NAs along the spectroscopic ππ* states. Substituent effect on Cyt (top): longer living ππ* states increase the fluorescence (5mCyt)[11] or intersystem crossing (5fCyt)[25] QYs. Color code: ππ* states in solid gray, nπ* dashed gray and other states (ICT or triplets) in pink. Solvent Effect (bottom): the environment modulates the interplay between ππ* and nπ* states in 5fluoroUracil.[12,19] Color code: ππ* states in solid gray, nπ* in purple. Data taken from refs. [11,12,19,25]

In NA, as in many MCA's, the focus is often on the processes involving multiple nucleobases (e.g. charge transfer, energy transfer). However, each single monomer can have an intrinsic very rich photophysics and photochemistry making the interpretation of TR experiments more complicated. Monomer-like decay routes and processes (like triplet generation) can be operative also in MCA and compete with inter-monomeric processes (e.g. charge-transfer), providing, in the case of NAs, effective deactivation routes to the GS.

The polymer (oligonucleotides): Large does not mean stiff; turtles and hares play together:

In oligonucleotides several bases are closely spaced and interact through stacking (intra-strand) or H-bonds (inter-strand) (Scheme 1). Consequently, their excited states can involve multiple bases[9,26] and their decay become much more complex, as witnessed by the several time components needed to fit the TR signals, with characteristic times spanning several orders of magnitudes.[6,7] The study of oligoAde[1,27,28] single strands, either

including[1,27] or not the effect of the backbone,[26,27] enabled us to define some of the key players providing a quite general overview of the chemical physical effects in play also in other sequences.

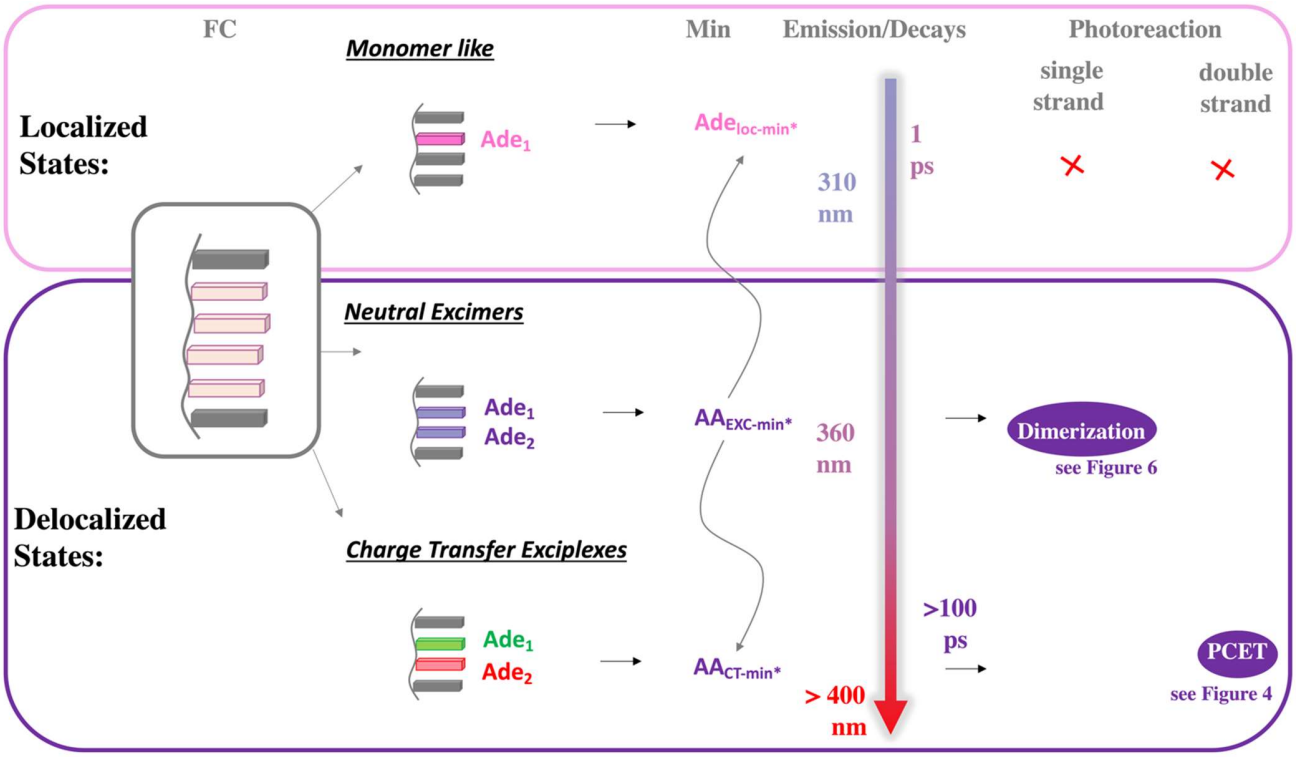


Figure 3. Schematic description of the main photophysical and photochemical processes involving oligoAdenine[1,27,28] tracts and of the associated excited states (involved bases colored). Localized excited states (top) and delocalized excited states (bottom). Data taken from refs. [27,28]

In our study of oligonucleotides, we have shown that despite the absorbing states are delocalized over multiple bases (2-4) they readily decay to localized states on a single base (monomer-like) or on two stacked bases (Figure 3).[1,28] In this latter case, both frontier orbitals can be delocalized on the two bases, in states we shall label as "neutral excimers' (Figure 3). Alternatively, the HOMO can be mainly localized on one base and the LUMO on the other, in an excited state with significant charge transfer (CT) character (CT exciplexes in Figure 3). These excited states have different spectral signatures and photochemical reactivity. Neutral excimers are indeed associated to the maximum of the fluorescence spectrum of oligoAde, significantly red-shifted with respect to that of Ade monomer-like.[1,28] Moreover, for suitable syn/anti arrangements of the bases (see next subsection) they can produce Ade/Ade photodimers.[29] CT states should be, instead, responsible of the long tail on the red-wing of the fluorescence spectra, giving account of the decrease of the fluorescence anisotropy with the increase of the emission wave-length.[1,28] Moreover, when Ade is base-paired in duplexes with Thy, (Figure 4) CT can trigger inter-strand Proton Transfer (PT) reactions, when the 'negatively' charged Ade^- accepts a proton from the H-bonded Thy pair, in a Proton Coupled Electron Transfer (PCET) reaction.[30-32]

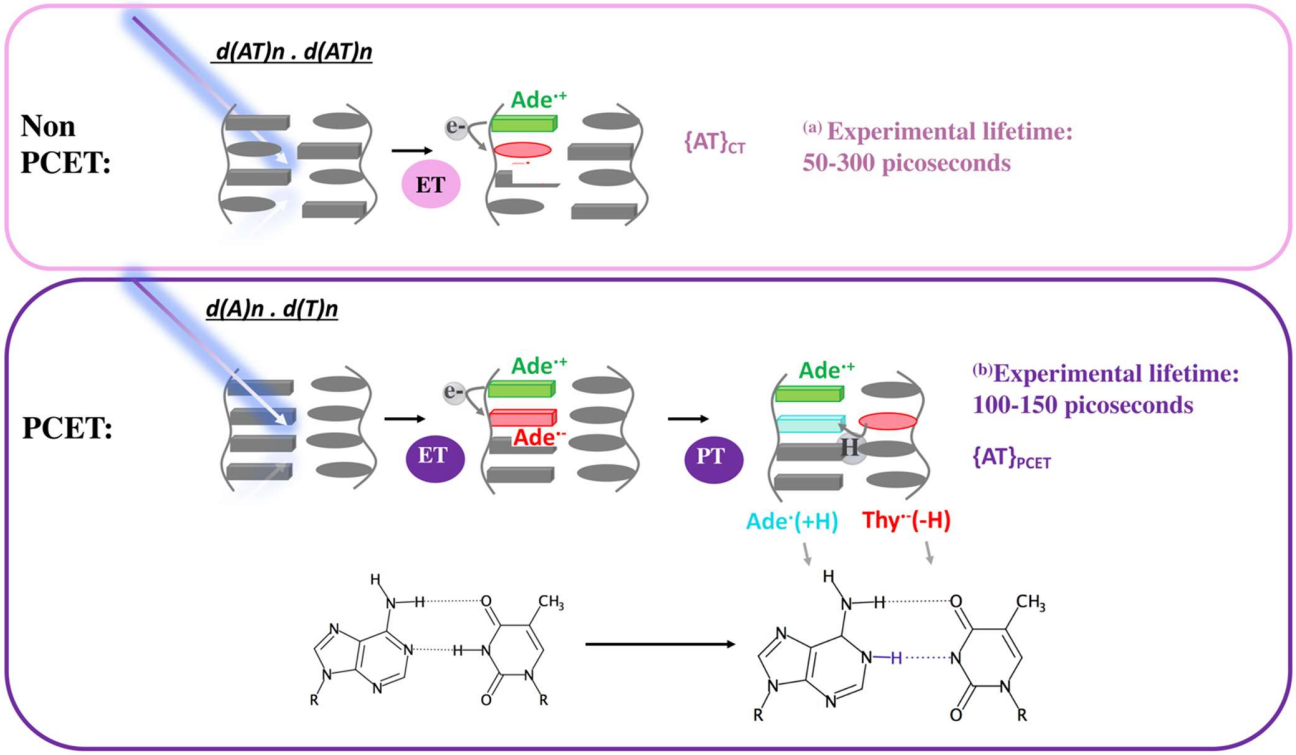


Figure 4. Schematic description of the CT and PCET processes occurring in alternated d(AT) (top) and homopolymer (dA)(dT) (bottom) DNA sequences.[31,32] Experimental lifetimes taken from refs (a)[32,33] and (b)[32,33]. Data taken from refs. [31-33]

The above picture provides a suitable interpretative framework for many other sequences. In Pyr/Pyr stacked homo- and hetero-dimers, 'neutral excimers' are those responsible for the formation of cyclobutane pyrimidine dimers (CPD), the most abundant DNA photolesion (Figure 5, top panel).[34] CT states are intermediates for the formation of 6-4 pyrimidine-pyrimidone photodimers (64-PP), following the formation of an oxetane intermediate (Figure 5, bottom panel), in Thy/Thy[34] and 5'Cyt/3'Thy steps, or without any intermediate in 5'Thy/3'Cyt steps.[35] PCET reactions are operative also in GC and AT duplexes.[31,32,36] Moreover, low-energy photoionization, observed in some DNA sequences, has been proposed to be initiated also by CT states.[10]

The complex photoactivated dynamics of G-Quadruplex helices, non-canonical structures formed by Gua rich sequences, can also be interpreted within a similar framework to that of duplexes, providing the co-existence of monomer-like, excimer-like and CT states.[3]

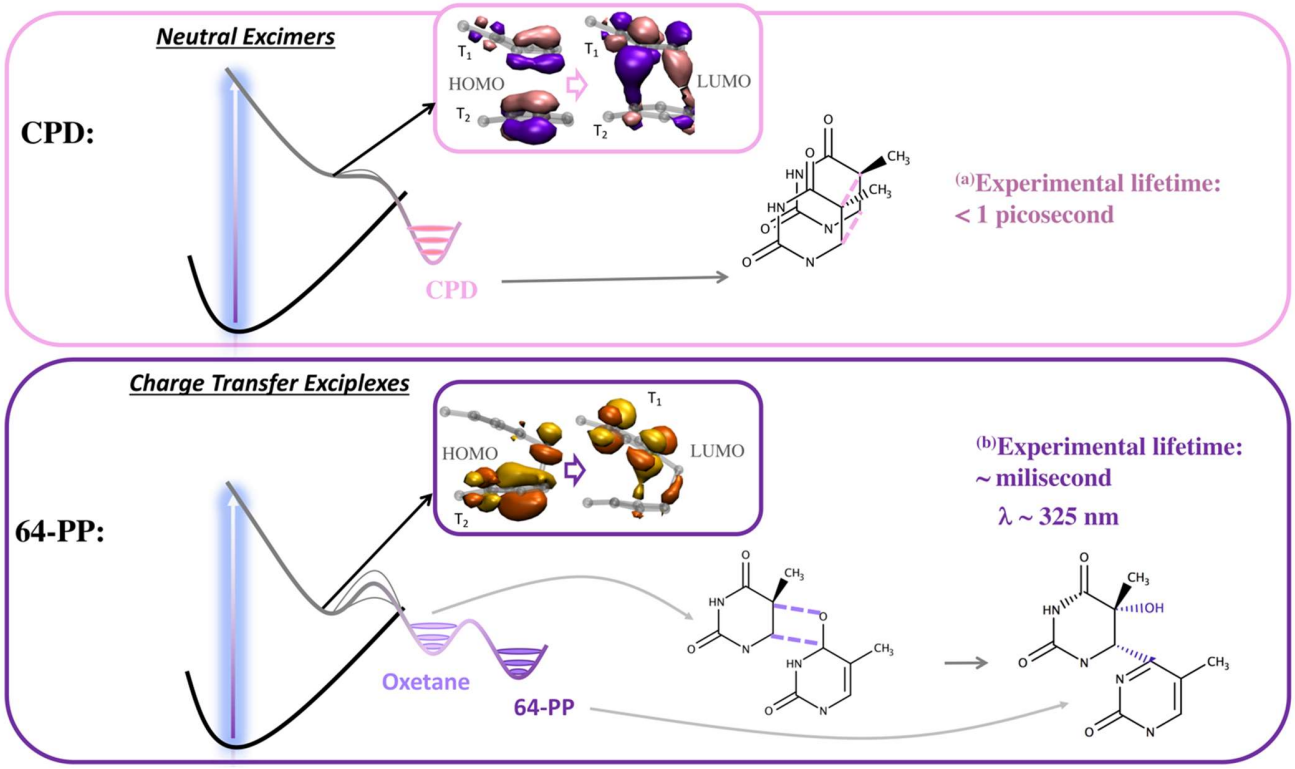


Figure 5. Schematic description of the main photochemical paths identified in the study of Pyr/Pyr steps[34] leading to CPDs (top) and 64-PP (bottom). Data taken from ref. [34], (a)[37], and (b)[38].

QD studies of the early photodynamics of GC and AT base pairs[39] in gas-phase based on Linear Vibronic Coupling (LVC) models parameterized with TD-DFT show that within 100 fs most of the initial photoexcited population on Gua and a substantial part of the one on Cyt decays to a G(+)-C(-) CT state, which should afterward lead to a PCET.[40] Such process is much less efficient in AT base-pair where most of the population follows intra-molecular decays to dark nπ* states.[41] A similar approach applied to GC stacked dimers predicts a population of the CT state in 50 fs,[42] and for GC tetramers indicates that the population of intra-strand CT is favored over the inter-strand one (unpublished results). Whereas, at the state of the art, QD studies are confined to the ultrafast regime, when the system has not time to undergo large distortions, semiclassical surface hopping approaches can investigate longer time-scales. A QM/MM simulation[43] of the photoexcited dynamics of a stacked tetramer of Ade in a longer oligomer of 20 Ade in water, describing 4 Ade bases at the QM level and the rest of the system at the MM level, found that the initially-populated delocalized excitons decay in monomer-like excitations (20 fs) or in neutral excimers (130 fs), which, then, progressively acquire a marked CT character (170 fs). The monomeric states decay either to the ground state (>2 ps) or convert to the same excimers (1100 fs) and subsequently to CT states.

In summary we identified 'monomer like' states, neutral excimers, and CT exciplexes as the key players for many of the photochemical and photophysical processes occurring in NAs. However, it is important to remind that, due to the peculiar properties of NAs, closely stacked fluctuating MCA, these labels are a useful interpretative framework but should not be taken 'literally'. In other words, CT states involving two bases with a stacking distance <4 Å, can have partial CT character, i.e. without involving the transfer of a full charge. Analogously,

neutral excimers perfectly delocalized on two bases should be considered a limiting case, which is impossible, for example, when the two bases are different, as it happens in AT steps. A more realistic picture of the complex photoactivated processes in DNA, thus, provides that these three pathways are in dynamic equilibrium ruled by different chemical physical effects and, in particular, by the interplay between fast and slow degrees of freedoms (DoFs). Population transfer between the different excited states mainly involve the fast stiff vibrational modes of the bases (bond lengths and angles). Slow DoFs on the contrary are related to the conformational behavior of the backbone, and to part of the environmental effects (see sections below). The conformation adopted by the strand at the moment of the excitation, ruled by the 'slow' fluctuations of the backbone, is surely an influential factor. For example, monomer-like decay would be favored for poorly stacked bases.

In NA, as in many MCA, the photoactivated dynamics is determined by the interplay between different kind of excited states, involving mainly one or two monomers, each one with its own spectral signature. When the chromophores, as in NA, are strongly coupled, any 'precise labeling' of the different excited states should be used cautiously and mainly for interpretative/modelling purposes. Depending on the time-scale considered, the motion along different degrees of freedom, associated both to the NAs and to the solvent, rules the equilibrium between the different excited states and their associated reactions.

The backbone: **silent does not mean innocent.**

Since the sugar and the phosphate groups absorb in the far UV (≤200 nm) their role in NA's photophysics/chemistry is usually overlooked. However, backbone conformations rule the mutual arrangement of the bases and, therefore, their electronic interactions. On this ground, although indirectly, the backbone can deeply impact on many of the NA's photoactivated reactions. For example, we have shown that the combination of the different sugar puckerings (Figure 6) along the chain modulates the stacking geometry (e.g. the inter-base distance) of two Thy's and, therefore, their photodimerization reaction,[34,44] i.e. the most frequently encountered photoproduct in DNA. Explaining the experimental trends, when compared to other combination (e.g. C2/C2, for which monomer-like decay is preferred), steps where both sugars have a C3-endo puckering exhibit a shorter interbase distance, and a stronger coupling between the excitations localized on the monomer, giving rise to the excimer state (Figure 6) responsible for Thy-Thy dimerization.

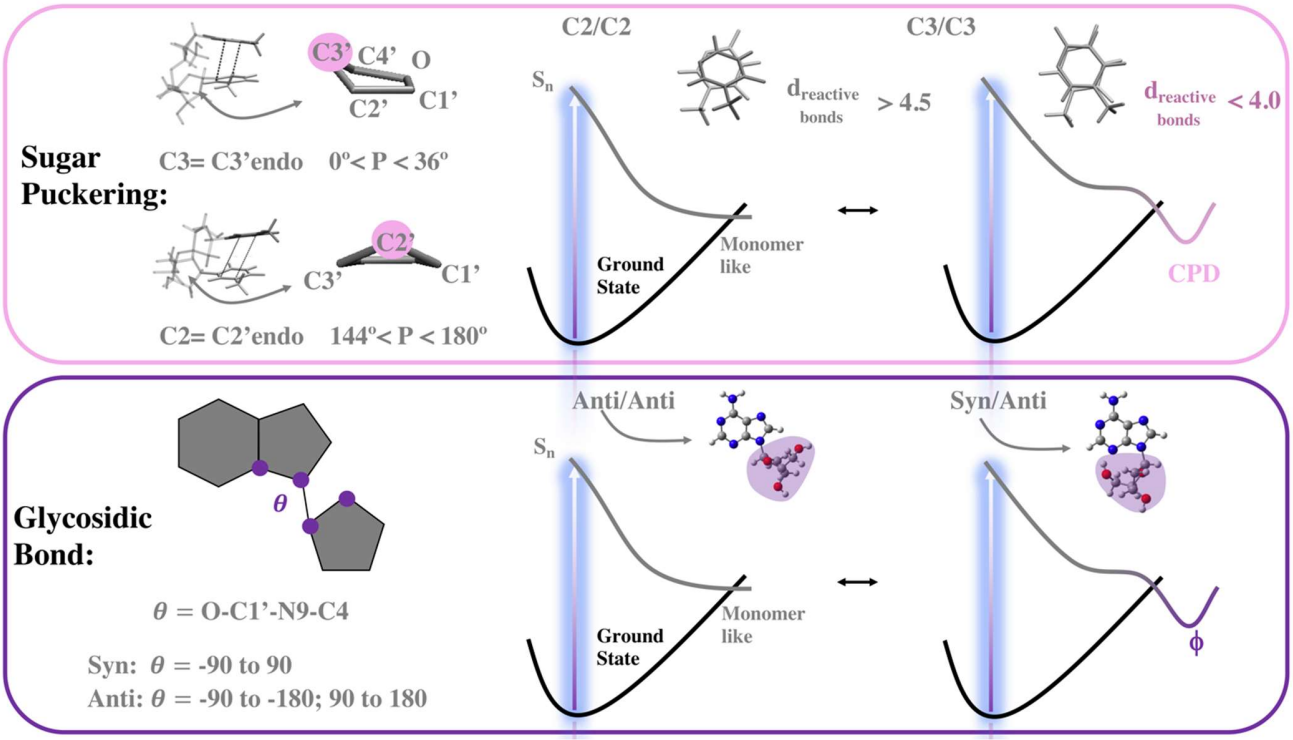


Figure 6. Schematic description of the PES associated to lowest energy excited state for different conformations of the sugar (P=phase angle) in a dinucleotide Thy/Thy step (top) and different syn/anti conformers of the Ade/Ade step (bottom). Data taken from refs.[29,34,44]

Ade/Ade steps offer another example of the importance of the orientation of the bases with respect to the backbone.[29] We have shown that when both bases adopt an *anti* conformation with respect to the glycosidic bond (Figure 6), the one typical of duplexes, a very large energy barrier prevents the dimerization reaction, explaining why in duplexes Ade-Ade dimerization yield is almost zero.[45] For single strands, syn/anti steps are possible and undergo a barrierless dimerization on the lowest energy excimer, in line with the observed formation of Ade-Ade dimer in single strands.[45]

There are surely other effects, not yet been investigated in detail, and not directly connected to the chromophores that impact the photoactivated dynamics of NAs. For example, the counter-ions modulate the energy of the excited states[46] and their motion could be crucial for the reactivity of CT states. Moreover, the sequence and order of nucleobases along a given strand can determine the stability of a given CT state and the photochemical reactivity. For example, in Thy-Thy and Thy-Cyt steps, (5'→3') CT states, where the charge is displaced from the base in 5' position towards that in 3', are more stable than those acting in the opposite direction, 3'→5,' and give rise to 64-PP photoproduct.[35,44]

In NA, as in many flexible MCAs, the photoactivated dynamics can be strongly affected by the behavior of components that, though not photoactive, can determine the structural arrangement of the chromophores. The electronic interaction between two nucleobases (or two chromophores in a generic MCA) are indeed determined by their relative orientation and, also, by their position within the MCA.

The solvent: **Solutions sometimes give problems**.

Biologically and technologically relevant processes occur in the condensed phase, making the inclusion of solvent effect very important. Our studies on NAs confirm that solvent can have a strong impact on the photoactivated dynamics, even when it does not directly take part to the reactions, as in the case of the deprotonation of Gua radical cation.[47] As anticipated above, the stability of the transitions involving the LP of nitrogen and, especially, of the carbonyl oxygen atoms strongly depends on the embedding medium and their possibility to be engaged in the H-bonds with solvent molecules. Consequently, $n\pi^*$ states are strongly destabilized since one electron is transferred from the LP in a π^* orbital in the ring. By using mixed models, where the molecules of the first solvation shell are explicitly included in the computations while bulk solvent effects are treated by the Polarizable Continuum Model (PCM), we have estimated that in water the lowest energy $n\pi^*$ state of Ura or Thy is destabilized by 0.7 eV with respect to the spectroscopic state, deeply affecting the photoactivated dynamics.[2]

Several studies also highlight the importance of dynamical solvation effects.[48] Indeed, while solvent electronic degrees of freedom can be considered always in equilibrium with the excited state density (fast polarization), the full equilibration of the solvent response, both the one mediated by the librational motions of the solvent molecules (important in water) and the 'collective' one due to the rearrangement of the solvation shells, require a finite time, which depends on the solvent. In our studies, we often treated dynamical solvation effects by resorting to a simple procedure available within continuum models. It considers two limiting situations, (short-time) non-equilibrium and (long-time) equilibrium, by simply dividing the solvent response in two components, ruled by two different dielectric constants (the optical and static ones, respectively).[49] This simple recipe suggests that solvent effects strongly modulate the equilibrium between neutral excimer and CT exciplexes in oligonucleotides.[1,27,28] The CT minima are indeed strongly stabilized by a full equilibration of solvent degrees of freedom. Solvent dynamical equilibration is also responsible for the appearance of an energy barrier in the path from a CT state to the formation of the oxetane intermediate in the reaction producing 64-PP photoproduct, stabilizing the minima of the CT state associated to this path. Even the application of continuum models is, in any case, challenging in oligonucleotides, because of their strongly anisotropic nature, and the presence of close-lying charged species (the phosphate groups, the counter-ions). Backbone conformational motions might be treated as 'environmental' degrees of freedom, whose rearrangement is important to fully stabilize CT states. However, this poses a further challenge increasing the time-window to be monitored, since the conformational rearrangements of the duplexes can be very slow (even on the ms time-scale).[50,51]

We recently proposed a hybrid dynamical approach to study the $\pi\pi^*/n\pi^*$ decay of Thy in water coupling the QD of wavepackets moving on the $\pi\pi^*/n\pi^*$ PES with the classical molecular dynamics (MD) of a solvent reacting to the nonadiabatic transition. Our results[2] (Figure 7) indicate that the $n\pi^*$ destabilization in water translates in a much smaller $n\pi^*$ yield than in gas phase. We document a large sensitivity of the predictions to the adopted solvent model, either implicit (LR-PCM or SS-PCM) or explicit (MD). In particular computations with explicit solvents indicate that[2] water solvent is fast enough to react to the ongoing nonadiabatic dynamics and modify its outcome; this was shown comparing the coupled dynamics (QD-MD), with the limiting case in which the solvent is considered too slow to move after photoexcitation (FluctΔE): the water dynamics tends to decrease the $n\pi^*$ yield. Finally, our results suggested that for close-lying electronic states mutual solvent/solute polarization may have a large impact on the nonadiabatic dynamics (compare QMsol and PCsol results). On the balance we predicted a negligible $n\pi^*$ population for Thy in water. This is in agreement with recent surface

hopping calculations in water with a QM/MM method where the QM part is described at RASPT2 level.[52] The latter work also pointed out that solvent slows down the decay to the ground state in thymidine, inducing a dynamical barrier in the movement toward the Conical Intersection.

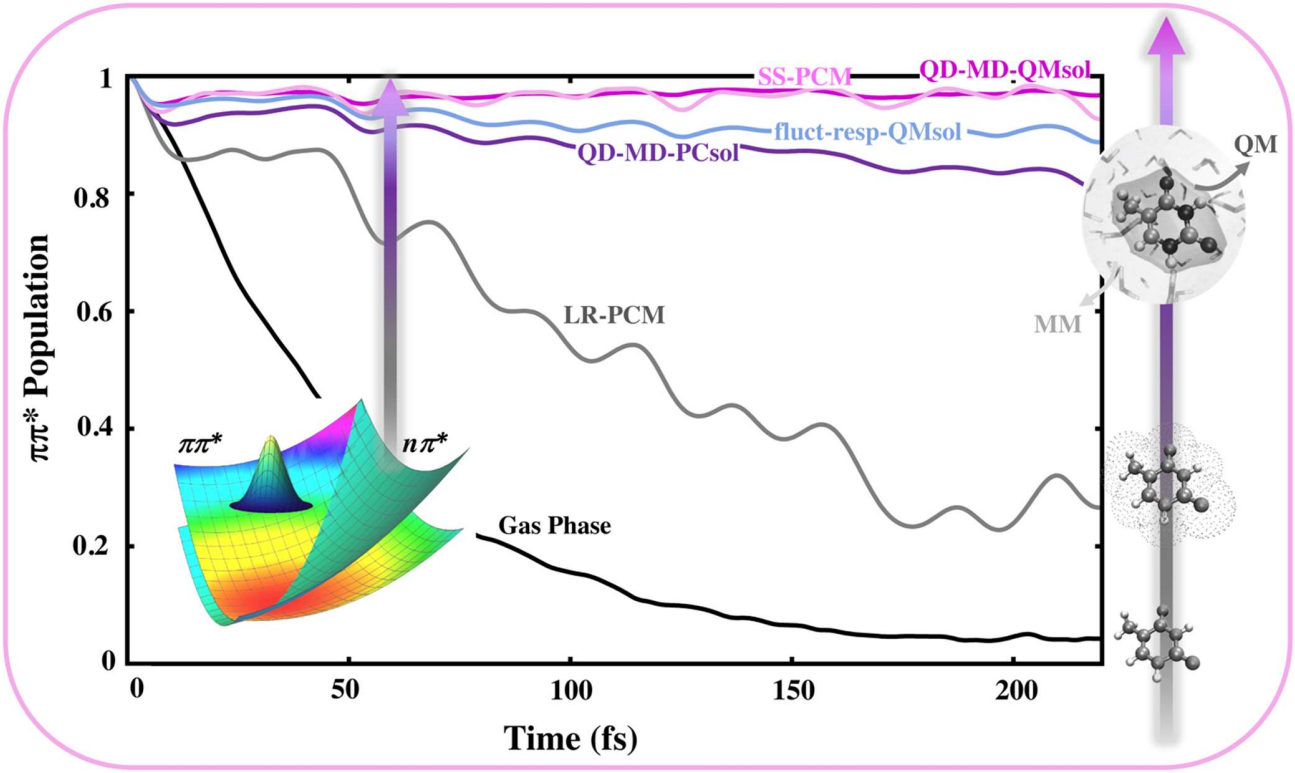


Figure 7. Quantum Dynamics of the ππ*/nπ* population transfer of Thymine. Results in gas phase are compared with those obtained in water with different solvent models: two different implementations of PCM, LR-PCM and SS-PCM and an explicit solvent model. In that case the solvent sampled with a number of representative configurations from an MD in the ground state is either moving according to classical MD coupled with the QD with a mean-field scheme (QD-MD) or is too-slow to move during the nonadiabatic transition (FluctΔE). In QMsol and PCsol the first solvent sphere around Thy is considered at quantum level or as point charges (PC). Data taken from ref.[2], where additional details can be found.

In NAs, as in many MCA, solvent can affect both intra-monomer and inter-monomer excited states, especially transitions involving Lone Pairs (thus affecting H-bonds) and CT states. An accurate treatment of the dynamical coupling between solute and solvent degrees of freedoms is very important.

The methodological approach: Two eyes are better than one

The above discussion clearly highlighted the main methodological challenges to be faced when studying the photoactivated dynamics of NAs and MCA in general. If attaining an accurate and balanced description of the dynamics of isolated nucleobases is not trivial, due to the coexistence and competition of different excited states, the same task is of course much more challenging for oligonucleotides. At the moment, accurate wave-function based methods can be practically applied to a maximum of two/three -bases, leaving DFT and TD-DFT as the only option for larger systems.[53] Despite the recent advances in DFT theory and the development of more and more

accurate functionals, there are problematic situations for TD-DFT as conical intersections with the GS, double excitations, etc.[54] In general, TD-DFT accuracy could not be sufficient to provide a correct picture of the photoactivated dynamics and therefore, based on our experience, comparative studies, using different computational approaches, are always extremely useful. Inclusion of solvent effects poses additional challenges, and the coupling between a given electronic method and a solvation model is also delicate and can give rise to error cancellation. Finally, QM/MM[55] approaches are very effective, but they should also be handled with care. The choice of the part of the systems to be studied at the QM level is not innocent and can dictate a major part of the final result. Just to make an example, any inaccuracy in describing the conformational equilibrium of sugar puckerings can impact on the stacking geometry and therefore on the predicted dynamics. There are other issues (e.g. the treatment of the conformational flexibility of the backbone, or of the electrostatic interaction with CT states etc.) for which the accuracy of the classical force fields cannot be taken for granted. Comparative studies, on smaller, yet meaningful systems, with full QM calculations are always very important.[43,52]

In DNA oligomers, the application of standard excitonic Hamiltonians, profitably used to simulate the photophysical properties of many MCA, is more challenging due to the proximity of many chromophores and the strong participation of CT transitions. In some recent contributions[56,57] we have successfully applied to the simulation of the spectral properties of NA a more refined approach, which exploits a fragment diabatization procedure (FrDEx) to parameterize the excitonic Hamiltonian, addressing the role of CT states and of the overlap between the MOs of the different chromophores. We have shown that such an approach can be also exploited to parameterize generalized LVC Hamiltonians suitable to study at fully QD level the competition between monomeric and inter-monomeric decay channels in MCA. As reported above, this methodology was recently applied to study the photodynamics of AT and GT base-pairs,[39] and it promises to be of general applicability.[58]

The integration between different techniques is also necessary to simulate the photoactivated dynamics, considering the interplay between elementary processes occurring on very different timescales. The population transfer between $n\pi^*$ and $\pi\pi^*$ states in the FC region occurs in a few dozens of fs, but its simulation requires an accurate treatment of the vibronic interactions and makes the inclusion of quantum nuclear effects desirable. The GS recovery in polynucleotides is instead characterized by several time components, from the sub-ps to the hundreds of ps and it is easier to describe it with semiclassical approaches. Finally, these electronic processes are affected by the conformational motions of the backbone, which requires a structural sampling on a longer time scale, by means of classical MD simulations.

Despite all the recent advances, the complexity of the processes to be studied makes very difficult to reach solid conclusions by resorting exclusively to computational studies. Based on our experience, the integration with the indications provided by the experiments is often necessary, and joint experimental and computational studies are extremely informative. In this respect, the availability of 'resolved' experimental spectra to be 'directly' compared with the computational results is particularly useful, as witnessed by the information on PCET processes in DNA duplexes gained through the assignment of the TR-IR spectra.[32]

Modeling NAs, as many MCAs, always involves multiple and delicate choices, starting from the selection of the 'meaningful' computational model, the level of treatment (classical, semiclassical, full quantum) of the different subsystems, and so on and initial choices can bias the final predictions of a computational study. Although these approximations are, nowadays, necessary since any reduction of the complexity often has a

strong modellistic potential, the limitations of any model should always be considered, so that cross-checks between different approaches (computational and experimental) are always recommended.

Though it is not possible to provide here a review, even partial, of the large variety of processes based on the interaction between light and a MCA, many of the conclusions we drawn on NA´s are relevant for other systems. Just to make a few examples, the interplay between exciton and CT states is crucial for organic solar cells[59] and in light harvesting systems.[60] Actually, the similarity, and the differences, between the processes involved in the photoactivated dynamics of NAs and light harvesting systems have been recently discussed in an interesting contribution.[60] Moreover, excited state electron transfer and PCET[61-63] which occur in photoexcited DNA play a key role in natural[64] and artificial photosynthesis.[65] It is well known that also these processes are strongly affected by the solvent [66-68]

Obviously, any complex system or process, as those involved in the photoactivated dynamics of MCA, exhibit its own peculiarities, whose detailed and careful consideration is necessary for a successful modeling. On the other hand, as we tried to demonstrate here based on study on NAs, the basic chemical physical effects into play are similar, as well as the related methodological issues. Further substantiating this perspective could make easier to the translation of approaches and interpretative frameworks from different research areas, allowing further advances in our mastering of processes fundamental for our life and society.

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Biography

Lara Martinez Fernandez, graduated in physical chemistry at the University of Oviedo (2009) and obtained her Ph.D. at Universidad Autónoma de Madrid (2014). After her PhD she became a postdoctoral fellow in Naples (IBB/CNR) and Paris (CEA/CNRS) and since 2018 she is an assistant professor at the Universidad Autónoma de Madrid. Her research interests focus on the study of any process initiated by UV light absorption in biologically relevant systems, as DNA or fluorescent probes, through quantum mechanical calculations and non-adiabatic molecular dynamics.

Fabrizio Santoro got his PhD in Chemical Sciences in 1999 in Perugia and had post-doc experiences in Siena and Würzburg. Since 2001 he joined the Italian National Research Council in Pisa, and is Research Director since 2019. His work focuses on the development of methodologies for studying photoinduced molecular processes, accounting for the quantum nature of vibrational motions. He is interested both in (chiro-)optical steady-state spectroscopies, and in the quantum dynamical description of nonadiabatic processes, studied in detail in DNA nucleobases. Recently, his focus is moving from single molecules to multichromophoric systems and from gas-phase to complex environments.

Roberto Improta, after receiving a Ph.D. in chemistry at the University Federico II of Naples and post-doc experiences at Pisa, Houston and Naples, in 2001 joined the Italian National Research Council (CNR), where he is

now Research Director. His main research interests involve the development, validation and application of theoretical models and quantum mechanical computational methods for the study of large size molecules, especially in their excited electronic states. In the last years he focused in particular on the study of the photophysics and photochemistry of nucleic acids.

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