DOE/ER/61604--1

Technical Progress Report

From: Dr. Thomas L. Netzel
Department of Chemistry
Georgia State University
University Plaza
Atlanta, GA 30303

Phone and FAX: 404-651-3129 [Alternate FAX: -1416]

To: Dr. Matesh N. Varma
Medical Applications and Biophysical Research Division, ER-73
Office of Health and Environmental Research,
Office of Energy Research

United States Department of Energy

ID No. DE-FG05-93ER61604

Project Title: An Exploration of Sequence Specific DNA-duplex/Pyrene
Interactions for Intercalated and Surface-associated Pyrene Species

Award Period: 05/15/93 to 04/30/94

Signature of Principle Investigator/
Program Director

Thomas L. Netzel
Thomas L. Netzel

1-7-94
date

MASTER

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DOE_rpt_1.msw

js
DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Table of Contents

1.0 General Goals of the Award.....	1
2.0 Summary of Overall Progress.	1
2.1 Personnel and Equipment Progress.	1
2.2 Progress Synthesizing Pyrene-labeled Nucleosides and Oligomers.	2
2.3 Progress Studying the Photochemistry of Pyrene-labeled Nucleosides, ss-DNA, and ds-DNA.	2
2.3.1 Emission Lifetime Results.	4
2.3.2 dT Versus dG Quenching of Pyrene*.	5
2.3.3 Pyrene* Quenching in Pyrene-labeled Duplexes.	6
2.3.4 Relationship of the Results to the General Goals of the Award. ..	9
2.3.5 Favorable Developments.	9
3.0 Summary of Work to be performed during the Succeeding Funding Period.	9
3.1 Pyrene-labeled Single Strand Studies.	10
3.2 Pyrene-labeled Duplex Studies.	10
3.2.1 Electron Trapping by dC Nucleotides.	11
3.2.2 Electron Mobility Within a DNA Double-helix.	12
4.0 Conclusion.	13
5.0 References	13
6.0 ER F 4620.1 - Budget for Year Two, 5/1/94 - 4/30/95	15
7.0 ER F 4620.1A - Summary Three Year Budget	16

1.0 General Goals of the Award. Covalent attachment of molecular labels to DNA duplexes (ds-DNA) of defined base content and sequence provides the basis for a large number of scientific inquiries and practical applications. In the context of the early effects of radiation on DNA chemistry, cellular metabolism, and carcinogenesis, attaching a strong photoredox agent such as pyrene to a DNA duplex can be thought of as constructing a point source of base ionization that can be activated with subnanosecond time resolution. This research proposal focuses on single photoexcitation and subsequent single ionization events. However, follow-on studies will in principle be able to examine the consequences of producing multiple ionization events on a single duplex at precisely defined locations.

The use of both short (5-atom) and long (12-atom) covalent linking chains to attach, respectively, a pyrenesulfonate or a pyrenebutyrate moiety to a central region of a DNA duplex allows construction of DNA-duplex/pyrene assemblies of two types. Long linking chains permit pyrene to intercalate within the DNA duplex, while the short chains constrain pyrene to remain in the outer-surface region of the major-groove of the duplex. Electrochemical data suggest that reductive electron-transfer (ET) quenching of photoexcited pyrene (pyrene*) labels will be most exothermic for guanosine (dG) than for the other three DNA nucleosides and that oxidative ET quenching of pyrene* will be most exothermic for thymidine (dT) than for the other three DNA nucleosides.¹⁻¹⁰ (If protonation in single strand DNA (ss-DNA) of reduced cytidine (dC⁻) by water is fast relative to ET quenching, oxidative quenching by cytidine (dC) can be competitive with that by dT. Additionally recent research suggests that in DNA duplexes dC⁻ protonation by hydrogen-bonded guanosine (dG) may make dC 0.2 V easier to reduce than dT.²) This research project combines two effects, (1) differential DNA/pyrene geometries in covalent assemblies with different length linking chains and (2) differential ET quenching reactivities among the DNA nucleotides to explore sequence specific and duplex/pyrene association specific effects on DNA-base ionization reactions.

2.0 Summary of Overall Progress. The first year of this research project has been concerned with four activities: (1) hiring and training personnel, (2) synthesizing target pyrene-labeled nucleosides and oligonucleotides, (3) commissioning our fluorescence lifetime measurement system, and (4) studying the photochemical behavior of pyrene-labeled nucleosides, single strands of DNA, and duplexes of DNA.

2.1 Personnel and Equipment Progress. A full time technician, Mr. David Nafisi was hired in the spring to develop custom software and to operate and maintain our extensive array of laser, electronics, and mechanical equipment. Mr. Nafisi has a bachelors of Electrical Engineering degree from the Georgia Institute of Technology. A one-half time technician, Mrs. Amy Zhao was also hired in the spring to pyrene-label and purify commercially synthesized DNA oligomers and to perform DNA-melting, emission quantum yield, and sample characterization experiments. Mrs. Zhao has a masters degree in bio-physical chemistry from Georgia State University (GSU). Additionally at no cost to this contract, a senior physics major, Mr. Joel Verbist, has been trained to measure CD spectra of pyrene-labeled DNA duplexes and a junior chemistry major, Mrs. Andrea Neil, has been trained to assist with the DNA-melting and emission quantum yield experiments.

Our fluorescence lifetime measurement system was commissioned by testing its ability to reproduce a number of published emission lifetimes. The agreement between the published values and our lifetime determinations was excellent. During the course of this work we completed the development of our data acquisition software and worked with the commercial

supplier of our lifetime-fitting and deconvolution software, Photon Technology International, to adapt their standard programs to our needs. We now have a completely optimized suite of acquisition and analysis software for emission kinetics, time-resolved emission spectra, and emission polarization anisotropy decays.

The new technicians are now proficient at all of the spectroscopic measurement and sample handling procedures required for the successful fulfillment of this research contract, including

CD, DNA-melting, FPLC purification, absorbance, fluorescence quantum yield, fluorescence lifetime, labeling reactions, and vacuum line techniques.

5'-OH-U(12)*-pyrene

5'-OH-T*(5)-pyrene

5'-OH-T*(0)-pyrene

Figure 1. Structures of Pyrene-labeled Nucleosides.

2.2 Progress Synthesizing Pyrene-labeled Nucleosides and Oligomers. Synthesizing pyrene-labeled nucleosides and fashioning them first into oligomers and then into duplexes is central to the conduct of this research program. Figure 1 lists the three complexes which have been made thus far. The first complex, 5'-OH-U(12)*-pyrene, has pyrene joined to uridine via a 12-atom linking chain to the 2'-position of the ribose group. This compound and several single strands and two duplexes incorporating it were synthesized by one of our research collaborators, Dr. Muthia Manoharan at Isis Pharmaceuticals. The second complex, 5'-OH-T*(5)-pyrene, has pyrene joined to the 5-position of thymidine via a 5-atom linking chain. This compound and several single strands and two duplexes incorporating it were synthesized in our research group. The third complex, 5'-OH-T*(0)-pyrene, has pyrene joined directly to the 5-position of thymidine (a 0-atom linking chain). This compound was synthesized by another of our research collaborators, Dr. Bruce E. Eaton at the Washington State University.

2.3 Progress Studying the Photochemistry of Pyrene-labeled Nucleosides, ss-DNA, and ds-DNA. As noted above dT and dC are the easiest nucleosides to reduce ($dN/dN^{\cdot-} = -1.35 \text{ V /SCE}$), and dG is the easiest nucleoside to oxidize ($dG^{\cdot+}/dG \geq 0.92 \text{ V /SCE}$).² With this in mind, we initially focused on pyrene*/dT interactions and the competition between dT and dG for pyrene* quenching. Figures 2-4 show absorbance and emission spectra for a series of two pyrene-labeled nucleosides and one short oligomer in which the linking chain is shortened

Figure 2.

energy release would locate the emission from the CT product near 494 nm (see the red-emission band in Figure 2). Since there is 2.5 eV of free energy stored in this CT state, relaxation to the ground state configuration, pyrene/dT, is expected to be an "inverted" ET process.²²⁻³⁰ This has two important consequences: (1) the CT product should have a lifetime of several nanoseconds, and (2) CT emission is a possible process.

The spectral results in Figures 2-4 strongly support the conclusion that we have observed *ET*

Figure 3.

from 12 - to 5- and then to 0-atoms. The striking result is that there is a progressive growth of a new emission band in the 450-600 nm spectral region. This new red-emission is very broad and unstructured. The same type of emission bandshape is commonly seen for exciplex and charge-transfer (CT) excited states.¹¹⁻¹⁶ Note that the free energy (ΔG^0) of oxidative quenching to form $\text{pyrene}^+/\text{dT}^-$ is -0.74 eV.^{1,17-21} This free

from pyrene to dT* followed by *reverse ET from dT* to pyrene**. This is an important finding which pins down the mode of pyrene/dT interaction. It is noteworthy that the reduction potential of dG^+ is less well known than that of dT. Thus ΔG^0 for the reductive quenching of pyrene* by dG is estimated to be ≥ -0.14 eV.^{1,2,17-21} In accord with this estimate, Geacintov has observed the reductive quenching

Figure 4.

oligonucleotides each five bases long. Also listed for comparison is the fluorescence lifetime of 1-pyrene butanoic acid. Note that all of the samples were degassed with three freeze-pump-thaw (FPT) cycles on vacuum line (10^{-5} torr) and sealed from the atmosphere with o-ring valves before being transferred to the laser system for kinetics measurements.

Table 1. Emission Lifetime (τ) and Average Lifetime ($\langle\tau\rangle$) Data for Several Pyrene-labeled Thymidines and DNA-oligomers.

Compound	τ , ns (400 nm)	$\langle\tau\rangle$, ns (400 nm)
5'-OH-U(12)*, ¹	36.4	36.4
5'-ODMT-U(12)*, ¹	29.4	29.4
TTT*(5)TT ²	0.489 (0.07)	7.88
	2.90 (0.27)	
	10.7 (0.66)	
5'-OH-T*(O) ¹	<0.20 (0.06)	-----
	4.07 (0.61)	
	>65 (0.33)	
TTU(12)*TT ²	0.306 (0.15)	12.4
	3.35 (0.31)	
	20.4 (0.55)	

product, pyrene⁻/dG⁺, by means of transient absorbance spectroscopy.¹⁶ We therefore expect that dT will be a much better ET quencher of pyrene* than dG.³¹ Note that for quenching by dT pyrene is *oxidized* and for quenching by dG pyrene is *reduced*.

2.3.1 Emission Lifetime Results.

Table 1 lists time-resolved emission results for three pyrene-labeled nucleosides and three pyrene-labeled

GGU(12)*GG ²	1.68 (0.11)	34.4
	8.31 (0.26)	
	30.2 (0.46)	
	107. (0.17)	
1-pyrene butanoic acid ¹	183.5±0.8	183.5

1. in MeOH

2. in phosphate buffer pH 7.0, 1.0 M NaCl.

In contrast to all of the pyrene-labeled oligomers and duplexes studied, 5'-OH-U(12)*-pyrene and 5'-ODMT-U(12)*-pyrene show single exponential decays (DMT= dimethoxytrityl). This indicates that there is configurational averaging on the time scale of <200 ps. That is, these complexes do not have any long-lived conformers which would produce multi-exponential emission decays. Presumably the slightly reduced lifetime of pyrene* in 5'-ODMT-U(12)*-pyrene versus 5'-OH-U(12)*-pyrene reflects the volume occupied by DMT from which pyrene is excluded. As result pyrene* comes into contact with uridine (dU) slightly more often in the 5'-ODMT substituted complex than in 5'-OH substituted one. Note that dU and dT should oxidatively quench pyrene* emission with similar rates, because of their similar molecular structures and identical reduction potentials.

In TTT*(5)TT pyrene* has an opportunity to encounter five different dT bases. This has two important consequences: (1) the lifetime of pyrene* is significantly shortened relative to 5'-OH-U(12)*-pyrene and 5'-ODMT-U(12)*-pyrene, and (2) the emission decay in the 400 nm region (where pyrene* fluorescence predominates) is multi-exponential. For 5'-OH-T*(0) the quenching of pyrene* occurs in <200 ps, and as a result the sharp vibrational features characteristic of the emission of the π - π^* state of pyrene* are not distinguishable in its time-integrated emission spectrum (see Figure 4). The rapidity of pyrene* quenching for 5'-OH-T*(0) has the benefit that the lifetime of the CT product state can be clearly determined as 4 ns. This complex is unstable which prevents us from completely purifying it. Thus we usually detect a small amount of long-lived pyrene* emission (>65 ns) in the 375-425 nm region. Note that long-lived emission from the decomposition product is absent in the 460-600 nm CT-region.

These results are consistent with our expectations that (1) the inverted nature of the reverse ET in the CT product, pyrene⁺/dT⁻, should slow its relaxation to several nanoseconds (4 ns was observed) and (2) the oxidative quenching reaction was sufficiently exergonic ($\Delta G^0 = -0.74$ eV) that this step would be extremely fast if pyrene* and dT could be brought sufficiently close to each other (<200 ps was observed). A corollary of these facts is that one would expect, all other things being equal, to observe CT-product emission in any pyrene-labeled oligomer or duplex in which <10 ns pyrene* quenching by dT is observed. In deed it is true for all three pyrene-labeled single strands and all four duplexes examined to date that (1) each exhibits some <10 ns pyrene* quenching and (2) each also produces detectable CT-product emission in the 460-600 nm region.

2.3.2 dT Versus dG Quenching of Pyrene*. Two oligomers in Table 1, TTU(12)*TT and GGU(12)*GG [written 5' to 3'], provide a remarkable comparison of the relative quenching efficiencies of dT and dG towards pyrene*. For the former 5-mer, quite rapid pyrene* quenching is observed at 400 nm; in fact quenching in this complex is not very different from that observed for TTT*(5)TT. In spite of the fact that dG is likely to quench pyrene* reductively, the average emission lifetime of pyrene* in GGU(12)*GG is 3-fold longer than in TTU(12)*TT, 34 ns versus

12 ns. Replacing the flanking-dT nucleotides with dGs not only produced less quenching of pyrene* but actually insulated pyrene* from quenching by the central uridine. Note that the lifetimes of pyrene* in 5'-OH-U(12)* and 5'-ODMT-U(12)* which lack flanking nucleotides are, respectively, 36 and 29 ns. In contrast, the longest-lived emission component is 20 ns for TTU(12*)TT, while it is 107 ns for GGU(12)*GG. How much reductive quenching the flanking dG nucleotides produce is an open question. One way of learning about this will be to compare the above quenching rates to quenching rates in oligomers with flanking dA and dC nucleotides. Adenosine is not expected to quench pyrene* either oxidatively or reductively.^{1,32} If protonation of dC⁻ by water in ss-DNA is sufficiently fast, dC will quench as well as dT. On the other hand if this is not the case, dC probably will not quench pyrene* at all.²

Table 2. Emission Lifetime (τ) and Average Lifetime ($\langle\tau\rangle$) Data for Four Pyrene-labeled DNA Duplexes.[†]

Compound	τ , ns (400 nm)	$\langle\tau\rangle$, ns (400 nm)	τ , ns (495 nm)	$\langle\tau\rangle$, ns (495 nm)
[18]-TTT*(5)TT-	0.090 (0.02)	20.0	0.092 (0.03)	5.92
[18]-AAA AA-	1.16 (0.07)		1.20 (0.17)	
	8.22 (0.32)		3.98 (0.52)	
	29.3 (0.59)		13.0 (0.28)	
[18]-GGT*(5)GG-	0.444 (0.03)	21.1	0.081 (0.04)	6.52
[18]-CCA CC-	3.88 (0.14)		1.62 (0.25)	
	16.6 (0.56)		3.85 (0.29)	
	41.5 (0.27)		11.9 (0.42)	
[18]-TTU(12)*TT-	0.401 (0.09)	55.9	0.19 (0.22)	3.58
[18]-AAA AA-	3.28 (0.10)		0.87 (0.27)	
	18.1 (0.29)		6.47 (0.51)	
	96.7 (0.52)			
[18]-GGU(12)*GG-	0.368 (0.08)	26.4	0.20 (0.14)	4.92
[18]-CCA CC-	2.95 (0.14)		1.17 (0.28)	
	17.1 (0.48)		5.36 (0.49)	
	59.2 (0.30)		21.5 (0.09)	

[†] Lifetimes were measured in sealed sample cells which were degassed with three freeze-pump-thaw cycles in phosphate buffer pH 7.0 with 1.0 M NaCl.

2.3.3 Pyrene* Quenching in Pyrene-labeled Duplexes. Table 2 lists lifetime data for both the pyrene* (400 nm) and CT-product (495 nm) emission regions for four duplexes. Each of the duplexes is 18 nucleotides long insuring full duplex formation at room temperature. Two of the duplexes have 12-atom linkers, and two have 5-atom linkers. The important distinction between these two sets of duplexes is that the longer linker permits pyrene to intercalate while the shorter one does not. Pyrene* quenching efficiency in duplexes can be influenced by both nucleotide-specific ET reactivity and sequence-specific intercalation phenomena. Thus a labeled duplex with less reactive nucleosides near the label can exhibit greater pyrene* quenching than one with more reactive nearby nucleosides if the former duplex promotes intercalation of pyrene more than the latter one. These competing effects on quenching efficiency make duplex studies with

long-chain linkers more complicated to interpret than ones with single strands. The two duplexes in each pair with the same length linker differ only by having either flanking-dT or flanking-dG nucleotides on either side of the labeled nucleotide. The basic 18-mer sequence for the labeled strands in these four duplexes is 5'-TAT ATA TTT*(5)TTT AAA TTT-3', which is termed [18]-TTT*(5)TT- in Table 2. The base sequences of the complementary unlabeled strands follow the usual DNA base-pairing rules.

For the T*(5)-containing duplexes in which pyrene intercalation is not possible, every lifetime component in the duplex with flanking dG nucleotides is longer than the corresponding component in the duplex with flanking dT nucleotides. This is reasonable on the basis of the earlier described reduced quenching efficiency expected for dG versus dT nucleotides. The average lifetimes of these two duplexes do not, however, differ very much because the relative weights of the two longest-lived emission components are interchanged. Presumably this reflects the relative populations of different conformations of the pyrene label in the two duplexes.

It is interesting that the 495-nm (CT) emission components for these two duplexes show a close correspondence with each other. Again there is an interchange of the relative weights of the two longest lived components, but the sense of this interchange is opposite to that found for the 400-nm (pyrene*) emissions. The relationship between the pyrene* quenching processes reflected in the 400-nm emission lifetimes and the CT relaxation processes reflected in the 495-nm emission lifetimes is not obvious. One possibility here is that a similar ensemble of pyrene⁺·/dT[·]- relative configurations is produced in each duplex, but more slowly in the one with flanking dG nucleotides than in the one with flanking dTs.

The longest emission components in the two T*(5)-containing duplexes are comparable to the lifetimes of the two U(12)* nucleosides. Figure 5 shows that, in contrast to the TTT*(5)TT single strand, the emission spectrum of the [18]-TTT*(5)TT- duplex shows sharp vibrational

Figure 5.

features at 376 and 397 nm as are found for both of the U(12)* nucleosides (see Figure 2). The emission and absorbance spectra for the [18]-GGT*(5)GG- duplex (not shown) are similar to those shown in Figure 5 with the difference that the relative amount of the CT emission in the 460-600 nm region is about a factor of two less. The sharp, vibrationally-resolved emission bands at 376 and 397 nm are due to

pyrene labels that are free of association with DNA. To underscore this point, note that the emission spectrum of 1-pyrene butanoic acid (not shown) is nearly identical to that shown in Figure 2 for 5'-OH-U(12)*.

For the T*(12)-containing duplexes in which pyrene intercalation is possible, the average pyrene* lifetime of the one with flanking dG nucleotides is one-half that of the one with flanking dTs, 26 ns versus 56 ns. If *opposite-strand* dC nucleotides can be shown in subsequent work to be unreactive with respect to pyrene* quenching, the fact that the duplex with the less reactive flanking-dG bases has the greater average quenching rate would be evidence for more favorable pyrene/duplex association in this duplex than in the one with flanking dT nucleotides. The large difference in average 400-nm emission lifetimes is due mainly to the almost factor of two difference in the lifetimes of the longest-lived emission components in these duplexes. In contrast the corresponding lifetimes of the first three emission components are very similar. Perhaps the most surprising result for the two U(12)*-containing duplexes is that 50% of the pyrene* emission lives for nearly 100 ns in the duplex with flanking dT nucleotides. Apparently this duplex positions pyrene* largely in dA-rich rather than in dT-rich regions and more in the solvent than in close association with the DNA duplex. As a consequence it is not surprising that the emission spectrum for this [18]-TTU(12)*TT- duplex (not shown) is indistinguishable from that of 1-pyrene butanoic acid in the same buffer. The reason is that the long-lived (97 ns) emission from solvent-associated pyrene labels completely dominates the much shorter (6.5 ns) CT emission in the time-integrated emission spectrum. Note also that the average lifetime of the pyrene* emission is 56 ns, while that of the CT-product emission is only 3.6 ns.

The 495-nm emission lifetimes in these two complexes have similar values for the corresponding first three components. This was the case for all four CT emission components in the T*(5)-containing duplexes. Presumably the 97-ns quenching process is too slow to produce a significant population of CT product for the duplex with flanking dTs. In contrast to the data for

Figure 6.

the duplex with flanking dTs, an exceptionally long-lived CT emission (22 ns) is found for the duplex containing flanking dGs. Figure 6 shows that the [18]-GGU(12)*GG-duplex has significant ground state absorbance in the 380-450 nm region. This red-absorbance feature is absent in TTT*(5)TT (see Figure 3) and in the T*(5)-containing duplexes which do not permit intercalation of the pyrene label.

Additionally, the molar extinction coefficient of the 380-450 red-absorbance is larger for the [18]-GGU(12)*GG- duplex than for [18]-TTU(12)*TT- duplex (spectrum not shown). These data are consistent with a larger amount of pyrene intercalation in the former duplex than in the latter.^{1,33} Increased intercalation in the U*(12)-duplex with flanking dGs on the labeled strand is therefore a likely explanation for the much shorter average pyrene* emission lifetime (400 nm) for this duplex compared to the one with flanking dTs. How much quenching is occurring in this duplex by dG and dC bases compared to dU is not yet known. Both transient absorbance measurements on labeled duplexes and additional emission kinetics experiments on labeled single strands with flanking dAs and dCs will help answer this question.

2.3.4 Relationship of the Results to the General Goals of the Award. The overall objective of this research proposal is *to establish a clear understanding of the base composition and sequence dependence of DNA ionization by powerful photoredox agents*. To date we have evidence of thymidine reduction by pyrene* labels. We will prove that this is the case by using picosecond transient absorbance spectroscopy (10-ps time resolution) to observe directly the formation of pyrene⁺ at 449 nm ($\epsilon = 60,000 \text{ M}^{-1} \text{ cm}^{-1}$).³⁴ We have also shown that guanosine is much less reactive with respect to pyrene* quenching than is thymidine. Future work will examine the relative quenching efficiencies of adenosine and cytidine nucleotides.

The results to date suggest that an appropriate understanding of ET quenching processes in labeled duplexes will rely heavily on the results obtained for labeled nucleosides and single strands. Also kinetics results for labeled duplexes will be simpler if the number of configurations that the label can adopt is significantly restricted. The most complicated labeled-duplexes to understand are not surprisingly the easiest to synthesize, ones with a long-chain linkers. For the duplexes which we have studied so far, the T*(5)-containing duplexes are the simplest to understand, and even they show multiple pyrene* and CT emission lifetimes.

2.3.5 Favorable Developments. Our discovery of CT emission in pyrene-labeled nucleotides, oligomers, and duplexes is a very favorable development. It means that transient absorbance experiments cannot fail due to ultrafast charge recombination in the CT product. The presence of CT emission also provides us with a very sensitive probe of the dynamics of the pyrene⁺/dT⁻ product. Emission kinetics measurements have much higher signal to noise ratios than do absorbance kinetics measurements. However, the latter are necessary to establish the identity of CT products. Equally important is the fact that thanks to the help of our research collaborators the number of kinds of pyrene-labeled nucleosides and phosphoramidite reagents presently available to us is significantly expanded relative to number of kinds of these compounds which we were able to make last year.

3.0 Summary of Work to be performed during the Succeeding Funding Period. During the coming year, we will focus on nucleoside/pyrene* and nucleotide/pyrene* quenching reactions for complexes with T*(5) and T*(1) thymidine subunits. The structure of the nucleoside used for T*(1) labeling is shown in Figure 7. This work can be grouped conceptually in terms of nucleoside, single strand and duplex studies. The work that needs to be done on the 5'-OH-T*(5) and 5'-OH-T*(1) nucleosides includes absorbance and emission spectroscopic studies, emission kinetics and quantum yield measurements, and picosecond transient absorbance measurements. One very important objective of this class of experiments is *to prove that the CT product in pyrene*/dT quenching is truly pyrene⁺/dT⁻*. Transient absorbance measurements will also provide a means of investigating ET reactions involving *adjacent* dC and dG nucleotides in DNA single stands and duplexes. Note that pyrene*/dG quenching will produce

5'-OH-T*(1)-pyrene

Figure 7.

the pK_a of reduced 3',5'-diphosphate-dT is 8.5 while that of reduced 3',5'-diphosphate-dC is >13 ,² at pH 7 dT nucleotides in single strands may be 0.09 eV easier to reduce than dCs, while at pH 6 dTs may be 0.15 eV more reducible. Finally at pH 8.5 dT and dC are expected to be equally reducible. Table 3 lists the single strands to be studied. For synthetic reasons the shortest length T*(5)-containing strand which we can make is 7-nucleotides long. (Our attempts to make GGT*(5)GG failed because this strand was too short to elute from a NAP-10 column after labeling with pyrene.) The synthesis of T*(1)-labeled oligomers is different from that for T*(5)-oligomers, i.e. it uses a pyrene-labeled phosphoramidite rather than an amine-labeled one. Consequently pyrene labels are incorporated into growing T*(1)-oligomers during automated synthesis, while pyrene labels are attached to amine-labeled oligomers after automated synthesis to fashion T*(5)-strands. Thus T*(1)-oligomers can be made with as few nucleotides as is desired.

Table 3. T*(5) and T*(1) Single Strands

TTT T*(5) TTT
GGG T*(5) GGG
CCC T*(5) CCC
AAA T*(5) AAA
T T*(1) T
G T*(1) G
C T*(1) C
A T*(1) A

3.2 Pyrene-labeled Duplex Studies. The results of the single strand studies described above will provide crucial information for the final selection of the nucleotide sequences to be synthesized for the pyrene-labeled duplex studies. Additionally because the number of conformational states of pyrene in T*(1)-duplexes is smaller than in T*(5)-duplexes, we will focus next year on T*(1)-labeled duplexes. The objective of these studies is *to clarify the roles of adjacent and opposite strand nucleotides on pyrene* quenching reactions as well as on subsequent ET processes*. Since we have no way of assessing yet the relative affects on ET rates of placing a given nucleotide in an adjacent same-strand versus an adjacent opposite-strand position with respect to a pyrene-labeled nucleotide, we will for the present discussion neglect primary ET-quenching events involving adjacent opposite-strand nucleotides. This is likely to be

pyrene⁻. Fortunately both the pyrene⁺ and pyrene⁻ ET products have sharp, signature absorbance bands, respectively, at 449 nm ($\epsilon = 60,000 \text{ M}^{-1} \text{ cm}^{-1}$) and 492 nm ($\epsilon = 95,000 \text{ M}^{-1} \text{ cm}^{-1}$).³⁴

3.1 Pyrene-labeled Single Strand Studies. The goal of the labeled single strand studies is *to assess the relative reactivities of dG, dC, and dA nucleotides versus dT with respect to pyrene* quenching for 5-atom and 1-atom linking chains*. Based on our own results and reports in the literature, we expect dAs to be unreactive,^{1,32} and dGs to be weakly reactive. If proton transfer from water is not rate limiting for ET quenching of pyrene*, dCs can be nearly as reactive as dTs in single strands. Because

a good assumption for T*(1)-labeled duplexes for two reasons: (1) the electronic coupling between pyrene* and dT is large due to the covalent carbonyl linker and (2) the pyrene*/dT quenching reaction is very exergonic, $\Delta G^0 = -0.74$ eV. It will be important to learn whether or not adjacent same-strand nucleotides are able to compete with the rapid primary-quenching event expected in T*(1)-labeled duplexes. *Even if the primary quenching event is unaffected by other nucleotides in a labeled duplex, neighboring nucleotides can significantly influence the lifetime of the primary CT product, pyrene⁺/dT⁻, and the lifetime of pyrene⁺ which may live longer than the primary CT product if the electron on dT⁻ can migrate to other dTs or be trapped by a dC.*

The simplest duplexes to study will not have dG or dC nucleotides. Thus it will be important to examine the following two duplexes to learn about modulations of either forward (quenching) or reverse (recombination) ET reactions within a T*(1)-nucleotide due to interactions between it and neighboring nucleotides.

[18]-TT T*(1) TT- and [18]-AA T*(1) AA-
[18]-AAA AA- [18]-TT A TT-

Delocalization of the electron initially on dT⁻ to other dTs could increase the lifetime of the primary CT product. It would probably also produce a difference between the lifetimes of the CT-product emission and the absorbance of pyrene⁺. If a difference between these two lifetimes is found, the sensitivity of electron delocalization to variation in the sequence of flanking dA- and dT-nucleotides can easily be studied. Such results would provide unique data on electron mobility in DNA.

3.2.1 Electron Trapping by dC Nucleotides. The strong tendency within a DNA duplex for dG to protonate dC⁻, forming dC(H)⁻, is estimated to make dC about 0.2 eV easier to reduce than dT.² Thus dC nucleotides are expected to act as electron traps within DNA duplexes. However, because of the strong electronic coupling between pyrene* and dT provided by the carbonyl linker within the T*(1)-nucleotide, pyrene* quenching by the covalently attached dT will likely dominate reactions of pyrene* with neighboring dCs. It is likely in a subsequent step, however, that dT⁻ will reduce dC. This subsequent electron-trapping step can be studied by comparing the lifetime of the CT-product emission to the lifetime of pyrene⁺. Electron trapping by dC will quench CT emission but will not shorten the lifetime of pyrene⁺. Both the increased distance of separation between dC(H)⁻ and pyrene⁺ and the lack of a short covalent coupling between them relative to covalently attached dT⁻ and pyrene⁺ will serve to lengthen the lifetime of pyrene⁺ in comparison to its lifetime in duplexes lacking dC nucleotides. Note that the charge recombination reaction within DNA duplexes between dC(H)⁻ and pyrene⁺ is also an inverted process due to its very exergonic ΔG^0 of -2.3 eV. *The observation of (either direct or subsequent) electron trapping by dC will provide important evidence in support of the generally accepted view that dC sites are better traps for excess electrons in DNA than are dT sites.*

Although dG nucleotides can reductively quench pyrene*, this reaction is at least 0.60 eV less favored than oxidative quenching by dT. For this reason and again because of the strong electronic coupling between pyrene* and dT within the T*(1)-nucleotide, pyrene* quenching by covalently attached dT is also expected to dominate pyrene* quenching by neighboring dG nucleotides.

3.2.2 Electron Mobility Within a DNA Double-helix. Recent work by Barton and Turro³⁵ has provided strong evidence based on emission quenching, but not yet proof based on transient absorbance ET-product identification, that excess electrons embedded within a DNA double-helix are extremely mobile. They showed in a ruthenium/rhodium-labeled duplex that the π -electron overlap of stacked DNA-bases enables an excess electron to traverse a distance of 40 Å to a trapping site at a high rate ($>10^9 \text{ s}^{-1}$) which generally is not found for donor/acceptor separations exceeding a few angstroms. Recall that electron transfer rates decrease exponentially with increasing donor/acceptor distance of separation.^{15,36-43} This is a very exciting result which needs to be verified as well as to have its generality tested. Fortunately *electron trapping by dC nucleotides in T*(1)-labeled duplexes provides precisely the required experimental format for measuring the mobility of an excess electron (alternately the strength of dT⁻/dC electronic coupling) within DNA double-helices. All that is required is to prepare a series of T*(1)-labeled duplexes in which the distance of separation between a dG:dC base-pair and the T*(1)-nucleotide is systematically varied.*

A final comment is in order concerning the likelihood of secondary oxidation of a neighboring dG by the primary quenching product, pyrene⁺. Our nucleotide sequences constructed for efficient trapping by dC of an electron initially on dT⁻ will locate the dC-trap on the same strand as the T*(1)-nucleotide. This choice will also locate the base-paired dG on the opposite strand from pyrene⁺. Given enough time pyrene⁺ will oxidize this paired dG nucleotide, since ΔG^0 for this reaction is $\geq -0.24 \text{ eV}$. [Note that the deprotonated dG anion, dG(-H)⁻, which would be present after intra-pair proton transfer to dC⁻ would be easier to oxidize than dG, but it is also likely to be protonated rapidly by a water molecule in competition with oxidation by pyrene⁺.] However, if dG and pyrene⁺ are about five angstroms apart, the rate for dG oxidation could be as slow as 10^5 s^{-1} .^{23,44-55} [The rate for dG(-H)⁻ oxidation could be as fast as 10^7 - 10^6 s^{-1} , but only after electron trapping by dC(H)[·].] Given the stiffness of the carbonyl linker between pyrene and dT in T*(1), it will not be hard to separate an opposite-strand dG and the pyrene label by this much distance. In fact it may be hard to get them closer together. In contrast dT⁻ and dC will be stacked on top of the each other in the double-helix (either directly or with intervening bases). In light of the recent Barton and Turro result, stacked dT⁻ and dC nucleotides are expected to have a phenomenal amount of electronic coupling. Thus the extent of oxidation of neighboring dGs by pyrene⁺ will depend on the lifetime of the dC(H)[·]/pyrene⁺ ET product, the exact dG/pyrene locations, and the rate of reprotonation of the paired dG(-H)⁻ nucleotide. Transient absorbance spectroscopy can observe the secondary oxidation of dG [or dG(-H)⁻] in the 360 and 449 nm regions. At 449 nm the absorbance of pyrene⁺ would decay, but at 360 nm the absorbance of dT⁻ would be replaced by that of dC(H)[·] indicating that the ground state configuration was not yet restored due to the formation of dG⁺/dC(H)[·] [or dG(-H)⁻/dC(H)[·], a radical pair].^{2,34} Additionally dG⁺ will have absorbance in the 390 nm region. GMP⁺ has a pK_a of 4, and therefore will deprotonate in the pH range 6-9. Pulse radiolysis and optical quenching studies have shown the deprotonated radical cation, GMP(-H)[·], to have the following absorptions (λ , ϵ): 320 nm, $2400 \text{ M}^{-1} \text{ cm}^{-1}$ and 475 nm, $1300 \text{ M}^{-1} \text{ cm}^{-1}$.^{3,16,56,57} Additionally, the driving force for interstrand ET within the dG(-H)⁻/dC(H)[·] radical-pair product is still large enough, $\Delta G^0 \leq -2.07 \text{ eV}$, for this reaction to be an inverted process.²

4.0 Conclusion. It is important to emphasize that both (1) demonstrating electron trapping by dC over dT in a DNA duplex and (2) measuring the mobility of an excess electron in a DNA duplex would be significant accomplishments. Once these studies are complete, there is the added bonus that the mechanisms of dG oxidation can also be studied. In light of current models of electronic coupling strengths between electron donors and acceptors,^{43,50,51,58-78} we expect to show also that dG:dC-pair placement within T*(1)-labeled duplexes can selectively retard opposite-strand dG oxidation by pyrene⁺ while maintaining facile electron trapping by dC.

5.0 References

1. Geacintov, N. E.; Zhao, R.; Kuzmin, V. A.; Seog, K. K.; Pecora, L. J. *Photochem. Photobiol.* **1993**, *58*, 185.
2. Steenken, S.; Telo, J. P.; Novais, H. M.; Candeis, L. P. *J. Am. Chem. Soc.* **1992**, *114*, 4701.
3. Lecomte, J. P.; Kirsch-De Mesmaeker, A.; Kelly, J. M.; Tossi, A. B.; Goerner, H. *Photochem. Photobiol.* **1992**, *55*, 681.
4. Kittler, L.; Lober, G.; Gollmick, F.; Berg, H. J. *Electroanal. Chem.* **1980**, *116*, 503.
5. Meites, L.; Zuman, P.; eds. *Electrochemical Data*; John Wiley and Sons, Inc.: New York, **1974**.
6. Meites, L.; Zuman, P.; eds. *CRC Handbook Series in Organic Electrochemistry*; CRC Press, Inc.: Cleveland, OH, **1977**.
7. Dryhurst, G.; eds. *Electrochemistry of Biological Molecules: Purines, Pyrimidines, Pteridines, Flavins, Pyrroles, Porphyrins, and Pyridines*; Academic Press, Inc.: New York, N. Y., **1977**.
8. Dryhurst, G.; Kadish, K. M.; Scheller, F.; Renneberg, R.; eds. *Biological Electrochemistry, Vol. 1*; Academic Press, Inc.: New York, N. Y., **1982**.
9. Dryhurst, G.; Nguyen, N. T.; Wrona, M. Z.; Goyal, R. N.; Brajter, T. A.; Owens, J. L.; Marsh, H. A. J. *J. Chem. Educ.* **1983**, *60*, 315.
10. Dryhurst, G. *Bioelectrochem. Bioenerg.* **1985**, *14*, 251.
11. Sisido, M.; Tanaka, R.; Inai, Y.; Imanishi, Y. *J. Am. Chem. Soc.* **1989**, *111*, 6790.
12. Borsarelli, C. D.; Cosa, J. J.; Previtali, C. M. *Langmuir* **1992**, *8*, 1070.
13. Hashimoto, S. *J. Phys. Chem.* **1993**, *97*, 3662.
14. Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1990**, *94*, 6237.
15. Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1991**, *95*, 3847.
16. Margulis, L.; Pluzhnikov, P. F.; Mao, B.; Kuzmin, V. A.; Chang, Y. J.; Scott, T. W.; Geacintov, N. E. *Chem. Phys. Lett.* **1991**, *187*, 597.
17. Loutfy, R. O.; Loutfy, R. O. *Can. J. Chem.* **1976**, *54*, 1454.
18. Gough, T. A.; Peover, M. E. *Polarography* **1964**; **1964**, 1017.
19. Perichon, J. in *Encyclopedia of Electrochemistry of the Elements, Organic Section*; Bard, A. J.; Lund, H.; eds., Marcel Dekker, Inc.: New York and Basel, **1978**; *11*, 108.
20. Pysh, E. S.; Yang, N. G. *J. Am. Chem. Soc.* **1963**, *85*, 2124.
21. Kubota, T.; Kano, J.; Uno, B.; Konse, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3865.
22. Bolton, J. R.; Archer, M. D. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, D. C., **1991**; *7*.
23. Closs, G. L.; Miller, J. R. *Science, Washington D. C.* **1988**, *240*, 440.
24. Irvine, M. P.; Harrison, R. J.; Beddard, G. S.; Leighton, P.; Sanders, J. K. M. *Chem. Phys.* **1986**, *104*, 315.

25. Liang, N.; Miller, J. R.; Closs, G. L. *J. Am. Chem. Soc.* **1990**, *112*, 5353.
26. Lu, H.; Prieskorn, J. N.; Hupp, J. T. *J. Am. Chem. Soc.* **1993**, *115*, 4927.
27. Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.
28. McCleskey, T. M.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **1992**, *114*, 6935.
29. Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966.
30. Sumi, H.; Marcus, R. A. *J. Chem. Phys.* **1986**, *84*, 4894.
31. Geacintov, N.; Prusik, T.; Khosrofian, J. *J. Am. Chem. Soc.* **1976**, *98*, 6444.
32. Lianos, P.; Georghiou, S. *Photochem. Photobiol.* **1979**, *29*, 13.
33. Slama-Schwok, A.; Jazwinski, J.; Bere, A.; Montenay-Garestier, T.; Rougee, M.; Helene, C.; Lehn, J.-M. *Biochemistry* **1989**, *28*, 3227.
34. Shida, T. *Electronic Absorption Spectra of Radical Ions*; Elsevier: New York, **1988**.
35. Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025.
36. Kavarnos, G. J. in *Topics in Current Chemistry*; Mattay, J.; eds., Springer-Verlag: Berlin, **1990**; 21.
37. Sutin, N. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, D. C., **1991**; 25.
38. Isied, S. S. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, D. C., **1991**; 230.
39. Miller, J. R. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, D. C., **1991**; 265.
40. Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1992**, *114*, 3656.
41. Mataga, N. in *Photochemical Energy Conversion*; Norris, J. R., Jr.; Meisel, D.; eds., Elsevier: New York, **1989**; 32.
42. Bowler, B. E.; Raphael, A. L.; Gray, H. B. *Prog. Inorg. Chem.* **1990**, *38*(Bioinorg. Chem.), 259.
43. Bowler, B. E.; Meade, T. J.; Mayo, S. L.; Richards, J. H.; Gray, H. B. *J. Am. Chem. Soc.* **1989**, *111*, 8757.
44. Sutin, N. in *Progress in Inorganic Chemistry*; Lippard, S. J.; eds., John Wiley & Sons: New York, **1983**; *30*, 441.
45. Brunswig, B. S.; Delaive, P.; English, A. M.; Goldberg, M.; Gray, H. B.; Mayo, S. L.; Sutin, N. *Inorg. Chem.* **1985**, *24*, 3743.
46. Newton, M. K.; Sutin, N. *Annu. Rev. Phys. Chem.* **1984**, *35*, 437.
47. Sutin, N.; Brunswig, B. in *Electron Transfer in Biology and the Solid State*; Johnson, M. K.; King, R. B.; Kurtz, D. M.; Kutal, C.; Norton, M. L.; Scott, R. A.; eds., American Chemical Society: Washington, DC, **1990**; 226, 65.
48. Siddarth, P.; Marcus, R. A. *J. Phys. Chem.* **1992**, *96*, 3213.
49. Marcus, R. A. *Chem. Phys. Lett.* **1988**, *133*, 471.
50. Cowan, J. A.; Upmacis, R. K.; Beratan, D. N.; Onuchic, J. N.; Gray, H. B. *Ann. N. Y. Acad. Sci.* **1988**, *550*, 68.
51. DeVault, D. *Q. Rev. Biophys.* **1980**, *13*, 387.
52. Jortner, J. *J. Chem. Phys.* **1976**, *64*, 4860.
53. Rips, I.; Klafter, J.; Jortner, J. *J. Phys. Chem.* **1990**, *94*, 8557.
54. Brunswig, B.; Sutin, N. *Comments in Inorganic Chemistry* **1987**, *6*, 209.
55. Liang, N.; Miller, J. R.; Closs, G. L. *J. Am. Chem. Soc.* **1989**, *111*, 8740.
56. Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094.
57. Janovic, S. V.; Simic, M. *Biochim. Biophys. Acta* **1989**, *1008*, 39.

58. Siddarth, P.; Marcus, R. A. *J. Phys. Chem.* **1993**, *97*, 6111.
59. Paddon-Row, M. N.; Shephard, M. J.; Jordan, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 3312.
60. Tang, R. *J. Phys. Chem.* **1993**, *98*, 6263.
61. Brun, A. M.; Harriman, A. *NATO ASI Ser., Ser. C* **1992**, *371*, 395.
62. Risser, S. M.; Beratan, D. N.; Meade, T. J. *J. Am. Chem. Soc.* **1993**, *115*, 2508.
63. Lianos, P.; Cremel, G. *Photochem. Photobiol.* **1980**, *31*, 429.
64. Hupp, J. T.; Kober, E. M.; Neyhart, G. A.; Meyer, T. J. *Nato Asi Ser., Ser. C* **1991**, *343*(Mixed Valency Syst.: *Appl. Chem., Phys. Biol.*), 51.
65. Cowan, J. A.; Upmacis, R. K.; Beratan, D. N.; Onuchic, J. N.; Gray, H. B. *Ann. N. Y. Acad. Sci.* **1989**, *550*, 68.
66. Beratan, D. N.; Onuchic, J. N.; Hopfield, J. J. *J. Chem. Phys.* **1987**, *86*, 4488.
67. Closs, G. L.; Calcaterra, N. J.; Green, N. J.; Penfield, K. W.; Miller, J. R. *J. Phys. Chem.* **1986**, *90*, 3673.
68. Therien, M. J.; Bowler, B. E.; Selman, M. A.; Gray, H. B.; Chang, I.-J.; Winkler, J. R. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, DC, **1991**; 228, 191.
69. Therien, M. J.; Selman, M.; Gray, H. B.; Chang, I. J.; Winkler, J. R. *J. Am. Chem. Soc.* **1990**, *112*, 2420.
70. Sola, M.; Cowan, J. A.; Gray, H. B. *J. Am. Chem. Soc.* **1989**, *111*, 6627.
71. Meade, T. J.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 4353.
72. Jacobs, B. A.; Mauk, M. R.; Funk, W. D.; MacGillivray, R. T. A.; Mauk, A. G.; Gray, H. B. *J. Am. Chem. Soc.* **1991**, *113*, 4390.
73. Gray, H. B. *Aldrichimica Acta* **1990**, *23*, 87.
74. Wasielewski, M. R.; Niemczyk, M. P.; Svec, W. A.; Pewitt, E. B. *J. Am. Chem. Soc.* **1985**, *107*, 1080.
75. Beratan, D. N.; Onuchic, J. N.; Winkler, J. R.; Gray, H. B. *Science, Washington D. C.* **1992**, *258*, 1740.
76. Beratan, D. N.; Onuchic, J. N.; Betts, J. N.; Bowler, B. E.; Gray, H. B. *J. Am. Chem. Soc.* **1990**, *112*, 7915.
77. Beratan, D. N.; Onuchic, J. N. in *Electron Transfer in Inorganic, Organic, and Biological Systems*; Bolton, J. R.; Mataga, N.; McLendon, G.; eds., American Chemical Society: Washington, D. C., **1991**; 72.
78. Beratan, D. N.; Betts, J. N.; Onuchic, J. N. *Science, Washington D. C.* **1991**, *252*, 1285.

6.0 ER F 4620.1 - Budget for Year Two, 5/1/94 - 4/30/95

not attached, ds

DATE

FILMED

6 / 2 / 94

END