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Introduce of Triplet Excited State in DNA and RNA

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1.1 Introduction of the Significance of Triplet Excited State in Nucleic Acids

Although the sunlight is essential for life on earth, it contains a large amount of ultraviolet (UV) photons, and the integrity of nucleic acids is jeopardized by the initial assault of those highly absorbent UV solar photons, posing a significant risk to genomic stability of life on Earth [1]. Fortunately, the unique chemical composition of DNA/RNA nucleobases as well as their second- and third-order structures facilitates the deactivation of excited singlet electronic states typically on the picosecond timescale, effectively dissipating the absorbed photon energy and reducing the likelihood of DNA/RNA photodamage. Indeed, excited state dynamics of nucleic acids has been extensively studied due to the development of state-of-the-art ultrafast time-resolved spectroscopy, more accurate computational methods, more efficient software, and the continuously increasing computing power. The research progress achieved in the past two decades has been timely reviewed from both experimental and theoretical aspects [2–11]. However, the main focus of those studies usually starts with excited singlet states because absorption of a UV photon in nucleic acids can only produce excited states with singlet multiplicity, and their decay pathways have to be fully understood before one can further study the intersystem crossing (ISC) process that generates triplet excited state as well as their dynamics.

Triplet excited states represent a distinct electronic configuration characterized by two electrons occupying different orbitals with parallel spin orientations. This spin alignment endows triplet states with specific quantum number features in spin configuration systems. The total spin angular momentum quantum number (S) equals 1 for triplet states, compared to $S = 0$ for singlet states. According to quantum mechanical principles, when $S = 1$, the magnetic quantum number (m_s) can take three

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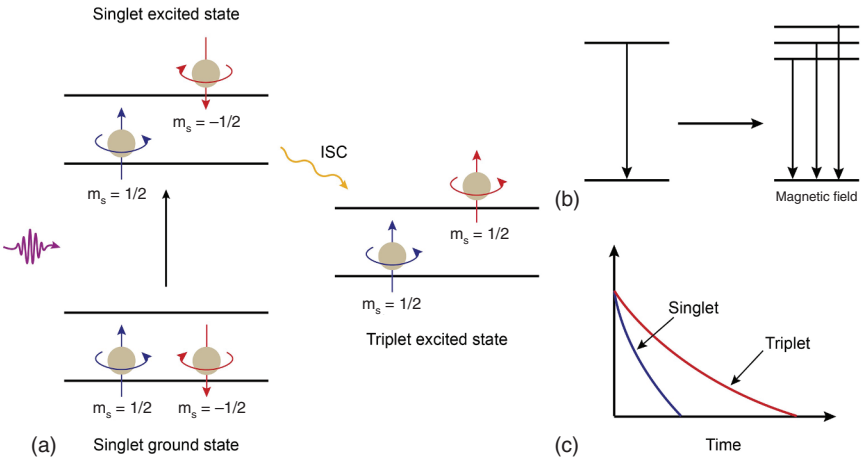
projection values: (−1), (0), and (+1), corresponding to three quantized orientations of spin angular momentum in a magnetic field. Under external magnetic fields, this spin degeneracy interacts with the field through the Zeeman effect, splitting the originally degenerate triplet energy levels into three nondegenerate sublevels—a phenomenon that directly accounts for the "triplet" nomenclature (Scheme 1.1).



Scheme 1.1 (a) Illustration of singlet and triplet excited states. (b) Illustration of Zeeman effect. (c) Comparison of lifetimes between singlet and triplet excited states.

Triplet states exhibit fundamental differences from singlet states in both spin configuration and spectroscopic transitions. Transitions between triplet and singlet states involve electron spin state alterations, making them subject to strict selection rules with distinct transition probabilities and rates. According to selection rules, transitions preserving spin multiplicity ($\Delta S = 0$) are allowed, while those altering multiplicity ($\Delta S \neq 0$) are forbidden. In ground states (S_0), electrons will usually occupy lower energy molecular orbitals with opposite spins (singlet configuration). Upon photon absorption, molecules undergo vertical transitions to singlet excited states (S_n), where electrons maintain identical spin multiplicity. Subsequent processes follow Kasha's rule: rapid vibrational relaxation (VR) and internal conversion (IC) lead to the lowest energy conformation of the first excited singlet state (S_1), which may differ geometrically from S_0. Spin-flipping through ISC then generates triplet excited states. Direct triplet excitation via photons is virtually impossible due to the spin–orbit coupling (SOC) prohibition, rendering triplet states typically "dark" in nature. It is worth noting that the transition from triplet state to the ground state ($\Delta S \neq 0$) is also prohibited, leading to much longer lifetimes (up to microseconds or even milliseconds) compared with singlet states (usually in nanoseconds).

Triplet excited state of nucleic acids and their participation in DNA photodamage after direct UV irradiation have discovered in the 1960s with the help of low-temperature spectroscopy techniques [12–16]. On the other hand, experimental spectroscopy study on the triplet excited state dynamics in nucleic acids at

room temperature is not straightforward because of its low extinction coefficient (~1000 M^{-1} cm^{-1}) and small quantum yields (~0.02) [17–20]. Therefore, a simplifying experimental approach, in which a triplet sensitizer is excited in the presence of nucleic acids at a wavelength where the latter one's absorption is negligible, is commonly used during the studies on the photophysics and photochemistry of nucleic acids when triplet excited states are involved [21–26]. Thanks to the development of advanced spectroscopic techniques and high-precision quantum chemical calculations, there is more intuitive evidence for the mechanistic understanding of the triple excited state dynamics as well as triple excited state-mediated photodamage of nucleic acids in the past decade [27–39]. Furthermore, triplet states have also been identified as playing a crucial role in the effectiveness of DNA-intercalated photosensitizing medicines [40–42]. Therefore, a comprehensive review focusing on the topic of triple excited state dynamics in DNA/RNA and the related photophysics and photochemistry is timely. In this book, we focus on those works that provide insight into the triplet excited states in DNA/RNA, which is a choice that represents our own research interests and also the limited perspective on the vast area of photophysics and photochemistry of nucleic acids [8, 11]. We review research progress in both experimental and theoretical studies on the triplet excited state dynamics in DNA/RNA and try to integrate the seemingly disparate results into a comprehensive model for explaining the remarkable triplet excited state-related photophysical properties.

1.2 Triplet Excited State Studies in the 1960–1970s with Low Temperature Spectroscopy

The study of triplet excited state in nucleotides by phosphorescence spectroscopy has been a subject of fascination within the fields of biochemistry and physical chemistry and nucleic acids. In this section, we briefly recall the main discoveries on DNA/RNA triplet excited state from the early 1960s through the 1970s, which is the first Golden Age of DNA photophysics. The first report on the phosphorescence in nucleic acids, which laid the foundation for understanding the origins of phosphorescence, was registered by Steele and Szent-Györgyi [43]. Then, the first results on isolated DNA/RNA monomers were obtained by Longworth in 1962 [44] and Bersohn in 1964 [45]. Phosphorescence from DNA/RNA at 77 K is typically observed as the sum of emissions from individual nucleotides, reflecting the triplet states of adenosine monophosphate (AMP), guanosine monophosphate (GMP), thymidine monophosphate (TMP), cytidine monophosphate (CMP), and uridine monophosphate (UMP), as shown in Figure 1.1 [16]. The phosphorescence quantum yields and lifetimes of the abovementioned monomers at different conditions are summarized in Table 1.1 [46]. It is worth pointing that the data in Table 1.1 are under the conditions of direct UV excitation of those nucleotides, which is the limitation of content in this chapter, and these values may vary under sensitization conditions. Indeed, triplet sensitization is an extremely important technique since it not only

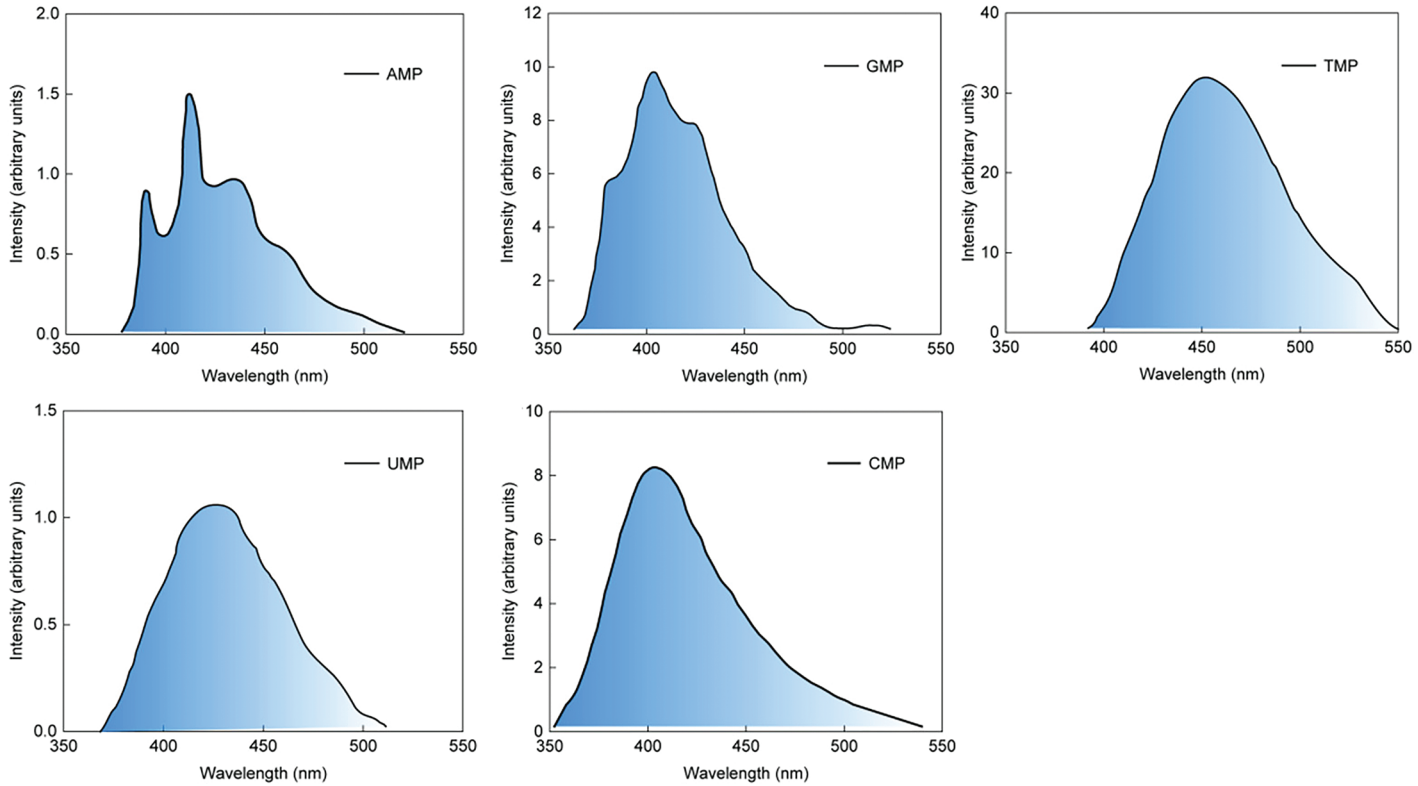


Figure 1.1 Phosphorescence of DNA/RNA nucleotides at 77 K under direct UV excitation.

Table 1.1 Phosphorescence quantum yields and lifetimes of DNA/RNA nucleotides at 80 K under direct UV excitation.

	pH	φ_p	φ_f	$^3\tau$(s)	Estimated triplet state energy (cm^{-1})
	pH = 2	0.005	—	1.75	27 350 (pH = 2)
AMP	pH = 7	0.025	0.01	2.4	26 700 (pH = 7)
	pH = 11	0.028	—	—	
	pH = 1	≤0.02	—	—	
GMP	pH = 7	0.095	0.13	1.3	27 200 (pH = 7)
	pH = 11	0.06	—	1.3	
	pH = 1	0.005	—	—	27 900 (pH = 7)
CMP	pH = 7	0.015	0.05	0.34	
	pH = 11	0.018	—	—	
	pH = 1	≤0.02	—	1.2	26 300 (pH = 7)
TMP	pH = 7	≤0.01	0.16	0.35	27 000 (pH = 12)
	pH = 12	0.03	—	0.45	
	pH = 1	≤0.006	—	—	
UMP	pH = 7	0.006	0.01	0.55	27 400 (pH = 7)
	pH = 12	0.006	—	—	28 400 (pH = 12)

makes it possible to study triplet states that could not be populated by direct excitation but also could selectively produce certain photolesions in DNA (see details in Chapter 5).

As shown in Figure 1.1 and Table 1.1, pyrimidine and purine nucleotides exhibit distinct phosphorescence behaviors. In general, the triplet states of pyrimidine nucleotides are less stable and exhibit lower quantum yields and shorter phosphorescence [16, 46, 47]. The main factor that affects the phosphorescence is the pH value. For example, the phosphorescence quantum yield of thymine base increases sharply in acidic conditions (pH = 1–2) while it remains low in neutral and basic environments. This is because deprotonation at the N3 position of the thymine ring under alkaline conditions disrupts the conjugated π-electron system and reduces molecular rigidity, which increases nonradiative decay rate (e.g. rotational/vibrational motions). In this case of TMP, the phosphorescence is strongly suppressed in acidic pH (pH < 4) while it intensifies near-neutral to mildly alkaline pH (pH = 6–9). Such observation is due to the protonation of the phosphate group in acidic media, leading to promoting collisional quenching because of the increased molecular solubility and flexibility. On the other hand, the negatively charged phosphate stabilizes the triplet state through electrostatic interactions and rigidifies the

structure at neutral or mildly alkaline pH. The pH can also induce tautomerization of thymine. In acidic conditions, the keto form of thymine is more stable, leading to a longer phosphorescence lifetime. In neutral and basic conditions, the enol form predominates, resulting in shorter lifetimes. The keto form of thymine is more stable in acidic conditions due to the stabilization of the thymine triplet state, which is facilitated by the protonation of the thymine ring [48, 49]. Furthermore, the presence of substituents on the pyrimidine ring can further reduce the phosphorescence quantum yield and lifetime by disrupting the electronic structure and the hydrogen bonding interactions [49]. Additionally, the phosphorescence of pyrimidine bases is more sensitive to the solvent environment, with polar solvents like ethanol and ethylene glycol enhancing the phosphorescence quantum yield [15, 46].

On the other hand, purine nucleotides such as AMP and GMP are known to show higher quantum yields and longer phosphorescence lifetimes, compared to pyrimidine nucleotides [45, 46, 49]. This difference is attributed to the electronic structure and the hydrogen bonding properties of the nucleotides. Specifically, the triplet state of AMP (adenine) is more stable due to its planar structure and the ability to form hydrogen bonds with neighboring bases, which enhances the phosphorescence quantum yield [16, 45, 47, 48]. Similar to pyrimidines, the pH value could affect the phosphorescence of purine nucleotides. For adenine base itself, the phosphorescence intensity decreases sharply in alkaline pH (e.g. pH > 7) but remains relatively stable in acidic to neutral conditions [45, 48]. This is because the deprotonation of the N9 position under alkaline conditions disrupts the rigid planar structure of adenine, enhancing nonradiative decay pathways (e.g. vibrational/rotational motions) and reducing triplet state lifetimes. Meanwhile, the phosphorescence of AMP is strongly quenched in acidic pH (e.g. pH < 4) but enhances significantly near-neutral pH (pH 6–8). In this case, protonation of the phosphate group in acidic media increases solubility and molecular flexibility, promoting collisional quenching. At neutral pH, the negatively charged phosphate group again stabilizes the triplet state through electrostatic interactions and rigidifies the structure, suppressing nonradiative decay [20, 49, 50].

1.2.1 Phosphorescence in Dinucleotides and Polynucleotides in 1960–1970s

The results of DNA/RNA monomers set the foundation for further research on phosphorescence in dinucleotides and polynucleotides. In dinucleotides, the observed phosphorescence spectra and decay kinetics are dominated by the monomer with the lowest triplet energy, reflecting efficient triplet–triplet energy transfer (TTET). For example, ApG (3′–5′) produces adenine-like phosphorescence with a lifetime of ~1.3 s, thus matching monomeric AMP. On the other hand, TpA (3′–5′) exhibits thymine-like phosphorescence with a lifetime of ~0.35 s, which again matches that of TMP. The TTET mechanism is proven by the observation that ApT (3′–5′) exhibits thymine-like phosphorescence at neutral pH while showing phosphorescence from both adenine and thymine anions (T^-) at pH 11.5, indicating that the deprotonation of thymine ($T \rightarrow T^-$) at high pH could disrupt base stacking and reduce TTET [49].

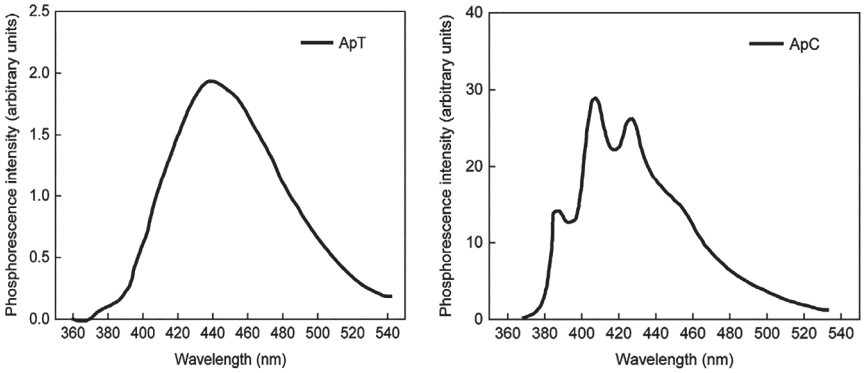


Figure 1.2 Phosphorescence spectra of representative dinucleotides (ApT and ApC) at 77 K.

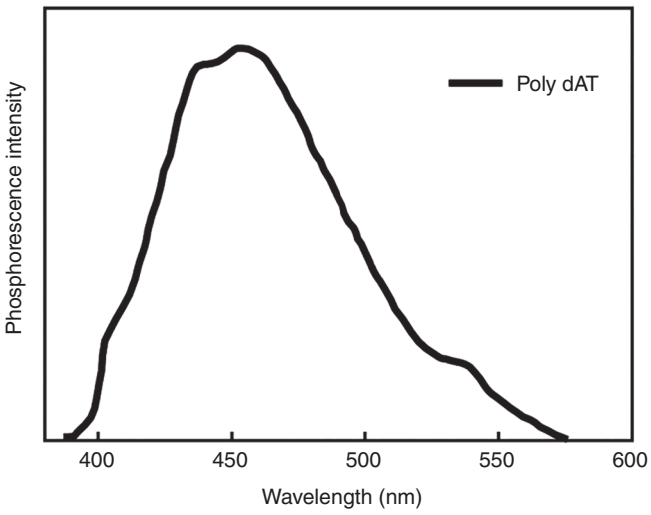


Figure 1.3 Phosphorescence spectra of representative polynucleotides at 80 K.

We have summarized the phosphorescence spectra of representative dinucleotides at 77 K in Figure 1.2 and the lowest triplet energies of DNA/RNA nucleotides in Table 1.1.

The phosphorescence spectra of representative polynucleotides are shown in Figure 1.3. For native DNAs and polynucleotides containing T (such as poly dAT:dAT) [51], they exhibit unstructured phosphorescence with a maximum at 450 nm and a lifetime of ~0.3 s at 80 K. The phosphorescence is attributed to T triplets, and it is confirmed by the sensitized phosphorescence of native DNAs using acetone or acetophenone as triplet energy donors. The underlying mechanism is similar to that found in dinucleotides, where triplet energy migrates from higher energy bases (A, G, C) to T via exchange interactions between stacked bases. For model polynucleotides that do not contain T base (such as poly rAU and poly rA),

they showed phosphorescence spectra resemble that of adenine, yet the lifetime could vary depending on the sequence [49, 51]. A special case is noted for poly rG:rC as it exhibits negligible phosphorescence due to complete quenching of G and C fluorescence at the singlet state level, which is proven due to efficient excited state proton-coupled electron transfer between G and C in more recent studies [52, 53].

1.2.2 The Understanding of Temperature Effect on Triplet Excited State Formation and Decay During 1960s–1970s

UV excitation of DNA/RNA nucleotides populates that their singlet states and triplet excited states are generated by ISC from the singlet states. However, the low phosphorescence quantum yields of DNA/RNA nucleotides summarized in Table 1.1 indicate that ISC is almost negligible in isolated nucleotide monomers. Meanwhile, fluorescence quantum yields of DNA/RNA nucleotides are also at the level of $\sim 10^{-2}$. Therefore, it is concluded that nonradiative decay dominates the excited state dynamics of DNA/RNA nucleotides even at 80 K (the values of $1\text{-}\boldsymbol{\varphi}_{\mathbf{f}}\text{-}\boldsymbol{\varphi}_{\mathbf{p}}$ are in the range of 0.72–0.99). Since there are only negligible changes in the absorption spectra of DNA/RNA nucleotides from 80 K to room temperature, it suggests that the energies necessary to populate the singlet states and the low-lying triplet states are essentially temperature independent [49]. Table 1.2 compares the ISC yields and the nonradiative decay rate constants of the singlet states of DNA/RNA nucleotides from the available data in the 1960s–1970s, revealing that the ratio of nonradiative decay rates of the singlet states increases by one to three orders of magnitude from 80 K to room temperature. Meanwhile, the ISC rates for GMP and CMP are in the same order as GMP and CMP from 80 K to room temperature. For AMP, UMP, and TMP, the ISC rates are two to three orders of magnitude higher at room temperature. Therefore, scientists have foreseen that the IC of DNA/RNA nucleotides is extremely fast at room temperature and that this is the key reason for their observed low fluorescence and phosphorescence at room temperature. However, this hypothesis was verified until the year 2000 using femtosecond time-resolved spectroscopy [54, 55].

Table 1.2 Comparison of ISC and nonradiative decay rate constants of the singlet states of DNA/RNA nucleotides with the data available in the 1960s–1970s.

	φ_{ISC} (80 K)	φ_{ISC} (298 K)	$\frac{k_{ISC}(298\,K)}{k_{ISC}(80\,K)}$	$\frac{k_{nr}(298\,K)}{k_{nr}(80\,K)}$
AMP	2×10^{-2}	4×10^{-4}	<30	<1500
TMP	$<3\times10^{-3}$	8×10^{-3}	≥530	300
CMP	3×10^{-2}	1.5×10^{-3}	5	100
GMP	1.5×10^{-1}	4.6×10^{-4}	1.3	450
UMP	$<3\times10^{-3}$	7×10^{-3}	≥30	13

1.3 Breakthroughs of Time-Resolved Spectroscopy Techniques and Progress of Triplet Excited State Study of DNA/RNA in 1980–2000

In 1980s, both time-resolved transient absorption and time-resolved emission experiments were carried out on DNA/RNA monomers at room temperature, even though most of the studies focus on singlet excited state dynamics. This is possible due to the development of picosecond lasers in the early 1980s. For example, Nikogosyan et al. performed the first transient absorption measurements on DNA/RNA bases and nucleosides at room temperature in 1981 with picosecond UV pulses and reported that the obtained S1 lifetimes are equal to or somewhat shorter than their instrumental time resolution [56]. The first time-resolved emission experiments on DNA/RNA nucleosides at room temperature were also reported by Ballini et al. in 1982 [57]. However, they were unable to distinguish emission decays from the instrumental response function and only estimated an upper limit of 100 ps for the monomer fluorescence lifetimes. Later, Kobayashi reported that the fluorescence lifetime for adenine is 6 ps in a room temperature aqueous solution with a streak camera in 1984. The abovementioned two experimental approaches (transient absorption and time-resolved emission by a streak camera) continued into the 1990s. Nikogosyan et al. extended their experiments to the femtosecond timescale [58, 59]. Although the instrumental response time is greatly improved by femtosecond excitation pulses, high pump intensities (20–200 GW cm^{-2}) used in the experiments could lead to substantial multiphoton absorption and ionization, resulting in errors in the detected singlet state lifetimes. To avoid such systematic uncertainty, Reuther et al. used visible wavelengths and estimated ∼1 ps lifetimes for adenine, cytosine, thymine, and uracil [60]. The use of femtosecond laser pulses did not improve the time resolution for streak camera detection in the 1990s. The measured lifetimes of DNA/RNA bases were estimated to be ∼4 ps, which is around the instrumental response time of the streak camera [61]. Therefore, the authors claimed that 1 ps is not a lower limit for IC in DNA/RNA bases.

Despite the advances in the time-resolved spectroscopy techniques and the progress in the studies of the singlet excited state in DNA/RNA monomers, studies on their triplet excited state are rarely reported during the 1980s–1990s. Kemp et al. used laser flash photolysis with 249 nm excitation to generate triplet excited states of thymine and uracil in acetonitrile and examined the quenching rates by various electron acceptors [62]. They found that the lifetimes of both triplet thymine and uracil were reduced upon addition of electron-accepting molecules and the correlations between rate constants and thermodynamics of electron-transfer quenching followed the Rehm–Weller relation, indicating an electron-transfer mechanism. Because of the low ISC yields of DNA/RNA monomers under direct UV excitation condition at room temperature, acetone sensitization emerged as a breakthrough. Acetone's high triplet energy (337 kJ mol^{-1}) and high Φ_{ISC} (∼1) efficient energy transfer to all DNA/RNA nucleotides, enhancing their triplet yields by two to three orders of magnitude [21, 22].

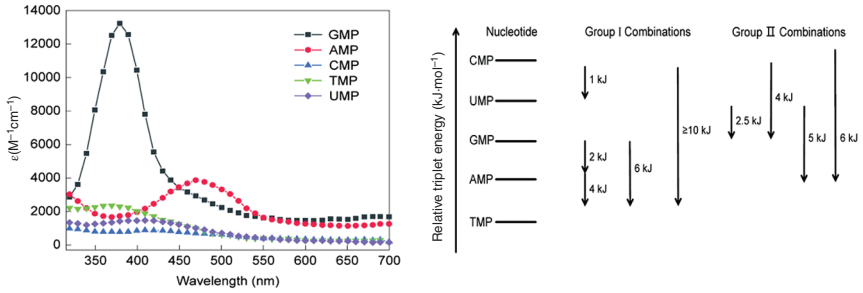


Figure 1.4 Triplet–triplet (T–T) absorption spectra of DNA/RNA nucleotides and scheme of their triplet state energy level.

For example, Song et al. demonstrated that acetone-sensitized triplet states of thymine components (Thy, Thd, TMP) in aqueous solution exhibited identical kinetic parameters to those from direct excitation, validating the method's reliability [23]. Enhanced signal amplitudes facilitated accurate spectral and kinetic measurements. Triplet–triplet (T–T) absorption spectra of all DNA/RNA bases and nucleotides were resolved using acetone sensitization [21–23, 63]. Notably, Zuo et al. first reported cytosine triplet spectra ($\varepsilon_T \approx 200$–$400\ M^{-1}\ cm^{-1}$), which are weaker than thymine ($\varepsilon_T \approx 4000\ M^{-1}\ cm^{-1}$) due to lower Φ_{ISC} and rapid self-quenching. Self-quenching rate constants (k_{sq}) for triplet excited states vary by base: thymine ($4.2 \times 10^8\ M^{-1}\ s^{-1}$) > cytosine ($2.4 \times 10^8\ M^{-1}\ s^{-1}$) > adenine ($1.9 \times 10^8\ M^{-1}\ s^{-1}$) ($k_{sq} \approx 1.8$–$4.2 \times 10^8\ M^{-1}\ s^{-1}$) [23, 63]. Redmond and coworkers established the triplet energy hierarchy for DNA/RNA nucleotides in aqueous solution at room temperature: CMP ($321\ kJ\ mol^{-1}$) > UMP ($320\ kJ\ mol^{-1}$) > GMP ($317\ kJ\ mol^{-1}$) > AMP ($314\ kJ\ mol^{-1}$) > TMP ($310\ kJ\ mol^{-1}$) [21, 22]. Triplet energy transfer equilibria were modeled using the Sandros equation, revealing that thymine acts as a partial energy sink, with an equilibrium ratio favoring triplet localization at lower energy bases. Moreover, in purine/pyrimidine pairs (e.g. GMP–UMP, GMP–CMP), triplet-mediated electron transfer occurs, producing purine radical cations (e.g. $G^{\bullet+}$) that deprotonate to neutral radicals ($G(-H)^{\bullet}$) [21, 22]. Electron transfer rate constant (k_{et}) for GMP–UMP reached $3.5 \times 10^8\ M^{-1}\ s^{-1}$, with radical yields doubling in 1:1 mixtures compared to pure GMP. This mechanism is absent in thymine-containing pairs due to its low triplet energy (Figure 1.4).

1.4 Current Research Frontiers (2000s–Present)

1.4.1 Ultrafast Spectroscopy Study

The development of femtosecond time-resolved spectroscopy in the 1990s has facilitated the research on the triplet state of DNA/RNA bases and oligonucleotides, and direct observations of the excited state dynamics of the initially populated singlet states have been achieved since 2000 [54, 55, 64]. The experimental research progress on the excited state dynamics of the singlet states in nucleic acids has been

well documented in the recent two decades [2–4, 6–8, 10]. Thanks to those studies, it is now possible to distinguish the ISC process in DNA/RNA bases and oligonucleotides. In this section, we go through the major discoveries after the year 2000 in order of time, and the detailed mechanism will be discussed in the next chapter.

As there is barely any triplet state observed after direct excitation of purine bases at room temperature, the studies are focused on the triplet excited state dynamics of pyrimidines. For thymine and its derivatives in solution, a low-lying $^1n\pi^*$ state is directly observed using UV pump and UV probe femtosecond transient absorption spectroscopy and such state is proposed to be a gateway to its lowest triplet state [65]. Such hypothesis was supported by a study on thymidine monomer and a single-stranded thymine oligonucleotide in solution, in which a doorway state with singlet nature is believed to facilitate bifurcation of the initially populated singlet state to either the ground state or the lowest triplet state [66]. Nevertheless, triplet states of thymine and thymidine are shown to be fully formed within the first 10 ps in acetonitrile-d_3 solution after UV excitation by time-resolved infrared (TRIR) spectroscopy[67] and such observation ruled out the possibility that the relaxed $^1n\pi^*$ state is the triplet precursor. In this case, the precursor of the triplet excited state is either the initially populated $^1\pi\pi^*$ state or the hot $^1n\pi^*$ state. Orrewing and coworkers revisit thymidine in chloroform solution and found that the triplet state is generated after 5–10 ps [68]. However, due to spectral overlap and anharmonic spreading of the hot band, they could not identify its precursor. Later, Pilles et al. revisited the decay pathways of thymine and TMP by time-resolved UV/vis and IR spectroscopy. They reported that the formation time of the lowest triplet state of thymine and TMP is ~10 ps, but the quantum yield is solvent dependent, ranging from 0.01 in D_2O to 0.1 in acetonitrile-d_3/methanol-d_4 [29]. Recently, our group reported that varying the excitation wavelength can significantly alter the branching of the excited state population at the Franck–Condon (FC) region, leading to different fluorescence and triplet state quantum yields of thymidine [38]. On the other hand, one study of thymine and its derivatives in the gas phase found dark states with sub-nanosecond to hundreds of nanosecond lifetimes and the nature of these states is proposed to be either the lower-lying singlet $n\pi^*$ state or triplet states generated through ultrafast ISC [69–72]. Although direct observation of the ISC process in thymine and its derivatives has not been successful yet, current evidence suggests that the ISC should be ultrafast and that the rate constant could reach the level of $\sim 10^{11}\ s^{-1}$.

As for uracil and its derivatives, the low-lying $^1n\pi^*$ state is also directly observed using UV pump and UV probe femtosecond transient absorption spectroscopy [65, 73]. Meanwhile, triplet excited states were observed in a few picoseconds after excitation, and they are reported not to form from the thermalized $^1n\pi^*$ state. Therefore, efficient ISC prior to vibrational cooling of the dark $^1n\pi^*$ state is proposed to be the reason for the wavelength- and solvent-dependent triplet yields seen in uracil and its derivatives. Brister and Crespo-Hernández used broadband transient absorption spectroscopy to study the triplet state population dynamics of 1-cyclohexyluracil, finding that SIC to the triplet state occurs in less than 1 ps [74]. They also investigated the excited state dynamics of UMP in aqueous solution using the same method, revealing that excitation at 267 nm leads to the simultaneous

population of the hot ground state, a long-lived $^1n\pi^*$ state, and a triplet state within 200 fs [75]. The quantum yield of the triplet in uridine was also reported to be significantly affected by the excitation wavelength, and ultrafast ISC ($k_{ISC} > 10^{11}\ s^{-1}$) is the key reason [38].

Compared to thymine/uracil and their derivatives, there is less experimental study on the triplet excited state of cytosine and its derivatives. Gas-phase studies by Lobsiger and Leutwyler revealed that the triplet energy of the T1 state of keto-amino cytosine is 3.26 eV above the ground state and the ISC rate is $\sim 10^{10}\ s^{-1}$ [76]. However, a more detailed study by the same group showed that if the S1 state excess energy is below 550 cm^{-1}, the ISC rate of keto-amino cytosine reduces to $0.4–1.5 \times 10^9\ s^{-1}$, which is at least one order of magnitude smaller than that of IC from S1 to S0 [77]. Such observation suggests that previously reported ultrafast ISC of keto-amino cytosine has to occur at the hot S1 state rather than at the energy minimum of the S1 state. Our group has pioneered in the study of ISC in cytidine in solution and directly observed its ultrafast ISC ($k_{ISC} > 10^{10}\ s^{-1}$) using femtosecond transient absorption spectroscopy. It is shown that the ISC in cytidine is sensitive to the excitation wavelength, and a spin-vibronic ISC mechanism at the FC region of the initial excited S1 state is proposed [37].

1.4.2 Theoretical Study

Theoretical studies on the photophysics and photochemistry of nucleic acids and nucleobases have also been extensively performed since 2000. Among those works, triplet states of pyrimidines are commonly involved. We also go through the major discoveries in order of time in this section, and the detailed mechanism can be found in Chapters 6 and 7.

For thymine/uracil and their derivatives, the most likely pathway for ISC is from the dark $^1n\pi^*$ state because a region of singlet/triplet degeneracy has been located near the minimum of this state according to the theoretical results at the CASPT2 (complete active space second-order perturbation)/CASSCF (complete active space self-consistent field) level [78, 79]. The SOC strength between the $^1n\pi^*$ and $^3\pi\pi^*$ states is reported to be $\sim 60\ cm^{-1}$, suggesting the high probability of ISC in the crossing region even though the experimental results suggest ISC should take from the hot $^1n\pi^*$ state rather than at its energy minimum [67]. Meanwhile, the coupling of the T1 ($^3\pi\pi^*$) state with S0 is only 2 cm^{-1}, which explains the long lifetime of the T1 state. The abovementioned mechanism has been corroborated with ISC rate calculations for the $^1n\pi^*$ to $^3\pi\pi^*$ transition using Fermi's golden rule approach [80]. Other pathways for the triplet state population of thymine to S0 have been found to be less favorable [78].

The general picture obtained for thymine has been confirmed for uracil [81]. Furthermore, a dynamic calculation using trajectory surface hopping at the CASSCF level confirms that ISC from the $^1n\pi^*$ to the $^3\pi\pi^*$ state is the favored mechanism for uracil [82]. They also found that the $^1n\pi^*$ state is formed after decay through the $^1\pi_O\pi^*$-min intermediate and further decay through the S2/S1 crossing. The triplet population of uracil is 20–25% after 1 ps of trajectory simulation time, depending on

the initial conditions. These results are in line with the solvent-dependent triplet quantum yields reported by Hare et al. [73]

For cytosine and its derivatives, the first computational study on the possible ISC mechanism was provided by CASPT2 calculations [83]. An energy region of S1/T1 degeneracy with substantial SOC (20 cm^{-1}) was located along the decay pathways to the S0. The large SOC values are explained by the mixed $\pi\pi^*/n_O\pi^*$ character induced by C6 pyramidalization and a small singlet–triplet energy gap at the S1/T1 crossing region. ISC from the singlet to the triplet states has been confirmed by simulations at the CASSCF(14,10) electronic structure level. Richter et al. reported that the ultrafast ISC should follow S1 ($^1n\pi^*$) → T2 ($^3n\pi^*$) → T1 ($^3\pi\pi^*$) path due to a small energy difference (<0.05 eV) between S1 and T2 states induced by pyramidalization of the NH2 group or the out-of-plane oscillation of the hydrogen in the NH group [84]. Mai et al. confirmed the above mentioned mechanism in their study and proposed that efficient ISC in keto-cytosine is achieved due to large SOC at a three-state (S1/T2/T1) energy near-degeneracy point [85]. The S1 → T2 ISC is enhanced by the mixed $\pi\pi^*/n_O\pi^*$ character of the states. However, these results seem to contradict the abovementioned experimental study of Lobsiger et al., in which ISC is not ultrafast when the excess energy of the S1 state is below ~550 cm^{-1} [77]. Pepino et al. pointed out that there are two efficient ISC regions in cytidine. One is $^1\pi\pi^*$ to $^3\pi^2\pi^*$ transition (S1 → T2) that occurs close to FC region with SOC value of ~12 cm^{-1}. The other one is close to the S1/S0 conical intersection, and it is $^1\pi\pi^*$ to $^3\pi\pi^*$ transition (S1 → T1) with SOC value of ~24 cm^{-1}. Meanwhile, they found efficient $^1n_O\pi^*$ to $^3\pi\pi^*$ ISC path with a larger SOC value (~42 cm^{-1}), which is believed to be the main ISC channel in all pyrimidine nucleosides [33]. Such mechanism is further supported by our experimental study of cytidine in solution [37].

1.4.3 Study on the Reaction Mechanism of Cyclobutane Pyrimidine Dimer (CPD) Photolesions in DNA/RNA

DNA/RNA photodamage can lead to a variety of photolesions, including cyclobutane pyrimidine dimer (CPD), the (6-4) lesion, and its Dewar valence isomer. Among them, CPD is the photoproducts that cause the most DNA damage under UVB and UVA irradiation in cellular DNA, as well as in DNA model systems [86]. Due to its relatively high abundance, CPDs between thymine bases became the most studied system in scientific research, and the reaction is expected to occur through either excited singlet or triplet states. However, the underlying reaction mechanism of CPD formation, especially those involving triplet excited states, has been under debate since the pioneering works in the 1960s [87–89]. This is because the importance of a triplet channel for CPD formation after the direct UV irradiation of DNA/RNA has been overlooked due to the explanation of the singlet excitation state reaction channel for CPD formation of thymine bases or nucleotides in frozen matrices and aggregates [15, 90–92]. In this section, we briefly recall the advances of the studies on the triplet excited state reaction mechanism of CPD formation by direct UV excitation, and the details of reaction mechanism by triplet photosensitization will be discussed in Chapters 4 and 5 (Figure 1.5).

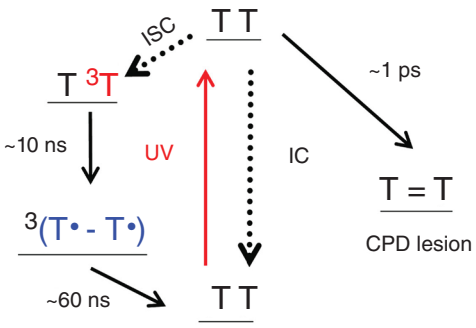


Figure 1.5 Schemes for cyclobutane pyrimidine dimer (CPD) formation in triplet states.

Through photosensitization method, Bosca et al. determined the triplet energy of thymine in DNA is 270 kJ mol^{-1} [25]. Meanwhile, the first transient absorption experiments that try to address CPD formation on the femtosecond to nanosecond timescale were performed in the UV–vis probe range. However, no triplet state was observed in all-thymine oligonucleotides [93]. Later, Banyasz et al. revisit this problem and reported wavelength-dependent quantum yields for CPD and triplet state formation [94]. They estimated that the contribution of triplet states to CPD formation in single-stranded DNA is below 10%. Pilles and coworkers measured decay of the triplet states in $(dT)_{18}$ and showed that triplet states predominantly decay to the electronic ground state via a biradical intermediate on a 10-ns timescale. Such assignment was supported by theoretical studies [95, 96], explaining the low efficiencies for CPD formation from triplet states as well as the low propensity of thymine triplets to form CPDs in sensitization experiments [97–99]. Very similar mechanism was also reported for the formation of CPDs in TpC and CpT dimers [31] as well as in Tp^{5m}C dimer (^{5m}C refers to 5-methyl-cytosine) [100, 101].

1.5 Book Structure Overview

This book is organized as follows: Chapter 1, "Introduce of Triplet Excited State in DNA and RNA," Chapter 2, "Triplet Excited State in Canonical Nucleobases and Their Sulfur Substituted Derivatives," Chapter 3, "Triplet States in Epigenetic Modified Nucleobases and DNA/RNA," Chapter 4, "Triplet-Mediated Photochemistry of Nucleic Acid Bases," Chapter 5, "Drug-Sensitized Triplet States Generation in DNA," Chapter 6, "Triplet Decay in Canonical and Noncanonical DNA/RNA Nucleobases," Chapter 7, "Triplet State Dynamics in DNA/RNA Nucleobases and DNA by Ab Initio Static and Surface-Hopping Dynamics Simulations," and Chapter 8, "The Photophysics and Photochemistry of Epigenetics and Natural Noncanonical Bases: Insights from Quantum Mechanical Calculations." Although comprehensive understanding of triplet excited state in DNA/RNA is not yet at hand, particularly for excitations in systems containing two or more nucleobases bases, it is our hope that this book will communicate the current achievement in this field and inspire future experimental and theoretical work.

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