

SUBNANOSECOND EMISSION FROM MODEL DNA OLIGOMERS
CHARACTERIZED THROUGH TIME-CORRELATED SINGLE-PHOTON
COUNTING SPECTROSCOPY

by

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DEDICATION

To my friends and family, for all their help and support.

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LIST OF ABBREVIATIONS

CT	Charge transfer
dAMP	2'-deoxyadenosine 5'-monophosphate
CD	Circular dichroism
EOM	Electro-optic modulator
FU	Fluorescence upconversion
FWHM	Full width half maximum
GF	Gel filtration
HPLC	High-performance liquid chromatography
IR	Infrared
IRF	Instrument response function
KG-TRF	Kerrgated time-resolved fluorescence
PCET	Proton-coupled electron transfer
TA	Transient absorption
TCSPC	Time-correlated single photon counting
TMP	Thymidine 5'-monophosphate
TRIR	Time-resolved infrared
UV	Ultraviolet
UV/Vis	Ultraviolet/ Visible

ABSTRACT

Exposure of DNA to UV radiation creates electronic excited states that can decay to mutagenic photoproducts. Excited states can return to the electron ground state through deactivation pathways, preventing photochemical damage. Understanding has significantly advanced over the last decade through the applications of time-resolved techniques capable of picosecond and femtosecond time-resolution. While significant strides have been made towards understanding monomeric deactivation pathways, unraveling the complex photophysics of base multimers still presents a significant challenge. This report uses time-resolved fluorescence and ultrafast transient absorbance to analyze model DNA oligomers to understand how fundamental interactions between monomeric constituents influences the dynamics of base multimers.

Model single- and double-stranded DNA oligomers were investigated using the time correlated single photon counting technique to address the uncertainty over how to compare results from time-resolved fluorescent and transient absorption techniques. Emission lifetimes ranging from 50 to 200 ps quantitatively agree with lifetimes measured from transient absorption experiments indicating emission observed on timescales greater than a few picoseconds is the result of excimer or charge recombination luminescence. In attempts to further characterize the time-resolved emission from model oligomers adenine oligomers consisting of 2 and 18 base constituents were examined in aqueous water and heavy water solutions. Differences in dynamics between the two oligomers revealed the average number of bases present within a stacked domain influence the dynamics of these systems. Lifetimes of the emission decays were assigned excimer-like states with various degrees of charge-transfer character. Finally, to further demonstrate the importance of base stacking domain length on the dynamics of these systems, time-resolved emission and absorption of the adenine dinucleotide and 18-mer were examined at temperatures ranging from 7 °C – 80 °C. It was observed that the kinetics between the oligomers was noticeably different at lower temperatures, but not at higher temperatures. It was concluded the domain length of the 18-mer was similar to the domain length of the dinucleotide at high temperatures, but not at low temperatures, demonstrating the domain length significantly impacts the photophysics of DNA.

CHAPTER ONE

INTRODUCTION

Excess exposure to sunlight damages DNA by forming UV induced photoproducts, which could lead to mutagenic or cancerous lesions.¹⁻³ Yet, the photoproduct yield is small even though the canonical nucleobases absorb strongly in the UV,⁴ making DNA a robust UV molecule. Because of this, the photophysics of DNA has been extensively examined to understand how energy is dispersed after UV excitation. The excited-state dynamics of the DNA base monomers have been studied since the 1960s,^{2,3} and the excited-state deactivation processes have been well characterized in the past 15 years, thanks to the emergence of ultrafast spectroscopic techniques and high-level theoretical methods. Upon UV excitation, these DNA monomers return to the electronic ground state by an ultrafast internal conversion process through conical intersections. As a result, the lifetimes associated with this process are generally less than 1 ps.

Since the first direct measurement of the excited-state lifetimes of nucleobase monomers, time-resolved studies have significantly advanced the understanding of excited-state dynamics of larger, more complex DNA systems such as single- and double-strands,^{5,6} G-quadruplexes⁷ and i-motif.⁸ The dynamics of DNA systems is diverse as the excited state properties depend strongly on the structure. Examination of model oligomers through these techniques provides a general description of how fundamental base interactions influence the photophysics of more complex systems such

as natural DNA. Higher-order DNA single- and double-stranded oligomers exhibit vastly different dynamics than those observed for single bases mainly due to base stacking (intrastrand) and base pairing (interstrand) interactions. Understanding the photophysical deactivation pathways in these higher order structures is essential to mapping energy dispersion in DNA.

Transient absorption (TA) studies report high yields of a long-lived component that lasts tens to hundreds picoseconds in numerous DNA multimers of varying lengths and base identity, observed only when bases are π -stacked.^{9, 10} Experimental and theoretical studies have assigned this long-lived component in various DNA model systems to a charge-transfer state.¹¹⁻¹⁶ Figure 1.1 shows an example of a model DNA oligomer and a basic deactivation pathway. The two adenine residues in Figure 1.1a are linked together by a deoxyribose phosphate backbone and are in close proximity and parallel to each other such that their π -orbitals overlap. The model energy diagram in Figure 1.1b shows that after UV excitation of the dimer an excitonic state is populated. From here the system can return to the ground state on a time scale less than 1 ps or populate an excimer-like state on an ultrafast time scale. This excimer or charge transfer (CT) state then deactivates back to the ground state on a time scale of 100 ps. The term excimer (same base identity) or exciplex (different base identity) is a more general term that describes various degrees of charge transfer and excitonic or monomeric character unlike the more restrictive term of CT, which is described as a dark state with no monomer character.

Contrasting the simple dynamics observed in TA experiments, time-correlated single photon counting (TCSPC) measurements reveal more complex kinetics. Ultrafast monomer-like lifetimes accompanied by multiple lifetimes spanning over 5 orders of magnitude are observed for DNA oligomer examined by TCSPC.^{6, 11, 17-21} Unlike fluorescence upconversion (FU), another time-resolved emission technique that provides femtosecond time resolution, TCSPC has an extended temporal range and much higher sensitivity. Compared to TA techniques which can detect virtually any excited state, including dark states (e.g., $n\pi^*$ states), TCSPC can only detect emissive states. The potential for the fs-TA and TCSPC technique to monitor different excited-state populations has been stressed in some publications,^{6, 18} while others have reported congruent results from both techniques.¹²

Using time-resolved fluorescent (FU or TCSPC) and absorption (fs-TA or TRIR) techniques in tandem provides powerful complementary information for elucidating DNA excited-state dynamics, yet very few studies have examined the same system in the same laboratory. The lack of tandem studies leads to conflicting conclusion even though the results themselves do not necessarily disagree with each other.

The first objective of this dissertation is to identify if the fs-TA and TCSPC technique are monitoring the same excited-state populations in higher order model DNA oligomers. Model single- and double-stranded DNA oligomers are examined by combined TCSPC and TA techniques. The technical details are outlined in Chapter 2. Chapter 3 presents the results. By comparing the signals from these two techniques, we found that the majority of the TCSPC signals matches those of the fs-TA signals,

concluding that TCSPC and TA are monitoring the same excited state populations for all systems. The identity of these TCSPC components decaying on a 100 ps timescale were identified as CT or excimer luminescence. Additional lifetimes, longer than those observed for fs-TA, were identified by the TCSPC technique. The ns lifetimes may be due to fluorescent impurities, but the identity of the intermediated lifetimes are discussed in Chapter 4.

The second objective of this dissertation is to completely characterize the TCSPC decays of simple model systems and use this additional information in conjuncture with fs-TA results to provide additional understanding to the dynamics of model adenine oligomers. Chapter 4 discusses adenine length dependent and kinetic isotope effect (KIE) studies of (dA)₂ and (dA)₁₈ adenine oligomers. It was found that, similar to the 100 ps TCSPC component, the intermediate TCSPC lifetime is also observed by the fs-TA decays. TCSPC, thanks to its enhanced sensitivity, resolves the single excimer/exciple lifetime observed in the TA experiments into two lifetimes. Both states were assigned as excimer states, but with varying degrees of CT character. A KIE was also identified for both adenine oligomers. This is the first study detailing KIEs in single stranded adenine systems, the presence of which suggest KIEs result from vibrational modes couple to the CT state.

Chapter 5 details the temperature dependence of the adenine oligonucleotides examined in Chapter 4. It was found that the differences in dynamics between adenine oligomers of different lengths highlighted in Chapter 4 are absent at higher temperatures with the two oligomers exhibiting similar TCSPC and fs-TA decays. The similar

dynamics between the adenine dinucleotide and the longer 18-mer at higher temperatures is explained by disrupting the weakly favorable base stacking interactions in the 18-mer producing domain lengths similar to that of the dinucleotide suggesting domain length significantly influences the dynamics of single stranded oligomers.

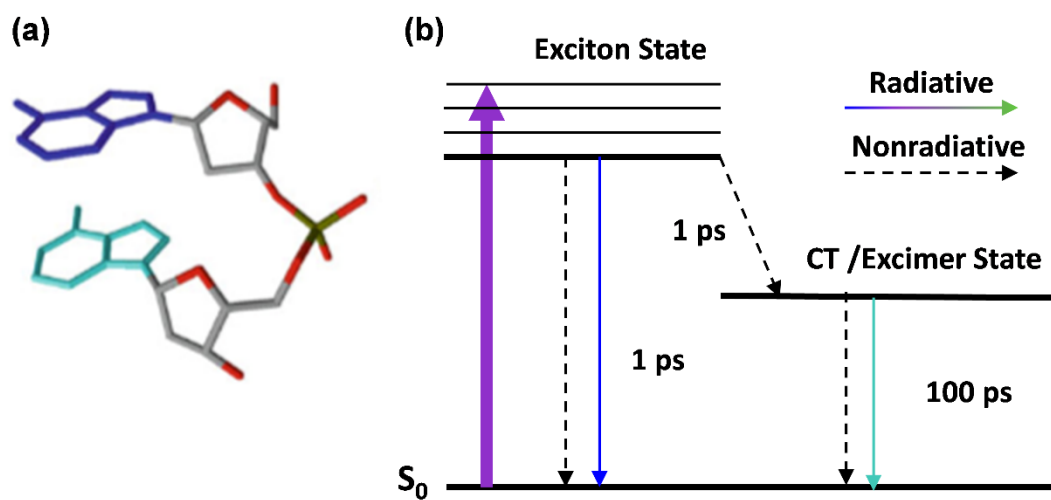


Figure 1.1 (a) An adenine dinucleotide linked by a deoxyribose phosphate backbone, (dA)₂, exhibits base stacking interactions. (b) Proposed deactivation pathways of simple model DNA oligomers after UV excitation.

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CHAPTER TWO

GENERAL METHODS

Preface

This chapter outlines the procedures used to analyze the excited-state dynamics of various DNA oligomers. The first two sections describe the time-resolved emission and absorption methods used for all samples. The sections following the time-resolved methods describe the steady-state methods, sample handling and data treatment.

2.1 Emission Lifetimes

Emission lifetimes were measured using the Time-Correlated Single Photon Counting (TCSPC) technique on the Picoquant FluoTime 200 commercial spectrometer (PicoQuant Photonics North America, West Springfield, MA). A schematic of the TCSPC apparatus is shown in Figure 2.1. The excitation source was provided by a tunable Ti:Sapphire laser (Chameleon Ultra II, Coherent Inc. Santa Clara, CA) with 180 fs pulses at a repetition rate of 80 MHz. A harmonics generator (SO3271 Harmonics, Coherent Inc. Santa Clara, CA) converted the fundamental pulse at 795 nm into 265 nm excitation pulses via third harmonic generation. The repetition rate of the excitation pulse was reduced to 4 MHz using an electro-optic modulator (M350-80, Conoptics, Danbury, CT) driven by a push-pull power amplifier (Model 25D, Conoptics, Danbury, CT). A synchronous countdown system (Model 305, Conoptics, Danbury, CT) triggered by the

laser was used to control the electro-optic modulator pick rate while simultaneously signaling the start of the timing electronics.

The excitation pulses were vertically polarized using a Glan-Thompson polarizer before the sample. The cuvette was oriented at a slight angle with respect to the incoming excitation beam to prevent back reflection. Fluorescence from the sample was filtered with a 300 nm long pass filter (model XLU0300, Asahi Spectra, Torrance, CA) placed before the emission monochromator and detected after passing through an analyzing polarizer oriented at magic angle (54.7°) relative to the polarization of the excitation pulses. Slit widths before and after the monochromator were set to 2 mm. The Picoquant FluoTime 200 spectrometer was equipped with the PicoHarp 300 timing electronics and a microchannel plate photomultiplier (model R3809U-5X, Hamamatsu Corp., Hamamatsu City, Japan). The IRF was determined by scattering from a diluted Ludox® HS-40 colloidal silica solution (Sigma Aldrich, St. Louis, MO) at 265 nm. The FWHM of the IRF was typically around 60 ps. A bin width of 8.0 ps was used for each TCSPC measurement.

The solution under study was held at 20.00 ± 0.02 °C using a temperature-controlled cuvette holder (TLC 50™, Quantum Northwest, Liberty Lake, WA) unless otherwise stated. The samples were held in a 10x10 (3-Q-10, Starna Cells, Atascadero, CA) or a 10x4 mm (9F-Q-10, Starna Cells, Atascadero, CA) Spectrosil® quartz cuvette. The samples in Chapters 3 and 5 were examined in the 10x10 cuvette while the samples in Chapter 4 were examined in the 10x4 mm cuvette. The 10x4 mm cuvette was oriented to provide a 4 mm path length. The solutions were constantly stirred using a 3x10 mm

magnetic stir bar (58948-375, VWR International, Radnor, PA) for the 10x10 mm cuvette and a 3x3 mm stir bar (SBM-3003-MIC, Big Science Inc., Huntersville, NC) for the 10x4 mm cuvette. The excitation fluence was attenuated to provide $\sim 10,000$ counts in the maximum channel after 5 minutes of acquisition from $(dA)_{15}$ or $(dA)_{18}$. The count rate produced from sample excitation was well below $< 1\%$ of the modulated repetition rate to prevent pile-up effects allowing the timing electronics an appropriate amount of time to reset after photon detection. Sample emission was then collected for 5 minutes at each emission wavelength. TCSPC decays were not corrected for background emission from a buffer-only solution because of the difficulty of distinguishing weak solute emission on the ns time scale from interfering signals.

2.2 Transient Absorption

The transient absorption experimental setup for UV-pump/Visible-probe is shown in Figure 2.2. The 800 nm laser fundamental at 1 kHz was produced by a Ti:sapphire regenerative amplifier (3.5 mJ, 80 fs; Libra-HE, Coherent, Santa Clara, CA) using chirped pulse amplification. About 20% of the fundamental was frequency tripled to produce the 266 nm pump pulses. The pump energy was attenuated to 0.9 μ J and the spot size was adjusted to 250 μ m (FWHM) at the sample. An optical chopper (New Focus, Santa Clara, CA) set to 333 Hz let through every third pulse. Approximately 40% of the fundamental was used to pump an optical parametric amplifier (OPerA Solo, Coherent, Santa Clara, CA) where 500 nm pulses were generated by sum-frequency mixing the signal beam and 800 nm fundamental. The visible pulses were subsequently frequency doubled to produce 250 nm pulses. A motorized IMS600PP translation stage (Newport

Inc., Irvine, CA) translation stage controlled the optical delay between the pump and probe pulses at the sample, giving 3 ns of total delay time. The relative polarization between the pump and probe pulses was set to the magic angle (54.7°) at the sample. Approximately 2 mL of sample was recirculated in a 1 mm path length temperature-controlled flow cell (TFC-M25-3, Harrick Scientific Products, Pleasantville, NY). The liquid was flowed between two CaF_2 windows separated by a 1 mm Teflon spacer from a 2 mL sample vial by a peristaltic pump (Masterflex 7524-00, Cole-Parmer, Vernon Hills, IL). The flow cell temperature was set to 20°C for the transients shown in Chapter 3 and left at room temperature for the transients shown in Chapter 4. After passing through the sample, the probe pulses were isolated by a monochromator and detected by a photomultiplier tube. The PMT signal was recorded with a lock-in amplifier (Model SR830, Stanford Instruments, Sunnyvale, CA) synchronized with the mechanical chopper. Transient signals were retrieved from the lock-in amplifier through a GPIB interface and recorded by a LabVIEW based data collection software.

2.3 Steady-State Measurements

2.3.1 UV/Vis Absorption Measurements

UV/Vis absorption spectra were recorded using a Lambda 25 spectrometer (PerkinElmer, Inc.) using either a 10x10 mm or 4 x10 mm Spectrosil® quartz cuvette in order to match the cuvette used to collect the TCSPC decays. The 4x10 mm cuvette was always positioned to produce a path length of 4 mm. A solution containing the buffer and salt but without the DNA sample was used for background correction.

2.3.2 UV/Vis Emission Measurements

The steady-state emission spectra were recorded using a fluoromax-4 spectrophotometer (HORIBA Jobin Yvon, Edison, NJ). Each sample was excited at 265 nm with a 4 nm excitation bandwidth. Emission was collected at 90° through a 4 nm emission bandwidth. Signal integration time was set to 0.5 sec and data were taken every 2 nm. The emission correction factor was supplied by the manufacture. Emission spectra were recorded using a 4x10 mm quartz cuvette positioned such that the excitation path length was 4 mm.

2.3.3 Circular Dichroism Measurements

Steady-state circular dichroism (CD) measurements were recorded using a J-815 spectrometer (JASCO, Easton, MD). Samples were held in the same 10x10 or 10x4 mm cuvette to provide the same path-length used for UV/Vis measurements. A solution containing the buffer and salt but without the DNA sample was used for background correction. For melting point measurements, a cuvette lid or stopper was used to prevent evaporation. The CD spectra were recorded from 10°C to 80°C with 10°C intervals. All measurements were repeated twice and averaged by the instrumentation software.

2.3.4 Fourier Transform Infrared Spectroscopy

Infrared absorption spectra were collected using a Fourier transform infrared spectrometer (FTIR 4200, Jasco, Easton, MD). Liquid samples were held in a custom built static cell which consists of a 100 μ m thick Teflon spacer sandwiched between two CaF₂ windows. Care was taken to prevent the formation of air bubbles between the

windows. The spectrometer was purged with dry air (6 SCFH) from a generator (Model 75-62, Parker Balston, Haverhill, MA) for ten minutes before taking the spectrum.

2.3.5 pH Measurements

The pH of the buffer solutions was adjusted to 7.1 for the Tris buffer using either HCl or DCl. The phosphate buffer was at a pH of 7.0. The pH of the sample was monitored using a MI-410 Micro Combination pH electrode (Microelectrodes Inc., Bedford NH) with a OrionStar A121 pH meter (Thermo Scientific, Waltham, MA). The pH probe was calibrated using three standard buffer solutions at a pH of 4.0, 7.0 and 10.0.

2.4 Sample Handling

2.4.1 Materials

All DNA oligonucleotides used were obtained from outside companies and laboratories in the form of lyophilized sodium salts and used as received. All purification of the oligonucleotides was carried out by the respective supplier. TMP (thymidine 5'-monophosphate, 99+%, Sigma Aldrich, St. Louis, MO), Tris (hydroxymethyl) aminomethane (99.9%, Sigma Aldrich, St. Louis, MO), NaCl (99.0 %, EMD Millipore, Billerica, MA), HCl (Fisher Scientific, Waltham, MA), DCl (99% D, Sigma Aldrich, St. Louis, MO), potassium phosphate monobasic (99.0 %, VWR International, Radnor, PA), and sodium phosphate (99.9%, Fisher Scientific, Waltham, MA) were used as received.

For the oligomers in Chapters 3 and 5, (dA)₁₅ and d(AT)₉ were purchased from Midland Certified Reagent Company (Midland, TX) and purified by a standard gel

filtration process. The $(dA)_{10} \cdot (dT)_{10}$ PEG-linked dumbbell was a gift from professor Fred Lewis (Northwestern University) and the synthesis procedure and characterization are described in more detail elsewhere.^[1] The $(dA)_{18}$ oligomers were purchased from Integrated DNA Technologies (Coralville, IA). One sample was purified using the standard desalting process, while a second one was purified by reversed-phase HPLC.

For the oligomers in Chapter 4, $(dA)_{18}$ was obtained from Integrated DNA Technologies (Coralville, IA) and purified by reversed-phase HPLC. The $(dA)_2$ oligomer was received as a gift from professor Mahesh Hariharan (Indian Institute of Science Education and Research Thiruvananthapuram), and was synthesized according to the procedure described below.

2.4.2 Sample Preparation

For the experiments described in Chapters 3 and 5, aqueous solutions for TCPSC measurements were prepared in 10 mM Tris-HCl buffer with 250 mM NaCl using 18 MΩ water from a water purification system (EMD Millipore, Billerica, MA). The pH was adjusted to 7.1 by dropwise addition of concentrated HCl solution. All sample concentrations were adjusted to an absorbance of 0.3 at 265 nm in a 10x10 mm quartz cuvette. Aqueous solutions for transient absorption measurements were prepared in 50 mM phosphate buffer and 100 mM NaCl. The $d(AT)_9$ oligomer was heated in a water bath to 90°C for 10 minutes then allowed to cool to room temperature while remaining in the water bath on the heating element.

For experiments described in Chapter 4, aqueous solutions for TCPSC measurements were prepared in 10 mM Tris-HCl buffer with 250 mM NaCl using either

18 M Ω water from a water purification system (EMD Millipore, Billerica, MA) or D₂O (99.9% D, Cambridge Isotope Laboratories, Tewksbury, MA) treated with activated charcoal (Sigma Aldrich, St. Louis, MO) by the procedure described below. The pH or pD was adjusted to 7.1 by addition of either concentrated HCl or DCl solution. All sample concentrations for TCSPC and SS emission measurements were adjusted to give an absorbance of 0.3 at 265 nm in a 10x4 mm quartz cuvette with the cuvette orientated to provide a 4 mm path length.

2.4.3 Fluorescent Impurities

The sensitivity of the TCSPC and steady-state fluorescence technique, coupled with the low fluorescence quantum yields ($\phi_f = 10^{-5}$ - 10^{-4}) of DNA systems,^[2] makes emission measurements of these systems especially susceptible to fluorescent impurities. Figure 2.3 shows the TCSPC decays of different components of the Tris Buffer. These decays were recorded under identical conditions to that of the oligonucleotides in Chapter 3. The decays in Figure 2.3 and the Tris-HCl buffer with 250 mM NaCl decays in Chapter 3 and 4 show that these components contribute a non-negligible amount of emission to TCSPC emission signals. To reduce emission from these fluorescent impurities, special precautions in addition to proper sample handling were taken.

To reduce the contribution from solvent emission the highest quality of available reagents was used, and contact with plastic material was avoided whenever possible. Clear borosilicate screw top vials with PTFE lined caps (VWR International, Radnor, PA) were used to prevent leaching of the cap material into solution, and contact between the solution and vial cap was prevented whenever possible. Tris (hydroxymethyl

aminomethane, 99.9%, Sigma Aldrich, St. Louis, MO) was used for all fluorescence measurements because it consistently produced less fluorescence than solutions of potassium phosphate monobasic (99.0 %, VWR International, Radnor, PA), or sodium phosphate (99.9%, Fisher Scientific, Waltham, MA) of equal concentrations dissolved in milipore H₂O. As seen in Chapter 4, a 10x4 mm quartz cuvette was used instead of a 10x10 mm quartz cuvette oriented so the excitation path length was 4 mm. By reducing the excitation path length and increasing the concentration to maintain a sample absorbance of 0.3 the number of photons absorbed by the solvent impurity decreases. This was done in response to the high level of emission from the Tris buffer dissolved in deuterium (Chapter 4). If these techniques were not suitable for reducing solvent emission, then an activated charcoal wash (Section 2.4.6) was used to reduce the solvent fluorescence.

Other methods of solvent purification were either unsuccessful in reducing solvent impurity. One method of note involved the use of nonpolar polystyrene adsorbents (Bio-beads SM-2 Resin, Bio Rad, Hercules, CA). Approximately 5 grams of Bio-beads SM-2 Resin was mixed with 25 mL of solution and stirred for 2 hours. The adsorbent was then separated from solution using a fritted glass filter. Steady-state emission measurements showed an increase in solvent emission after the first washing. Fresh solvent (25 mL) was then added to the previously used adsorbent and stirred for two hours. After the second washing the intensity of the solvent emission was reduced by approximately 50% compared to the emission intensity of the neat solvent before the first wash.

2.4.4 Photoproducts

To ensure DNA photoproducts were not formed by the 265 nm excitation beam during TCSPC data collection, initial successive measurements were recorded in triplicate for each sample. The reproducibility of the sample decays ensured photoproducts were not forming over the course of data collection.^[3] Additionally, steady-state emission and absorption spectra were taken before and after TCSPC measurements. Change in the spectra due to irradiation time would suggest the formation of photoproducts or sample degradation. To determine spectroscopic markers of adenine photoproducts (dA)₁₅ dissolved in Tris-HCl buffer with 250 mM NaCl (see section 2.4.1 for specifications) was irradiated with 254 nm light in a UV photoreactor (Srinivasan-Griffin Rayonet Type, The Southern New England Ultraviolet Co., Middletown, CT) in a sealed 10x10 mm quartz cuvette for 60 minutes. Irradiation was performed with 6 out of the 13 Hg lamps present. Figure 2.4 shows the UV-Vis spectra (a) and the steady-state emission (b) of (dA)₁₅ after irradiation (dashed trace) and a control (dA)₁₅ sample (solid trace).

2.4.5 Adenine Dinucleotide Synthesis

The (dA)₂ dinucleotide was received from professor Mahesh Hariharan (Indian Institute of Science Education and Research Thiruvananthapuram). For the synthesis of the (dA)₂ oligomer examined in Chapter 4 the reagent 2'-deoxyadenosine phosphoramidite (Glen Research, Sterling, VA) was used as received. Oligonucleotides were synthesized on a DNA synthesizer (K&A Laborgeraete, Schaafheim, Germany) employing the automated solid-phase phosphoramidite chemistry. The synthesis of the

deoxyadenosine oligomers was carried out on 1 μ mol scale with appropriate controlled pore glass (CPG) beads used as 3' solid support as reported elsewhere.^[4-6] Oligonucleotides synthesized were deprotected and isolated from the solid-phase support using concentrated ammonium hydroxide and stirred for 24 hours at room temperature. The purification of the (dA)₂ oligomer was then carried out using reverse-phase HPLC (Prominence Liquid Chromatographer, Shimadzu, Kyoto, Japan) with a Luna 5u C18(2) 100A column (250 x 10 mm, Phenomenex, Torrance, CA) at a gradient of 20 mM ammonium acetate buffer and acetonitrile (flow rate of 1 ml/min) as the mobile phase.

2.4.6 Activated Charcoal Treatment

D₂O (99.9% D, Cambridge Isotope Laboratories, Tewksbury, MA) contains fluorescent impurities that emit over the same emission wavelengths as DNA oligomers when excited at 265 nm (Figure 4.1 in Chapter 4). To remove these fluorescent impurities, D₂O was treated with activated charcoal. First the powdered activated charcoal was heated at 260°C for 3 hours. The charcoal was then suspended in a 10% v/v HCl solution, and stirred for 24 hr. The acid washed charcoal was separated using a coarse fritted glass filter, washed with 18 Ω M H₂O and kept at 80°C for 6 hours. The dried washed charcoal was combined with D₂O to make an approximate 0.5 % m/m solution, and stirred for 16 hours. The charcoal was separated from the D₂O using a fine fritted glass filter. The steady-state IR absorbance of the D₂O solution before and after treatment was compared to ensure the isotope purity after treatment (Figure 2.5). The peak around 3400 cm⁻¹ is slightly greater for D₂O after activated charcoal treatment (blue trace) in comparison to D₂O before treatment (red trace). The 3400 cm⁻¹ band is typically

associated with the symmetric and asymmetric stretching vibration of H₂O, indicating some of the isotopic purity was lost during the treatment process. However, the saturation of both spectra in the range of 2100 cm⁻¹ to 2700 cm⁻¹ indicates D₂O is still present in high concentrations after the activated charcoal treatment.

2.5 Data Treatment

2.5.1 Modeling Emission Lifetimes

All emission decays were analyzed using a global analysis program (FluoFit Version 4.5, PicoQuant, Berlin, Germany). A detailed description explaining the modeling of TCSPC decays can be found in Chapter 3, and is briefly discussed here. The emission decays were fit to a model function consisting of a sum of n exponentials convoluted with the normalized instrument response function, $IRF(t)$, and compared with the data:

$$S(t) = \int_{-\infty}^t IRF(t' - t_s) \sum_{i=1}^n A_i e^{-\frac{t-t'}{\tau_i}} dt' + N_0 IRF(t' - t_s) \quad (2.1)$$

where A_i and τ_i are the amplitude and lifetime, respectively, of the i th decay component, t is the bin width (0.008 ns), and the adjustable parameter, t_s , accounts for any temporal offset between the emission decay and the IRF. In Equation (2.1), $IRF(t)$ is the signal measured from a scattering solution (see section 2.1). The N_0 parameter accounts for all detected photons that are not in the decay components represented by the sum of exponentials in Equation (2.1). All uncertainties reported are twice the standard error produced from the global analysis program.

2.5.2 Modeling Transient Absorption Lifetimes

The ground-state bleach recovery signals were fit to a biexponential function with a constant offset:

$$\Delta A = A_1 e^{-\frac{t}{\tau_1}} + A_2 e^{-\frac{t}{\tau_2}} + A_3 \quad (2.2)$$

convoluted with a Gaussian instrument response function using IGOR Pro version 6.3.4 A program (Wavemetrics Inc., Portland, OR). All uncertainties reported are twice the standard error produced from the fitting program.

2.5.3 Emission Spectral Corrections

Both steady-state and time-resolved emission spectra were corrected for difference in instrumentation responses through the use of a correction spectrum (Figure 2.6). The correction factor for steady-state emission spectra was provided by the fluorometer manufacture. The TCSPC correction factor was collected by Dr. Charles Stark and is described in more detail elsewhere.⁷ In summary, the correction factor was acquired using five laser dyes as emission standards through the process described by Gardecki and Maroncelli.⁸ The time-resolved emission spectra was recorded for each dye at multiple wavelengths for identical periods of time. The steady-state emission spectrum from each dye was constructed by integrating the decay signal at each emission wavelength. The ratio of these spectra and those of the respective standard spectra were scaled by arbitrary constants such that they created a smooth curve.

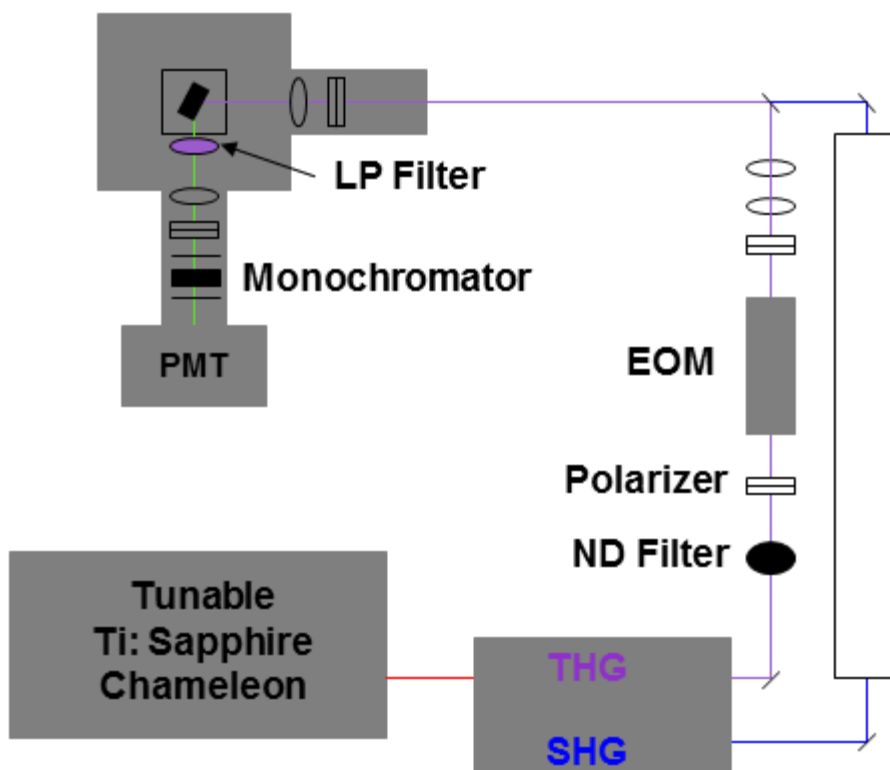


Figure 2.1. TCSPC apparatus showing the configuration of the third harmonic (purple) excitation pathway. The configuration of the SHG excitation pathway (blue) mirrors that of the THG configuration. The cuvette depicted is representative of the 10x4 mm cuvette oriented to provide the shortest (4 mm) of the two possible path lengths and rotated to prevent back reflection of the excitation pulses.

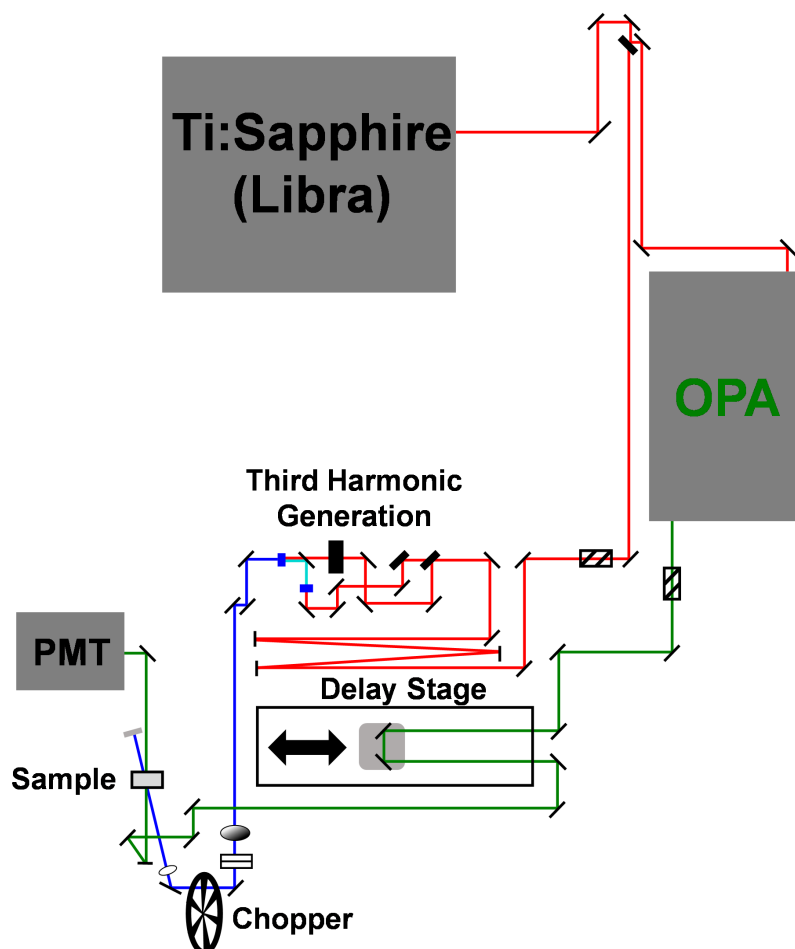


Figure 2.2 Transient absorption schematic displaying pathways for the 265 nm pump (blue) and 250 nm probe (green). The schematic was produced by Dr. Ashley Beckstead. The original along with a detailed description of the TA schematic can be found in ref [9].

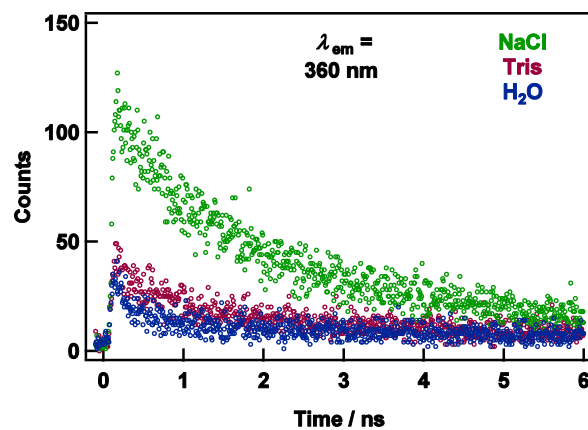


Figure 2.3. Fluorescence decays of neat water (blue), 0.25 M NaCl aqueous solution (green), and 10 mM Tris-HCl buffer solution without NaCl (red) collected at 360 nm following 265 nm excitation. The traces are shown on a linear scale. All signals were collected under identical conditions.

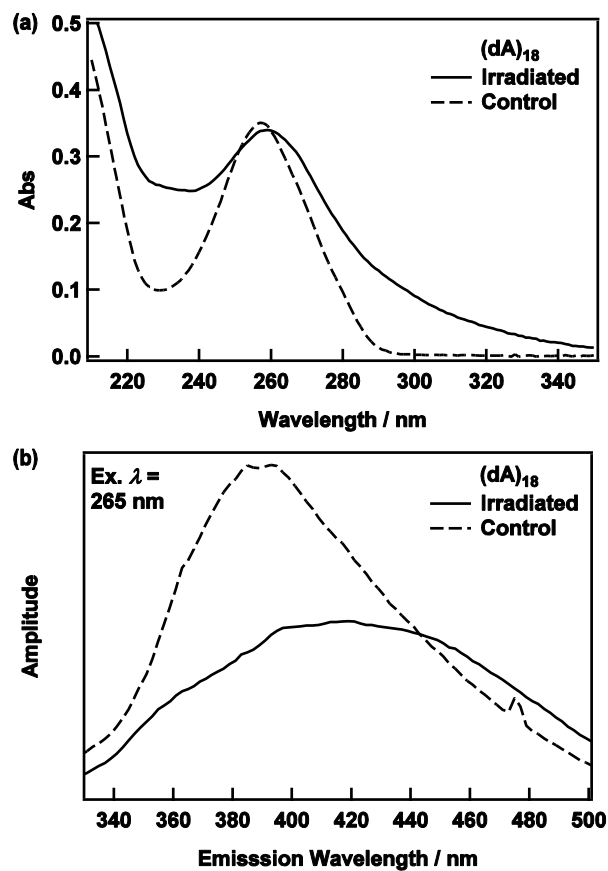


Figure 2.4. UV-Vis absorption (a) and corrected steady-state emission (b) of $(dA)_{15}$ after 60 minutes of UV irradiation (solid trace) and a control $(dA)_{15}$ sample (dashed trace). Both samples were dissolved in 10 mM Tris-HCl buffer with 250 mM added NaCl. Both graphs are shown on a linear axis.

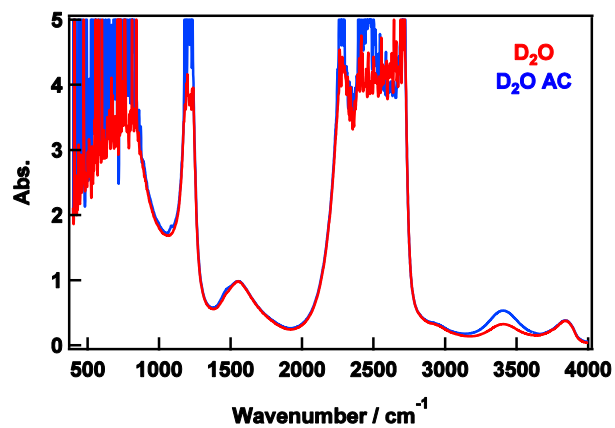


Figure 2.5. The IR absorption spectra for neat D₂O before activated charcoal treatment (red trace) and neat D₂O treated with activated charcoal (blue trace) per the procedure detailed in section 2.4.4.

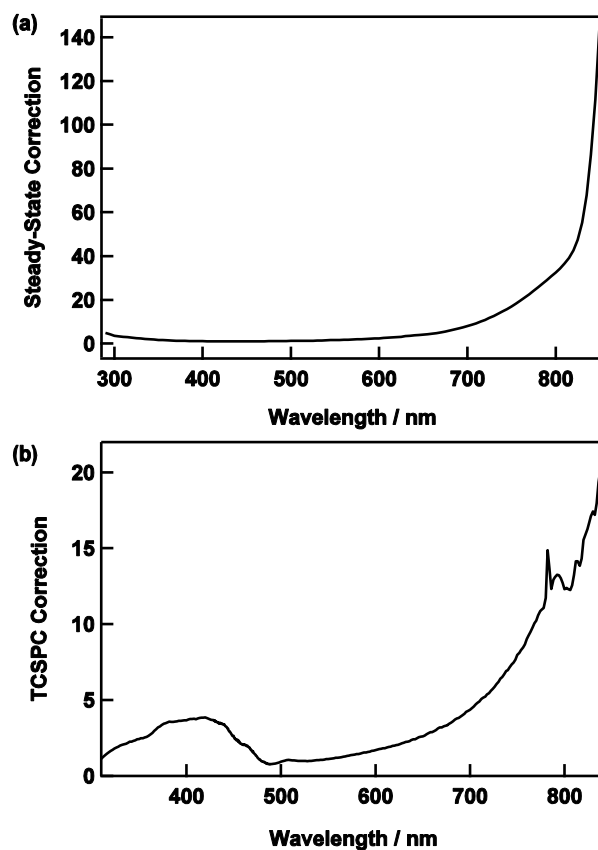


Figure 2.6. Correction factors for emission spectrometers. This shows the built-in correction factor for the fluoromax4 steady-state emission spectrometer provided by the manufacturer (a) and the TCSPC correction factor using the five laser dyes (b) described in section 2.5.3.

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CHAPTER THREE

SUBNANOSECOND EMISSION DYNAMICS OF AT DNA OLIGONUCLEOTIDES

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: David J. Skowron

Contributions: Collected and analyzed TCSPC data on all oligonucleotides. Acquired all steady state measurements. Generated figures and drafted the initial manuscript.

Co-Author: Yuyuan Zhang

Contributions: Performed transient absorption studies on d(AT)₉·d(AT) oligonucleotide. Assisted with writing process.

Co-Author: Ashley A. Beckstead

Contributions: Performed transient absorption studies on d(AT)₉·d(AT) oligonucleotide. Assisted in final proofs of the manuscript.

Co-Author: Jacob M. Remington

Contributions: Assisted with TCSPC analysis and proof reading the final manuscript.

Co-Author: Madison Strawn

Contributions: Assisted in sample preparation, and steady-state data collection.

Co-Author: Bern Kohler

Contributions: Provide important direction and insight for data collection and analysis. Wrote and aided in the preparation of the manuscript.

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SUBNANOSECOND EMISSION DYNAMICS OF AT DNA OLIGONUCLEOTIDES

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Abstract

UV radiation creates excited electronic states in DNA that can decay to mutagenic photoproducts. When excited states return to the electronic ground state, photochemical injury is avoided. Understanding of the available relaxation pathways has advanced rapidly during the past decade, but there has been persistent uncertainty, and even controversy, over how to compare results from transient absorption and time-resolved emission experiments. Here, emission from single- and double-stranded AT DNA compounds excited at 265 nm was studied in aqueous solution using the time-correlated single photon counting technique. There is quantitative agreement between the emission lifetimes ranging from 50 to 200 ps and ones measured in transient absorption experiments, demonstrating that both techniques probe the same excited states. The results indicate that excitations with lifetimes of more than a few picoseconds are weakly emissive excimer and charge transfer states. Only a minute fraction of excitations persist beyond 1 ns in AT DNA strands at room temperature.

3.1 Introduction

The intrinsic fluorescence from natural and synthetic DNA strands is challenging to observe due to ultralow fluorescence quantum yields ($\phi_f=10^{-5} - 10^{-4}$).^[1] Although time-resolved emission from DNA has been studied for many years (see ref.[2] for a review of experiments carried out before 2000), understanding has advanced dramatically over the past decade with the application of techniques with picosecond and femtosecond time resolution.^[3–9] During this time, many DNA systems have also been studied by the femtosecond transient absorption (fs-TA) technique. Time-resolved emission measurements monitor excited states that decay radiatively to the ground state, while the fs-TA technique can detect any excited state—even a non-emissive dark state—that is capable of reaching a higher-lying excited state by absorption. The potential for these two techniques to monitor different excited-state populations has been stressed in some publications,^[8,10] while others have reported congruent results from both techniques.^[11–13] To date, only a handful of studies have examined the same system in the same laboratory using these complementary techniques,^[11,13,14] and additional experiments are needed.

The femtosecond fluorescence upconversion technique provides the highest time resolution for studying emission. Initial studies on DNA strands using this technique revealed subpicosecond lifetimes that are similar to—or even shorter than—ones observed from monomeric nucleobases.^[6,15–17] These ultrafast emission components, which have been assigned to excitons, are accompanied by much weaker emission that extends out to much longer times. Often, the limited dynamic range of the fluorescence upconversion technique makes it difficult to detect the latter emission. On the other hand,

the sensitive time-correlated single-photon counting (TCSPC) technique excels at detecting even very weak emission on timescales longer than about 50 ps. The exquisite sensitivity of single-photon counting detection has revealed emission on the many ns timescale where it is typically difficult to detect signals in fs-TA experiments.^[7,18] Consequently, literature descriptions of long-lived excited states do not always refer to the same states.^[19] Instead, “long-lived excited states” are often the states with the longest lifetimes that can be detected by a given technique—nanosecond in the case of TCSPC studies^[8] and subnanosecond for fs-TA experiments. As is evident from their very different lifetimes, these states may have little in common.

Here, we study emission from the three AT-oligonucleotides depicted in Figure 3.1 (see Figures S1–S4 and accompanying text in the Supporting Information, SI, for characterization details) using the TCSPC technique and compare with results from fs-TA experiments. We carefully compare dynamics from several tens of ps to a few ns where both techniques have overlapping measurement windows. We show that the subnanosecond lifetimes ranging from 50 to 200 ps measured by the TCSPC technique agree quantitatively with ones measured for the same substrates by the fs-TA technique and that this agreement holds for both single and double strands. This agreement indicates that the states probed in fs-TA experiments are not completely dark, but can decay radiatively. Because ground-state bleach recovery signals measured in fs-TA experiments indicate that most excited states (95% or more) decay before 1 ns, emission detected at longer times originates from a small minority of excitations.

3.2 Experimental Methods

3.2.1 Chemicals

Tris (hydroxymethyl) aminomethane (Sigma Aldrich, 99.9%), NaCl (EMD Millipore, 99.0%), HCl (Fisher Scientific), potassium phosphate monobasic (VWR International, 99.0%), sodium phosphate (Fisher Scientific, 99.9%), TMP (thymidine 5'-monophosphate, Sigma Aldrich, 99+%) were used as purchased. The oligonucleotides (dA)₁₅ and d(AT)₉ were purchased as lyophilized sodium salts from Midland Certified Reagent Company (Midland, TX). These oligonucleotides were purified by the supplier by gel filtration (GF grade) and used as received. The (dA)₁₀·(dT)₁₀ PEG-linked dumbbell (structure shown in Figure 3.1) was a gift from Prof. Fred Lewis (Northwestern University) and is described in more detail in ref. [51]. The d(AT)₉·d(AT)₉ duplex was formed by annealing the d(AT)₉ sample at 80 °C for 10 minutes before allowing it to slowly cool down to room temperature. Formation of the d(AT)₉·d(AT)₉ duplex was confirmed by circular dichroism (CD) spectroscopy (Figure A1 in Appendix A). A melting point of 80 °C was determined for the (dA)₁₀·(dT)₁₀ PEG-linked dumbbell by temperature-dependent CD spectroscopy (Figure A3 in Appendix A), consistent with previous reports.^[21]

Aqueous solutions for TCPSC measurements were prepared in 10 mM Tris-HCl buffer with 0.25 M NaCl using 18 MΩ water from a water purification system (EMD Millipore). Tris-HCl buffer was used for all measurements because it was found to have significantly less emission when excited at 265 nm than phosphate buffer solution. The pH was adjusted to 7.1 by dropwise addition of concentrated HCl solution. Aqueous

solutions for transient absorption measurements contained 50 mM phosphate buffer and 0.1 M NaCl.

3.2.2 Steady-State Spectroscopy

UV/Vis absorption spectra were recorded using a Lambda 25 spectrometer (PerkinElmer, Inc.). CD measurements were recorded using a J-815 spectrometer (Jasco, Inc.). UV absorption spectra were recorded using either 1 or 10 mm path length Spectrosil[®] quartz cuvettes. A solution containing the pH buffer and salt but without the DNA sample was used for background correction. All samples studied by TCSPC were adjusted to have an absorbance of 0.3 at 265 nm (Figure A4 in Appendix A).

3.2.3 TCSPC Measurements

A tunable Ti:Sapphire laser (Chameleon Ultra II, Coherent Inc.) provided 180 fs pulses at a repetition rate of 80 MHz with a fundamental wavelength of 795 nm. A harmonics generator supplied by the laser manufacturer converted the fundamental pulse into 265 nm excitation pulses. The repetition rate was reduced to 4 MHz using an electro-optic modulator (M350-80, Conoptics) driven by a pushpull power amplifier (Model 25D, Conoptic Inc.). A synchronous countdown system (Model 305, Conoptics Inc.) triggered by the laser was used to control the electro-optic modulator pick rate while simultaneously signaling the start of the timing electronics.

The TCSPC setup consists of a commercial fluorescence lifetime spectrometer (Picoquant model FluoTime 200 equipped with the Picoharp 300 timing electronics) and a microchannel plate photomultiplier (MCP-PMT model R3809U-5X, Hamamatsu).

Sample emission was filtered with a 300 nm long pass filter (Asahi model XLU0300) placed before the emission monochromator. The excitation pulses were vertically polarized using a Glan-Thompson polarizer. Fluorescence from the sample was detected after passing through an analyzing polarizer oriented at magic angle (54.78°) relative to the polarization of the excitation pulses. The IRF was determined by scattering from a diluted LudoxS HS-40 colloidal silica solution (Sigma Aldrich) at 265 nm. The FWHM of the IRF was typically around 60 ps. A bin width of 8.0 ps was used for each TCSPC measurement. The solution under study was held in a 10V10 mm SpectrosilS quartz cuvette with constant stirring at 20.000 ± 0.02 °C utilizing a temperature-controlled cuvette holder (TLC 50TM, Quantum Northwest). The excitation power was attenuated to provide approx. 10,000 counts in the maximum channel after 5 minutes of acquisition from (dA)₁₅. The count rate produced from sample excitation was well below <1% of the modulated repetition rate to prevent pile-up effects. Initial successive measurements were recorded for each sample to ensure the absence of fluorescent photoproducts.^[50] Sample emission was then collected for 5 minutes at each emission wavelength. TCSPC decays were not corrected for background emission from a buffer-only solution because of the difficulty of distinguishing weak solute emission on the ns timescale from interfering signals (see Section 3.5).

3.2.4 Transient Absorption Measurements

The fs-TA laser system has been described elsewhere,^[21] and is only briefly outlined here. The 800 nm laser fundamental was produced by a 1 kHz repetition rate Ti:sapphire regenerative amplifier using chirped pulse amplification (Libra-HE,

Coherent). About 20% of the laser output was frequency tripled to produce pump pulses with a center wavelength of 267 nm. The pump pulse energy was attenuated to 0.9 mJ with a spot size of 250 μ m (FWHM) at the sample, and an optical chopper set to 333 Hz to let through every third pulse. Approximately 40% of the fundamental was pumped into an optical parametric amplifier (OPerA Solo, Coherent) where 500 nm pulses were generated by sum-frequency mixing the signal and 800 nm. The visible pulses were subsequently frequency doubled to produce the 250 nm probe pulses. A motorized translation stage controlled the optical delay of the pump and probe pulses at the sample, giving 3 ns of total delay. The relative polarization between the pump and probe pulses was set to the magic angle (54.78°) at the sample. Approximately 2 mL of sample was flowed through a flow cell to avoid photodegradation. The flow cell temperature was set to 20 °C. After passing through the sample, the probe pulses were isolated by a monochromator and detected by a photomultiplier tube. Samples for fs-TA measurements were recirculated in a flow cell with a path length of 1 mm.

3.2.5 Data Fitting

Using a global analysis program (FluoFit Version 4.5, PicoQuant), emission decays were fit to a model function [Eq. (3.1)] consisting of a sum of exponentials convoluted with the normalized instrument response function, $IRF(t)$, measured as described above. The fs-TA signals were fit to a similar expression, but the IRF was modeled by a Gaussian function with a 200 fs FWHM. Fitting in this case was done with the IGOR Pro program version 6.34A (Wavemetrics). All uncertainties are reported as twice the standard deviation reported by the fitting program.

3.3 Results

3.3.1 TCSPC Decay Signals

In the TCSPC technique, all emitted photons are detected by the instrumentation, but decay times significantly shorter than that of the instrument response function (IRF) cannot be determined accurately. Photons emitted on the shortest timescales make a contribution to the total signal that has the same temporal characteristics as the IRF, while photons emitted at later times (typically at times greater than a few tens of ps) contribute to measurable decay components. A response function consisting of a delta function to represent the fastest emission and a sum of n exponentials was convoluted with the measured IRF and compared with the data [Eq. (3.1)]:

$$S(t) = \int_{-\infty}^t \text{IRF}(t' - t_s) \sum_{i=1}^n A_i e^{-\frac{t-t'}{\tau_i}} dt' + N_0 \Delta t \cdot \text{IRF}(t - t_s) \quad (3.1)$$

where A_i and τ_i are the amplitude and lifetime, respectively, of the i th decay component, Δt is the bin width (0.008 ns), and the adjustable parameter, t_s , accounts for any temporal offset between the emission decay and the IRF. In Equation (3.1), $\text{IRF}(t)$ is the signal measured from a scattering solution (see the experimental section) normalized to have unit area. Finally, the parameter N_0 accounts for all detected photons that are not in the decay components represented by the sum of exponentials in Equation (3.1).

The time integral of Equation (3.1) divided by the bin width gives the total number, N , of counted photons in the decay signal and can be written as [Eq. (3.2)]:

$$N = \frac{1}{\Delta t} \int_{-\infty}^{+\infty} S(t) dt = N_0 + N_1 + N_2 + \cdots + N_n \quad (3.2)$$

where N_0 is defined in Eq. (3.1), and each N_i with $i > 0$ measures all counts contributed by the i th exponential decay component and is equal to $\frac{A_i \tau_i}{\Delta t}$. Note that N_0 includes emission from lifetime components that are too short to be resolved by the TCSPC instrumentation together with any photons scattered by the pump pulse that reach the detector. Because emission in our experiments is detected at a wavelength far removed from the pump wavelength, the amount of scattering is negligible, and we assume that the N_0 signal arises solely from excited states that decay too quickly for their lifetimes to be quantified by the TCSPC technique. Hereafter, we will refer to this signal contribution as the prompt emission. It will be contrasted with emission from resolvable lifetime components, that is, emission that can be clearly distinguished from emission with the temporal shape of the IRF.

Emission decays from (dA)₁₅ in buffer solution were recorded at emission wavelengths between 330 and 480 nm with excitation at 265 nm. Normalized emission traces at 330 and 420 nm are compared in Figure 3.2 (the same traces are shown on a linear scale in Figure A5 in Appendix A). Emission counts drop by two orders of magnitude during the first few nanoseconds before decreasing more slowly. The ratio of emission at 3 ns to the maximum emission at short times is greater at 420 nm than at 330 nm. Figure 3.2 also shows that the slope of each decay curve is somewhat greater for the shorter emission wavelengths at both early and late times.

Emission was recorded from a Tris-HCl buffer solution containing 0.25m NaCl but without added oligonucleotide (dark gray dots in Figure 3.2). This trace was recorded with identical experimental parameters (acquisition time, excitation fluence, etc.) as the

(dA)₁₅ trace at 330 nm in Figure 3.2. The decay curves with and without the oligonucleotide approach each other at times greater than 3 ns, but somewhat more emission is observed from the (dA)₁₅ solution. Emission from the Tris-HCl buffer differs only slightly from the very weak background emission detected from neat water from the Millipore water ultrapurifier (Figure A6 in Appendix A). Adding 0.25 m NaCl increased emission threefold compared to the Tris-HCl buffer solution alone. The emission from the buffer plus 0.25 m NaCl solution, recorded at wavelengths from 330 nm to 480 nm with 265 nm excitation, is described in Figure A1 and Table A3 in Appendix A.

Emission decays at 360 and 450 nm from the d(AT)₉·d(AT)₉ duplex in Tris-HCl buffer solution with 0.25m NaCl are shown in Figure 3.3 (see Figure A8 in Appendix A, for TMP emission at these wavelengths). Time-integrated emission from the NaCl-containing buffer solution is maximal near 330 nm, while that from the duplex is greatest near 420 nm. As with (dA)₁₅, the emission decays quickly in the first nanosecond and more slowly thereafter. After 1 ns, the signal at 360 nm decays somewhat more rapidly than at 450 nm. Relative to (dA)₁₅ at the same detection wavelength, emission from d(AT)₉·d(AT)₉ is strongly quenched during the first 2 ns (Figure 3.4). Emission from the (dA)₁₀·(dT)₁₀ dumbbell is reduced even further, but otherwise has a similar slope (cyan curves in Figure 3.4). All signals in Figure 3.4 were acquired for the same amount of time under identical experimental settings from solutions with identical absorbance of 0.3 at the excitation wavelength of 265 nm (Figure A4 in Appendix A).

All decays in this study were fit to three exponentials and the N_0 parameter that models prompt emission [Eq. (3.1)]. Adding a fourth exponential term did not

significantly reduce the χ^2 value. Best-fit parameters for all three substrates are listed in Table 3.1. For (dA)₁₅, the fits confirm that shorter lifetimes are seen at shorter emission wavelengths. The percent of all emitted photons that are prompt photons is shown in the final column of Table 3.1. For (dA)₁₅, prompt photons constitute a small fraction of all photons detected from 330 to 480 nm, but they make a larger contribution to the duplex signals. For all samples, the prompt emission is greatest at the shortest emission wavelengths.

The τ_1 decay component in Table 3.1 accounts for approximately 90% of the decay amplitude from the resolvable lifetime components in both the single- and double-stranded systems. The longest decay component has a lifetime of between 4 and 9 ns. It accounts for less than 1 or 2% of the multiexponential decay amplitude for (dA)₁₅, but makes a somewhat larger contribution to the signals from the two duplex samples. For (dA)₁₅, all three lifetimes increase at longer wavelengths. The percentage increase from shortest to longest emission wavelength is largest for τ_3 and smallest for τ_1 . For the two duplexes, the best-fit values of the three time constants agree within experimental uncertainty at the various emission wavelengths with only one or two exceptions. For both d(AT)₉·d(AT)₉ and the (dA)₁₀·(dT)₁₀ dumbbell, the τ_2 lifetimes decrease slightly as the emission wavelength increases, but this trend may not be statistically significant. As shown in Table A1 in Appendix A, linking the τ_2 lifetime across all emission wavelengths produces nearly equally good fits for the two duplex samples, according to the Akaike information criterion.^[20]

Time-resolved emission spectra for $(dA)_{15}$ were constructed from discrete emission decays recorded at wavelengths between 330 and 480 nm (Figure 3.5). Care was taken to maintain constant excitation intensity and the signals were corrected for the spectral sensitivity of the instrument (see Appendix A). At the earliest delay time (50 ps), the emission is peaked near 360 nm. During the first nanosecond there is little change in the shape of the time-resolved emission spectrum, but the emission maximum gradually shifts to 420 nm after 5 ns. Time-resolved emission spectra from the two double strands are shown in Figures 3.6 and 3.7. At early times, emission from the non-alternating dumbbell is peaked at 390 nm, while that of the alternating $d(AT)_9 \cdot d(AT)_9$ duplex is maximum at 420 nm. As with $(dA)_{15}$, the time-resolved emission spectra shift to longer wavelengths at delay times greater than 1 ns.

The time-integrated emission was calculated using Equation (3.2). Integrating the fluorescence decays vs. time at each emission wavelength yields the time-integrated spectra shown in Figure 3.8 (gray area). This figure also shows the contributions to the total emission (N_0). All parameters used to construct Figure 3.8 are listed in Table A5 in Appendix A.

3.3.2 Transient Absorption Signals

Both $(dA)_{18} \cdot (dT)_{18}$ and the 10-mer linked dumbbell were studied recently by the fs-TA technique.^[21,22] fs-TA measurements on the alternating duplex $d(AT)_9 \cdot d(AT)_9$ were first reported in 2005,^[23] but were later shown to have been recorded at a temperature of approximately 40 °C due to heating by the pump laser, and not at room temperature as originally stated.^[19,21] For this reason, the fs-TA signal from $d(AT)_9 \cdot d(AT)_9$ was re-

measured using a temperature-controlled flow cell apparatus to rigorously maintain a temperature of 20 °C. Figure 3.9 shows the ground state bleach recovery signal (267 nm pump/ 250 nm probe) recorded for d(AT)₉·d(AT)₉. A biexponential fit yields lifetimes of 5.6 ± 1.4 ps ($A_1 = 52\%$) and 84 ± 14 ps ($A_2 = 45\%$) with a slight offset ($A_3 = 3\%$). Table A1 in Appendix A summarizes the fitting parameters.

3.4 Discussion

The time window of our TCSPC measurements extends over more than two decades from 60 ps (the FWHM of the IRF) to 6 ns and beyond. In the following discussion, our focus is on emission that is resolvable by the TCSPC instrumentation [i.e. the non-prompt emission described by the sum of exponentials in Eq. (3.1)] unless otherwise noted. Decays recorded at a number of discrete emission wavelengths provide kinetic and spectral data, which are used to understand excited-state deactivation pathways. We first discuss the fluorescence decay kinetics as a function of emission wavelength in section 3.4.1 before turning to the time-resolved and time-integrated emission spectra in section 3.4.2. In section 3.4.3, the emission decay traces are compared with fs-TA experiments carried out on the same substrates and this comparison forms the basis of the assignments to be discussed in section 3.4.4. Finally, in section 3.4.5, emission occurring beyond 1 ns is briefly discussed before conclusions are summarized.

3.4.1 Emission Kinetics

The emission from all three samples decays multiexponentially. The subnanosecond decay component (τ_1) accounts for approx. 90% of the decay amplitude of

the multiexponential decay that can be resolved by fitting the TCSPC decay trace (see Table 3.1). According to Table 3.1, all three time constants for single-stranded (dA)₁₅ increase as the emission wavelength increases. The progressive slowing down of the dynamics from this sample is also evident in the average decay time, $\langle\tau\rangle$, calculated from the best-fit exponential lifetimes and amplitudes in Table 3.1 using Equation (3.3):

$$\langle\tau\rangle = \sum_i A_i \tau_i / \sum_i A_i \quad (3.3)$$

The value of $\langle\tau\rangle$ for (dA)₁₅ systematically increases, doubling from 220 ps at 330 nm to 440 ps at 480 nm. Due to evidence that the nanosecond decay component (τ_3) contains contributions from fluorescent impurities (see below), we do not attach much significance to $\langle\tau\rangle$, but it does capture the trend of wavelength-dependent dynamics for (dA)₁₅ and the wavelength-independent dynamics observed for the two duplexes (see below).

Markovitsi and co-workers studied emission from (dA)₂₀ and several AT duplexes similar to our compounds using the TCSPC technique.^[10,24] They reported exponential time constants from fits to emission decays from (dA)₂₀ at 330 nm (3.2, 37.5, 186, and 748 ps), 360 nm (11.6, 101, 253, and 1,830 ps), and 420 nm (39, 198, 551, and 5,050 ps).^[10] It is difficult to compare these results with ours because neither amplitudes nor details about the fitting procedure were reported in ref.[10]. Nevertheless, the three slowest lifetimes at 420 nm agree reasonably well with our measured lifetimes for (dA)₁₅ at the same wavelength (Table 3.1). In agreement with our results for (dA)₁₅, Markovitsi and co-workers find that emission decays from (dA)₂₀ recorded by the TCSPC technique become progressively slower at longer emission wavelengths.^[10,25] However, the fitting

parameters in Table 3.1 indicate that the lifetimes increase more slowly with emission wavelength than is suggested by the lifetimes reported in ref.[10]. This is particularly true for the τ_1 component responsible for most of the subnanosecond emission amplitude.

Phillips and co-workers also observed that decay times increase at longer emission wavelengths for (dA)₂₀, but they chose to fit their signals globally at several wavelengths with three time constants of 0.39, 4.3, and 182 ps.^[11] Multiexponential fits can lack uniqueness, particularly when two or more of the time constants are not widely separated. In this case, global fitting with fixed time constants, but varying amplitudes, can produce fits of nearly equal quality to ones obtained when all time constants are allowed to vary. Clearly, only the latter approach can detect a time constant that slowly increases with wavelength. We obtain the best fit to our emission transients for (dA)₁₅ when all time constants are unlinked (these are the parameter values shown in Table 3.1), but the fit obtained when the τ_1 parameter is linked across all emission wavelengths is only slightly inferior (see Table A1 in Appendix A), according to the Akaike information criterion.

The majority of emission from d(AT)₉·d(AT)₉ and the (dA)₁₀·(dT)₁₀ dumbbell recorded with TCSPC instrumentation also decays on the subnanosecond timescale (Figure 3.4). In contrast to single-stranded (dA)₁₅, the average decay times calculated for the duplexes using Equation (3.3) remain nearly constant and show no clear trend with emission wavelength. For the (dA)₁₀·(dT)₁₀ dumbbell, the value of t_1 is approximately 65 ps and independent of emission wavelength within experimental uncertainty (Table 3.1). In a TCSPC study, Markovitsi and co-workers measured substantially longer decay times

for poly(dA)·poly(dT), the polymer analog of our (dA)₁₀·(dT)₁₀ duplex,^[24] but these differences might be due to the length of the helix. At each emission wavelength, the τ_1 time constant for the (dA)₁₀·(dT)₁₀ dumbbell is about 60% as large as that for d(AT)₉·d(AT)₉. Slower dynamics were reported previously on the TCSPC timescale for alternating vs. non-alternating AT polymers.^[8]

3.4.2 Emission Spectra

Time-resolved spectra from (dA)₁₅ between 50 and 500 ps are similar in shape with an emission maximum near 360 nm (Figure 3.5). The spectra resemble that of (dA)₂₀ at 20 ps after excitation reported by Kwok et al.^[11] As noted previously,^[11,25] the maximum steady-state emission from (dA)_n oligomers occurs at longer wavelengths than from 2'-deoxyadenosine 5'-monophosphate (dAMP), which is maximal at 306 nm.^[26] At times between 2 and 3 ns, the maximum in the emission spectrum (λ_{max}) increases to 390 nm, and finally shifts to 420 nm after 5 ns.

Time-resolved emission from the duplexes (Figures 3.6 and 3.7) occurs at much longer wavelengths than from the monomers TMP and dAMP, which have maximum emission at 330 nm and 306 nm, respectively.^[26] Time-resolved emission from d(AT)₉·d(AT)₉ at times between 100 and 500 ps is peaked at 420 nm, while λ_{max} at later times is at 450 nm (Figure 3.6). For comparison, Kwok et al.^[11] observed an emission maximum at 430 nm from d(AT)₁₀·d(AT)₁₀ 18 ps after excitation by the Kerrgated time-resolved fluorescence (KG-TRF) technique. Because the time resolution of the KG-TRF technique is <400 fs, the 18 ps emission spectrum is undistorted by the instrument response function. At each time throughout our observation window (50 ps - 10 ns), λ_{max}

of the (dA)₁₀·(dT)₁₀ dumbbell is consistently 30 nm shorter than λ_{max} of the d(AT)₉·d(AT)₉ duplex (compare Figures 3.6 and 3.7). For all three samples, the maximum in the time-resolved emission spectra at times less than 1 ns is very similar to the maximum in the time-integrated spectrum of the resolvable decay components for each DNA compound (filled black circles, Figure 3.8). This agreement demonstrates that the subnanosecond emission is the dominant contribution to the resolvable emission. The fit amplitudes in Table 3.1 confirm this finding.

The time-integrated spectra shown in Figure 3.8 also agree reasonably well with steady-state emission spectra from the literature for all three DNA compounds. As an added check, we compare the time-integrated spectrum for a (dA)₁₈ oligomer with the steady-state spectrum in Figure A1 in Appendix A. Markovitsi and co-workers observed a maximum at 370 nm and a weak shoulder near 425 nm for both poly(dA) and (dA)₂₀ with excitation at 265 nm, in good agreement with our results.^[7,25] The time-integrated spectrum constructed from our TCSPC emission traces for the (dA)₁₀·(dT)₁₀ dumbbell rises monotonically towards shorter wavelengths, suggesting that λ_{max} occurs below 360 nm. This is consistent with the literature data indicating maximum emission at 330 nm from (dA)₂₀·(dT)₂₀.^[15] The steady-state fluorescence spectrum of poly(dAdT)·poly(dAdT) has a distinctive shape with two maxima—a more intense band near 425 nm is accompanied by a weaker maximum near 320 nm.^[12,16,27] The maximum in the time-integrated emission spectrum from d(AT)₁₀·d(AT)₁₀ determined by Kwok et al.^[12] occurs at 460 nm with a weaker band at 323 nm. A recent study reported maxima at 324 nm and 430 nm in the steadystate emission spectrum from the same substrate.^[28] Our time-

integrated spectrum agrees well with the steady-state spectrum at longer emission wavelengths reported in both studies. The short wavelength peak, which is likely dominated by emission from short-lived excitons or monomer-like excited states, was not investigated in our study.

The agreement between the time-integrated spectra of $(dA)_{15}$ and $d(AT)_9 \cdot d(AT)_9$ in Figure 3.8 with the steady-state emission spectra at times greater than a few tens of ps are responsible for much of the steady-state emission. However, prompt emission contributes more to the steady-state spectrum of the $(dA)_{10} \cdot (dT)_{10}$ dumbbell. The difference between the $\lambda_{\max}$ of the time-integrated spectrum (gray area in Figure 3.8b) and the time-resolved emission spectrum 100 ps after excitation (Figure 3.7) provides evidence for this as does the high percentage of photons emitted at short wavelengths on timescales faster than the IRF (Table 3.1).

TA experiments indicate that stacked and paired bases in double strands can decay via a monomer-like channel,^[21] resulting in ultrashort excited-state lifetimes that are too short to accurately characterize by the TCSPC technique. These fast decays are not the focus of our study.

3.4.3 Comparison of Emission Decays with fs-TA Signals

As the signal in Figure 3.9 illustrates, fs-TA signals typically probe dynamics over several nanoseconds with a minimum time resolution on the order of 100 fs. TCSPC measurements with a micro-channel plate-PMT detector can observe dynamics from a few tens of ps to a maximum time set by the period between excitation pulses. The latter time can easily reach the microsecond timescale, but there is very little emission from

DNA strands 5 ns after excitation as seen in Figures 3.2–3.4. Comparing dynamics from the fs-TA and TCSPC techniques in their common overlap window from approximately 10 ps to a few ns provides additional insight into the nature of long-lived excited states formed in DNA oligonucleotides.

Ground state bleach signals measured by fs-TA experiments on DNA strands typically recover biexponentially.^[19,29] A fast component of several picoseconds is assigned to vibrational cooling following ultrafast relaxation to the electronic ground state.^[19] Additionally, a slow component is observed with a lifetime that can exceed 100 ps. The short time constant of 5.6 ± 1.4 ps from the fit to the fs-TA signal of $d(AT)_9 \cdot d(AT)_9$ in Figure 3.9 agrees well with the lifetime of 5.2 ± 0.5 ps determined for the duplex sequence isomer $(dA)_{18} \cdot (dT)_{18}$ at the same pump and probe wavelengths.^[21] This suggests that vibrational cooling is insensitive to base sequence in these AT double strands, possibly because the loss of excess vibrational energy is determined primarily by hydration, which is similar in both cases. Hydrogen bonding to solvent molecules is known to play an important role in mediating the relaxation of excess vibrational energy.^[30] Although vibrational cooling contributes strongly to fs-TA bleach recovery signals, no such decay components are expected in emission signals because vibrational cooling takes place on the electronic ground state.

The long-lived component of 84 ± 14 ps in Figure 3.9 is somewhat longer than the 66 ± 14 ps lifetime reported by Crespo Hernandez et al. in 2005 for the same duplex.^[23] However, pump laser-induced heating of the sample in the spinning cell used in the 2005 experiments is now recognized to have produced a steady-state temperature of

approximately 40 °C.^[21] Preliminary experiments show that lifetimes in the alternating duplex decrease as the temperature increases, and a lifetime of 66 ps is not unreasonable at 40 °C.

To facilitate comparison of the emission decays to fs-TA signals, the τ_1 emission lifetimes from Table 3.1 were averaged using Equation (3.4):

$$\langle \tau_1 \rangle_\lambda = \sum_i A_{1,i} \tau_{1,i} / \sum_i A_{1,i} \quad (3.4)$$

where the index i runs over all emission wavelengths, and the absolute amplitudes $A_{1,i}$ are from Table A5 in Appendix A. The results are summarized in Table 3.2 together with lifetimes from fs-TA experiments. The wavelength-averaged lifetime of 110 ± 26 ps determined for the d(AT)₉·d(AT)₉ duplex compares favorably with the lifetime of 84 ± 14 ps determined from the fs-TA signal on the same substrate. Even better agreement is observed for (dA)₁₅ and the (dA)₁₀·(dT)₁₀ dumbbell. The excellent agreement indicates that emission due to the shortest resolvable decay component in the TCSPC experiments (τ_1) originates from the same long-lived excited states detected in the fs-TA experiments.

Other researchers have previously noted good agreement between fs-TA and time-resolved emission experiments.^[11–14] Kwok, Ma and Phillips determined that lifetimes measured by the fs-TA and KG-TRF techniques are similar for single-stranded (dA)₂₀^[11] and for double-stranded d(AT)₁₀·d(AT)₁₀.^[12] Temps and co-workers observed similar lifetimes in their femtosecond fluorescence upconversion and fs-TA experiments on the dApdG dinucleotide in aqueous solution^[13] and from aggregates of guanosine in hexane.^[14] Despite these reports, there has been debate over how to compare results from fs-TA and TCSPC measurements. Markovitsi and co-workers asserted that time constants

obtained from fits of TCSPC data are phenomenological and cannot be associated with particular excited states.^[10,24] It has also been stated that time constants measured in fs-TA experiments need not agree with ones from fluorescence measurements due to the possibility that the fs-TA technique probes completely dark states.^[8] Although it is possible in principle that the two techniques could probe different excited-state populations, this study and earlier ones^[11–13] strongly indicate that this is not the case.

3.4.4 Assignments

The good agreement between emission and fs-TA lifetimes suggests that conclusions from fs-TA experiments are germane to the interpretation of the TCSPC signals. The fs-TA experiments have shown that excited states decaying on the subnanosecond timescale only form when two or more bases are well stacked.^[11,29,31] Experiments on variable-length (dA)_n oligonucleotides (n=2–18) have shown that there is little difference between the signals observed in dinucleotides and those seen in longer strands.^[29] This supports the conclusion that excitations are localized in two stacked bases. These excitations constitute excited nucleobase dimers and are known as excimers. The red-shifted fluorescence of oligonucleotides that decays much more slowly than emission from single nucleotides.^[33]

A variety of experiments have presented evidence that the long-lived excimer states in single strands have strong charge transfer (CT) character.^[34–37] Recent fs-TA studies with mid-infrared probing have detected CT states in DNA strands when two different bases are p-stacked.^[22,35–37] For example, Doorley et al.^[35] observed vibrational marker bands consistent with electron transfer from A to T in the single-stranded

dinucleotide dApdT and observed a similar CT state in duplex poly(dAdT)·poly(dAdT). Electron transfer is reasonable in heterodimers where one base is a better electron donor or acceptor, but it is less obvious that (partial) electron transfer can occur in a homodimer like AA. Nevertheless, AA stacks have been intensively studied computationally, revealing excited dimer states with CT character.^[33,38–41]

Electronic structure calculations have revealed multiple types of excimer states in (dA)_n oligonucleotides.^[33,39,41] Olaso Gonzalez et al.^[33] classified excimers by their degree of CT character as either neutral or CT excimers. The DNA backbone influences the overlap between adjacent adenines and profoundly affects excimer stability.^[33,39] Neutral CT excimer states were found to lie at lower energy than CT excimer states even when stabilization of the latter states by a polar solvent was taken into account.^[33] Improta and Barone assigned emission from single-stranded (dA)_n near 360 nm to a neutral excimer, which is characterized by a twist angle of approx. 0°. ^[39] Banyasz et al.^[25] proposed that neutral excimers are responsible for the higher energy fluorescence on the subnanosecond timescale in (dA)_n oligomers, while CT excimers give rise to the lower energy fluorescence seen beyond 1 ns. Conti et al.^[40] concluded that a CT excimer in an AA stack can deactivate via the same nuclear motions responsible for deactivation of the L_a excited state associated with a single adenine.

Several investigators have discussed the possibility that two or more kinds of excimer states are populated during deactivation.^[11,28,33,39,41] Kwok, Ma, and Phillips proposed that the initial excited state in (dA)₂₀ is localized on a single base. This state is said to decay on a subpicosecond timescale to an excimer state localized on two bases.

The excimer subsequently decays with a 4.3 ps lifetime to an “excimer-like” excitation that is delocalized over more than two bases, and which has a 182 ps lifetime.^[11]

However, the observation that the fs-TA bleach recovery signal for the dinucleotide (dA)₂ decays with a lifetime of 183 ± 6 ps^[29] calls into question delocalization over more than two bases. In the model presented in ref.[11], an initially localized excitation progressively delocalizes over a larger number of residues. This picture is difficult to reconcile with the tendency of excitations in other multichromophoric systems to localize as a result of structural fluctuations. In fact, it has been suggested that the opposite process of dynamic localization from more extended excitons to excited dimer states takes place in DNA strands.^[29,34]

The formation of an excimer state is driven in large measure by covalent interactions between the two aromatic molecules.^[32,42] Consequently, nucleobase excimers can be distinguished not only by the degree of CT character, but also by the extent of covalent bonding between the interacting bases. Spata and Matsika distinguish between regular excimers and bonded excimers.^[41] In the latter species, the C6 atoms of the neighboring adenines approach to a distance of approx. 2 Å, while this distance is approximately 3 Å in the other excimers. Bonded excimers have been identified in gas-phase calculations of 9-methyladenine dimers.^[43] In contrast to the results of Improta and co-workers,^[25,39] Spata and Matsika predict similar emission energies for neutral and CT excimers that lack a strong bonding interaction.^[41] They find further that the neutral non-bonded excimers can form with a twist angle very close to that found in B-DNA. Bonded

excimers are predicted to have the greatest degree of CT character and the lowest emission energies.^[41]

The trend seen in $(dA)_{15}$ of slower dynamics at longer emission wavelengths may be the result of greater conformational heterogeneity in the single strand than in the duplex structures, which exhibit emission wavelength-independent decays. The energy stabilization of base stacking is modest compared to $k_B T$ ^[19,44] and single strands like $(dA)_n$ contain both stacked and unstacked bases, leading to a distribution of stacked domains with a variable number of stacked bases.^[45] A stack of just two bases is expected to be hydrated more strongly than a longer stacked domain. Excimer state energies are predicted to be lowered through interactions with the polar solvent, particularly for excimers with significant CT character,^[25,33,46] Given the clear trend in our measurements in which the longest-lived excited states have the most red-shifted emission we propose that emission from better-solvated excimers is red-shifted and longer-lived than emission from excimers that are less accessible to the solvent such as those located in longer stacked domains. Temperature-dependent studies of emission from variable-length $(dA)_n$ oligonucleotides are needed to further explore this question.

A number of computational studies have considered how the dynamics of excimers in double-stranded DNA differ compared to ones in single-stranded DNA.^[39–41] Improtà and Barone suggested that the structure of a double helix like $(dA)_n \cdot (dT)_n$ inhibits the deformations needed to achieve the fully eclipsed stacking structure, which they identified as the minimum energy geometry of the neutral excimer.^[39] In this case, the neutral excimer pathway is blocked and longer wavelength emission from a CT excimer

($\lambda_{\text{max}}=420$ nm) is predicted to dominate, consistent with the longer wavelength emission seen from the non-alternating AT double strand compared to (dA)₁₅ (Figure 3.8).

Improta and Barone proposed further that reduced solvent accessibility in double strands would increase the driving force for charge recombination, resulting in somewhat slower decay times for an AA excimer in duplex (dA)_n·(dT)_n.^[39] However, Table 3.2 clearly shows that shorter, not longer, lifetimes are seen in both fs-TA and emission experiments on (dA)_n·(dT)_n double strands compared to single-stranded (dA)_n. To date, most computational studies have assumed that an AA excimer is responsible for emission in duplex (dA)_n·(dT)_n. These studies, which employ a QM/MM approach, have included only stacked bases in the QM region, and are therefore unable to capture deactivation channels unique to base pairs such as interstrand proton transfer.

Recent experimental evidence strongly suggests that alternative decay channels are important in duplex DNAs.^[22] The integrated emission of the resolvable components from both duplex systems studied here is red-shifted compared to singlestranded (dA)₁₅ (Figure 3.8). Differences in both lifetimes and emission spectra strongly suggest that distinct excited states are populated in each of the three systems. If intrastrand charge separation were dominant in the non-alternating dumbbell, then there should be an emission component similar to that seen in single-stranded (dA)₁₅. However, no such component is seen. Instead, the emission from the dumbbell occurs at lower energy than from (dA)₁₅ (Figure 3.8).

Zhang et al.^[22] recently identified a multisite PCET pathway in (dA)₁₈·(dT)₁₈. Upon UV excitation, A^{•+} and A^{•-} are formed via intrastrand electron transfer, and the

latter acquires a proton from its H-bonded partner T, forming the $A^{\cdot+}$ radical cation stacked with the $A(+H)^{\cdot}$ neutral radical. The energy of this state is lower than that of the $A^{\cdot+} A^{\cdot-}$ state formed by charge separation in a single strand. The higher energy of the latter state increases the driving force for charge recombination, leading to a longer emission lifetime because charge recombination takes place in the Marcus inverted region. This explains why a longer emission lifetime is seen in the $(dA)_{18}$ single strand than in the $(dA)_{10} \cdot (dT)_{10}$ dumbbell.

3.4.5 Emission on the ns Timescale

Nanosecond emission components like the ones in Figures 3.2–3.4 were first detected in the 1980 s. Ballini et al.^[18] measured lifetimes of 0.31 ns (69%) and 4.6 ns (31%) for poly(A) emission at 406 nm upon excitation at 271 nm. These authors speculated that the several-ns component is excimer phosphorescence. Time-resolved emission experiments performed during the past decade have confirmed the existence of ns lifetimes,^[10,24,25,47] and multiple explanations have been advanced for the nanosecond component. It was proposed that the electron and hole created by photon absorption can separate, and then recombine on the ns timescale, re-forming a $^1\pi\pi^*$ state of a single base, which decays via delayed fluorescence.^[48] Banyasz et al.^[25] suggested that CT excimers are responsible for the ns emission, while Spata and Matsika^[41] predict that bonded excimers are responsible for the longest wavelength emission. In our view, the nature of excitations with emission lifetimes >1 ns is still uncertain.

Because intrinsic DNA fluorescence is extremely weak, it is essential to carefully assess any contributions from trace amounts of impurities. Interference from background

emission is one of the most common difficulties encountered in fluorescence spectroscopy.^[49] Impurity emission could contribute significantly to the very weak signals seen on the ns timescale where count rates are several orders of magnitude lower than at time zero. Impurities can be present in concentrations too low to affect the absorption spectrum of a DNA sample, but they can contribute to emission signals detected with single-photon counting sensitivity.

Figure 3.2 shows that emission from a buffer-only solution 3 ns after excitation is approximately one third as large as that from the (dA)₁₅ sample. This is clear evidence that fluorescent impurities are present in the buffer, which may contribute to the ultraweak nanosecond emission observed when a DNA sample is present. A substantial portion of the impurity emission appears to be introduced by added NaCl, but weak background emission is detected even from water taken directly from the water ultrapurifier (Figure A6 in Appendix A). The buffer emission is greatest at 330–360 nm and tails off to longer wavelengths (Figure A7 in Appendix A, and gray trace in Figure 3.5b). In contrast, λ_{max} for (dA)₁₅ emission 3 ns after excitation is approximately 420 nm (Figure 3.5b). Time-resolved spectra of the buffer emission also show no sign of the steady shift to longer emission wavelengths observed as a function of time for the DNA strands. These differences suggest that the impurities responsible for emission from the buffer differ from the ones responsible for emission when the DNA sample is additionally present.

Although there is evidence for impurity emission, strong evidence for solute emission from the DNA-containing solutions is seen in the clear increase in emission

from these solutions compared to the buffer-only one (Figures 3.2 and 3.3). If emission on the ns timescale were due solely or primarily to fluorescent impurities in the buffer, then there should be less, not more, emission from the (dA)₁₅ solution. This follows because strong absorption of the UV excitation laser by the DNA strand reduces the excitation rate of any buffer impurities. These impurities could still be excited via Förster energy transfer from the DNA sample, but this is unlikely because emission from the DNA samples on the several ns timescale occurs at longer wavelengths than is seen from the buffer (Figures 3.5, 3.6 and 3.7).

Of course, the increased emission seen when DNA is present could also arise from a fluorescent impurity present in the lyophilized oligonucleotide sample that is dissolved in the buffer. A lower count rate is observed beyond 3 ns from the (dA)₁₀·(dT)₁₀ dumbbell compared to the other two oligonucleotides when solutions with matching absorbance at 265 nm are excited under identical experimental conditions (Figure 3.4). This could be because the dumbbell provided by Prof. Fred Lewis was more extensively purified than the two other substrates, which were obtained commercially. Figure A1 in Appendix A compares emission from two (dA)₁₈ substrates obtained from the same supplier, but differing in purity grade (see Appendix A). Decay traces recorded for the two samples under identical experimental conditions are very similar at times less than 1 ns, but clear differences are seen at longer times. It was reported earlier that oligomers sometimes contain significant levels of fluorescent impurities that vary by synthesis batch.^[50]

Overall, the evidence suggests that there is at least some genuine emission from the oligonucleotide. This is supported by the fact that the ns emission is most intense (and most different from the buffer-only emission) at the longest emission wavelengths (Figures 3.2 and 3.3). For all of the AT-containing DNA strands studied here, the longest-lived excitations emit at the longest wavelengths. Nevertheless, emission recorded more than one or two ns after excitation includes non-negligible contributions from both substrate impurities and solvent and/or buffer impurities, making it very difficult to interpret the ultraweak ns emission and assign the τ_2 and τ_3 lifetimes.

An examination of the lifetimes that are resolvable by the TCSPC technique shows that τ_3 emission from the two duplexes is somewhat greater in relative amplitude (2% to 3%) than from (dA)₁₅ (1%), and emission occurring 1 or 2 ns after excitation is red shifted for all three samples with respect to the subnanosecond emission (Figures 3.5–3.7). The time-resolved emission profiles for (dA)₁₅ (Figure 3.5) show that the wavelength of maximum emission shifts from 360 nm at approximately 50 ps after excitation to 420 nm several ns later. From 2 ns onwards, timeresolved emission spectra from d(AT)₉·d(AT)₉ have the same shape with a maximum at 450 nm. For (dA)₁₀·d(T)₁₀ maximum emission is at 420 nm at times greater than 2 ns.

The bleach recovery signal in Figure 3.9 recovers almost to the baseline within a few ns. The residual offset determined by fitting has an amplitude that is 3% as large as the maximum signal (Table A4 in Appendix A). Similar results are observed for (dA)_n strands^[29] and the (dA)₁₀·(dT)₁₀ dumbbell.^[21] The weak offset observed in the fs-TA signals indicates that there is a small residual population of excited states. Neglecting

excited-state absorption, the relative amplitude of the bleach recovery signal at time t measures the fraction of excited states that have yet to decay. The evidence suggests that the vast majority of excited states in (dA)_n single strands returns to the ground state prior to 1 ns.^[13,29] Earlier, Kwok et al. reached the same conclusion that ns emission components are due to deactivation by a minority of states in d(AT)₁₀·d(AT)₁₀.^[12]

3.5 Conclusions

Subnanosecond and nanosecond emission from two AT duplexes and single-stranded (dA)₁₅ were studied by the TCSPC technique. In all three systems, the maxima in the time-resolved emission spectra move steadily to longer wavelengths as time increases, indicating that the longest-lived excited states have the most red-shifted emission. The fluorescence decay times are independent of emission wavelength for the duplexes, but increase slightly with emission wavelength in the case of (dA)₁₅. The longer lifetimes seen from (dA)₁₅ may be associated with emission from shorter stacked domains that are better solvated.

Major experimental challenges (weak emission from DNA substrates, laser-induced denaturing of double-stranded samples in fs-TA experiments) have made it difficult to compare results from time-resolved emission and absorption experiments, resulting in controversy about how the signals should be interpreted. This work shows that there is extensive agreement between lifetimes determined by the fs-TA technique and the fluorescence decay times that are resolvable by the TCSPC technique on the subnanosecond timescale. The results also suggest that fundamentally different

excitations form in the double strands compared to the single strand. This is consistent with the recent proposal that intrastrand electron transfer can initiate interstrand proton transfer in duplex DNA. Our experiments reveal that very few excitations remain beyond 1 ns, but more study of these very long-lived excitations is needed in order to learn whether they have an increased propensity to photochemically damage DNA.

■ ASSOCIATED CONTENT

Supporting Information can be found in Appendix A.

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■ DEDICATION

Dedicated to Professor Wolfgang Zinth on the occasion of his 65th birthday

Table 3.1. Best-fit parameters for emission decays from $(dA)_{15}$, $d(AT)_9 \bullet d(AT)_9$, and the $(dA)_{10} \bullet (dT)_{10}$ dumbbell.

Wavelength / nm	τ_1 / ps	% A_1	τ_2 / ps	% A_2	τ_3 / ns	% A_3	% N_0/N [a]
$(dA)_{15}$							
330	165 ± 6	86	412 ± 24	13	4.3 ± 0.3	0.62	30
360	173 ± 5	85	431 ± 16	15	3.7 ± 0.3	0.40	15
390	185 ± 6	87	507 ± 25	13	4.1 ± 0.3	0.54	9
420	197 ± 9	86	562 ± 43	13	6.1 ± 0.6	0.85	7
450	200 ± 13	85	618 ± 60	14	7.9 ± 0.7	1.4	4
480	210 ± 23	82	648 ± 100	15	8 ± 1	2.1	4
$d(AT)_9 \bullet d(AT)_9$							
360	180 ± 100	78	1400 ± 600	14	5.1 ± 1.2	7	59
390	110 ± 30	92	1000 ± 400	5.6	4.7 ± 1.2	2.2	36
420	105 ± 24	93	930 ± 400	5.6	6.8 ± 1.4	1.8	19
450	105 ± 24	91	920 ± 400	6.3	8.5 ± 1.2	3.0	9
480	101 ± 28	92	760 ± 400	6.0	7.8 ± 2.0	2.1	8
$(dA)_{10} \bullet (dT)_{10}$ dumbbell							
360	61 ± 36	93	930 ± 660	4.1	5.2 ± 1.6	1.9	76
390	64 ± 30	93	800 ± 500	5.3	5.2 ± 1.4	1.9	47
420	70 ± 36	90	700 ± 500	7.3	5.3 ± 1.2	3.0	23
450	73 ± 44	88	600 ± 550	8.4	5.2 ± 1.4	3.5	11
480	63 ± 58	84	340 ± 400	13	4.6 ± 1.6	3.6	8

[a] Percent of prompt photons to all photons, see Equation 3.2.

Table 3.2. Model DNA subnanosecond decay constants from fs-transient absorption and time-resolved emission experiments.^a

Sample	Transient Absorption Decay Constant (ps) ^b	Emission Decay Constant (ps)
(dA) ₁₅	183 ± 6 ^c	183 ± 6
(dA) ₂₀	182 ^d	182 ^d
d(AT) ₉ •d(AT) ₉	84 ± 14	110 ± 26
d(AT) ₁₀ •d(AT) ₁₀	72 ^e	72 ^e
(dA) ₁₀ •(dT) ₁₀ dumbbell	62 ± 17 ^f	66 ± 37

^aResults are from this study unless otherwise noted. TCSPC decay constants reported are the result of an amplitude-weighted average over the collected emission range.

^bDecay constants measured in ground state bleach recovery signals using 266/250 nm pump/probe.

^cGlobal fit to (dA)_n oligonucleotides with *n* values between 2 and 18 from ref. [28]

^dRef. [11]. Global fit to emission and TA signals.

^eRef. [12]. Global fit to emission and TA signals.

^fRef. [21].

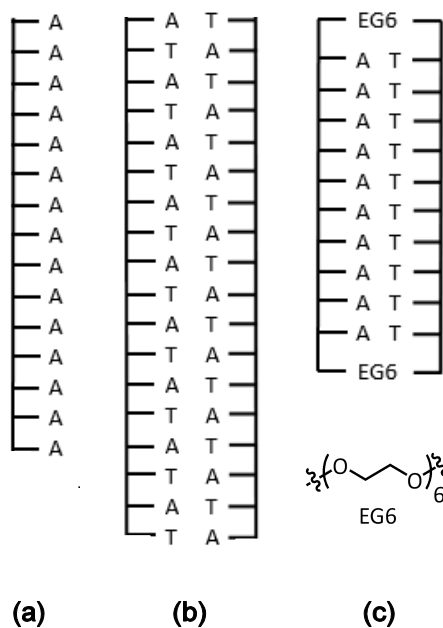


Figure 3.1. Model AT DNA oligonucleotides studied: (a) $(\text{dA})_{15}$, (b) $\text{d(AT)}_9 \cdot \text{d(AT)}_9$, and (c) the $(\text{dA})_{10} \cdot (\text{dT})_{10}$ dumbbell with hexaethyleneglycol linkers.

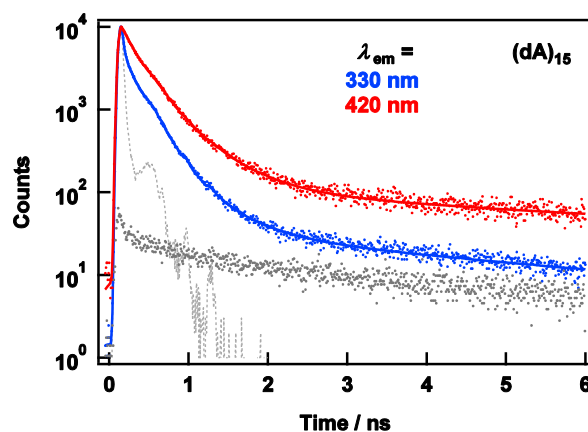


Figure 3.2. Fluorescence decays (dots) and fits (solid curves) from (dA)₁₅ in 10 mM Tris-HCl buffer with 0.25M NaCl at emission wavelengths of 330 nm (blue) and 420 nm (red) shown on a logarithmic scale. The decay at 420 nm has been scaled to have the same maximum amplitude as the decay at 330 nm. Emission at 330 nm from a solution containing only Tris-HCl buffer and 0.25M NaCl and recorded under identical conditions as the blue curve is shown by the dark gray trace. The light gray trace is the instrument response function.

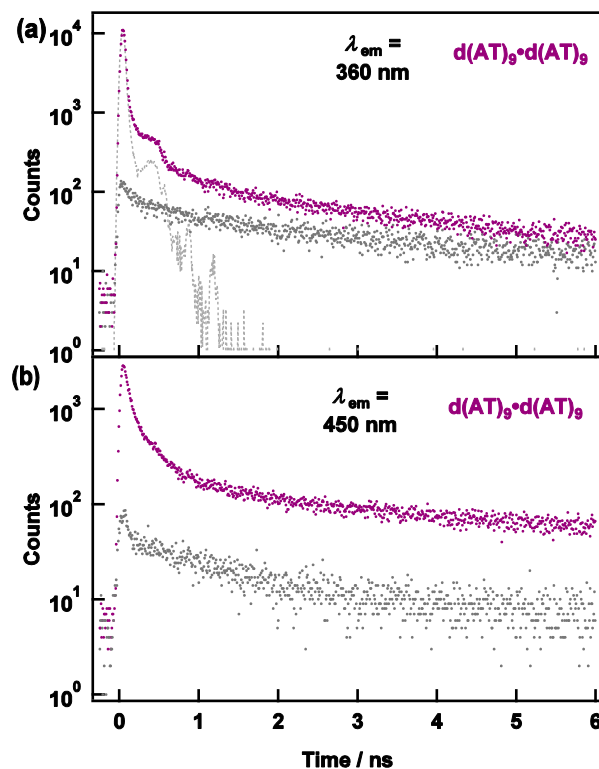


Figure 3.3. Fluorescence decays from $d(AT)_9 \cdot d(AT)_9$ in Tris-HCl buffer solution with 0.25m NaCl (purple curves) at emission wavelengths of (a) 360 nm and (b) 450 nm shown on a logarithmic scale. Decays from just the Tris-HCl buffer solution with 0.25m NaCl recorded under identical conditions at the same wavelengths are shown by the dark gray curves. The instrument response function (light gray dots in panel a) was scaled to the maximum of the $d(AT)_9 \cdot d(AT)_9$ decay curve.

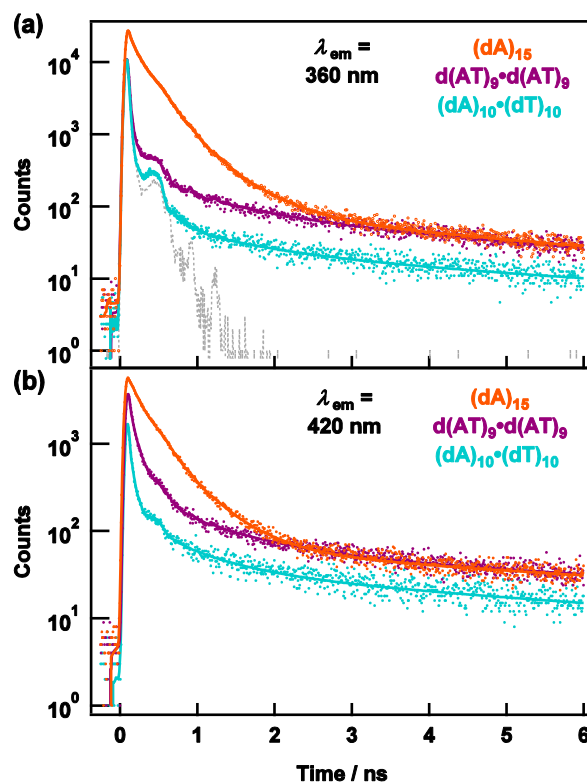


Figure 3.4. Fluorescence decays (dots) and fits (solid curves) from $(dA)_{15}$ (orange), $d(AT)_9 \cdot d(AT)_9$ (purple), and the $(dA)_{10} \cdot (dT)_{10}$ dumbbell (cyan) in a Tris-HCl buffer with 0.25m NaCl at emission wavelengths of 360 nm (a) and 420 nm (b) shown on a logarithmic scale. The instrument response function (gray dots in panel a) was scaled to the maximum of the $d(AT)_9 \cdot d(AT)_9$ decay curve.

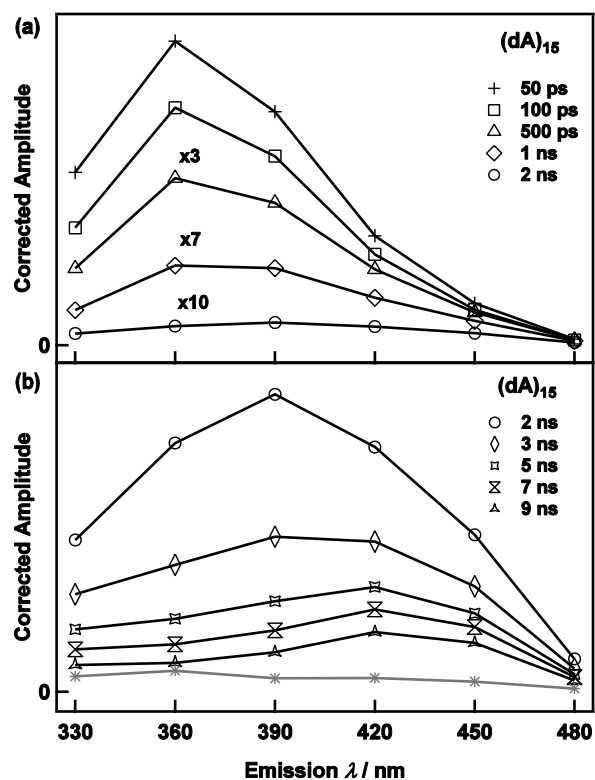


Figure 3.5. Time-resolved emission spectra on a linear scale from $(dA)_{15}$ at 50 ps to 2 ns (a) and 2 to 9 ns (b) after excitation at 265 nm. The gray trace in panel b is emission from Tris-HCl buffer with 250 mM NaCl at 9 ns after excitation. The vertical scale differs in the two panels.

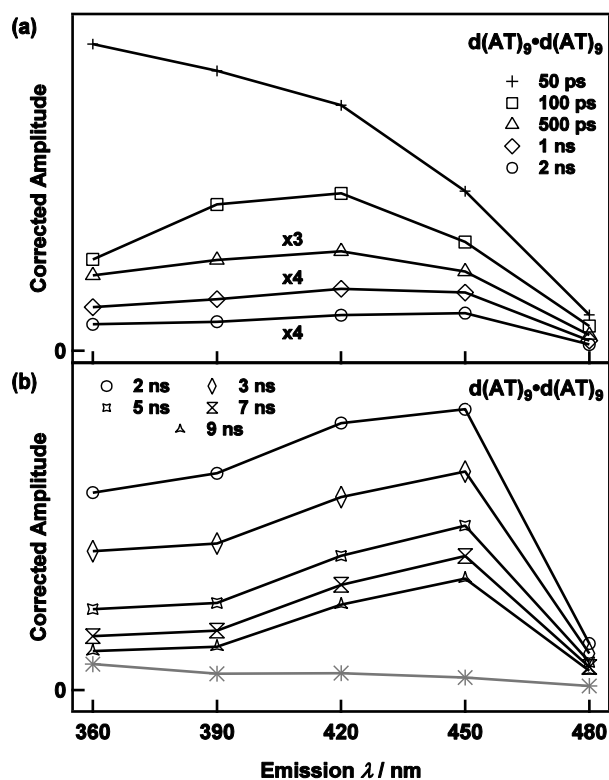


Figure 3.6. Time-resolved emission spectra on a linear scale from $d(AT)_9 \cdot d(AT)_9$ at 50 ps to 2 ns (a) and 2 to 9 ns (b) after excitation at 265 nm. The gray trace in panel b is emission from a buffer-only solution at 9 ns after excitation. The vertical scale differs in the two panels.

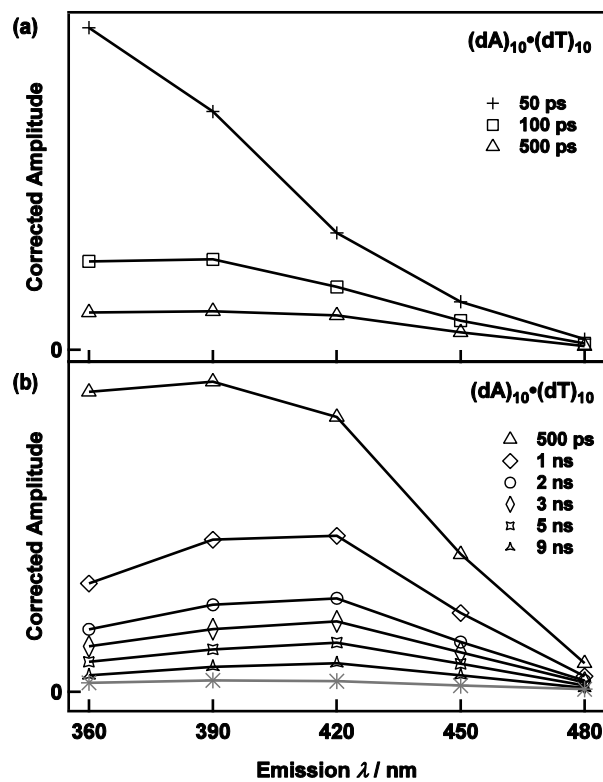


Figure 3.7. Time-resolved emission spectra on a linear scale from $(dA)_{10} \cdot (dT)_{10}$ at 50 ps to 500 ps (a) and 500 ps to 9 ns (b) after excitation at 265 nm. The gray trace in panel b is emission from a buffer-only solution at 9 ns after excitation. The vertical scale differs in the two panels.

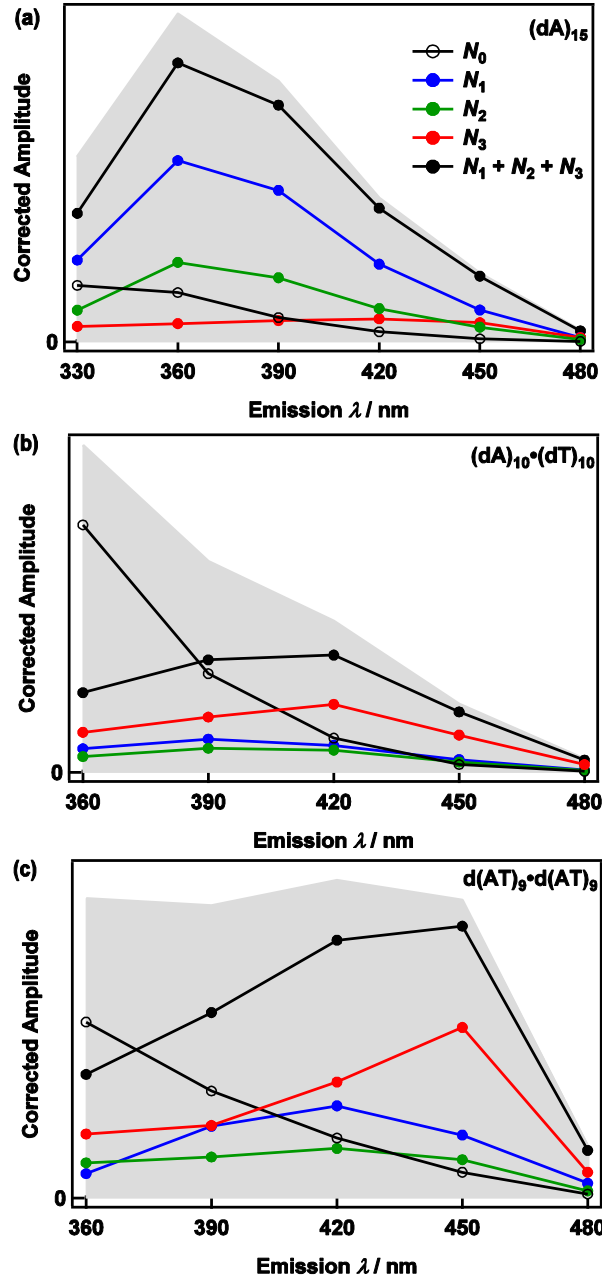


Figure 3.8. Time-integrated emission spectra shown with a linear vertical axis for (a) $(dA)_{15}$, (b) the $(dA)_{10} \cdot (dT)_{10}$ dumbbell, and (c) $d(AT)_9 \cdot d(AT)_9$. The gray shading represents the total integrated emission ($N_0 + N_1 + N_2 + N_3$). The open black circles show the contribution from prompt photons emitted, N_0 , while the solid black circles are all photons emitted in resolvable decay components, that is, $N_1 + N_2 + N_3$. The separate contribution of each resolvable component is also shown: N_1 (blue), N_2 (green), and N_3 (red).

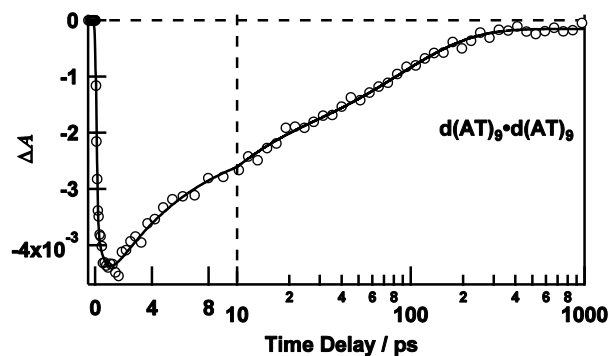


Figure 3.9. fs-TA signal (267 pump/ 250 probe) of $d(AT)_9 \cdot d(AT)_9$ in aqueous buffer solution containing 0.1m NaCl at 20°C (circles). The solid curve is a nonlinear least-squares fit to two exponentials plus an offset. The vertical dashed line denotes the transition between the linear and logarithmic portions of the time axis.

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CHAPTER FOUR

KINETIC ISOTOPE EFFECT AND LENGTH DEPENDENT DYNAMICS OF
ADENINE OLIGOMERS4.1 Introduction

The photophysics of DNA has been extensively examined due to its ability to undergo UV induced photodamage.¹ Of special importance are single-stranded oligomers exhibiting intrastrand base stacking forces in the absence of base pairing interactions.² These systems provide an important model to examine the photophysical implications of π - π interactions between monomer constituents. The dynamics of these systems are complicated by the large distribution of structures adopted in aqueous solution, and the resulting heterogeneous local environments.^{2, 3} Nonetheless, understanding of the processes governing the dynamics in single strands has been greatly advanced through the use of ultrafast techniques. Upon UV excitation, single-stranded oligomers deactivate through an ultrafast femtosecond channel similar to that of its free base monomer constituents.⁴⁻⁸ Accompanying this deactivation process, which has been assigned monomeric deactivation of unstacked bases, is a process or processes decaying in times of 10 ps to multiple nanoseconds,⁹ present only if bases are stacked.^{5, 8} The exact lifetimes depends on both the base constituents,^{10, 11} and the time regions highlighted by the technique used (See reference [12] for a detailed discussion). Some studies present a monolithic description of this long lived decay, which involves excimer or charge transfer state (CT) formation,^{5, 7, 13} while others present a more complex picture detailing

a mixture of both excimers and CT states,^{4, 6} or even a charge separated states delocalized over multiple bases.¹⁴ While some differences in these assignments can be attributed to differences in the identity constituent oligomer bases, there is still uncertainty as to of the exact nature of the deactivation processes described by these long-lived signals, and additional experiments are needed.

The most commonly studied single-stranded system by ultrafast techniques is the adenine oligomer, $(dA)_n$, in which n denotes the number of phosphate-linked adenosine bases. While $(dA)_n$ oligomers of different lengths, or number of base constituents, have been previously examined, there are no time-resolved fluorescent studies detailing the difference in dynamics of oligomer of various lengths. A recent fs-TA study of $(dA)_n$, in which n was varied from 2 to 18, shows no difference in the lifetimes of these systems, only relative amplitudes, leading the authors to conclude that a common excited state localized on two bases is present in all oligomers regardless of length.⁵ The aim of the work described in this chapter is to use the highly sensitive time-resolved emission technique to obtain new insights into excited states formed in DNA single strands.

Adenine oligomers produce an emission band that is red-shifted in regards to the constituent monomer emission.^{4, 6, 12, 15} Time-resolved studies generally assign this red-shifted emission to excimer emission, and the emission decays show multiexponential kinetics, decaying on times 3-4 orders of magnitudes longer than the monomer emission.^{4, 6, 9} Some studies report that these lifetimes depend on emission wavelength,^{6, 9, 12} while others describe global lifetimes from emission at all wavelengths.⁴ The complicated kinetics of this oligomer band is usually attributed to differences in base

stacking geometry and local solvation environments, both of which are likely to affect excimer or CT states in poorly understood ways.^{4, 6, 7} Although it was argued in a previous TA study that long-lived excited states are dynamically indistinguishable in various length (dA)_n oligomers, clear differences have been reported from emission experiments. Thus, steady-state fluorescence of the dinucleotide p(dA)₂ reveals a red-shifted emission band in comparison to the adenine 19-mer (dA)_n.¹⁶ However, there has been no systematic study of how emission dynamics change with oligomer length for (dA)_n.

In addition to a lack of detailed length-dependent studies, there have been only a few reports of a kinetic isotope effect (KIE) on excited state lifetimes in DNA compounds.^{11, 17-19} The excited state behavior of DNA bases and base analogs in D₂O have been examined by fluorescence upconversion (FU), revealing a small inverse KIE on the lifetime of the lowest singlet excited state.¹⁷ A study examining monomers and monomer analogs, utilizing the fs-TA technique, reported a KIE of 2-3 on the rate of vibrational cooling after internal conversion, which is attributed to differences in solute solvent energy transfer.¹⁸ Examination of double-stranded DNA oligomers in D₂O and H₂O showed variable KIEs depending on the base sequence.^{11, 19} The KIE in these oligomers was proposed to result from the coupling of hydrogen bonds in the DNA duplex to the decay of an excimer state, with KIEs observed for alternating double-stranded systems and absent for single-stranded or nonalternating double-stranded systems.^{11, 19} However, recent time-resolved IR investigations have provided evidence of interstrand proton transfer in a nonalternating AT duplex, but this process is not seen in

the alternating AT duplex,²⁰ calling into question the assignment of the KIE to interstrand proton transfer in these systems. Indeed, we report for the first time that KIEs are clearly observable in time-resolved emission decays recorded from single-stranded oligomers that lack base pairing interactions altogether. The interpretation of these findings are the principal objective of this chapter.

Here, we study emission from (dA)₂ and (dA)₁₈, using the time-resolved single photon counting (TCSPC) technique. Decays from these systems are multiexponential and vary according to emission wavelength and strand length. The excited-state dynamics of these strands when dissolved in D₂O are also examined. All TCSPC measurements are supported by fs-TA measurements. We provide experimental evidence detailing the effects of oligomer length on the excited state dynamics. In addition, a pronounced KIE is observed in both oligomers. We explain the length dependence through differences in the length of stacked domains and the KIE is attributed to vibrational modes coupled to the CT process. The effects of domain length on the dynamics are ascribed to differences in the solvation environments influencing the CT character of the adenine excimers. The length dependent and KIE observations lead to a proposed excited-state model of adenine oligomers describing emission from excimer-like states with varying degrees of CT.

4.2 Results

4.2.1 Steady-State Absorption and Emission Spectra

The concentration of (dA)₂ and (dA)₁₈ in both H₂O and D₂O Tris buffers was adjusted to provide an absorbance of 0.3 at 265 nm. The absorption spectra of both oligomers in H₂O and D₂O Tris buffer are shown in Figure 4.1.

Steady-state emission from neat D₂O and H₂O was recorded after 265 nm excitation (Figure 4.2). All emission spectra were recorded back-to-back under identical experimental conditions to ensure a meaningful comparison of the different signal amplitudes. The D₂O produces significantly more emission than the 18 MΩ H₂O over the emission range of 300 – 400 nm. This emission is an order of magnitude greater than that of the neat H₂O background emission at the emission maximum of the D₂O solvent. To reduce background emission D₂O was treated using activated charcoal according to the procedure outlined in Chapter 2. The treated D₂O (magenta trace in Figure 4.2) was significantly less fluorescent than the untreated D₂O, producing a signal no greater than twice the neat H₂O emission. The spectra, uncorrected for the instrument response, are reported with the Raman peaks in Figure 4.2 to provide a scale of the relative emission intensity. The difference in energy between the Raman peaks and the 265 nm excitation is approximately 3600 and 2650 cm⁻¹ for H₂O and D₂O respectively, corresponding to the frequency of the OH and OD symmetric stretches, respectively.

Steady-state emission of (dA)₁₈ and (dA)₂ in H₂O and D₂O Tris buffer with 250 mM NaCl were recorded after 265 nm excitation (Figure 4.3). Emission from (dA)₁₈ in H₂O peaks around 350 nm and continues to around 500 nm. The emission from (dA)₂ in

H₂O buffer is red-shifted with respect to that from (dA)₁₈. The former has an emission maximum around 420 nm, and is less emissive than the latter by a factor of three. Both samples have similar emission spectral shapes when examined in D₂O buffer with the emission maximum of (dA)₁₈ appearing redshifted in D₂O and slightly blue shifted for (dA)₂ in D₂O in comparison to the emission from the respective oligomer measured in H₂O. The integrated emission of (dA)₁₈ in D₂O is 1.5x greater in comparison to H₂O and 2x greater for (dA)₂. The H₂O and D₂O Tris-HCl/DCl buffers with 0.25 M NaCl and without oligonucleotides are shown by the black and gray traces, respectively. The amplitude of the treated D₂O buffer is approximately 3x greater than the H₂O buffer at the emission maximum (350 nm) of the D₂O buffer.

4.2.2 TCSPC Decays

A general discussion of TCSPC decays recorded using the experimental setup is given in Section 3.3.1. Emission decays from (dA)₁₈ and (dA)₂ in H₂O buffer solution were recorded at emission wavelengths between 310 and 480 nm with excitation at 265 nm. Emission traces recorded at 360 and 420 nm for both oligonucleotides are compared in Figure 4.4. Emission counts from both samples drop by about two orders of magnitude during the first few ns before decreasing more slowly. Figure 4.4 also shows the slope of each decay curve is somewhat greater for the shorter emission wavelengths at both early and late times. Emission recorded from the Tris-HCl buffer with 0.25 M NaCl but without any oligonucleotide is shown by the black traces. The oligonucleotide traces approach the buffer trace at latter times, but slightly more emission is observed from the oligomer trace.

The area under the $(dA)_{18}$ trace in Figure 4.4 is greater than that of $(dA)_2$ at both emission wavelengths, consistent with the steady-state emission results in Figure 4.3. The slope of the $(dA)_2$ decay at 360 nm is noticeably greater than $(dA)_{18}$ at early times, and then begins to resemble that of $(dA)_{18}$ after 200 ps. When examining the normalized emission traces (Figure 4.5) it appears that, with the exception of the first 200 ps at 360 nm, the slopes of the $(dA)_2$ traces are smaller than those of the $(dA)_{18}$ traces during the first 2 ns. After 2 ns the slopes become similar with little differences in amplitude between the two decays.

Decays from $(dA)_2$ and $(dA)_{18}$ in D_2O buffer were also recorded at emission wavelengths between 310 and 480 nm with excitation at 265 nm. Figure 4.6 compares emission traces for $(dA)_2$ and $(dA)_{18}$ in H_2O and D_2O buffer at 360 and 420 nm. The area under the $(dA)_2$ D_2O decay is greater than that of the $(dA)_2$ H_2O decay, which again is consistent with the steady-state fluorescence. The initial maximum of each decay is similar for both emission wavelengths. The slopes of the $(dA)_2$ D_2O decays are noticeably smaller than the $(dA)_2$ H_2O decays with the exception of the first 100 ps at 360 nm, in which the slopes appear similar. Emission counts drop by two orders of magnitude in the first 5-6 ns for $(dA)_2$ D_2O trace unlike the $(dA)_2$ H_2O trace which drops by two orders of magnitude in the first 2 ns. Both tails begin to approach each other at later times with the emission of the $(dA)_2$ in D_2O remaining slightly greater.

The slope of the $(dA)_{18}$ decay in D_2O is noticeably smaller than that of $(dA)_{18}$ in H_2O for the first 4 ns at both emission wavelengths. Afterwards, the traces approach each other with the $(dA)_{18}$ D_2O trace eventually dropping below the $(dA)_{18}$ trace in H_2O at 360

nm. The difference in slopes between (dA)₁₈ in D₂O and H₂O is noticeably smaller than that is seen with (dA)₂ in D₂O and H₂O.

The gray trace in Figure 4.6 represents the D₂O Tris-DCI with 0.25 M NaCl but without oligonucleotide. The (dA)₂ and (dA)₁₈ emission in D₂O remains much greater than the D₂O buffer emission at early times with the oligonucleotide traces approaching the D₂O buffer decay at later times, but consistently remaining greater.

All signals were fit to a sum of exponentials with an additional parameter, N_0 , describing the contributing number of photons from the TCSPC signals decaying on time scales much shorter than the FWHM of the IRF as described in more detail in Chapter 3. Adding a fourth exponential term did not significantly reduce the χ^2 value. A complete discussion of the TCSPC fitting parameters can be found in Chapter 3. Best-fit parameters for all four substrates at 360 and 420 nm are listed in Table 4.1. A complete list of the fitting parameters at each recorded emission wavelength for all four samples are listed in Tables 4.2 – 4.5. Values for 470 and 480 nm are not reported due to the large errors associated with the lifetimes. Additionally, due to the weak signals at these emission wavelengths (especially for (dA)₂ in H₂O) there was little difference in χ^2 values between a two and three exponential fit. A two exponential fit was used to model the decays at 470 and 480 nm, but still produced large error relative to the fitting parameters. Thus, models describing the decays from these weak emission wavelengths are insignificant and are not reported.

For (dA)₂ and (dA)₁₈ in H₂O, the fits confirm that shorter lifetimes are seen at shorter emission wavelengths. The percent of prompt photons emitted over all photons

emitted are shown in the last column. The greatest percentage of prompt photons emitted are observed at shorter emission wavelengths, and are greater for (dA)₂ in comparison to (dA)₁₈. The τ_1 decay component for (dA)₂ comprises about half of the decay amplitude of the resolvable lifetimes, while τ_1 for (dA)₁₈ consistently comprises greater than half of the decay amplitude. The τ_3 decay component comprises no more than 3% of the decay amplitude for both samples at all emission wavelengths (Tables 4.2 and 4.3). All (dA)₂ lifetimes, with the exception of τ_1 at emission wavelengths below 360 nm, are longer than the respective (dA)₁₈ lifetimes, which is in agreement with the observations above.

Similar to (dA)₂ and (dA)₁₈ in H₂O, shorter lifetimes are observed at shorter emission wavelengths for both (dA)₂ and (dA)₁₈ in D₂O. Again, all lifetimes, with the exception of τ_1 at 360 nm, are longer for (dA)₂ in comparison to (dA)₁₈, and the percentage of prompted photons emitted are greater for (dA)₂. When comparing the same oligonucleotides in H₂O and D₂O longer lifetimes are observed for the oligonucleotide dissolved in heavy water, consistent with the traces in Figures 4.4 and 4.6. The τ_2 decay component for (dA)₂ displays the greatest difference in lifetime when comparing decays in H₂O and D₂O.

For simplicity Figure 4.7 shows τ_1 and τ_2 for (dA)₂ H₂O (a), (dA)₁₈ D₂O (b), (dA)₂ D₂O (c), (dA)₂ H₂O (d), and the kinetic isotope effect (KIE) of τ_1 and τ_2 for (dA)₂ (e) and (dA)₁₈ (f). The KIE is defined as,

$$\text{KIE} = \frac{\tau_n(\text{D}_2\text{O})}{\tau_n(\text{H}_2\text{O})} \quad (4.1)$$

where $n = 1$ or 2 , allowing the KIE to be defined for each of the two observed decay components.

The KIE values for both oligomers increase with increasing emission wavelength, although this trend might not be statistically significant for the $(\text{dA})_2 \tau_1$ KIE due to large errors associated with these values. The largest KIE values are exhibited by τ_2 of $(\text{dA})_2$ with a range of 1.7 - 2.3. These values are significantly greater than the KIE values for τ_1 of $(\text{dA})_2$, which range between 0.8 and 1.6. The KIE values for $(\text{dA})_2 \tau_2$ are greater than both $(\text{dA})_{18} \tau_1$ and τ_2 KIE values at most emission wavelengths. The $(\text{dA})_{18} \tau_1$ KIE values are below 1 at short wavelengths and grow to around 1.5 at longer wavelengths. The $(\text{dA})_{18} \tau_2$ KIE values are slightly greater than the τ_1 values at around 1.5 for short emission wavelengths, but are statistically indistinguishable at longer wavelengths.

The time-integrated emission was calculated using Equation (3.2). Integrating the fluorescence decays vs. time at each emission wavelength yields the time-integrated spectra shown in Figure 4.8 (gray area). This figure also shows the contributions to the total emission from the various components, including from the prompt emission (N_0).

4.2.3 Transient Absorption Signals

Both $(\text{dA})_{18}$ and $(\text{dA})_2$ in H_2O were previously studied by the fs-TA technique. The fs-TA measurements on the oligomers were reported in 2012,⁵ but were later shown to have been recorded at a temperature of approximately 40°C due to heating by the pump laser, and not at room temperature as originally stated.^{21, 22} For this reason, the fs-TA signals from $(\text{dA})_{18}$ and $(\text{dA})_2$ in H_2O were re-measured. Transient absorption signals shown in this chapter were recorded by Yuyuan (Tom) Zhang and Ashley Beckstead.

Figure 4.9 shows the normalized ground state bleach recovery signal (267 nm pump/ 250 nm probe) recorded for $(\text{dA})_2$ and $(\text{dA})_{18}$ in H_2O , (b) $(\text{dA})_2$ in H_2O and D_2O ,

and (c) (dA)₁₈ in H₂O and D₂O in aqueous buffer solution containing 0.1 M NaCl at room temperature. Initially the slope of the fs-TA signals for (dA)₂ H₂O appears to be greater than that of (dA)₁₈ in H₂O, but after 10 ps the (dA)₂ slope becomes smaller than the (dA)₁₈ slope. When comparing both strands in H₂O vs. D₂O the initial slope of the fs-TA signal appears similar for both (dA)₂ and (dA)₁₈. After the first 10 ps, the slope representing (dA)₂ and (dA)₁₈ in D₂O appears to be smaller in comparison to the slope from the corresponding oligomer in H₂O.

The fitting parameters corresponding to the fs-TA signals in Figure 4.9 are reported in Table 4.2. A biexponential fit yields a lifetime consisting of a few ps and a longer lifetime on the order of 100-1000 ps, with a slight offset consisting of no more than 4% of the decay amplitude for all four signals. Again, the fitting parameters confirm the observations above showing that τ_2 is longer for (dA)₂ in H₂O in comparison to (dA)₁₈ in H₂O. Additionally, both τ_1 and τ_2 are longer for strands examined in D₂O when compared to their counterpart dissolved in H₂O.

4.3 Discussion

4.3.1 (dA)₂ and (dA)₁₈ in H₂O

4.3.1.1 Steady-state emission. The steady-state fluorescence from (dA)₂ and (dA)₁₈ in H₂O buffer after 265 nm excitation reveals similar spectral shapes (Figure 4.3). The (dA)₂ oligomer is less emissive than (dA)₁₈ by a factor of 3 and the emission maximum is red-shifted to 420 nm compared to (dA)₁₈, which has an emission maximum

near 350 nm. The emission spectrum of (dA)₁₈ roughly agrees with previously reported steady-state emission spectra of (dA)₂₀, (dA)₁₉ and (dA)₁₈.^{4, 6, 12, 16}

Very few studies have examined (dA)₂ in room temperature aqueous solution (see reference [15] for a review of studies carried out before 1983, and ref [1] for studies before 2000). Recently Temps and coworkers reported steady-state emission spectra from (dA)₁₉ and from the adenine dinucleotide d(pApA).¹⁶ The emission from (dA)₁₉ and d(pApA) peaks around 360 and 420 nm, respectively. This approximate 60 nm shift is consistent with the shift seen in our emission maxima. However, in ref. 16 the emission intensity of the dinucleotide appears to be greater than that of the (dA)₁₉ oligonucleotide by about a factor of 3 in contrast to the smaller amount of emission from (dA)₂ observed in this study and shown in Figure 4.3. The authors of ref. 16 did not propose an explanation for the greater emission they observed for the dinucleotide compared to the 19-mer. The d(pApA) dinucleotide examined by Temps and coworkers contains a terminating phosphate group on the 5' end and a hydroxyl group on the 3' end unlike the dinucleotide studied here, (dA)₂, which contains hydroxyl groups on both terminating ends. Terminal phosphorylation of a dinucleotide is reported to slightly decrease the propensity for base stacking. However, the ionic strength established by the addition of a salt like NaCl also has a large effect on the percent stacking in a dinucleotide. As the authors of ref. 16 did not state whether additional salt was added to their solution of d(pApA) it is difficult to compare with the results here, which nonetheless agree in terms of lifetimes and emission maxima.

4.3.1.2 TCSPC Kinetics. Emission from (dA)₂ and (dA)₁₈ in H₂O buffer decays multiexponentially. The subnanosecond decay components (τ_1 and τ_2) account for over 95% of the decay amplitude that describes the resolvable decay components observed after the prompt emission characterized by N_0 (see Tables 4.1 and 4.2). Per Tables 4.2 and 4.3, all three time constants for (dA)₂ and (dA)₁₈ increase as the emission wavelength increases. The progressive slowing down of the dynamics from these samples is also evident in the average decay time, $\langle\tau\rangle$, calculated from the best-fit exponential lifetimes and amplitudes in Table 4.2 and 4.3 using Equation (4.2):

$$\langle\tau\rangle = \sum_i A_i \tau_i / \sum_i A_i \quad (4.2)$$

The value of $\langle\tau\rangle$ for (dA)₁₈ and (dA)₂ systematically increases with increasing emission wavelength, with (dA)₁₈ increasing approximately 100 ps and (dA)₂ increasing 300 ps over the 330 – 460 nm emission range. Due to evidence that the nanosecond decay component (τ_3) contains contributions from fluorescent impurities, we do not attach much significance to $\langle\tau\rangle$, but it does capture the trend of wavelength-dependent dynamics for adenine oligomers as noted in a previous study.¹²

The average lifetimes over all emission wavelengths shows that (dA)₂ decays on a slower timescale than (dA)₁₈ (Table 4.7). The slower dynamics seen in (dA)₂ are illustrated by the normalized emission decays at 360 and 420 nm (Figure 4.5). Additionally, both τ_1 and τ_2 for (dA)₂ at its emission maximum of 420 nm are longer than τ_1 and τ_2 of (dA)₁₈ at its respective emission maximum of 360 nm (Table 4.1).

A detailed comparison of the time-resolved emission signals from (dA)₁₅ recorded using the TCSPC technique can be found in Chapter 3.¹² As many observations here are similar to those previously reported for (dA)₁₅, we will compare the TCSPC signals observed here for (dA)₁₈ with those reported for (dA)₁₅ in Chapter 3.

Emission lifetimes were recently reported by Skowron et al.¹² for (dA)₁₅ between 330 and 480 nm with 30 nm increments. The decays were fit in the same manner as mentioned above with three exponentials and a prompt emission component. All lifetimes for (dA)₁₈ reported here appear to be faster than those reported for (dA)₁₅ at shorter emission wavelengths. At wavelengths longer than 420 nm, there is no statistical difference between τ_1 components of (dA)₁₅ and (dA)₁₈, however τ_2 and τ_3 are shorter for (dA)₁₈. The difference in dynamics between these two oligomers is subtle, and not much significance is attached to this difference. Each sample was synthesized by a different manufacture using a different purification process, which has been previously shown to impact the observed dynamics of the oligomer.¹² Assuming that the subtle differences in lifetimes between (dA)₁₈ and (dA)₁₅ are related to genuine difference in dynamics the following discussion (see below) provides a potential explanation.

Temps et al. previously reported time-resolved emission of an adenine dinucleotide through fluorescence upconversion (see above for discussion of the steady-state emission).¹⁶ The authors reported fluorescent time profiles for a d(pApA) dinucleotide at 310, 350, 430, and 470 nm. These time profiles consist of a 0.36, 5.1 and 278 ps lifetime, with the 278 ps component comprising a greater portion of the emission transients at longer (430 and 470 nm) emission wavelengths. The 278 ps lifetime

identified by Temps and coworkers matches nicely with the τ_1 components reported for (dA)₂ in Table 4.2 at longer emission wavelengths. In agreement with our observations, the 278 ps component measured by Temps is longer than corresponding lifetimes reported for (dA)₁₈ (182 ps) using similar fluorescence techniques.⁴

4.3.1.3 Transient Absorption. The ground state bleach recovery signals (267 nm pump/ 250 nm probe) of (dA)₂ and (dA)₁₈ (Figure 4.9) are modeled by a two exponential decay with a slight offset comprising less than 3% of each decay. The long-lived τ_2 components of the transient decays show (dA)₂ decaying on a time scale 2x longer than (dA)₁₈. These slower dynamics observed for (dA)₂ in comparison to (dA)₁₈ by the fs-TA method agree with the dynamics seen from the TCSPC signals.

Previously, Su et al.⁵ studied adenine oligomers with various numbers of base constituents ranging between 2 – 18 using the fs-TA technique. The authors reported a 2.7 and 183 ps lifetimes for all systems. This 183 ps component agrees with 183 ± 13 ps component for (dA)₁₈ reported in Table 4.6. However, this lifetime contrasts with the longer 311 ± 44 identified for (dA)₂. As mentioned above the samples from the Su et al. study were recorded at temperatures around 40°C due to heating by the pump laser. Temperature dependent studies, as reported in Chapter 5, have shown a decrease in the τ_2 lifetimes at higher temperatures. The lifetime of (dA)₂ exhibiting a greater dependence on temperature than that of (dA)₁₈, thus the lifetimes from the two oligomers begin to resemble one another higher temperatures. This would justify the global analysis used to model the oligomers of different lengths, but does not accurately describe the dynamics of adenine oligomers of varying length at room temperature. The fs-TA decays shown in

Figure 4.9 were recorded with a flow cell apparatus, which does not suffer the same heating affects by the pump laser. The observation that the fs-TA decay for (dA)₂ is slower than that of (dA)₁₈ is also confirmed by a recent TA study by Temps and coworkers which report a 400 ps ESA lifetime for (dA)₂.²³

4.3.2 Length Dependent Dynamics

The steady-state fluorescence maximum of (dA)₂ is redshifted by approximately 60 nm in comparison to (dA)₁₈ (Figure 4.3). Examining the time-resolved emission reveals (dA)₂ and (dA)₁₈ decay on noticeably different time scales. The fs-TA decays also reveal differences between (dA)₂ and (dA)₁₈ with (dA)₂ decaying on slower time scales. The difference in the steady-state emission and time-resolved lifetimes between (dA)₂ and (dA)₁₈ could result from differences in the average base stacking domain length, which is discussed in detail in Chapter 5. The discussion to follow attempts to describe the differences seen between the two oligomers because of differences in solvation environment resulting from differences in domain length. While there are various explanations as to how domain length can impact the dynamics of these systems, such as charge delocalization, a detailed discussion of these mechanisms is present in Chapter 5. Given the observations here and the many unanswered questions still associated with the mechanism described in Chapter 5, we have chosen to describe the differences in terms of solvation environment.

As previously discussed, emission from adenine oligomers occurring on the 100 ps time scale is attributed to excimer-like states with varying degrees of CT.¹² There is strong experimental evidence suggesting electron transfer occurs in nucleobase

heterodimers,^{13, 14, 24} but homodimers have not been studied as extensively. Electron transfer is a reasonable assumption for heterodimers, where one base is a better electron donor or acceptor, but it is less obvious that (partial) electron transfer can occur in a homo-dimer like AA. Nevertheless, computational studies of stacked AA systems reveal excited dimer states with CT character and multiple types of excimer states.²⁵⁻²⁹ The formation of multiple types of excimers in adenine oligomers is supported experimentally by multiexponential emission decays and the dependence of these decays on emission wavelength.¹² A recent joint theoretical and time-resolved fluorescent study of (dA)₂₀ assigned emission on the 100 ps and nanosecond time-scales to neutral excimers and CT states respectively.⁶ The assignments described by these authors only represent two limiting cases and further experimental evidence is needed to describe the true heterogeneous nature of adenine oligomer dynamics as modeled by the fitting parameters in Tables 4.1-4.5.

The heterogeneous nature of the adenine oligomer in aqueous solution could give rise to distinct excimer-like states. As discussed in Chapter 3, single strands like (dA)_n contain both stacked and unstacked bases, as base stacking is weakly favorable in room-temperature aqueous solution. In an adenine oligomer, stacked bases are distributed throughout stacked domains of various lengths.³⁰ Neighboring stacked bases are separated vertically by 3.4 Å, excluding water from in-between the stacked bases. This exclusion is proposed to affect the solvation energy of bases in different length domains because a base stacking domain of just two bases is expected to experience a greater

degree of hydration than two stacked bases, which are present in a longer stacked domain.

To examine the effect of domain length on the dynamics of adenine oligomers the (dA)₂ oligomer was chosen as it provides an excellent model for observing the dynamics of a base stack in a well-solvated environment. As discussed previously, the steady-state fluorescence maximum of (dA)₂ is redshifted by approximately 60 nm in comparison to (dA)₁₈ (Figure 4.3). Since both emission maxima (360 and 420 nm) are well red-shifted of adenine monomer emission (305 nm) it is assumed that these spectra arise from distinct excimers present in the two oligomers.

On average, an excitation in two stacked bases in (dA)₁₈ is likely to be found in a less polar environment than would be the case in (dA)₂. Effects of polarity on exciplex (acceptor and donor pairs of different chemical compositions) and charge separated excited state complexes are well documented,³¹⁻³⁵ and could provide an explanation to the differences in dynamics between (dA)₁₈ and (dA)₂. Gould et al. documented the effect of oxidation potential and solvent polarity on the steady-state emission from various acceptor/donor species known to form excited-state CT complexes.^{32, 33} The authors observed that emission energy decreased as solvent polarity increased and/or as acceptor donor abilities increased. The authors explained the excited-state dynamics as a mixture of three states, the ground state AD, the locally excited acceptor complex A*D, and the ion-pair complex (A⁻D⁺). As the solvent polarity increases and/or the strength of the electron acceptor and donor increase, the thermodynamic driving force for electron transfer, $-\Delta G_{\text{et}}$, becomes greater in magnitude and the percent CT character increases.

More recent experimental observations by Vauthey et al.³⁵ have assigned specific spectroscopic IR markers to these exciplex states based on separation in frequencies between these bands and ionic products along with the variability in intensity of these markers following the same trends of solvent polarity and acceptor donor abilities.

The effects of solvent polarity on the dynamics of the acceptor/donor systems are uniform; as polarity increases the emission energy is lowered as the percent of CT character increases. The trends of the exciplex studies compare nicely with the observations made for (dA)₂ and (dA)₁₈. The environment surrounding two stacked bases in (dA)₂ is expected to be more polar than the average environment surrounding two stacked bases in (dA)₁₈. The more polar environments resulting from increased solvation would result in a greater degree of CT character and characterized by red-shifted emission as seen for (dA)₂ (Figure 4.3).

Previous studies have found longer lifetimes for adenine oligomers at red-shifted vs. blue-shifted wavelengths^{4, 6, 16, 36} in agreement with our results. Additionally, the majority of emission lifetimes reported for (dA)₂ are longer than those reported for (dA)₁₈ especially at longer emission wavelengths (Table 4.1), which is confirmed by the fs-TA lifetimes (Table 4.6). These observations suggest that longer lifetimes are associated with a greater degree of CT in agreement with similar conclusions by Markovitsi et al.⁶

Importantly, differences in solvation environment fail to explain the longer lifetimes observed for (dA)₂ at lower energy emission wavelengths as we would expect hydration of this oligomer to remain uniform unlike (dA)₁₈. Additionally, it doesn't explain why two distinct lifetimes (τ_1 and τ_2) are observed for (dA)₂ at all emission

wavelengths. Computational studies suggest that the orientation of the stacked bases plays a significant role in the nature of the excited state. Thus, we considered the possibility that different base stacking conformations result in different CT states. To address these issues and support the further examine the conclusion of our current model we examine next the effects of isotopic substitution on the excited-state dynamics of adenine oligomers.

4.3.3 (dA)₂ and (dA)₁₈ in D₂O

To our knowledge this is the first report of a KIE on the sub-nanosecond emission from DNA oligomers. The steady-state emission of (dA)₂ and (dA)₁₈ in D₂O resembles the emission spectral shape of the respective oligomer in H₂O. The integrated emission in D₂O is approximately 2x and 1.5x greater for (dA)₂ and (dA)₁₈, respectively. Each D₂O emission spectrum is slightly red-shifted with respect to the H₂O emission spectrum.

As in H₂O, emission from the (dA)₂ and (dA)₁₈ oligomers in D₂O buffer solution decays exponentially with the τ_1 and τ_2 lifetimes. These components account for the majority of the decay the ensues after the prompt emission that cannot be resolved by the TCSPC technique (Tables 4.3 and 4.4). The τ_3 component of (dA)₂ comprises a noticeably greater portion of the decay amplitude (approximately 10%) at short emission wavelengths than τ_3 at longer emission wavelengths or τ_3 for (dA)₁₈ component in H₂O or D₂O. This could be attributed to both the lower quantum yield of (dA)₂ and the greater amount of background fluorescence seen from the D₂O buffer.

The KIE, as described by equation 4.1, shown in Figure 4.7 (panels e and f) describes the difference in kinetics for the τ_1 and τ_2 lifetimes at each emission wavelength

for (dA)₂ and (dA)₁₈. The magnitude of the KIE increases with increasing emission wavelength for both oligomers. The KIE for τ_2 is consistently greater than for τ_1 in both oligomers, although this difference is not statistically significant at all emission wavelengths. In comparing the KIE for both oligomers, it is clear that τ_2 for (dA)₂ is consistently 1.5-2 x greater than the KIE of τ_1 or τ_2 for (dA)₁₈. The error associated with the τ_1 KIE for (dA)₂ is too large to make any statistically significant observations, although this too does appear to increase in magnitude at longer emission wavelengths.

To further clarify the KIEs, we performed transient absorption measurements on the same samples as the two methods probe the same excited-state dynamics at times less than a few nanoseconds as discussed in Chapter 3.¹² The ground state bleach recovery signals (267 nm pump/ 250 nm probe) of (dA)₂ and (dA)₁₈ in D₂O buffer are modeled by a two exponential decay with a slight offset (Table 4.6). Both lifetimes of both oligomers exhibit a clear KIE. The τ_2 KIE for (dA)₂ is approximately 3 while the KIE for (dA)₁₈ is approximately 2. The observation of a KIE for both oligomers, and the larger KIE magnitude observed for (dA)₂, mirror the results seen in the emission experiments. Note that the τ_1 and τ_2 lifetimes from the TA signals are not meant to be compared directly with the same named lifetimes from the TCSPC experiments. The τ_1 decay is assigned to vibrational cooling of the hot ground state following internal conversion (see section 4.3.4).¹⁸ These dynamics, which are too fast to be observed in TCSPC experiments, are not important for understanding emissive excited states and will not be considered further.

Previously, de La Harpe et al.¹¹ examined various GC oligomers in H₂O and D₂O buffer solution using the fs-TA technique. The authors observed a pronounced KIE in the

ground state bleach recovery and excited state absorption transients of an (GC)₉·(GC)₉ duplex, however no KIE was observed for a similar single-stranded, alternating GC oligomer with methylation at the N3 position of cytosine introduced to prevent WC base pairing. The observation of a KIE in the double-stranded oligomer and not the single-stranded oligomer led the authors to conclude that interstrand proton transfer in the duplex was responsible for the observed KIE. Additionally, the authors did not observe a KIE for the nonalternating d(C₄G₄)·d(C₄G₄) duplex. This was in agreement with previous fs-TA studies examining alternating and nonalternating AT duplexes in H₂O and D₂O. In this study by Crespo et al.¹⁹ a kinetic isotope effect was observed in the alternating d(AT)₉·d(AT)₉ duplex while none was observed in the nonalternating d(A)₁₈·d(T)₁₈ duplex. Again, the authors assumed that proton coupling to the excited state is responsible for the KIE and that excited-state proton transfer occurs in d(AT)₉·d(AT)₉, but not in d(A)₁₈·d(T)₁₈.

A recent time-resolved IR (TRIR) study of d(AT)₉·d(AT)₉ and d(A)₁₈·d(T)₁₈ reported the first observation of excited-state proton transfer in DNA duplexes.²⁰ The authors of this study identified vibrational markers, which indicate a proton-coupled electron transfer deactivation pathway in the d(A)₁₈·d(T)₁₈ oligomer, while only excited-state CT was seen in the d(AT)₉·d(AT)₉ oligomer. Additionally, the long-lived TRIR lifetimes, recorded in D₂O, were approximately 150 ps for d(AT)₉·d(AT)₉ and 300 ps for d(A)₁₈·d(T)₁₈. These are 2 and 3 times longer than the lifetimes observed for the same or similar oligomers recorded in H₂O as measured by TA ground state bleach recovery signals (267 nm pump/ 250 nm probe).¹² Assuming that TRIR is probing vibrational

markers associated with decay of the CT state, the longer lifetimes observed for both oligomers suggest a KIE is present in both the alternating and nonalternating double strands.

4.3.4 KIE Assignment

The observation of a KIE of 2-3 in singled-stranded adenine oligomers indicates that a KIE does not always provide evidence of excited-state proton transfer as traditionally thought.¹¹ Proton transfer is not expected for excited states of adenine single strands. However, a recent two-photon ionization study identified a significantly shorter excited-state lifetime for adenosine in comparison to adenine.³⁷ In response to this study, MD simulations have identified intramolecular hydrogen bonding between the sugar and nucleobase, and explain the faster lifetime by deactivation through a barrierless conical intersection that involves proton transfer.^{38, 39} However, examining these oligomers in solution reveals that this hydrogen bonding interaction is in competition with solvent hydrogen bonding. It seems unlikely that proton transfer is a high quantum yield deactivation pathway in an aqueous environment.

Given the observed KIE in adenine single strands, and the contradictions mentioned in Section 3.3, we propose a new mechanism to describe the observed KIE in adenine single-stranded oligomers. While this discussion is focused on adenine single-stranded oligomers, the conclusions are thought to apply to other single strands. It is important to start by identifying the locations of deuterium substitution on the adenine ring. Substitution of the hydrogens in the amino group of adenine occur in both mononucleotides and polymeric DNA.⁴⁰ Substituting deuterium for hydrogen at other

locations generally requires chemical synthesis or a catalyst.^{41, 42} It has been reported that hydrogen on the C8 can be exchanged at elevated temperatures,⁴³ but the exchange rate at room temperature is negligible under the current experimental conditions. We conclude that the only substantial deuterium substitution occurs on the amino group of adenine.

Some important insights into the origin of the KIE are given by studies examining mononucleotides in deuterated solution. Previously, Markovitsi et al.¹⁷ identified an inverse KIE from fs-FU signals of thymine and 5-fluorouracil monomers. The authors found a 20% reduction in the 5-fluorouracil fluorescent lifetime and attributed this to a less effective solute-solvent energy transfer process in D₂O. This provides the monomer with a greater amount of additional energy, accelerating molecular motion towards the S _{π} /S₀ conical intersection. In contrast, Middleton et al.¹⁸ observed no KIE in signals monitoring the decay of excited-state absorption from nucleobase monomers, suggesting that internal conversion may not be highly sensitive to deuteration.

Deuterium substitution of hydrogen has been widely used to study CT complexes.^{44, 45} In these studies, the authors typically use specific deuterium substitutions to identify the coupling of particular vibrational modes to the CT process.⁴⁴⁻⁴⁷ These reported KIE magnitudes are generally around 1.2 -2, and have been shown to increase in magnitude with increasing $-\Delta G_{\text{et}}$.⁴⁵ We therefore assign the KIE seen in the adenine oligomers to coupling of vibrational modes to the CT state. Following this conclusion and the location of the substitution, we can attribute the KIE specifically to coupling of the amino vibrational modes to the back-electron transfer process that causes the CT state to decay to the ground state.

4.3.5 Assignments

We have presented here various new observations of the emission dynamics of adenine oligomers. The most notable of these is the KIE observed in single-stranded systems. While not the first study to report that the steady-state emission from (dA)₂ is substantially red-shifted with respect to that of (dA)₁₈, we are able to correlate this effect with differences in emission lifetimes. In the new TA measurements reported here, differences in long lived fs-TA lifetimes are noted between (dA)₂ and (dA)₁₈, whereas a previous study globally fit TA signals to d(A)_n oligomers with $n = 2 - 18$ to a single length-independent lifetime.

Using this information, we now attempt to identify differences between the τ_1 and τ_2 emission lifetimes. While the TCSPC τ_1 from (dA)₁₅ was assigned to excimer-like luminescence in Chapter 3, the identity of the τ_2 lifetime was not addressed. The TCSPC decays from (dA)₂ and (dA)₁₈ are not accurately described by the same emission lifetimes. Furthermore, τ_2 for (dA)₂ consistently decays on slower timescales than τ_2 for (dA)₁₈, and exhibits the greatest KIE (Figure 4.7). The time-integrated emission spectra for (dA)₁₈ in H₂O (Figure 4.8b) show that τ_1 and τ_2 contribute approximately an equal percentage of photons to the overall steady-state emission, while τ_2 for (dA)₂ contributes the majority of photons to its steady-state emission spectrum (Figure 4.8a). We also observe a substantial increase in the percent photon contribution from τ_2 when both adenine oligomers are examined in a deuterated solvent (Figure 4.8).

To fully understand the contribution of the τ_2 component to the overall dynamics of the oligomer, we turn to the fs-TA data. It was previously reported in Chapter 3 that

the shortest resolvable TCSPC lifetimes of model AT oligonucleotides, including (dA)₁₅, match the lifetimes reported in fs-TA experiments, leading to the conclusion that the excited states with these TCSPC lifetimes decay via excimer or charge recombination luminescence. The τ_3 or nanosecond components identified in Chapter 3 contained contributions from background emission and oligomer impurities obscuring any intrinsic oligomer emission on the ns timescale. Changing the strand length and the solvent isotope identity clearly change the population of excited states deactivating through channels characterized by τ_2 . Understanding what gives rise to the τ_2 decay component is needed to determine why this component has not been observed previously in fs-TA measurements.

The wavelength-average lifetimes for (dA)₂ and (dA)₁₈ in H₂O and D₂O (calculated as previously discussed in Chapter 3 using equation 3.4) are shown in Table 4.7 along with the fs-TA τ_2 lifetimes reported in Table 4.6. The sample with the smallest percent photon contribution from τ_2 (Figure 4.8) is (dA)₁₈ in H₂O, and as previously reported in Chapter 3, the fs-TA τ_2 lifetime most resembles the TCSPC $\langle\tau_1\rangle_\lambda$. As the percent photon contribution from the τ_2 component increases, the more the TCSPC $\langle\tau_2\rangle_\lambda$ resembles that of the fs-TA absorption lifetime. The sample with the greatest percent photon contribution from τ_2 is (dA)₂ in D₂O, and the TCSPC $\langle\tau_2\rangle_\lambda$ is statistically identical to the τ_2 fs-TA lifetime in this case.

The agreement between the fs-TA absorption and TCSPC signals provides further evidence that the two methods are monitoring the same excited-state populations. Changes in the TCSPC signals as the strand length and solvent isotopes are changed

mirror the changes observed in the fs-TA signals. In the case of the TCSPC signals, two components are needed to describe the subnanosecond dynamics typically assigned to excimer states, while only one component is used to describe the fs-TA signals. This could be the result of the increased sensitivity of the TCSPC technique in comparison to the fs-TA technique. However, the fact that the time-resolved signals of the two techniques mirror each other suggest we can use the sensitivity and wavelength resolution of the TCSPC to expand on the information obtained from the fs-TA technique.

We see that both τ_1 and τ_2 for (dA)₂ and (dA)₁₈ depend on the length of the strand, emission wavelength, and the isotope identity of the solvent. In Chapter 3, the TCSPC signals from (dA)₁₅ showed that the emission lifetimes depend on emission wavelength. As the energy of the emission wavelength decreased the emission lifetimes became slower. The same trend is also observed here for (dA)₁₈ and (dA)₂. In Chapter 3, this observation was attributed to differences in solvation environments as a result of stacking domains of various lengths. Since exposure to the polar solvent is expect to lower the energy of the CT state, it was proposed that the longer lifetimes at red-shifted emission wavelengths were the result of short solvated stacked domains.

Differences in domain length could also impact the number of bases over which the excited state could delocalize. A more detailed review on delocalization is presented in Chapter 5 and is briefly summarized here. A previous time-resolved broadband fs-TA study assigned the long-lived excited states in (dA)₂₀ to delocalized excitations,⁴⁸ while a later study by Su et al. provided evidence that excitations responsible for the long-lived components are localized on only two bases.⁵ A recent TRIR study by Zinth and

coworkers assigned long-lived components to charge recombination from a delocalized charge separated state.¹⁴

As mentioned above, τ_1 and τ_2 for (dA)₂ and (dA)₁₈ depend on the length of the strand, emission wavelength, and isotope identity of the solvent. We observe that τ_2 is slower for (dA)₂ in comparison to τ_2 for (dA)₁₈, and the integrated emission represented from τ_2 is greater in (dA)₂. One potential difference between (dA)₁₈ and (dA)₂ is the difference in solvation environment arising from differences in domain length as discussed in Chapter 3 and Section 4.3.2. Two stacked bases present in a polar environment are expected to experience a greater extent of CT, in comparison to a less polar environment in a longer stacked domain. Increasing the polarity of the surrounding environment would lower the energy of the CT state increasing the percent of CT character used to describe the excited state. Therefore, we would expect bases present in a more polar environment to exhibit greater CT character than bases present in a less polar environment. It is also possible that solvation environment influences the type of excited state formed. Various computational studies suggest multiple types of excimer states are formed in adenine oligomers and are distinguished by their degree of CT.^{25, 27, 28}

We can conclude that the population of excited states deactivating through the pathways modeled by the TCSPC τ_2 lifetime are greater in (dA)₂ than (dA)₁₈. This suggests that the pathways deactivating with lifetime τ_2 are favored for shorter domain lengths. If we were to attribute this to differences in solvation it could be concluded deactivation of states described by τ_2 might exhibit a greater degree of CT character than τ_1 . However, solvation alone does not explain the multiple lifetimes observed for (dA)₂,

nor the dependence of these lifetimes on emission wavelength. While domain length might vary in longer adenine oligomers giving rise to different solvation environments, the domain length of (dA)₂ remains consistent. To help explain the multiple lifetimes identified for (dA)₂ we turn to the meaning of the KIE observed in these systems. It is important to point out that a KIE does not always arise because of a proton transfer reaction. Instead, KIEs as large as ~2 are relatively commonplace in many outer-sphere electron transfer reactions. {cite M. H. V. Huynh and T. J. Meyer, Chem. Rev. 2007, 107, 5004-5064} Because nonradiative decay of a CT excited state can also be understood in terms of back electron transfer, KIEs have also been noted in CT complexes.⁴⁵ In such complexes the magnitude of the KIE increases with increasing $-\Delta G_{et}$,⁴⁵ suggesting that the magnitude of the KIE in a DNA oligomer can be used to qualitatively characterize the degree of CT character in an excimer state. Given that τ_2 of (dA)₂ exhibits the greatest KIE, we propose that the excited state that decays on this time scale has the greatest degree of CT character. This corresponds nicely with the observation that deactivation through such states becomes favored in more polar environments.

With this information, we assign τ_1 and τ_2 to two kinds of excimer-like states with the longer τ_2 lifetime assigned to an excimer state exhibiting a greater degree of CT character. The proposal of two types of excimer states was also made in a joint experimental and computational study by Markovitsi and coworkers.⁶ However, in that study the authors proposed that neutral excimers, excimers that do not exhibit any significant CT character, are responsible for the subnanosecond emission (i.e., the emission measured by our τ_1 components), while CT states are responsible for lower

energy ns emission. Our analysis and our observed KIEs also suggest that longer lifetimes arise from excited states having a greater degree of CT, but our results strongly suggest that CT states contribute to the subnanosecond emission and not merely to emission on ns timescales. We do not examine the ns emission studied by Markovitsi and coworkers due to interference in our measurements by emission from the buffer and impurities introduced present in the DNA strand samples. While models have been proposed describing ns emission as the result of electron and hole separation and recombination,^{49, 50} these models have only been used to describe dynamics in double-stranded DNA involving blue-shifted emission (310 nm). In any event, it is difficult to accurately measure intrinsic ns emission from adenine oligomers.

According to our model, an excitation localized in a stack consisting of at least two adenine bases results in a long-lived signal assigned to an excimer state, while excited states of unstacked bases decay as rapidly as the short-lived excited states observed in monomers. The domain length heterogeneity present in longer adenine oligomers means that excimers can experience different solvation environments and these different environments can in turn give rise to classes of excimers that differ in their CT character. The observation that decay times for (dA)₂ depend on emission wavelength suggests that domain length is not the only factor influencing relaxation. In other words, there could already be two or more types of emissive excimers in the dinucleotide.

A recent fs-TA study of adenine dinucleotides with linkers of various lengths was conducted to observe the effect of different base stacking interactions on the dynamics of these systems.⁸ The authors used circular dichroism spectroscopy to argue that different

linkers give rise to quite different stacking geometries, yet the dynamics were nevertheless virtually identical in all systems. It would be interesting to re-investigate some of these same systems in emission using the sensitive TCSPC technique, which is able to more accurately resolve multiple subnanosecond decay components than is the case in TA measurements. As a further advantage over the single-wavelength TA measurements emphasized in past work from the Kohler Group, the TCSPC technique is able to accurately measure kinetics at various emission wavelengths. It is furthermore possible that the emission signals could be more sensitive to changes in wavelength than TA signals.

As discussed in Chapter 3, computational studies have identified excimer states with varying degrees of CT between two stacked adenine bases.^{6, 25, 27, 28} These states are distinguished by differences in base stacking or geometry. States with greater CT character were found to be lower in energy than neutral excimer states. Given the multiple lifetimes of the (dA)₂ TCSPC decays and differences in the KIE of the τ_1 and τ_2 lifetimes, we assign both lifetimes to excimer-like states with the excimer state with the τ_2 lifetime exhibiting a greater degree of CT than that with lifetime τ_1 .

Compiling all the observations made here along with conclusions from previous computational studies, we propose the following model to explain deactivation kinetics in adenine single strands. The two lifetimes, τ_1 and τ_2 , identified by the TCSPC technique describe two different types of excimer-like states with varying degrees of charge transfer character with states modeled by τ_2 exhibiting a greater CT character than states modeled by τ_1 . Given that τ_1 and τ_2 increase in lifetime as the emission wavelength increases, it is

proposed that τ_1 and τ_2 do not represent excited states of a single population but more a distribution of states that exhibit a similar degrees of CT characteristics with states emitting at longer emission wavelengths exhibiting a greater extent of CT than states emitting at shorter emission wavelengths. Studies have identified charge recombination in DNA occurs in the Marcus inverted region, however the trend of longer lifetimes observed at longer emission wavelengths is indicative of charge recombination in the Marcus normal region. Instead of describing stacked adenine bases in terms of pure CT states we can described them in terms of excimer states with varying degrees of CT. States with less CT character exhibit greater monomer like kinetics, fast lifetimes and blue shifted emission, while states with greater CT character are described by redshifted emission and longer lifetimes. This is inferred from the redshifted steady-state emission and longer τ_2 lifetimes observed for (dA)₂ in comparison to (dA)₁₈.

4.4 Conclusion

Emission from (dA)₂ is substantially red-shifted in comparison to emission from (dA)₁₈. Time-resolved emission and absorption lifetimes reveal that long-lived (dA)₂ excited states decay on a slower time scale than similar states in (dA)₁₈. The differences in emission and lifetimes between the two systems suggest that the length of stacked domains influences the dynamics. The red-shifted emission of (dA)₂ in comparison to (dA)₁₈ is proposed to be the result from differences in solvation environments caused by differences in the average stacked domain length. Longer domain lengths in (dA)₁₈ are

expected to exclude solvent molecules making the surrounding environment less polar than in the case of the solvated (dA)₂.

This is the first report describing a KIE on excited-state relaxation in single-stranded adenine oligomers. A substantial KIE is observed for both systems and is most prominent for (dA)₂. The magnitude of the KIE increases as the decay time increases. The distinct KIE values observed for the τ_1 and τ_2 lifetimes in both (dA)₂ and (dA)₁₈ strongly suggest that these decay components arise from different excited-state populations. The detection of a KIE in the absence of base pairing is suggested to arise because deuteration retards the decay rate of an excimer state by back electron transfer. Specifically, this reflects the influence of high-frequency N-H/N-D stretch modes of the amino group of adenine on the excimer lifetime.

The τ_1 and τ_2 components of the TCSPC decays were assigned to two excimer-like states with the state with lifetime τ_2 , exhibiting a greater percent CT character than the excimer state with the τ_1 lifetime. Both τ_1 and τ_2 lifetimes of the TCSPC decays mirrored the kinetics modeled by the fs-TA τ_2 lifetimes depending on the percent photon contribution from each lifetime, suggesting that the TCSPC decays are modeling significant deactivation channels in the adenine oligomers. The sensitivity of components described by τ_2 to differences in (dA)₂ and (dA)₁₈ coupled with the differences in steady-state emission was described by differences in solvation resulting from different domain lengths. The multiple lifetimes identified for (dA)₂ by the TCSPC and the differences in the KIE associated with each lifetime suggest multiple states are present that can be distinguished by various degrees of CT. These multiple lifetimes suggest solvation or

domain length is not the only factor contributing to the heterogeneity of the adenine systems.

Table 4.1. Best-fit parameters for emission decays from (dA)₂ and (dA)₁₈ in H₂O and D₂O at emission wavelengths of 360 and 420 nm.

Emission λ / nm	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	τ_3 (ns)	% A_3	% N_0/N ^[a]
(dA) ₂ H ₂ O							
360	56 ± 18	53	377 ± 20	45	2.3 ± 0.4	1.5	20.9
420	267 ± 34	48	512 ± 26	51	6.7 ± 1.2	1.1	2.7
(dA) ₂ D ₂ O							
360	77 ± 25	48	0.77 ± 0.43	38	2.0 ± 0.1	13.7	11.2
420	395 ± 97	25	1.2 ± 0.3	74	9 ± 2	1.1	1.1
(dA) ₁₈ H ₂ O							
360	128 ± 6	59	305 ± 7	40	2.9 ± 0.3	0.7	6.9
420	196 ± 11	79	417 ± 27	20	4.0 ± 0.8	0.6	1.2
(dA) ₁₈ D ₂ O							
360	136 ± 12	43	435 ± 8	54	1.3 ± 0.1	2.6	5.2
420	245 ± 24	47	611 ± 20	49	1.7 ± 0.1	4.0	0.4

[a] Percent of prompt photons to all photons.

Table 4.2. Best-fit parameters for emission decays from (dA)₂ in H₂O

Wavelength (nm)	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
310	47 ± 21	88	470 ± 170	9	2.8 ± 0.7	2.7	75.5
320	47 ± 15	87	432 ± 100	10	2.5 ± 0.5	2.1	68.6
330	45 ± 13	82	397 ± 55	16	2.6 ± 0.5	1.6	58.0
340	54 ± 15	72	390 ± 39	26	2.3 ± 0.5	1.7	46.0
350	80 ± 21	60	413 ± 30	38	3.1 ± 0.6	1.4	33.0
360	56 ± 18	53	377 ± 20	45	2.3 ± 0.4	1.5	20.9
370	93 ± 34	38	405 ± 20	61	2.8 ± 0.5	1.4	14.9
380	211 ± 32	48	474 ± 24	51	4.4 ± 1	1.0	10.0
390	215 ± 34	44	468 ± 23	55	4.7 ± 0.9	1.1	6.6
400	228 ± 31	46	484 ± 22	53	5.4 ± 1.2	0.9	4.5
410	195 ± 52	29	456 ± 19	70	3.3 ± 0.6	1.5	3.4
420	267 ± 34	48	512 ± 26	51	6.7 ± 1.2	1.1	2.7
430	244 ± 37	45	508 ± 25	54	6.7 ± 1.4	1.1	2.1
440	232 ± 50	37	483 ± 26	62	8.8 ± 2.3	1.1	1.1
450	252 ± 56	35	475 ± 27	64	5.5 ± 1.5	1.2	0.7
460	299 ± 43	51	512 ± 38	48	6.1 ± 1.7	1.2	0.0

[a] Percent of prompt photons to all photons.

Table 4.3. Best-fit parameters for emission decays from (dA)₁₈ in H₂O

Wavelength (nm)	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
310	102 ± 12	69	339 ± 25	28	2.7 ± 0.2	3.0	33.4
320	93 ± 9	62	291 ± 13	36	2.7 ± 0.2	2.1	24.6
330	96 ± 7	60	287 ± 9	38	2.9 ± 0.2	1.5	17.4
340	123 ± 6	62	304 ± 9	37	3.0 ± 0.2	1.1	13.5
350	99 ± 7	53	279 ± 6	46	2.8 ± 0.2	0.9	8.4
360	128 ± 6	59	305 ± 7	40	2.9 ± 0.3	0.7	6.9
370	148 ± 7	65	328 ± 9	34	2.8 ± 0.3	0.6	5.2
380	147 ± 7	65	334 ± 10	35	3.0 ± 0.3	0.5	3.7
390	165 ± 8	67	349 ± 13	32	2.6 ± 0.4	0.5	3.1
400	182 ± 8	79	415 ± 20	21	3.3 ± 0.6	0.4	2.5
410	164 ± 10	68	374 ± 17	31	3.2 ± 0.6	0.5	1.3
420	196 ± 11	79	417 ± 27	20	4.0 ± 0.8	0.6	1.2
430	183 ± 14	71	398 ± 27	28	3.6 ± 0.8	0.7	0.0
440	196 ± 14	80	486 ± 38	19	6.3 ± 1.3	0.7	0.0
450	171 ± 23	57	357 ± 30	42	3.4 ± 0.9	1.2	0.0
460	203 ± 20	79	470 ± 61	19	3.2 ± 0.9	1.3	0.0

[a] Percent of prompt photons to all photons.

Table 4.4. Best-fit parameters for emission decays from (dA)₂ in D₂O

Wavelength (nm)	τ_1 (ps)	% A_1	τ_2 (ns)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
310	57 ± 29	74	0.87 ± 0.14	18	3.8 ± 0.3	7.5	53.1
320	48 ± 14	81	0.77 ± 0.11	13	3.2 ± 0.2	5.8	43.8
330	55 ± 16	74	0.77 ± 0.80	19	3.0 ± 0.2	6.8	35.9
340	54 ± 14	70	0.76 ± 0.60	23	2.8 ± 0.2	6.8	25.3
350	63 ± 18	59	0.73 ± 0.50	30	2.2 ± 0.1	10.4	17.2
360	77 ± 25	48	0.77 ± 0.43	38	2.0 ± 0.1	13.7	11.2
370	126 ± 42	37	0.87 ± 0.41	48	1.9 ± 0.1	15.1	7.2
380	198 ± 56	34	1.1 ± 0.3	63	3.2 ± 0.4	3.3	4.6
390	244 ± 75	27	1.1 ± 0.3	69	2.9 ± 0.4	3.9	3.2
400	224 ± 75	25	1.1 ± 0.3	70	2.5 ± 0.3	4.8	2.0
410	326 ± 97	24	1.2 ± 0.3	75	11 ± 2	1.2	1.4
420	395 ± 97	25	1.2 ± 0.3	74	9 ± 2	1.1	1.1
430	286 ± 110	21	1.2 ± 0.3	77	12 ± 3	1.2	0.2
440	426 ± 118	25	1.2 ± 0.4	74	19 ± 8	1.1	0.4
450	400 ± 150	21	1.2 ± 0.4	78	27 ± 10	1.4	0.1
460	490 ± 173	21	1.2 ± 0.4	78	18 ± 6	1.4	0.2

[a] Percent of prompt photons to all photons.

Table 4.5. Best-fit parameters for emission decays from (dA)₁₈ in D₂O

Wavelength (nm)	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
310	87 ± 15	56	399 ± 18	43	1.9 ± 0.3	1.3	33.2
320	84 ± 11	53	383 ± 12	45	1.3 ± 0.1	2.0	22.2
330	99 ± 11	49	403 ± 9	49	1.4 ± 0.1	1.6	15.0
340	100 ± 11	44	388 ± 8	53	1.1 ± 0.1	3.2	10.1
350	135 ± 12	44	425 ± 8	54	1.3 ± 0.1	2.5	7.8
360	136 ± 12	43	435 ± 8	54	1.3 ± 0.1	2.6	5.2
370	168 ± 14	45	464 ± 9	53	1.4 ± 0.1	2.5	4.3
380	179 ± 15	46	485 ± 11	52	1.5 ± 0.1	2.6	2.9
390	216 ± 16	51	538 ± 13	47	1.6 ± 0.1	2.2	2.5
400	244 ± 15	56	609 ± 17	41	1.8 ± 0.2	2.1	1.5
410	243 ± 20	51	612 ± 18	46	1.8 ± 0.2	3.0	0.6
420	245 ± 24	47	611 ± 20	49	1.7 ± 0.1	4.0	0.4
430	280 ± 27	51	705 ± 24	46	1.9 ± 0.2	3.3	0.0
440	275 ± 32	49	702 ± 27	48	2.2 ± 0.3	2.5	0.0
450	292 ± 38	51	679 ± 35	46	2.0 ± 0.3	3.3	0.0
460	306 ± 43	54	721 ± 45	43	2.2 ± 0.5	2.9	0.0

[a] Percent of prompt photons to all photons.

Table 4.6. Best-fit parameters for the (dA)₂ and (dA)₁₈ in H₂O and D₂O transient absorption signals in Figure 4.7. The ground-state bleach recovery signal (negative) was fit to a biexponential function with a constant offset: $\Delta A = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3$.

Sample	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	% A_3
(dA) ₂ H ₂ O	2.5 ± 0.2	75.1	311 ± 44	22.3	2.6
(dA) ₂ D ₂ O	3.7 ± 0.2	76.5	1100 ± 270	19.6	3.9
(dA) ₁₈ H ₂ O	2.9 ± 0.2	59.2	183 ± 13	38.5	2.4
(dA) ₁₈ D ₂ O	4.3 ± 0.4	61.9	354 ± 42	34.8	3.2

Table 4.7. Amplitude weighted average τ_1 and τ_2 TCSPC lifetimes and τ_2 fs transient absorption lifetime for (dA)₂ and (dA)₁₈ in H₂O and D₂O buffer

Sample	TCSPC $\langle\tau_1\rangle_\lambda$	TCSPC $\langle\tau_2\rangle_\lambda$	fs-TA τ_2
(dA) ₂ H ₂ O	142 ± 20	428 ± 45	311 ± 44
(dA) ₂ D ₂ O	174 ± 40	1104 ± 82	1100 ± 270
(dA) ₁₈ H ₂ O	144 ± 15	325 ± 30	183 ± 13
(dA) ₁₈ D ₂ O	169 ± 21	517 ± 37	354 ± 42

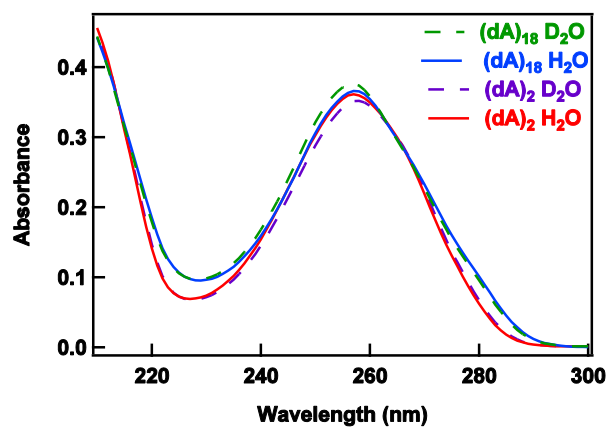


Figure 4.1. UV/Vis absorption spectra for (dA)₁₈ and (dA)₂ in Tris-HCl and Tris-DCl buffer with 0.25 M NaCl. Concentrations were adjusted to yield an absorbance of 0.3 at 265 nm for all four samples.

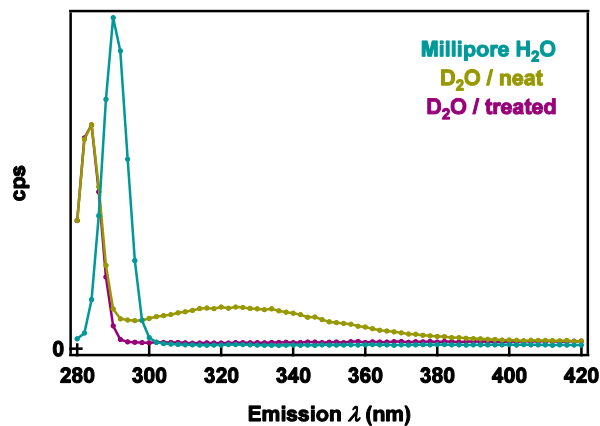


Figure 4.2. Steady-state emission spectra of 18 MΩ H₂O (cyan), neat D₂O used as purchased (gold), and treated D₂O (magenta) after 265 nm excitation shown on a linear scale. The treated D₂O was purified with activated charcoal according to the procedure outlined in the Methods Section.

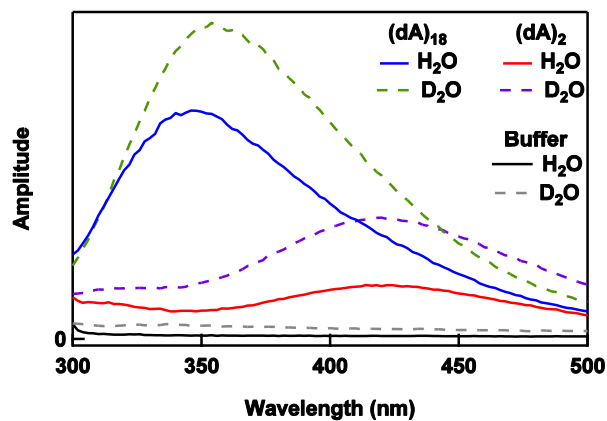


Figure 4.3. Corrected steady-state emission of (dA)₂ and (dA)₁₈ in Tris H₂O (solid lines) and D₂O buffer (dashed lines) with 250 mM NaCl after 265 nm excitation shown on a linear scale. The Tris H₂O and Tris D₂O buffer without the oligomers are shown by the solid black and dashed gray traces, respectively.

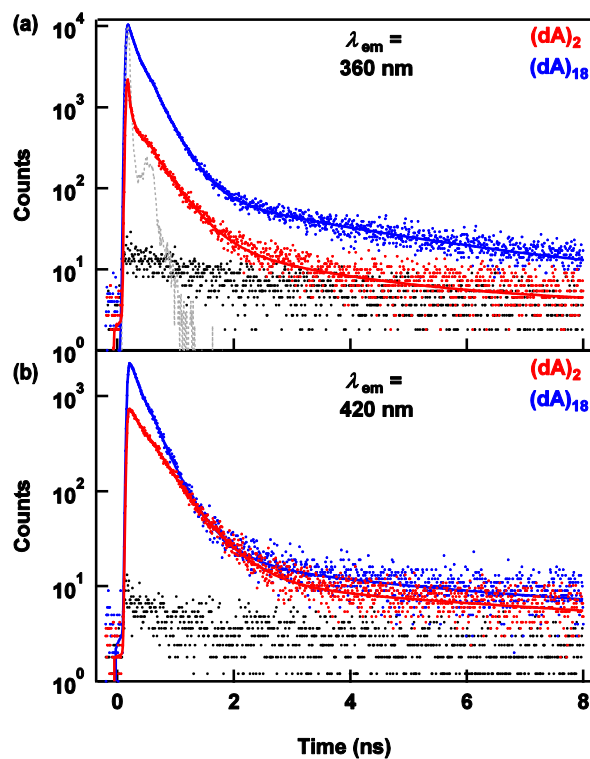


Figure 4.4. Fluorescent decays (dots) and fits (solid curves) of $(dA)_{18}$ (blue trace) and $(dA)_2$ (red trace) in Tris-HCl H_2O buffer with 0.25 M NaCl recorded at 360 nm (a) and 420 nm (b) shown on a logarithmic scale. The instrument response function is shown by the gray dashes in panel a. Emission from the Tris-HCl buffer with 0.25 M NaCl in the absence of oligonucleotide recorded under identical conditions is shown in black for each respective wavelength.

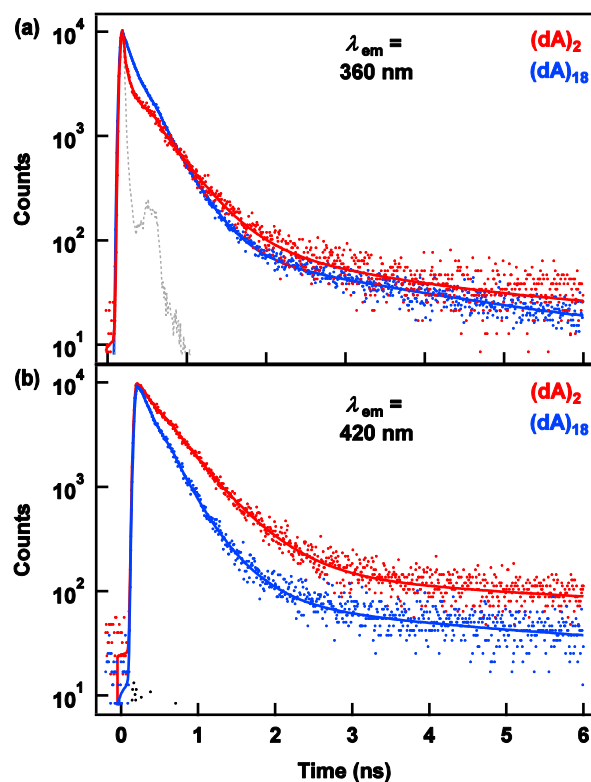


Figure 4.5. Normalized fluorescent decays (dots) and fits (solid curves) of $(dA)_{18}$ (red trace) and $(dA)_2$ (purple trace) in Tris-HCl H_2O buffer with 0.25 M NaCl at 360 nm (a) and 420 nm (b) shown on a logarithmic scale. The instrument response function is shown by the gray dashes in panel a. Each decay has been scaled to have a maximum count of 10,000.

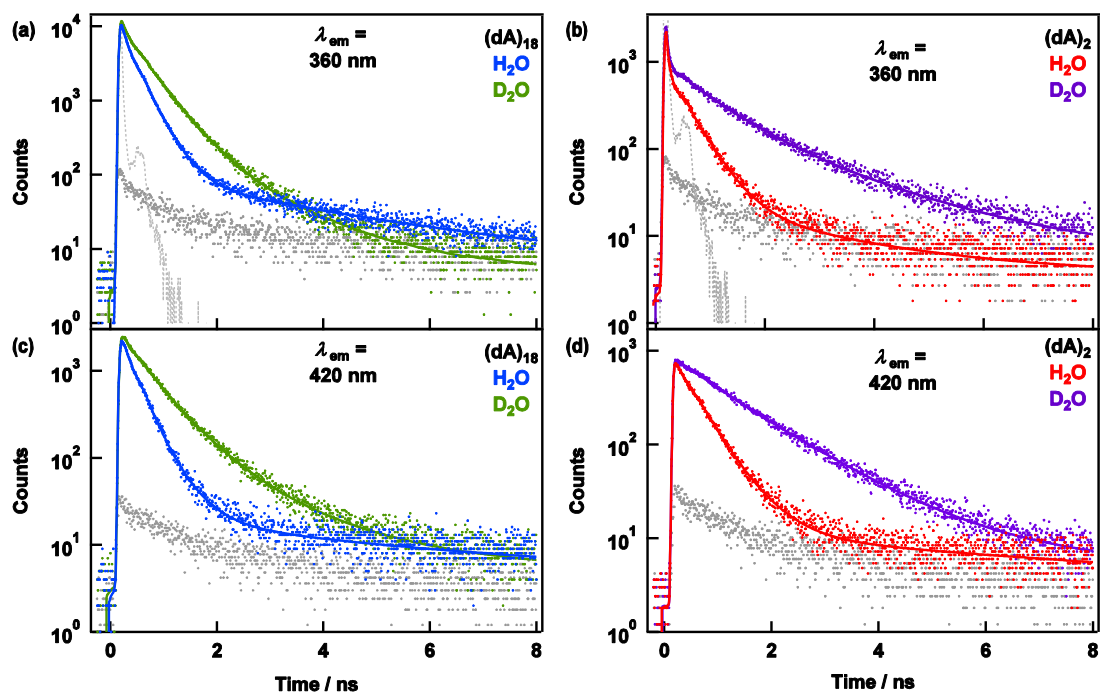


Figure 4.6. Fluorescent decays (dots) and fits (solid curves) of (dA)₁₈ in Tris-HCl H₂O (blue) and Tris-DCl D₂O (green) buffer with 0.25 M NaCl at emission wavelengths of (a) 360 nm and (c) 420 nm and (dA)₂ in Tris-HCl H₂O (red) and Tris-DCl D₂O (purple) buffer with 0.25 M NaCl at emission wavelengths of (b) 360 nm and (d) 420 nm. All traces are shown on a logarithmic axis. The Instrument response function is shown by the gray dashes in panels a and b. Emission from the Tris-DCl buffer with 0.25 M NaCl in the absence of oligonucleotide recorded under identical conditions is shown by the gray dots for each respective wavelength.

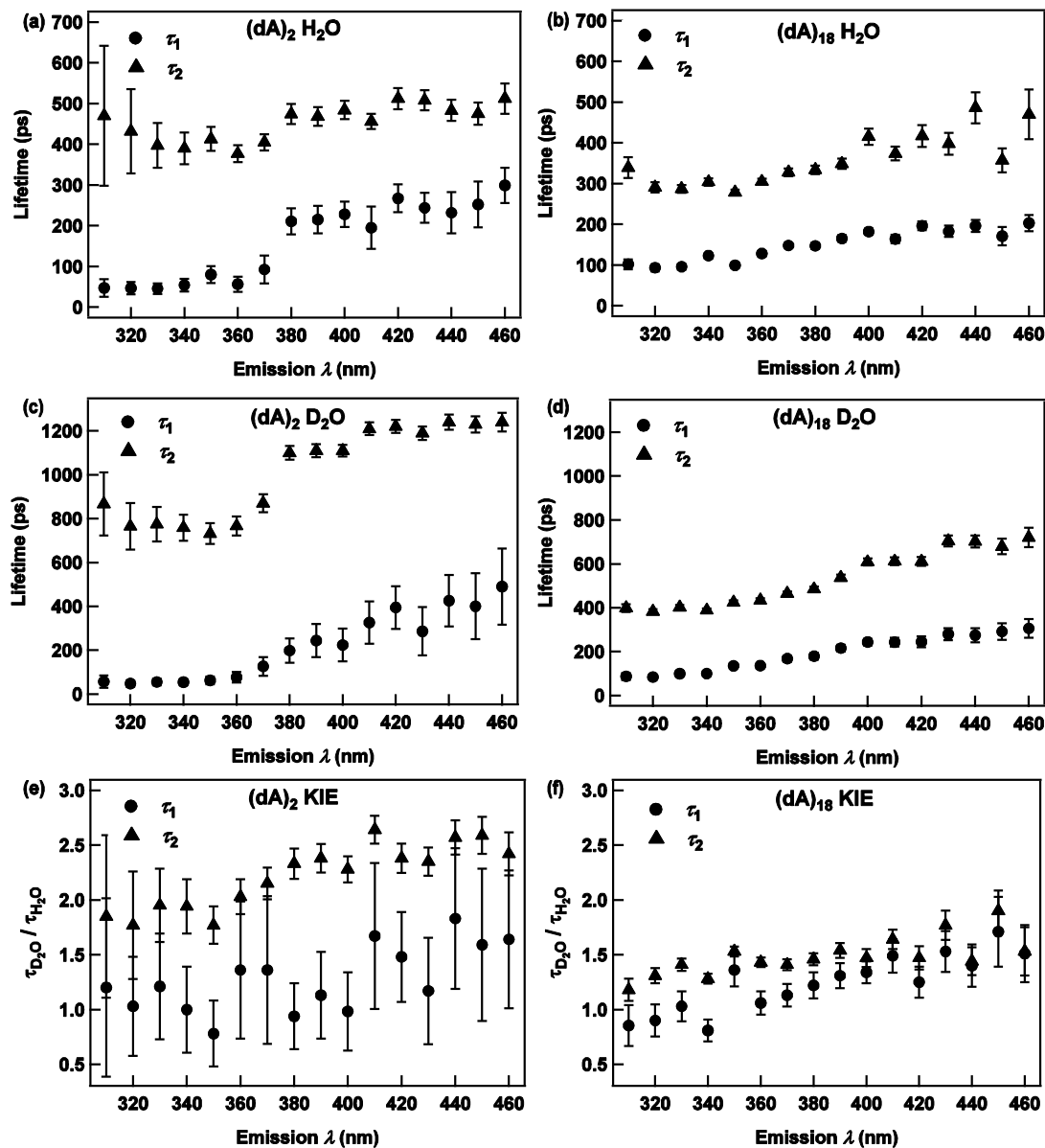


Figure 4.7. Values of τ_1 and τ_2 of the best parameters used to fit the TCSPC decays of $(dA)_2$ in H₂O buffer (a), $(dA)_{18}$ in H₂O buffer (b), $(dA)_2$ in D₂O buffer (c), $(dA)_{18}$ in D₂O buffer (d), and the KIE of each lifetime for $(dA)_2$ (e) and $(dA)_{18}$ (f). The complete fitting parameters are reported in Tables 4.2–4.5. The KIE is calculated according to Equation 4.1. All errors represented by the error bars are 2σ .

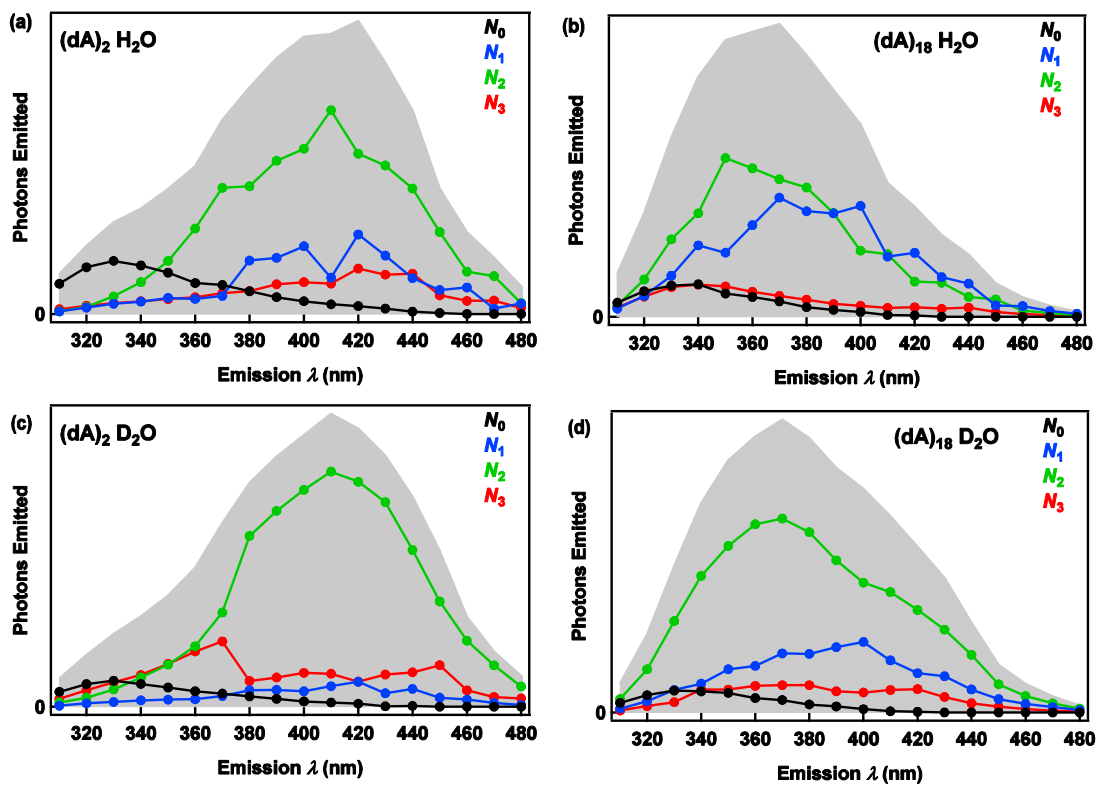


Figure 4.8. Time-integrated emission spectra shown with a linear vertical axis for (a) $(dA)_2 H_2O$, (b) $(dA)_{18} H_2O$, (c) $(dA)_2 D_2O$, and (d) $(dA)_{18} D_2O$. The gray shading represents the total integrated emission ($N_0 + N_1 + N_2 + N_3$). The black circles show the contribution from prompt photons emitted, N_0 . The separate contribution of each resolvable component is also shown: N_1 (blue), N_2 (green), and N_3 (red).

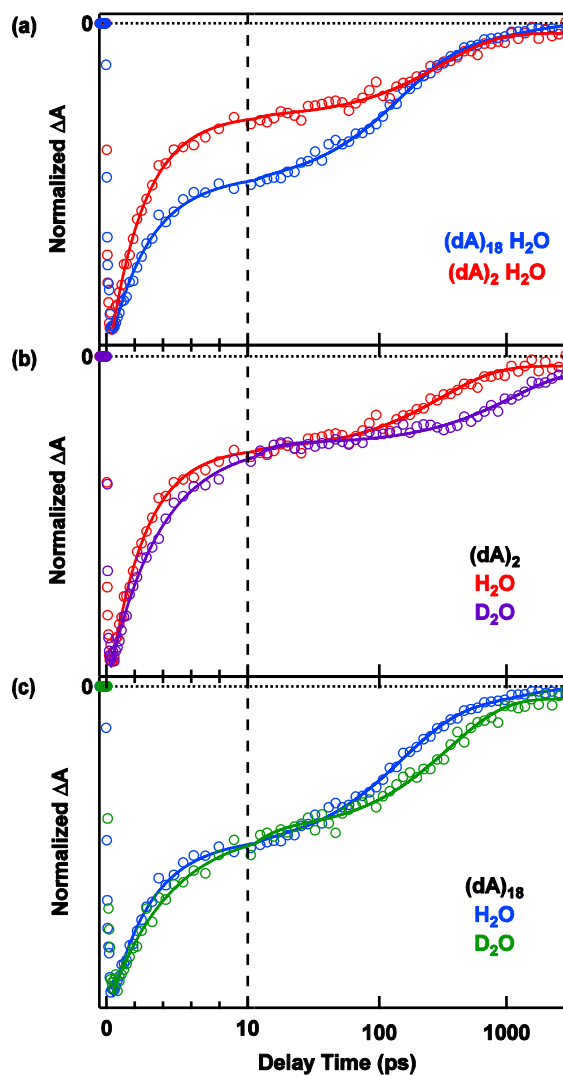


Figure 4.9. Normalized fs-TA signal (267 pump/ 250 probe) of (a) $(dA)_2$ and $(dA)_{18}$ in H_2O , (b) $(dA)_2$ in H_2O and D_2O , and (c) $(dA)_{18}$ in H_2O and D_2O in aqueous buffer solution containing 0.1 M NaCl at room temperature (circles). The solid curve is a nonlinear least-squares fit to two exponentials plus an offset. The vertical dashed line denotes the time axis transitions between a linear and logarithmic scale after 10 ps.

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CHAPTER FIVE

TEMPERATURE DEPENDENT DYNAMICS OF SINGLE STRANDED ADENEINE
OLIGOMERS IN SOLUTION5.1 Introduction

Single stranded oligomers are commonly used as a model system to examine how base stacking interactions influences the photophysics of DNA. Single-stranded adenine oligomers are chosen to model base stacking effects in the absence of base pairing as they exhibit the greatest propensity of base stacking in comparison other conical nucleotide single-stranded oligomers comprised of a single base identity.¹ Although these oligomers are homogenous in terms of their constituent base identity, base stacking interactions stabilizing these single strands are only weakly favored in room temperature solution resulting in a distribution of structural conformations.²

Base stacking interactions in DNA are generally characterized by two parallel neighboring bases in van der Waal contact giving rise to overlapping $\pi\pi$ orbitals. These stacking interactions have been shown to play an important role in the deactivation of excited-state dynamics in DNA.^{3, 4} Systems exhibiting base stacking interactions decay on time scales two orders of magnitude slower than unstacked oligomers. While largely accepted that base stacking directly influences the dynamics of DNA systems, there remains uncertainty in how to describe the excited process responsible for long-lived signals resulting from these base stacking interactions. Some time-resolved studies present a simplistic view of the processes governing deactivation kinetics in adenine

oligomers by demonstrating the deactivation kinetics are independent of stacking geometry or oligomer length.^{3,4} More complex deactivation kinetics reported for similar systems using different techniques suggest the excited state dynamics are influenced by factors such as domain length or stacking geometry.⁵

Many studies have estimated the probability of stacking in adenine dinucleotides to be around 70 to 90%.^{4, 6-8} Assuming this probability applies to any two adjacent bases in longer oligomers allows an estimation of the average stacking domain length of an oligomer comprised of n bases.⁹ The domain length is described by the number of neighboring stacked bases and is suggested to increase with the number of bases present in the oligomer. The average stacked domain length in an adenine dinucleotide can be no longer than two while the average domain length of an 18-mer is estimated to be 5.6 bases.⁹ Since base stacking is a weakly favored interaction in room temperature aqueous solution, it is reasonable to infer multiple domain lengths are present in longer oligomers like the 18-mer.

It is important to consider how stacking domains of various lengths would impact the excited-state dynamics of these systems. TCSPC and FU studies of (dA)₁₈ and (dA)₂₀ reveal a multitude of long-lived lifetimes decaying on the subnano- to nanosecond time-scales all displaying a strong dependence on emission wavelength.^{5, 10-12} Additionally, steady-state emission reveals drastic differences in dynamics between the adenine dimer and longer oligomers as detailed in Chapter 4 and other studies.¹³ Both experimental and computational studies suggest that these long-lived states observed for adenine oligomers are the result of excimer states with various degrees of CT.^{5, 12 14-16} The mounting

evidence suggesting the long-lived dynamics are influenced by factors such as oligomer length and can potentially be described by excited states of various identities calls for additional investigation into the factors governing the deactivation kinetics.

The heterogeneous nature of the adenine oligomer could be explained by distributions of stacking domains of different lengths. Since base stacking is weakly stabilized it is assumed the probability of base stacking exhibits a strong temperature dependence. Temperature dependent studies have played an important role in identifying dynamics associated with base stacking interactions.⁶⁻⁸ Time-resolved absorption studies of adenine oligomers reveal the fractional amplitudes from the long-lived signal decrease as temperature increases.^{2, 17} The authors interpreted this as a reduction in the fraction of stacked bases at higher temperatures. Another fs-TA study on the adenine dinucleotide reveals long-lived signals still present at higher temperature leading the authors to suggest base stacking is reduced at higher temperatures, but not completely eliminated.² Based on the description of domain length above, decreasing the fraction of stacked bases should also decrease the average stacked domain length. Changing the domain length could impact the dynamics of these systems in various ways. As discussed in Chapter 4, the solvation environment of two stacked adenines could be influenced by the number of bases in the stacking domain. Other studies reveal excited CT states are not localized to two bases with charges delocalizing over multiple bases.¹⁸⁻²⁰

If domain length is the factor influencing the differences in dynamics observed for the adenine dinucleotide and 18-mer then a careful time-resolved comparison between these two oligomers at different temperatures could provide valuable insight. However,

no such temperature dependent study has been reported, which could be due to the lack of time-resolved studies highlighting the differences in kinetics between (dA)₂ and (dA)₁₈. The results present in Chapter 4 detailing the differences in between (dA)₂ and (dA)₁₈ provides a unique opportunity to address the effects of temperature dependence on the kinetics of these systems.

In this work, we detail the effects of temperature on the excited state dynamics of (dA)₂ and (dA)₁₈ using the TCSPC and fs-TA techniques. We find that the dynamics of these systems in solution are strongly dependent on the temperature. While the deactivation kinetics of (dA)₂ and (dA)₁₈ are noticeably different at lower temperatures, the TCSPC signals from these oligomers at higher temperatures are virtually indistinguishable. This is described by a smaller fraction of stacked base at higher temperatures resulting in a reduced average stacking domain length in the (dA)₁₈ oligomer. The similarity in kinetics suggest domain length significantly influences the dynamics of adenine oligomers.

5.2 Results

5.2.1 TCSPC Signals

A general discussion of TCSPC decays recorded using the experimental setup is discussed in Chapters 2 and 3. Emission decays from (dA)₁₈ and (dA)₂ in Tris-HCl buffer solution with 0.25 M NaCl were recorded at 10 nm intervals between emission wavelengths of 330 and 480 nm after excitation at 265 nm. All TCSPC decays were recorded under identical experimental conditions including excitation power. This allows

for a meaningful comparison between the relative decay intensities of the unnormalized TCSPC decays. Figure 5.1 shows emission traces recorded at 7 °C (blue), 30 °C (green), 55 °C (gold), and 80 °C (red) at emission wavelengths of 360 nm and 420. As with the traces recorded at room temperature in Chapters 3 and 4, the emission amplitude drops by 1.5 – 2 orders of magnitude within the first nanosecond at all temperatures. As the temperature increases the relative intensity of the TCSPC signals decreases while the slopes of the decays increase. This trend describes all of the TCSPC signals except those of (dA)₁₈ recorded at 80 °C and longer (420 - 470 nm) emission wavelengths. This one exception is suspected to be the result of a solute impurity and is discussed in section 5.4.4. TCSPC signals from the Tris-HCl buffer with 0.25 M NaCl without added oligonucleotide (not shown) are similar in relative intensity and slope as those seen in Chapter 3. As the temperature increases the intensity of the Tris-HCl buffer TCSPC signals decrease at all emission wavelengths.

Comparison of the (dA)₂ and (dA)₁₈ TCSPC signals recorded at 7 °C and 80 °C at 360 and 420 nm are shown in Figure 5.2. As observed in Chapter 4 for (dA)₂ and (dA)₁₈ at 20 °C, there is a clear difference between the TCSPC signals of (dA)₁₈ and (dA)₂ recorded at 7 °C within the first 2 ns after excitation. Differences in the TCSPC decays of (dA)₂ and (dA)₁₈ at 7 °C are similar to those described in Section 4.3.2, except for the higher intensity of (dA)₂ relative to that of (dA)₁₈ at 420 nm. Of important note for this discussion is the difference in the slope and intensity between (dA)₂ and (dA)₁₈ recorded at 7 °C and 360 nm emission within the first 2 ns after excitation. Although the two signals approach each other in slope and intensity after 2 ns, initially the intensity of the

(dA)₁₈ signal remains 4-8x greater than that of (dA)₂. Comparing signals from the same oligomers at the same emission wavelength, but at 80 °C (Figure 5.3c) reveals none of the differences observed at 7°C, with the signals appearing virtually identical in signal intensity and slope during the first 2 ns after excitation. The same observation is made for signals recorded at 80 °C and 420 nm emission wavelength for the first ns, after which the long-lived signal is slightly greater in intensity and slope for (dA)₁₈. Although all signals in Figure 5.1 decrease in intensity and slope with increasing temperature it appears that the change in (dA)₁₈, especially at 360 nm, is more drastic than that of (dA)₂.

Time-resolved emission spectra for (dA)₁₈ (Figure 5.4) and (dA)₂ (Figure 5.5) were constructed from discrete emission decays recorded at wavelengths between 330 and 480 nm. At the earliest delay times (100 – 500 ps), the emission is peaked around 370 nm at all temperatures for (dA)₁₈. The traces at 7 °C are similar to the corresponding traces in Chapter 3 for (dA)₁₅. As the temperature increases, the shape for the trace begins to broaden, and an additional peak appears around 400 nm at 80°C. This broadening is particularly noticeable at times 200 and 300 ps after excitation. The time-resolved emission spectra from (dA)₂ 100 ps after excitation at 7 °C is noticeably red-shifted in comparison to that of (dA)₁₈ with an emission maximum around 420 nm. Again, as the temperature increases the spectral shapes of the time-resolved traces of (dA)₂ begin to broaden, except in this case the shape broadens toward the blue. As with the TCSPC traces it appears that the time-resolved spectra of (dA)₁₈ and (dA)₂ begin to approach each other as the temperature increases. Figure 5.5 compares the time-resolved emission 100 ps after excitation for (dA)₂ and (dA)₁₈ at all recorded temperatures. As with the TCSPC

decays, the initial time-resolved emission spectra are vastly different in amplitude and emission maximum at 7 °C (Figure 5.6a). As the temperature increases, the time-resolved emission of (dA)₁₈ begins to resemble that of (dA)₂ with both shapes appearing nearly identical at 80 °C (Figure 5.6d). These spectra were constructed from the TCSPC signals recorded under identical experimental conditions, and the relative amplitudes of the spectra are applicable.

A discussion of TCSPC decay modeling using the experimental setup is discussed in Chapters 2 and 3. All signals were fit to a sum of exponentials with an additional parameter, N_0 , describing the contributing number of photons from the TCSPC signals decaying on time scales much shorter than the FWHM of the IRF. The best-fit parameters of (dA)₁₈ and (dA)₂ recorded at 360 and 420 nm emission wavelengths are shown in Table 5.1 and 5.2 respectively. All traces were fit individually and these models only provide a general description of how (dA)₂ and (dA)₁₈ TCSPC decays change with emission wavelength and temperature. At 7 °C for (dA)₁₈, τ_1 comprises approximately 70% of the decay amplitude with τ_2 comprising the majority of the remaining decay amplitude. As temperature increases τ_1 decreases while τ_2 increases, however, at temperatures higher than 7 °C τ_1 comprises the majority (95%) of the decay amplitude. At 7 °C for (dA)₂, τ_1 comprises about 65% of the decay amplitude with τ_1 again comprising the majority of the remaining decay amplitude. As with (dA)₁₈, τ_1 for (dA)₂ comprises approximately 90% of the decay amplitude at higher temperatures. There is an initial increase in τ_1 and τ_2 when increasing the temperature from 7 °C to 30 °C then τ_1 decreases as the temperature increases to 80 °C. This seemingly contradicts the decays shown in

Figure 5.1, but can be explained by the contribution of each component to the overall emission decay as discussed in section 5.3.2

5.2.2 Transient Absorption Signals

The ground state bleach recovery signals (267 nm pump/ 250 nm probe) recorded for (dA)₂ (a) and (dA)₁₈ (b) in aqueous buffer solution with 0.1 NaCl at 7 °C and 80 °C are shown in Figure 5.6. Similar to the TCSPC traces in Figure 5.2 the amplitudes of the signals decrease with increasing temperature. The decay of all signals appears to be nearly complete 1000 ps after excitation.

The fitting parameters from fits to the fs-TA signals in Figure 5.6 are reported in Table 5.2. A biexponential fit yields a lifetime of a few ps and a longer lifetime on the order of 100-1000 ps, with a slight offset consisting of no more than 2% of the decay amplitude for all four signals. As the temperature increases the lifetimes represented by τ_2 become faster. At 7 °C the (dA)₂ τ_2 lifetime is distinguishably longer than the (dA)₁₈ τ_2 lifetime. As temperature is increased to 80 °C there is no statistical difference between the lifetimes of the two oligomers.

5.3 Discussion

5.3.1 Temperature Dependent Dynamics

Emission from (dA)₂ and (dA)₁₈ in solution decays on faster timescales and drops in intensity as the temperature increases (Figure 5.1). The TCSPC decays from (dA)₁₈ and (dA)₂ at 7 °C display vastly different dynamics with (dA)₁₈ exhibiting greater emission intensity and faster decay times. These observations agree with the fs-TA

decays in Figure 5.6. Modeling the fs-TA decays shows the τ_2 component of (dA)₂ recorded at lower temperature is approximately 2x slower than τ_2 of (dA)₁₈. As the temperature increases the τ_2 components from each sample decrease to a point where they are no longer statistically distinguishable.

Temperature-dependent studies of DNA oligomers have been traditionally used to identify the fraction of stacked bases. Utilizing steady-state measurements like NMR, CD and UV hypochroism and a simple two state model (stacked or unstacked), base stacking interactions were found to be weakly stabilized at room temperature with the ΔG of stacking between two adenine bases estimated at $-0.9 \text{ kcal mol}^{-1}$.²¹⁻²³ The weak nature of these base stacking interactions in aqueous solution suggest a distribution of stacked domain lengths in (dA)₁₈.

Time-resolved temperature dependent studies of single stranded oligomers have been traditionally used to disrupt base stacking in an attempt to identify dynamics linked to base stacking interactions. A temperature dependent fs-TA study on poly(A) showed that as temperature decreases the fractional amplitude from the long-lived fs-TA signal decreased as the fractional amplitude of the faster component increased.¹⁷ The authors concluded that base stacking interactions were responsible for the long-lived component. The increase in the fractional amplitude from the ultrafast portion of the signal was the result of enhanced monomer decay from portions of the strand that corresponded to unstacked bases.

While temperature dependent studies are useful for determining the impact of base stacking on the deactivation kinetics, other methods to disrupt base stacking can also

be employed. Different solvents, such as methanol, or decreasing the pH have been used to identify dynamics of base stacking interactions in adenine single strands.²⁻⁴ A fs-TA study comparing the effectiveness of temperature and methanol as means for eliminating base stacking interactions shows methanol concentrations of 50% completely removes any contribution from the long-lived component while a long-lived signal was still present, although attenuated, at temperatures of 75 °C in aqueous solution.²

The decrease in relative intensity or fractional contribution from TCSPC and fs-TA decays on the 100 ps timescale with increasing temperature (Figures 5.1 and 5.6) agree with the aforementioned studies. As discussed in Chapter 3, the TCSPC technique and the fs-TA technique have been shown to monitor the same excited-state dynamics on the 100 ps time scale. Given the lack of time-resolved fluorescent studies detailing the temperature dependence of adenine oligomers, the TCSPC results will be compared with previous fs-TA results. As temperature increases the intensity of the TCSPC signal on the 100-500 ps time scale decreases in comparison to signals at lower temperatures (Figure 5.1), presumably due to the decrease in fraction of stacked bases. It is important to note the intensities of the TCSPC signals shown here are applicable as the signals were collected back-to-back under identical experimental conditions. The % N_0/N component describes the percentage of photons emitted on timescales faster than the FWHM of the instrument response function (approximately 50 ps) compared to all photons emitted. As temperature increases the % N_0/N component increases, suggesting that the fractional emission from the monomer is increasing as base stacking is disrupted. Although reduced, there is still a significant amount of emission decaying within the first ns at the

highest temperatures of 80 °C for both oligomers, suggesting that base stacking interactions are still present. These results are in agreement with previous fs-TA temperature dependent measurements of adenine dinucleotides.²

The changes in the deactivation kinetics of (dA)₁₈ as temperature increases can be explained by changes in the average domain length. Base stacking is modest compared to $k_B T$,^{9, 24} and given the probability of stacking it can be inferred that a variety of stacking domains of different lengths exist in a single stranded oligomer like (dA)₁₈. The adenine dinucleotide in this study models a stacking domain consisting of 2 bases, while the (dA)₁₈ oligomer models longer, yet still variable, stacking domains with the average length extending 5-6 bases.⁹ As the temperature increases the fraction of stacking is suggested to decrease. Since the average number of bases in a stacked domain is estimated by the probability of base stacking between two bases it can be inferred that decreasing the fraction of stacked bases would reduce the average domain length.

The average number of base present in a stack domain could explain the difference in dynamics between the (dA)₂ and (dA)₁₈ oligomer at 7 °C (Figure 5.2). While (dA)₁₈ can exhibit stacked domains of 2 bases, base stacking probability suggest the average stack is longer. When the temperature of the solution is increased to 80 °C the TCSPC signals from (dA)₂ and (dA)₁₈ are virtually indistinguishable in contrast to signals collected at lower temperatures. The similarity between the TCSPC signals at higher temperatures in contrast to the differences observed at low temperatures, coupled with the assumption the fraction of bases present in a stacking conformation decrease with increasing temperature, suggest the average stacked domain length in (dA)₁₈ is also

decreasing with increasing temperature. From these results we can conclude the average number of bases in a stacked domain of (dA)₁₈ is close to 2 at 80 °C. More importantly, we can infer domain length significantly influences the deactivation kinetics of adenine oligomers.

Concluding domain length significantly influences the excited state dynamics of adenine hinges on the observation that (dA)₂ and (dA)₁₈ exhibit significantly different deactivation kinetics at lower temperatures, but not at higher temperatures. Differences in dynamics between (dA)₂ and (dA)₁₈ detailed by the fs-TA and TCSPC decays reported here are difficult reconcile with a previous fs-TA study on adenine oligomers of varying length. In this study Su et al.³ reported a global lifetime modeling the long-lived fs-TA signals from adenine oligomers ranging in size from 2 to 18 bases. The global lifetime suggest there is no significant difference in the lifetimes exhibited by adenine oligomers of various lengths leading the authors to propose excitations responsible for the long-lived fs-TA are localized to two bases. The differences in the observations reported by Su et al.³ and those reported here and in Chapter 4 can be explained by the sample apparatus used in the fs-TA experiments. The temperatures of the samples in the study by Su et al. were estimated to be around 40 °C due to heating by the pump laser. Given the time-resolved signals of adenine oligomers of different length are nearly indistinguishable at higher temperatures, it is possible that global modeling of the long-lived component is an accurate description of the dynamics recorded at these higher temperatures. However, the differences in lifetimes and dynamics of (dA)₂ and (dA)₁₈ are distinguishable at room temperature and 7°C and cannot be accurately described by global lifetimes linking

lifetimes of the two samples as determined by the Akaike information criterion (see Chapter 3).

5.3.2 Domain Length

The similar kinetics observed from (dA)₂ and (dA)₁₈ at higher temperatures in contrast to the differences in kinetics observed at low temperatures can be explained by the dependence of the average domain of (dA)₁₈ on temperature. Chapter 4 describes the possible implications domain length has on the polarity of the surrounding environment and how that could influence the dynamics of these oligomers. However, it is important to address other possible excited state deactivation pathways which would also be effected by differences in domain length.

It has been established that long-lived signals from single stranded oligomers measured by fs-TA and time-resolved emission techniques, decaying on time-scales 2-5 orders of magnitude slower than the constituent monomers, result from base stacking interactions.^{3, 4, 10} While base pairing does influence the dynamics of double stranded systems a detailed description of base pairing interactions on the deactivation kinetics is outside the scope of this discussion. There are multiple experimental and computational studies that provide evidence the long-lived signals in DNA oligomers are the result of excimer or CT states.^{12, 14-16} However, the extent to which these states are delocalized is still debated. Some studies provide evidence that CT (or excimer) states are localized to two stacked bases while other studies provide evidence of excited state delocalization over multiple bases of a well stacked domain.

DNA has been shown to mediate CT over long distances of stacked domains.²⁵⁻²⁷

The mechanisms of charge transport in DNA are described by charge hopping, super exchange or charge delocalization over stacked domains.²⁶ Recently a study by Zinth and coworkers examined various single stranded systems of varying length composed of deoxyadenine, the modified nucleobase 5-methyl2'-deoxycytidine, and deoxyuridine.¹⁹ Vibrational marker bands of adenine consistent with a CT state were observed after selective UV excitation of 5-methyl2'-deoxycytidine. Contributions from the adenine anion were still observed upon examination of longer single stranded systems in which multiple deoxyuridine bases separated the deoxyadenine and 5-methyl2'-deoxycytidine. The authors proposed a model describing the formation of a charge separated state on an ultrafast time-scale followed by delocalization of the electron over multiple bases occurring on timescales around 5 ps. The delocalized electron would then recombine with the hole on the 100 ps timescale.

Delocalized states over well stacked domains have also been proposed for adenine oligomers,²⁸ while others suggest states responsible for the long-lived dynamics are localized to two bases.³ The fs-TA study by Su et al.³ discussed above reported a global lifetime describing the long-lived component of adenine systems ranging from 2 to 18 bases. Additionally, comparing signals from the adenine dinucleotide in neutral and acidic solutions showed the long-lived fs-TA signal from the dinucleotide in neutral solution was not present in acidic solution. The authors concluded that the long-lived fs-TA lifetime was attributed to base stacking and given the same lifetime was identified for oligomers of various lengths, including (dA)₂, it was also concluded excitation of the

states responsible for the long-lived lifetime were localized to two stacked adenine bases. As discussed in Section 5.3.1, while the model of the fs-TA signals described by Su. et al. is accurate at higher temperatures it is not representative of the dynamics at lower (7 °C – 25 °C) temperatures. Given the difference in dynamics between (dA)₂ and (dA)₁₈ was not recognized by Su et al. due to the elevated temperature of the sample solution it is important to consider the possibility that excimer or CT states can delocalize over multiple (>2) bases.

Charge separation and transport over multiple bases followed by charge recombination recombining have also been proposed.^{18, 20, 29, 30} Described as high-energy long-lived mix states, these states are proposed to be characterized by ns emission at high emission energies similar to that of monomer emission. Domain length could influence the charge transport in these systems, however, these states have only been described in double stranded DNA. Additionally, any intrinsic high energy long lived emission observed from these decays is masked by the buffer signal. A discussion of background emission and buffer signals can be found in Chapter 3.

Yet another way domain length can influence the excited-state dynamics of oligomers is observed in Chapter 4. Differences in solvation environments as a result of differences in average stacked domain lengths could explain the differences in dynamics observed between (dA)₁₈ and (dA)₂. These different solvation environments could result in excimer states with various degrees of CT, with more polar environments resulting in greater CT character and red-shifted emission as shown by the steady-state emission data in Chapter 4. The red-shifted emission maximum of (dA)₂ in comparison to (dA)₁₈ is

also observed by the time-resolved emission spectra shown in Figure 5.3 and 5.4. The time-resolved emission spectra for (dA)₂ is clearly red-shifted in regard to (dA)₁₈, with (dA)₂ peaking around 420 nm and (dA)₁₈ peaking around 360 nm. This matches quite well with the SS emission at 20 °C discussed in Chapter 4 and other SS emission studies.¹³

The spectral maxima of the time-resolved emission spectra in Figures 5.3 and 5.4 are similar for the first ns after excitation for all temperatures with the exception of (dA)₁₈ at 80 °C (See Section 5.3.3). As the temperature increases the time-resolved spectra of (dA)₁₈ begins to broaden, maintaining the same emission maximum at 360 nm while expanding towards the red. The (dA)₂ exhibits similar behavior as temperature increases, but in this case the spectra remain peaked at 420 nm and expand towards the blue. Comparing the time-resolved spectra 100 ps after excitation from (dA)₁₈ and (dA)₂ at each temperature (Figure 5.5) shows that like the TCSPC decays in Figure 5.2, the spectra begin to resemble each other in amplitude and shape as temperature increases.

Increasing the temperature decreases the fraction of stacked bases and therefore the average domain length of (dA)₁₈. The broadening of the (dA)₁₈ time-resolved emission spectra to the red could be the result of increased solvation because of shorter average stacked domains. The τ_1 lifetime reported for the TCSPC signals (Table 5.1) becomes faster with increasing temperature while τ_2 becomes slower. The trend observed in Figure 5.1 is that the lifetimes become shorter with increasing temperature. This suggest that τ_1 is the dominant decay component at higher temperatures as this most closely resembles the TCSPC decays. It is also observed τ_1 significantly increases in

fractional amplitude as the temperature is increased from 7 °C to 30 °C providing further support τ_1 describes the majority of the emission kinetics at higher temperatures.

Chapter 4 provides evidence τ_2 is related to states with a greater amount of CT character. The longer lifetimes seen at higher temperatures for τ_2 of (dA)₁₈ agrees with the observation that states with greater degrees of solvation or CT character decay on longer time scales. As the average domain length of (dA)₁₈ decreases the solvation should become more polar resulting in states with a CT character. This is also demonstrated by the red-shifted broadening of the time-resolved emission spectra with increasing temperature.

These observations correspond nicely with those made in Chapter 4, providing further evidence that the solvation environment significantly effects the deactivation kinetics of adenine single strands. This however does not discount the possibility that these effects are not the result of delocalized excimers. Well stacked domains are a requirement of delocalization or charge transport. If delocalized excimers are populated upon excitation, changing the average domain length in (dA)₁₈ would impact the number of bases which the excimer could delocalize over. It is possible that both solvation and delocalization effect the excited state dynamics of adenine.

Regardless of the deactivation mechanism dependent on domain length, the red-shifting of the (dA)₁₈ time-resolved emission spectra provide additional evidence the average number of bases in a stacked domain influence the dynamics of these systems. In addition to lifetimes, (dA)₁₈ and (dA)₂ exhibit significantly different steady-state and time-resolved emission maxima at lower temperatures, but produce similar time-resolved

emission spectra. Again, it is suggested domain length significant influences the dynamics of these systems.

5.3.3 Assignment

A detailed lifetime assignments as conducted in Chapter 4 becomes challenging because of the drastic change in TCSPC decay parameters for (dA)₂ (Table 5.2). The heterogeneous nature of the (dA)₂ TCSPC decay suggest that solvation is not the only factor governing the excited state dynamics of stacked adenines. Increasing the temperature decreases the average lifetime of (dA)₂ as evidence by the TCSPC decays in Figure 5.1, the fs-TA decays in Figure 5.6 and fitting bleach recovery parameters in Table 5.3. The TCSPC fitting parameters in Table 5.2 show that as temperature increases from 7 °C to 30 °C both τ_1 and τ_2 become slower, contradicting the emission decays in Figure 5.1. Like the (dA)₁₈ TCSPC fitting parameters, the fractional contribution from τ_1 for (dA)₂ increases significantly when the temperature is increased from 7 °C to 30 °C. As seen in Chapter 4, the longer τ_2 lifetime represents the majority of the TCSPC decay intensity for (dA)₂. As the temperature increases from to 30 °C τ_1 begins to represents the majority of the TCSPC decay intensity for (dA)₂. Comparing the τ_2 lifetime at 7 °C with the τ_1 lifetime at 30 °C shows that the lifetimes contributing to the majority of the decay intensity decrease with increasing temperature. At temperatures higher than 30 °C τ_1 contributes to the majority of emission on decaying within the first ns after excitation.

As the temperature increases the time-resolved emission of (dA)₂ decreases and the time-resolved emission spectra begin to broaden shifting towards the blue. As suggested by multiple computational studies,^{12, 14, 15, 31} excimer states with various

degrees of CT or bonding character can exist between two stacked adenines. This is supported by the heterogeneous TCSPC decays observed for (dA)₂, as the solvation environment is assumed to be unchanging around the average stacked base pair in the (dA)₂ oligomer unlike the longer (dA)₁₈ oligomer. The increase in temperature could shift the distribution of states towards higher energy excimers. These higher energy excimers would result in greater monomer-like and less CT character. Building off the conclusions in Chapter 4 this would shift the emission to the blue and result in faster lifetimes.

The dependence of the (dA)₂ lifetimes on temperature suggest additional factors in addition to domain length influence the dynamics of these systems. Given the observations from Section 5.3.1 and 5.3.2 we propose domain length is the main factor governing the deactivation in (dA)₁₈. While additional factors, such as base stacking geometry, responsible for the temperature dependence of the (dA)₂ lifetimes might influence the dynamics of dA₁₈ we have to consider dA₂ has only one stacking domain and might exhibit more sensitivity to differences in base stack geometry.

5.3.4 Fluorescent Impurities

As mentioned above the dynamics in Figure 5.1 all follow the same trend. As temperature increases the slope and amplitude decreases, with the exception of the ns emission of (dA)₁₈ at 80 °C. Additionally, there is a maximum observed at 430 nm for time-resolved emission spectra 500 ps and 1 ns after excitation (Figure 5.3). These spectral features could be the result of increased solvation, however this could also be the result of a solute impurity. As shown in Chapter 3 different purification methods resulted in different emission intensities from the ns component. The (dA)₁₈ sample used in this

investigation was supplied by Midland with GF grade purity. GF grade is the standard purification process and is of lower quality than the HPLC grade. Previous adenine samples supplied by Midland with GF grade purification revealed stronger emission around 440 nm in comparison to other suppliers or purity grades. Emission from this ns component dominates the integrated (dA)₁₈ emission at 80 °C and is therefore excluded from this analysis. It is possible a highly fluorescent impurity present in low concentrations in the solute are contributing to this emission. Repeat experiments utilizing higher grade oligomers are needed in order to determine if the spectral features of (dA)₁₈ at 80 °C are the result of a solute impurity or are the result of intrinsic (dA)₁₈ fluorescence.

5.4 Conclusion

Subnanosecond emission from (dA)₂ and (dA)₁₈ becomes faster and loses intensity as the temperature of the solution is increased. The loss in emission intensity is interpreted as a decrease in the average number of stacked bases as base stacking is a weakly favorable interaction in room temperature solution. TCSPC and fs-TA decays show (dA)₁₈ and (dA)₂ exhibit very different dynamics at 7 °C, but begin to show similar dynamics at higher temperatures. Increasing the temperature not only disrupts base stacking interactions, but reduces the average domain length of (dA)₁₈. The similarity in dynamics between (dA)₂ and (dA)₁₈ at higher temperatures suggest the average domain length of (dA)₁₈ begins to approach that of (dA)₂.

By changing the domain length, the solvation of the average stacked base pair in $(dA)_{18}$ is expected to increase resembling the solvation of $(dA)_2$. Examination of the emission lifetimes reveals complex multiexponential dynamics for both $(dA)_{18}$ and $(dA)_2$ at low and high temperatures. This suggest domain length is not the only factor influencing the dynamics of these systems, and other explanations such as differences base stacking geometries should be considered.

There are multiple proposed deactivation mechanisms that could be influenced by the average domain length. Additional investigations are needed to identify if charge delocalization, solvation effects or other mechanisms are responsible for these long-lived lifetimes. Systems with well characterized stacking domains such as the PEG-linked dumbbell discussed in Chapter 3 provide promising opportunities. Such systems can be synthesized to different lengths and the stability of these systems in room temperature solution would stabilize the stacking domain length.

Table 5.1 Best-fit parameters for emission decays from (dA)₁₈ at emission wavelengths of 360 and 420 nm.

Emission λ / nm	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
(dA) ₁₈ 7°C							
360	207 ± 9	66	480 ± 14	33	4.7 ± 0.2	1	10
420	264 ± 14	75	640 ± 32	23	5.8 ± 0.4	1	5
(dA) ₁₈ 30°C							
360	167 ± 6	95	740 ± 88	4	4.6 ± 0.2	1	15
420	186 ± 10	92	720 ± 100	7	6.1 ± 0.5	1	7
(dA) ₁₈ 55°C							
360	115 ± 6	95	830 ± 120	3	4.3 ± 0.3	1	22
420	138 ± 10	94	900 ± 200	4	6.7 ± 0.6	2	13
(dA) ₁₈ 80°C							
360	91 ± 8	94	950 ± 140	5	4.6 ± 0.4	1	27
420	103 ± 12	91	1100 ± 350	5	11 ± 0.6	4	5

[a] Percent of prompt photons to all photons.

Table 5.2 Best-fit parameters for emission decays from (dA)₂ at emission wavelengths of 360 and 420 nm.

Emission λ / nm	τ_1 (ps)	% A_1	τ_2 (ns)	% A_2	τ_3 (ns)	% A_3	% $N_0/N^{[a]}$
(dA) ₂ 7°C							
360	155 ± 7	67	0.33 ± 0.01	31	3.3 ± 0.2	0.7	19
420	170 ± 12	62	0.36 ± 0.02	37	3.2 ± 0.3	1.6	5
(dA) ₂ 30°C							
360	285 ± 20	89	1.5 ± 0.2	8	5.8 ± 0.4	3.5	23
420	285 ± 12	89	0.72 ± 0.8	10	5.5 ± 0.4	1.8	7
(dA) ₂ 55°C							
360	188 ± 20	88	1.3 ± 0.2	9	6.5 ± 0.5	3.3	28
420	188 ± 12	94	0.96 ± 0.2	4	5.7 ± 0.5	2.0	12
(dA) ₂ 80°C							
360	141 ± 20	85	1.1 ± 0.2	10	6.8 ± 0.6	4.3	33
420	127 ± 14	93	1.1 ± 0.3	5	6.3 ± 0.6	2.4	15

[a] Percent of prompt photons to all photons.

Table 5.3. Best-fit parameters for the (dA)₂ and (dA)₁₈ transient absorption signals in Figure 5.6 recorded at 7 °C, 30 °C, 55 °C and 80 °C. The ground-state bleach recovery signal (negative) was fit to a biexponential function with a constant offset: $\Delta A = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3$.

Sample	τ_1 (ps)	% A_1	τ_2 (ps)	% A_2	% A_3
(dA) ₂ 7 °C	2.3 ± 0.2	71	502 ± 48	28	1
(dA) ₂ 80 °C	2.9 ± 0.6	84	280 ± 150	15	2
(dA) ₁₈ 7 °C	3.1 ± 0.6	59	271 ± 28	39	2
(dA) ₁₈ 80 °C	2.9 ± 0.6	69	129 ± 40	29	2

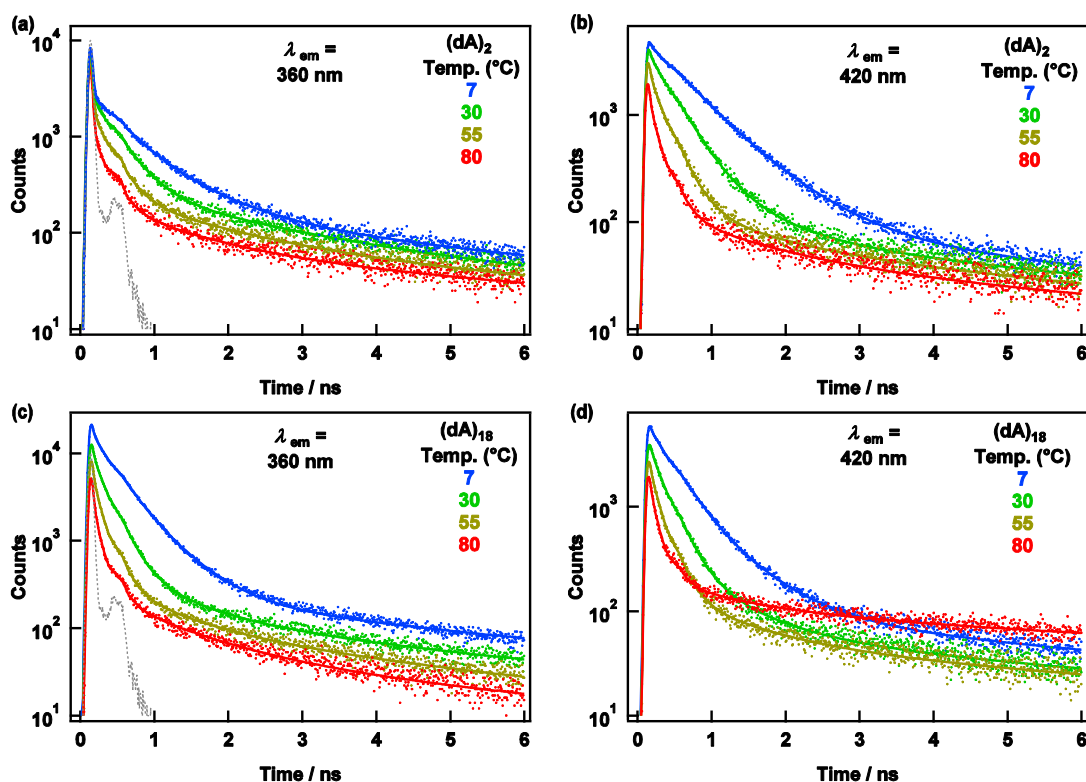


Figure 5.1. Fluorescence decays (dots) and fits (solid curves) from (dA)₂ at emission wavelengths of 360 nm (a) and 420 nm (b) and (dA)₁₈ at emission wavelengths of 360 nm (c) and 420 nm (d) in 10 mM Tris-HCl buffer with 0.25M NaCl recorded at 7 °C (blue), 30 °C (green), 55 °C (gold), and 80 °C (red). All traces are shown on a logarithmic scale. The light gray trace in panels (a) and (c) is the instrument response function.

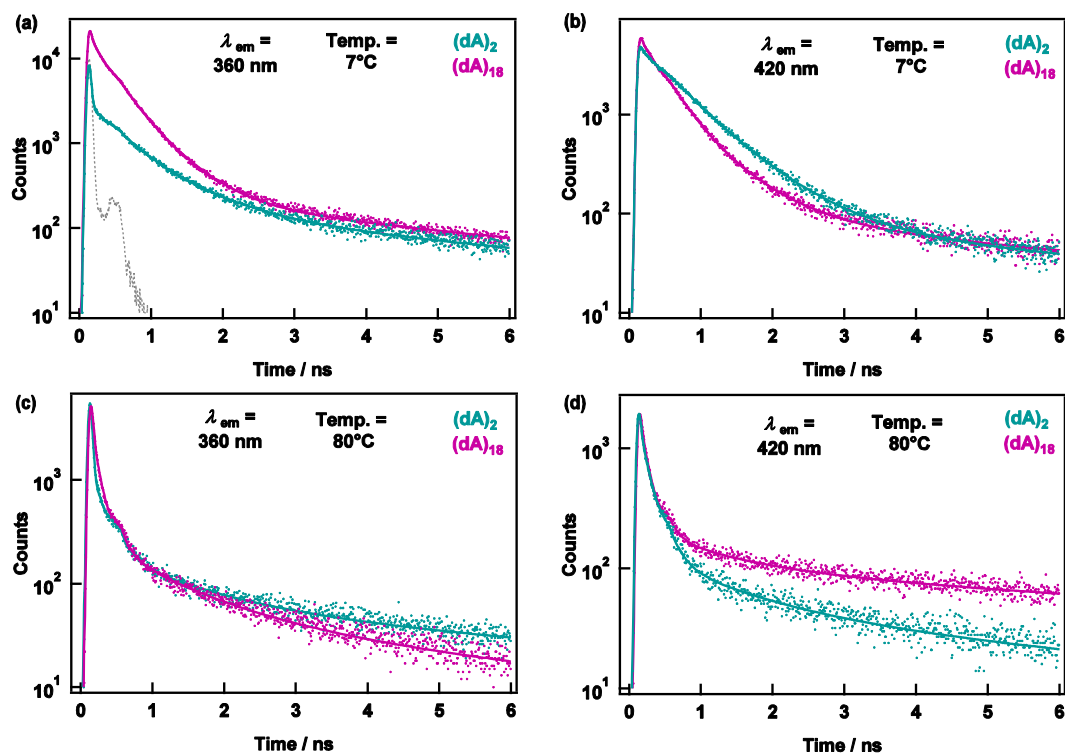


Figure 5.2. Fluorescence decays (dots) and fits (solid curves) from $(dA)_2$ and $(dA)_{18}$ at emission wavelengths of 360 nm (a) and 420 nm (b) recorded at 7°C and emission wavelengths of 360 nm (c) and 420 nm (d) recorded at 80°C in 10 mM Tris-HCl buffer with 0.25M NaCl. All traces are shown on a logarithmic scale. The light gray trace panel (a) is the instrument response function.

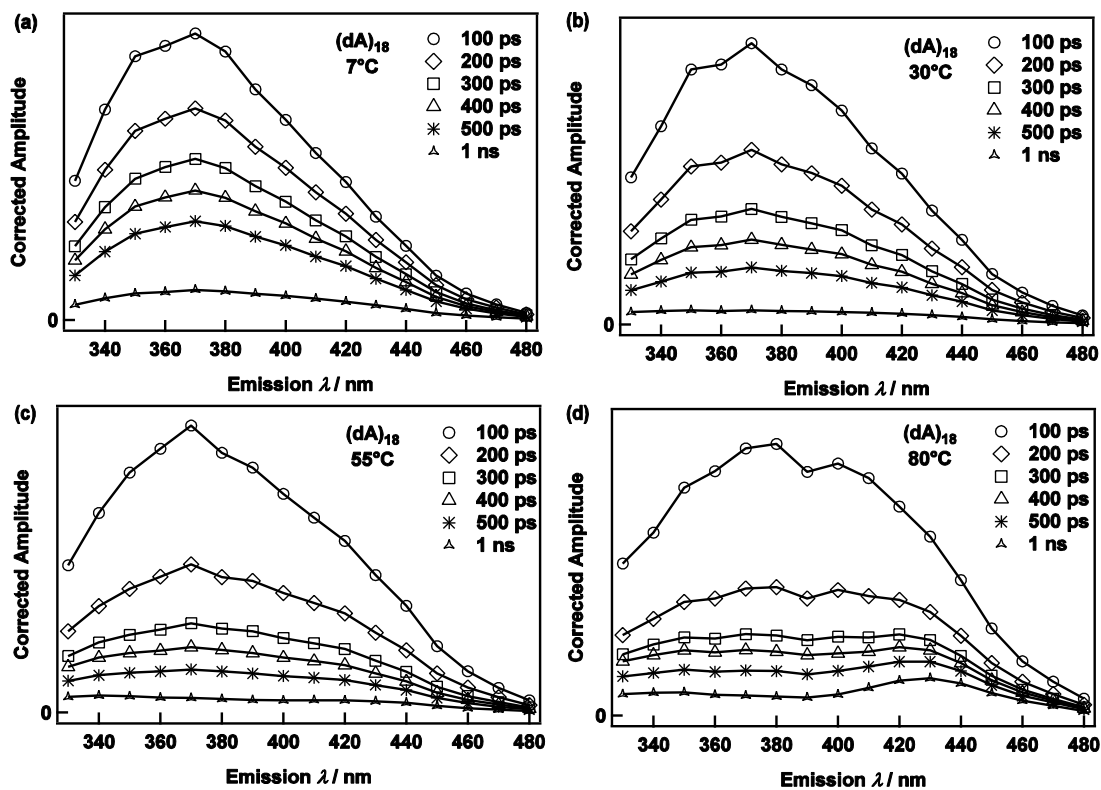


Figure 5.3. Time-resolved emission spectra on a linear scale from $(dA)_{18}$ at 7 °C (a), 30 °C (b), 55 °C (c), and 80 °C (d) from 100 ps to 1 ns after excitation at 265 nm. Shown on a linear scale. The vertical scale differs in all four panels.

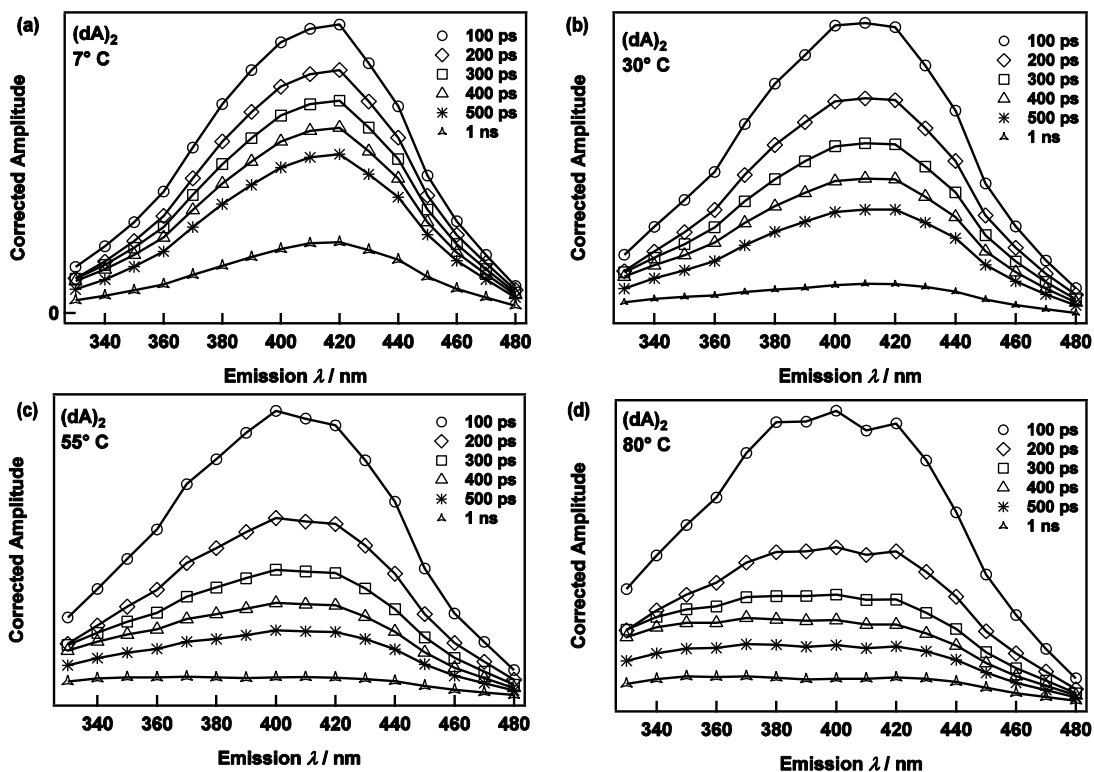


Figure 5.4. Time-resolved emission spectra from $(dA)_2$ at 7 °C (a), 30 °C (b), 55 °C (c), and 80 °C (d) from 100 ps to 1 ns after excitation at 265 nm shown on a linear scale. The vertical scale differs in all four panels.

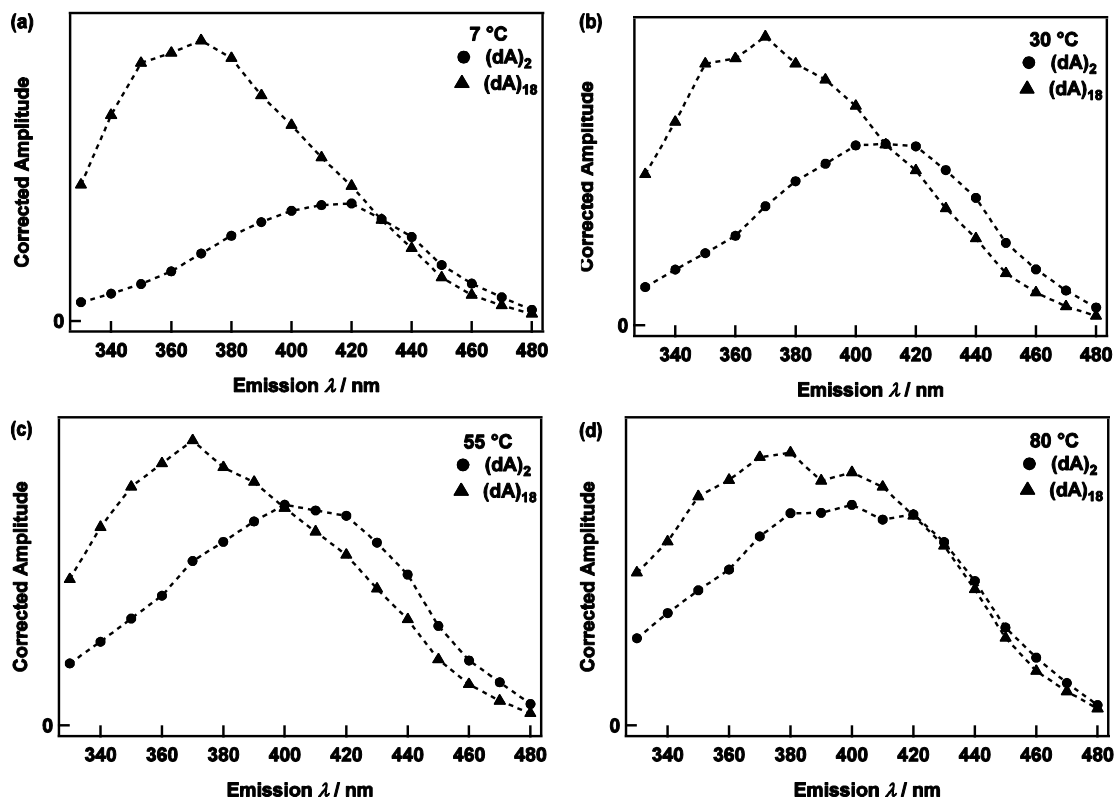


Figure 5.5. Time-resolved emission spectra from $(dA)_2$ (circles) and $(dA)_{18}$ (triangles) at 7 °C (a), 30 °C (b), 55 °C (c), and 80 °C (d) from 100 ps after excitation at 265 nm show on a linear scale. The vertical scale differs in all four panels.

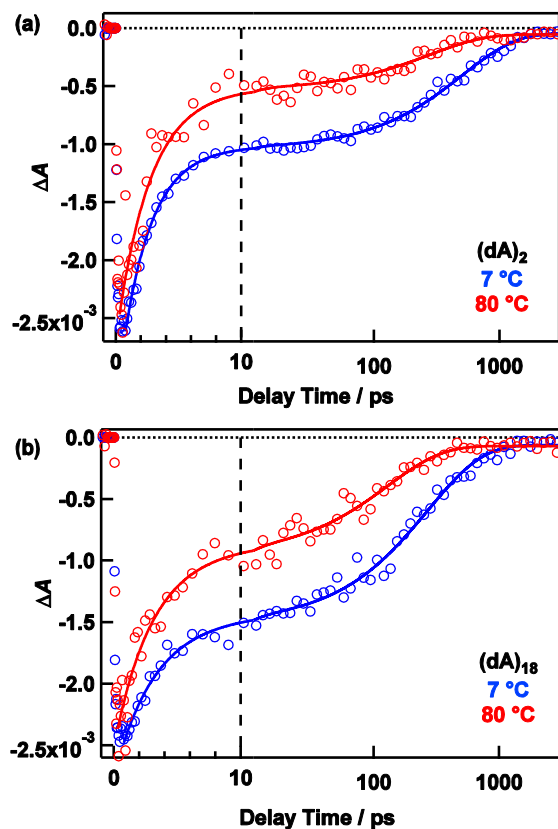


Figure 5.6 fs-TA signal (267 pump/ 250 probe) of $(dA)_2$ (a) and $(dA)_{18}$ (b) in aqueous buffer solution containing 0.1 M NaCl recorded at 7 °C (blue) and 80 °C (red). The solid curve is a nonlinear least-squares fit to two exponentials plus an offset. The vertical dashed line denotes the transition between the linear and logarithmic portions of the time axis. The fs-TA signals were collected by Dr. Yuyuan Zhang and Dr. Ashley Beckstead.

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CHAPTER SIX

SUMMARY AND OUTLOOK

The time-resolved emission from model AT DNA systems was examined using the time-correlated single-photon (TCSPC) technique. Special attention was devoted to the single-stranded adenine oligomer to identify the fundamental processes governing excited-state deactivation. Characterization of the TCSPC signals and insights into the dynamics of the model oligomers can be used as a basis for explaining more complex DNA multimers.

In Chapter 3, $(dA)_{15}$, $d(AT)_9 \cdot d(AT)_9$, and the $d(A)_{10} \cdot d(T)_{10}$ dumbbell were examined using the TCSPC technique. Emission from each oligomer was described by multiexponential decays ranging from 50 ps to multiple nanoseconds. The lifetimes decaying on the order of 50-200 ps were shown to match lifetimes identified by the transient absorption technique (fs-TA). The agreement suggests the two techniques were monitoring the same excited states. Emission on the 50 – 200 ps time was assigned to excimer-like or charge recombination luminescence.

The sensitivity of the TCSPC technique along with a time observation window extending from the ps to μ s time scale often highlights complex dynamics typically not highlighted by other time-resolved techniques. Often result from the fs-TA are hard to reconcile with results from the TCSPC technique which have led to conflicting views of how to describe the excited state dynamics of DNA. Identifying the emission lifetimes identified from the TCSPC technique mirrored the fs-TA absorption lifetimes showed

these two techniques complement each other allowing us to move past the unspoken, but implied, debate of which technique more accurately describes the excited state dynamics of DNA systems.

While the lifetimes between the TCSPC and fs-TA were shown to agree on the 50- 200 ps time scale, the TCSPC technique decaying identified additional lifetime decaying out to the ns time region. The lifetimes identified for (dA)₁₅ also experienced a wavelength dependence. The multiple lifetimes and emission energy dependence suggested excited states of multiple identities were decaying on the subns to ns timescale.

To understand the factors influencing the emission lifetimes from adenine systems an adenine dinucleotide and 18-mer were examined by the TCSPC technique (Chapter 4). In addition to length dependence Chapter 4 also details the dynamics of these systems in D₂O buffer solution. A KIE of 1.5-2.5 was observed for both adenine systems. This is the first time a KIE has been identified in adenine single stranded systems, which are not typically associated with proton transfer. In absence of proton transfer the KIE was suggested to be the result of coupling of the adenine vibrational modes to the CT state. Traditionally the observation of a KIE in DNA systems is linked to excited state proton transfer, but the systems described as undergoing proton transfer conflicted with the systems identified by time-resolved infrared studies. The observation of a KIE in the adenine oligomer suggest that excited state proton transfer does not have to be present for systems to exhibit a KIE.

The length was shown to significantly impact the dynamics of adenine oligomers. The steady-state emission reveals $(dA)_2$ was red-shifted in respect to $(dA)_{18}$, and TCSPC and fs-TA signals revealed $(dA)_2$ decays on slower time scales than $(dA)_{18}$. The difference in dynamics between $(dA)_2$ and $(dA)_{18}$ was attributed to differences in average base stacking domain length between the two oligomers. There are many proposed excited state mechanisms which could be effected by domain length such as solvation, charge delocalization and charge hopping. In the case of Chapter 4 the differences in dynamics between were attributed to differences in solvation.

Shorter stacking domain lengths were expected to give rise to a more polar surrounding environment than longer stacking domain lengths. Stacked bases present in a more polar environment were expected to give rise to excimer states described by a greater degree of CT than states in a less polar environment. It was assumed states with more CT character exhibited a KIE of greater magnitude than states described by less CT and more monomer like character. Using this information, the τ_1 and τ_2 emission lifetimes were described as excimer-like states with varying degrees of CT character.

The differences between the subnanosecond emission of $(dA)_2$ and $(dA)_{18}$ was suggested to be the result of differences in domain length. The literature presents conflicting information suggesting the subns dynamics are the same between adenine oligomers of different lengths. The observations in Chapter 4 suggest stacking domain length does significantly impact the dynamics of adenine oligomers.

To further explore the dependence of the subns dynamics of adenine oligomers on stacking domain length, $(dA)_2$ and $(dA)_{18}$ were examined at temperatures of 7 °C, 30 °C,

55 °C and 80 °C. It was observed that the TCSPC and fs-TA decays of (dA)₁₈ and (dA)₂ exhibit very different dynamics at 7 °C, similar to the observations made at 20 °C in Chapter 4. When the temperature of the solution is increased to 80 °C the TCSPC signals from (dA)₂ and (dA)₁₈ are virtually indistinguishable in contrast to signals collected at lower temperatures. It was concluded the difference in dynamics between (dA)₂ and (dA)₁₈ at lower temperatures was the result of domain length. As the temperature increases the probability of base stacking decreases resulting in shorter domain lengths. While different at lower temperatures the stacking domain length of (dA)₂ and (dA)₁₈ were suggested to be equal at higher temperatures.

There many proposed excited state mechanisms which could be effected by domain length such as solvation, charge delocalization and charge hopping. Giving the discussion in Chapter 4 assignments in Chapter 5 were also described in terms of differences in solvation. While the evidence in Chapter 4 suggest solvation does influences the subns dynamics of adenine oligomers, it does not rule out the possibility of charge delocalization or charge hopping. Additional experiments are needed in order to address the deactivation mechanisms dependent on domain length.

Systems with well characterized stacking domains such as the PEG-linked dumbbell discussed in Chapter 3 provide promising opportunities. Such systems can be synthesized to different lengths and the stability of these systems in room temperature solution would stabilize the stacking domain length. Examining these systems in deuterated buffer solution also provides promising possibilities.

While AT duplexes were examined in deuterated solution the results are not present here. The complexity coupled with the error associated with these signals did not provide any meaningful insight. Double-stranded systems exhibit effects such as end fraying that produce regions of bases that experience hydrogen bonding base pairing while other bases in other regions are unpaired. Given the sensitivity of the TCSPC technique it is theorized the heterogeneity of the double stranded systems are responsible for the complex TCSPC signals. It is suggested that linked dumbbells be used to investigate double stranded systems with the TCSPC technique.

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APPENDIX A

SUPPORTING INFORMATION FOR SUBNANOSECOND EMISSION DYNAMICS
OF AT DNA OLIGONUCLEOTIDES

Supporting Information**Subnanosecond Emission Dynamics of AT DNA Oligonucleotides**

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Supplementary Methods

The (dA)₁₈ samples were purchased from Integrated DNA Technologies. One sample was purified using the standard desalting process (DS), while the second was purified by reversed-phase HPLC. Both were used as received and dissolved in 10 mM Tris-HCl buffer with 0.25 M NaCl. The sample absorbance at 265 nm was adjusted to 0.3. Fresh samples were prepared for each TCSPC experiment. The emission decay curves of (dA)₁₈ that had been purified by the different methods (shown in Figure A1 in Appendix A) were recorded under identical experimental conditions, including the excitation pulse fluence. These experimental conditions were also identical to those used to acquire the (dA)₁₅ signals shown in the main text.

The time-resolved emission spectra shown in Figures 5 – 8 in the main text were corrected for the wavelength sensitivity of the detection system. The correction factors for different wavelengths were determined by a standard comparative method, where the spectrum recorded from an incandescent light bulb using the TCSPC instrumentation was compared to the known spectrum.

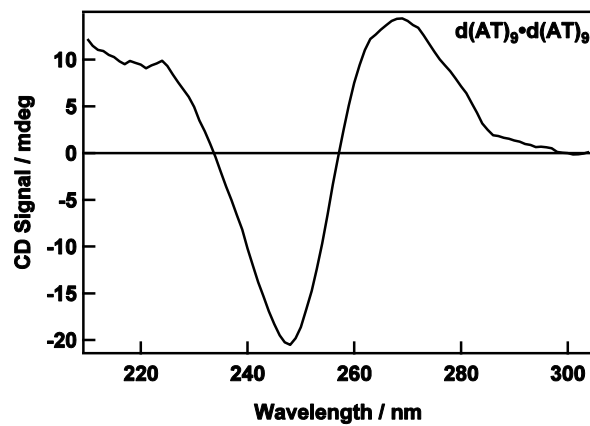


Figure A1. CD spectrum of the $d(AT)_9 \cdot d(AT)_9$ duplex in Tris-HCl buffer with 0.25 M NaCl measured at 20 °C.

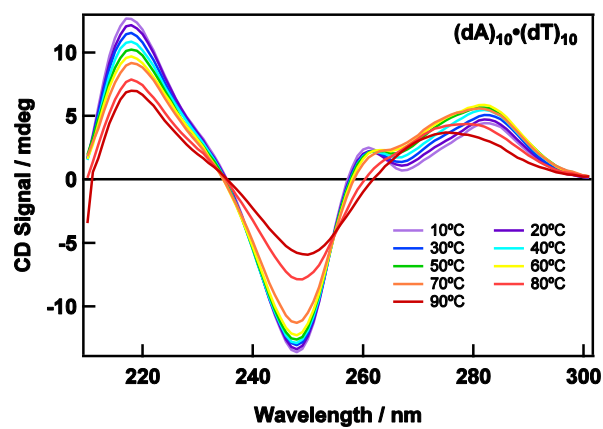


Figure A2. CD spectra of the $(dA)_{10} \cdot (dT)_{10}$ dumbbell in Tris-HCl buffer with 0.25 M NaCl measured at temperatures between 10 °C and 90 °C.

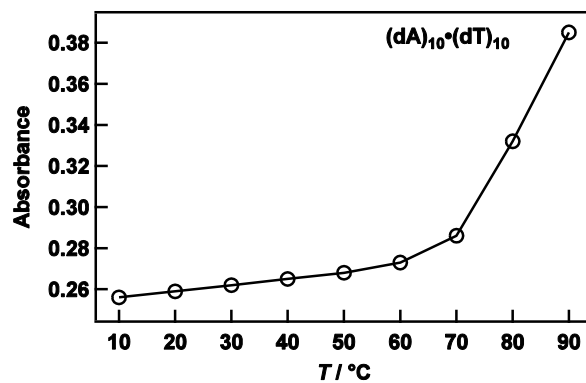


Figure A3. Melting curve for the $(dA)_{10} \cdot (dT)_{10}$ dumbbell in Tris-HCl buffer with 0.25 M NaCl measured at $\lambda_{\text{abs}} = 250$ nm.

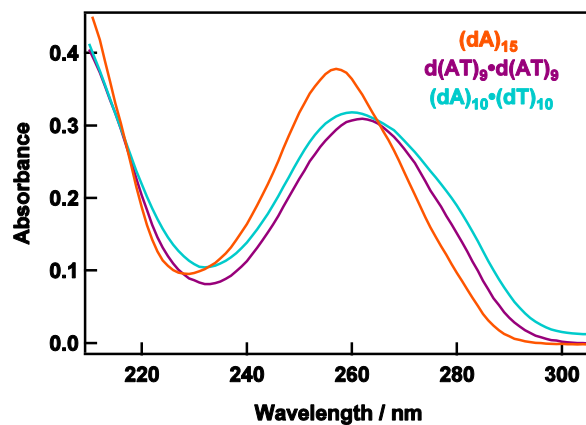


Figure A4. UV-visible absorption spectra for $(dA)_{15}$, $d(AT)_9 \cdot d(AT)_9$, and the $(dA)_{10} \cdot (dT)_{10}$ dumbbell in Tris-HCl buffer with 0.25 M NaCl. Concentrations were adjusted to yield an absorbance of 0.3 at 265 nm for all three samples.

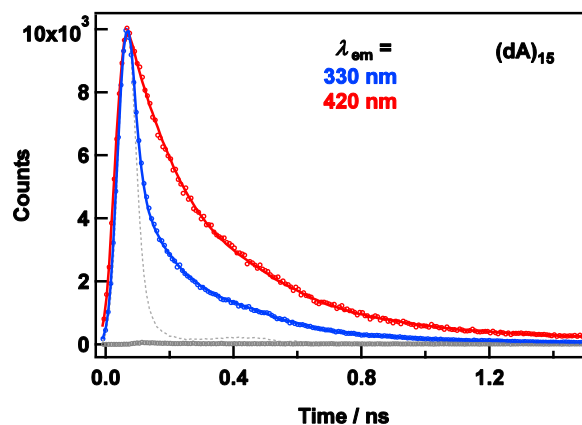


Figure A5. Fluorescence decays (markers) and their best fits (solid lines) at emission wavelengths of 330 nm (blue) and 420 nm (red) for $(dA)_{15}$ in Tris-HCl buffer with 0.25 M NaCl. The excitation wavelength is 265 nm. The traces are shown on a linear scale. The 330 nm emission from a solution containing only Tris-HCl buffer and 0.25 M NaCl is shown as the dark gray trace. The light gray trace is the instrument response function (IRF). The $(dA)_{15}$ emission at 330 nm and 420 nm and the IRF were scaled to have the same maximum peak intensity.

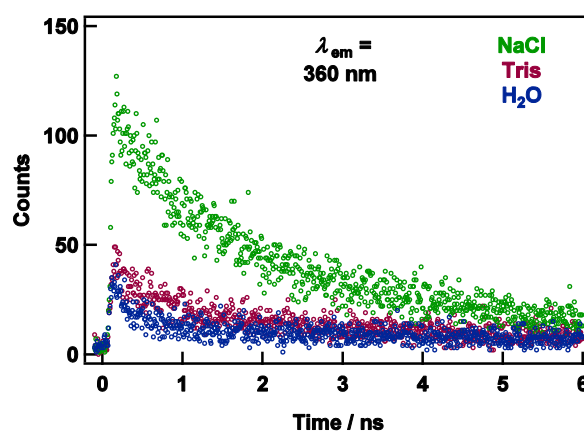


Figure A6. Fluorescence decays of neat water (blue), 0.25 M NaCl aqueous solution (green), and 10 mM Tris-HCl buffer solution without NaCl (red) collected at 360 nm following 265 nm excitation. The traces are shown on a linear scale. All signals were collected under identical conditions.

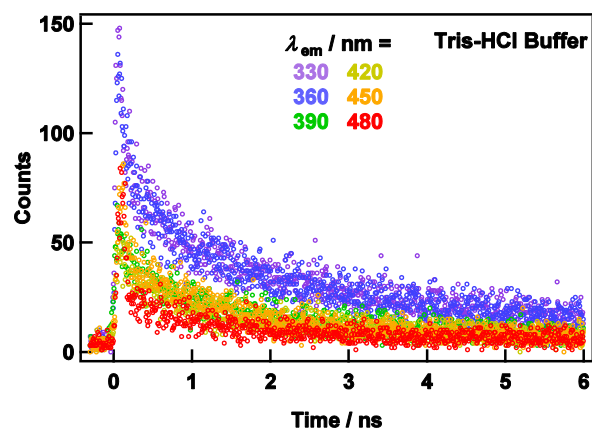


Figure A7. Fluorescence decays for a Tris-HCl buffer solution containing 0.25 M NaCl at the indicated emission wavelengths after 265 nm excitation. The traces are shown on a linear scale. All decays were collected under identical conditions.

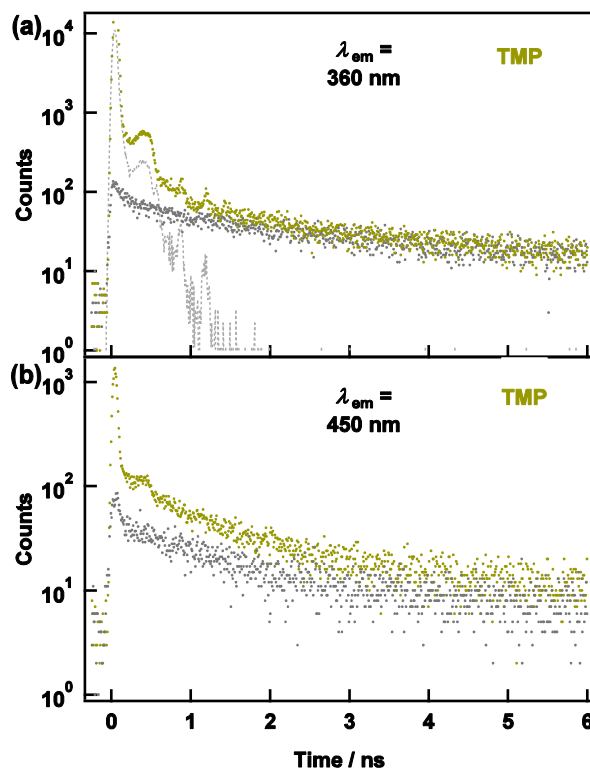


Figure A8. Fluorescence decays for TMP (gold) and Tris-HCl buffer containing 0.25 M NaCl (dark gray) at emission wavelengths of 360 nm (a) and 450 nm (b) after 265 nm excitation. The traces are shown on a logarithmic scale. The light gray trace in (a) is the instrument response function. All decays were collected under identical conditions.

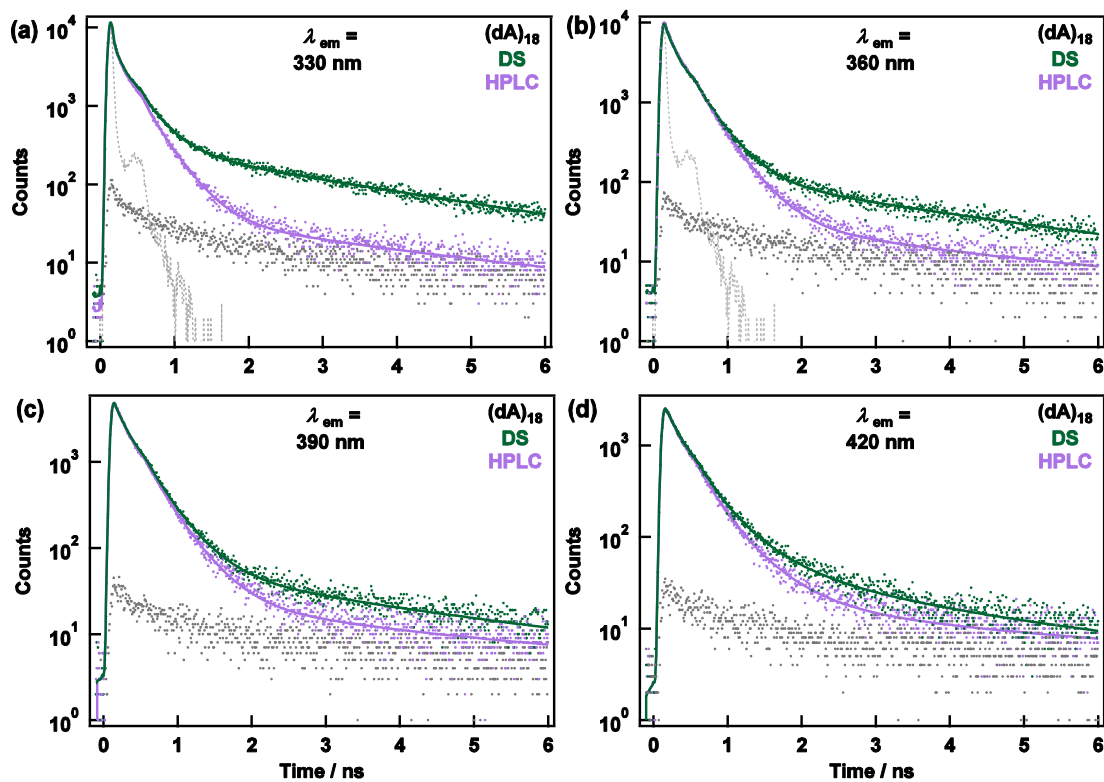


Figure A9. Fluorescence decays (markers) and fits (solid curves) for $(dA)_{18}$ purified by a standard desalting process (green) and by reverse-phase HPLC (purple) in Tris-HCl buffer containing 0.25 M NaCl at emission wavelengths of 330 (a), 360 (b), 390 (c), and 420 nm (d) after 265 nm excitation. All decays are shown on a logarithmic scale. The instrument response function (light gray) is shown in (a) and (b). The dark gray markers are the buffer signal at each emission wavelength.

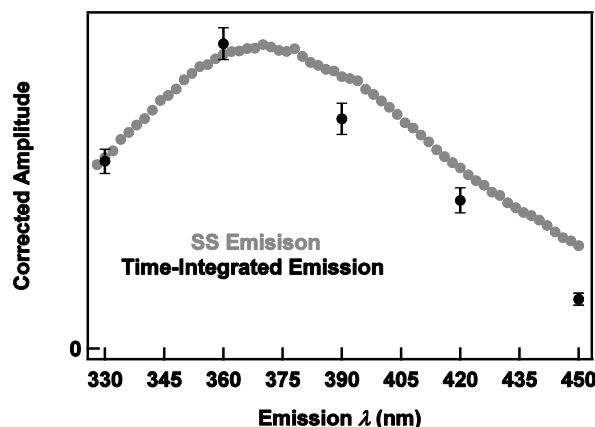


Figure A10. Emission spectrum obtained by integrating the emission decays at each emission wavelength (black circles with error bars) compared to the steady-state emission spectrum (gray circles) of (dA)₁₈ in Tris-HCl buffer.

Table A1. The reduced sum-of-squares (χ^2) and corrected Akaike information criterion (AICc) metric for global fits to decays from each of the three substrates at all emission wavelengths. Time constants shown in the first column were linked in fits for each substrate.^a

	(dA) ₁₅		d(AT) ₉ ·d(AT) ₉		(dA) ₁₀ ·(dT) ₁₀ Dumbbell	
Global Parameters ^b	χ^2	AICc	χ^2	AICc	χ^2	AICc
none	0.981	-295	0.9765	-330	0.9029	-155
τ_1, τ_2, τ_3	1.030	605	1.0195	334	0.9077	-148
τ_1	0.986	-215	0.983	-230	0.9029	-155
τ_2	0.986	-199	0.9783	-305	0.904	-153
τ_3	1.02	410	0.9819	-247	0.903	-155
τ_1, τ_2	0.988	-175	0.9832	-231	0.9063	-150
τ_1, τ_3	1.02	470	0.9971	-10	0.903	-156
τ_2, τ_3	1.02	507	1.0165	292	0.9047	-153

^a The lowest AICc value (shown in red font) indicates the best fit. See ref [2] for additional details.

^b τ_1 , τ_2 , and τ_3 represent the shortest, intermediate, and longest lifetime, respectively. Each parameter shown in this column was constrained to have the same value at each emission wavelength.

Table A2. Fitting parameters for emission decays from the (dA)₁₀·(dT)₁₀ dumbbell with τ_1 and τ_3 globally linked at all emission wavelengths.^a

Emission λ / nm	τ_1 / ps	% A_1	τ_2 / ps	% A_2	τ_3 / ns	% A_3	% N_0
(dA)₁₀·(dT)₁₀ Dumbbell							
360	66 ± 16	93	940 ± 700	4.5	5.1 ± 0.8	2.1	75
390		93	810 ± 400	5.5		2.0	46
420		90	650 ± 400	7.4		3.0	21
450		88	550 ± 420	8.6		3.3	10
480		86	400 ± 400	11		3.4	8

^a Lifetimes (τ_n), percent amplitudes (% A_n), and the percent of photons emitted on time scales shorter than the IRF (% N_0) were obtained by fitting the emission decays to eq. 1 in the main text.

Table A3. Lifetimes for the Tris-HCl buffer with 0.25 M NaCl emission decays obtained from biexponential fits (see eq. 1 in the main text).

Emission λ / nm	τ_1 / ps	τ_2 / ns
Tris-HCl Buffer		
330	360 ± 160	3.5 ± 0.3
360	290 ± 140	3.4 ± 0.3
390	420 ± 250	3.2 ± 0.5
420	420 ± 250	3.3 ± 0.7
450	80 ± 40	1.6 ± 0.2
480	50 ± 20	1.6 ± 0.4

Table A4. Best-fit parameters for the d(AT)₉·d(AT)₉ transient absorption signal in Figure 9. The ground-state bleach (GSB) recovery signal (negative) was fit to a biexponential function with a constant offset: $\Delta A = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3$.

Sample	τ_1 / ps	A_1	τ_2 / ps	A_2	A_3
d(AT) ₉ ·d(AT) ₉	5.6 ± 1.4	-0.0026	84 ± 14	-0.0023	-0.00015

Table A5. Best-fit parameters for emission decays from $(dA)_{15}$, $d(AT)_9 \cdot d(AT)_9$ and the $(dA)_{10} \cdot (dT)_{10}$ dumbbell with absolute amplitudes.^a

Em. λ / nm	τ_1 / ps	A_1	τ_2 / ps	A_2	τ_3 / ns	A_3	N_0 (10^{-3})
$(dA)_{15}$							
330	165 ± 6	17900 ± 800	412 ± 24	2800 ± 250	4.3 ± 0.3	130 ± 10	250 ± 10
360	173 ± 5	28700 ± 900	431 ± 16	5000 ± 250	3.7 ± 0.3	130 ± 15	170 ± 10
390	185 ± 6	16600 ± 650	507 ± 25	2600 ± 200	4.1 ± 0.3	110 ± 15	61 ± 5
420	197 ± 9	7400 ± 400	562 ± 43	1100 ± 120	6.1 ± 0.6	70 ± 10	23 ± 5
450	200 ± 13	4100 ± 300	618 ± 60	600 ± 100	7.9 ± 0.7	64 ± 6	10 ± 4
480	210 ± 23	1600 ± 200	648 ± 100	290 ± 60	8 ± 1	40 ± 5	5 ± 1
$d(AT)_9 \cdot d(AT)_9$							
360	180 ± 100	800 ± 500	1400 ± 600	140 ± 60	5.1 ± 1.2	70 ± 20	125 ± 20
390	110 ± 30	2800 ± 900	1000 ± 400	170 ± 80	4.7 ± 1.2	60 ± 20	60 ± 10
420	105 ± 24	3500 ± 980	930 ± 400	200 ± 100	6.8 ± 1.4	70 ± 20	30 ± 10
450	105 ± 24	3500 ± 920	920 ± 400	200 ± 120	8.5 ± 1.2	120 ± 15	18 ± 8
480	101 ± 28	2300 ± 800	760 ± 400	150 ± 100	7.8 ± 2.0	50 ± 15	8 ± 6
$(dA)_{10} \cdot (dT)_{10}$ dumbbell							
360	61 ± 36	4000 ± 3500	930 ± 660	200 ± 160	5.2 ± 1.6	80 ± 30	325 ± 40
390	64 ± 30	4000 ± 2000	800 ± 500	240 ± 170	5.2 ± 1.4	90 ± 30	90 ± 20
420	70 ± 36	3000 ± 1800	700 ± 500	230 ± 180	5.3 ± 1.2	100 ± 30	30 ± 10
450	73 ± 44	2000 ± 1200	600 ± 550	200 ± 160	5.2 ± 1.4	80 ± 30	10 ± 9
480	63 ± 58	1200 ± 1000	340 ± 400	170 ± 200	4.6 ± 1.6	50 ± 25	4 ± 6

^aThe parameters are defined in eq. 1 in the main text

Table A6. Emission amplitudes corrected for the instrumentation wavelength sensitivity at times ranging from 50 (A_{50}) to 9000 ps (A_{9000}) for $(dA)_{15}$, $d(AT)_9 \cdot d(AT)_9$ and the $(dA)_{10} \cdot (dT)_{10}$ dumbbell after 265 nm excitation.^a

Emission λ / nm	A_{50}	A_{100}	A_{500}	A_{1000}	A_{2000}	A_{3000}	A_{5000}	A_{7000}	A_{9000}
$(dA)_{15}$									
330	28657	19480	4248	829	191	123	79	54	38
360	50470	39422	9240	1886	314	160	92	60	41
390	38768	31369	7875	1827	375	196	114	77	55
420	18164	15061	4197	1121	309	189	132	104	84
450	6945	5868	1816	571	198	133	99	82	68
480	1000	850	285	101	41	29	22	19	16
$d(AT)_9 \cdot d(AT)_9$									
360	9438	2817	773	337	203	143	83	56	40
390	8613	4507	932	398	223	151	90	61	45
420	7557	4845	1019	477	275	199	138	108	88
450	4909	3352	813	448	289	225	169	138	115
480	1101	757	161	80	48	38	29	24	20
$(dA)_{10} \cdot (dT)_{10}$ dumbbell									
360	6928	1897	400	145	83	61	40	29	22
390	5122	1940	414	203	116	84	56	43	33
420	2512	1346	367	208	125	94	66	49	38
450	1026	622	184	105	66	53	37	28	22
480	221	133	38	21	15	12	9	6	5

^a Values shown are the corrected amplitudes obtained from fitting using eq. 1 in the main text.

Table A7. Best-fit parameters for emission decays from (dA)₁₈ subject to different purification procedures.

Em. λ / nm	Purification Process ^a	τ_1 / ps	% A_1	τ_2 / ps	% A_2	τ_3 / ns	% A_3	% N_0
330	DS	130 ± 10	64	321 ± 16	32	2.7 ± 0.2	3.6	28
	HPLC	128 ± 11	61	295 ± 12	39	2.7 ± 0.4	0.6	26
360	DS	146 ± 8	65	325 ± 10	34	2.8 ± 0.4	1.2	14
	HPLC	149 ± 8	64	316 ± 10	36	2.5 ± 0.4	0.4	13
390	DS	160 ± 10	63	349 ± 12	36	3.0 ± 0.4	1.0	8
	HPLC	163 ± 12	66	342 ± 14	33	2.3 ± 0.4	0.5	8
420	DS	207 ± 10	83	586 ± 40	15	2.9 ± 0.4	1.3	5
	HPLC	199 ± 10	80	457 ± 40	20	3.0 ± 0.6	0.7	5
450	DS	202 ± 16	80	505 ± 50	19	4.1 ± 1.4	1.1	4
	HPLC	234 ± 15	88	765 ± 80	12	4.5 ± 1.0	0.9	4

^a Lifetimes (τ_n), corresponding percent amplitudes (% A_n), and the percentage of all photons emitted on time scales shorter than the IRF (% N_0) were obtained from fitting emission decays to eq. 1 in the main text.

^b DS: standard desalting purification. HPLC: reversed-phase high performance liquid chromatography purification.