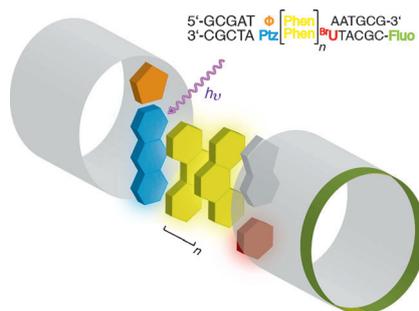


EET grown ohm: Excess electron transfer (EET) was observed within a DNA duplex containing π -stacked phenothiazine as an electron donor, phenanthrenes as electron carriers and 5-bromouracil as an electron trap. Increasing the number of phenanthrenyl base pairs increased EET efficiency.



Electron Transfer

*P. Roethlisberger, F. Wojciechowski,
C. J. Leumann* ■■■■-■■■■*

**Enhancement of Excess Electron
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Containing a Phenothiazine Donor
and Multiple Stable Phenanthrenyl
Base Pairs**



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Enhancement of Excess Electron Transfer Efficiency in DNA Containing a Phenothiazine Donor and Multiple Stable Phenanthrenyl Base Pairs

Pascal Roethlisberger,^[a] Filip Wojciechowski,^[a, b] and Christian J. Leumann*^[a]

Electron transport through the π -stacked DNA base pairs has attracted considerable interest and continues to be intensively studied.^[1] A better understanding of photoinduced negative charge transfer, often called excess electron transfer (EET), could allow to develop DNA nanoscale devices, such as biological sensors used in, for example, single-nucleotide polymorphism detection.^[2] The electron conducting properties of DNA are determined by the physicochemical properties of the natural nucleobases and for certain applications, such as DNA nanowires, enhancement of the conducting properties would be highly desirable. One envisioned way to enhance EET efficiency is to replace the natural base pairs with non-natural hydrogen bonding partners or other aromatic residues with favorable conductive properties.^[3]

In earlier work we investigated EET through a stacked phenanthrenyl (Phen–Phen) pair in a DNA duplex containing 5-(pyren-1-yl)uridine as an electron injector and 5-bromouracil (^{Br}U) as an electron acceptor.^[3a] This provided the first example of electron transfer through a stable, interstrand π -stacked, aromatic base pair. However, the reduction potential of Phen is more negative than that of the pyrene donor, which excludes electron transfer to occur through a hopping mechanism. This, however, would be necessary to increase efficiency and distance of EET. Indeed, we observed lower efficiency through a Phen–Phen pair compared to an A–T base pair. To remedy and to explore the scope and limitations of EET through such interstrand stacked aromatic residues, devoid of hydrogen-bonding capability, we decided to use a stronger electron donor, such as phenothiazine (PTZ; $E_{ox}^* \approx -2.7$ V vs. SCE; Figure 1)^[4] and to use up to three Phen–Phen pairs in between the electron injector and acceptor in an otherwise similar setup.

PTZ has been used previously as an electron donor in DNA. Using a flexible, acyclic linker PTZ was placed at the 5' end of DNA or at internal positions facing the natural

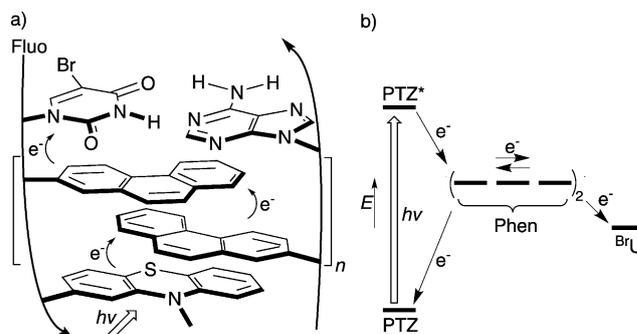


Figure 1. a) Schematic representation highlighting EET from a photoexcited PTZ- β -C-nucleoside (PTZ) placed against an abasic site (ϕ) to an interstrand stacked phenanthrenyl (Phen) base pair and 5-bromouracil (^{Br}U); the long arrows represent a phosphodiester backbone; b) an EET pathway from photoexcited (PTZ*) to three Phen base pairs.

bases/an abasic site.^[3b,5] We reasoned that using a rigid PTZ- β -C-nucleoside the PTZ unit could intercalate well against an abasic site and stack favorably with a planar Phen residue, thus enabling efficient EET. On the example of duplexes containing one or three Phen–Phen pairs we show here that EET becomes more efficient as compared to duplexes with the same number of A–T base pairs. Moreover, we demonstrate for the first time that EET can occur over multiple Phen–Phen pairs.

Although a PTZ- β -C-nucleoside has previously been synthesized by Grinstaff and co-workers, they were unable to protect its 5'-hydroxyl with DMT-Cl, and consequently PTZ was placed at the 5' end of the DNA.^[6] Our attempts at protecting the 5'-hydroxyl with standard DMT-Cl/pyridine also failed. Fortunately, the 5'-hydroxyl could be protected with DMT-triflate,^[7] a reagent occasionally used to protect sterically hindered hydroxyl groups.^[8]

The DMT protected nucleoside was subsequently phosphorylated using standard conditions yielding the corresponding phosphoramidite (Supporting Information, Scheme S1). PTZ-, Phen-,^[9] and 5-bromouracil phosphoramidites were used in the synthesis of modified oligodeoxynucleotides (ODNs) **1–14** (Table 1). The ODNs were purified by RP-HPLC and characterized by ESI-MS.

Thermal denaturation experiments were performed with duplexes **D1–D9** to judge the stability of DNA containing PTZ and multiple Phen–Phen pairs. The T_m data are summarized in Table 1. PTZ placed against an abasic site (PTZ- ϕ , duplex **D2**) leads to a duplex that is less stable by 5.8 °C

[a] P. Roethlisberger, Dr. F. Wojciechowski, Prof. C. J. Leumann
Department of Chemistry and Biochemistry
University of Bern, Freiestrasse 3, 3012 Bern (Switzerland)
E-mail: leumann@ioc.unibe.ch

[b] Dr. F. Wojciechowski
Present address: Department of Chemistry
University of Konstanz, Universitätsstrasse 10
78457 Konstanz (Germany)

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Table 1. Sequence and thermal stability (T_m) data for DNA duplexes 1–9.^[a]

No.	ss ^[a]	Duplex sequence	T_m [°C] ^[b]
D1	1	5'-GCGAT ϕ (A) ₁ AATGCG-3'	49.0
	2	3'-CGCTAPtz (T) ₁ ^{Br} UTACGC-Fluo	
D2	3	5'-GCGAT ϕ (Phen) ₁ AATGCG-3'	48.0
	4	3'-CGCTAPtz (Phen) ₁ ^{Br} UTACGC-Fluo	
D3	5	5'-GCGAT ϕ (A) ₃ AATGCG-3'	51.0
	6	3'-CGCTAPtz (T) ₃ ^{Br} UTACGC-Fluo	
D4	7	5'-GCGAT ϕ (Phen) ₃ AATGCG-3'	54.8
	8	3'-CGCTAPtz (Phen) ₃ ^{Br} UTACGC-Fluo	
D5	9	5'-GCGATA (Phen) ₁ AATGCG-3'	53.8
	10	3'-CGCTAT (Phen) ₁ ^{Br} UTACGC-Fluo	
D6	3	5'-GCGAT ϕ (Phen) ₁ AATGCG-3'	49.8
	11	3'-CGCTAPtz (Phen) ₁ TTACGC-Fluo	
D7	12	5'-GCGATA (Phen) ₃ AATGCG-3'	56.3
	13	3'-CGCTAT (Phen) ₃ ^{Br} UTACGC-Fluo	
D8	7	5'-GCGAT ϕ (Phen) ₃ AATGCG-3'	51.4
	13	3'-CGCTAT (Phen) ₃ ^{Br} UTACGC-Fluo	
D9	7	5'-GCGAT ϕ (Phen) ₃ AATGCG-3'	57.0
	14	3'-CGCTAPtz (Phen) ₃ TTACGC-Fluo	

[a] Single-stranded oligonucleotide; [b] $c=1.2 \mu\text{M}$ duplex in 10 mM NaH_2PO_4 , 0.15 M NaCl, pH 7.0; estimated error in $T_m = \pm 0.5^\circ\text{C}$.

than duplex **D5** containing an A–T base pair instead. However, PTZ is accommodated much better in sequences containing three Phen–Phen base pairs; here duplex **D4** containing PTZ– ϕ is only -1.5°C less stable than the duplex **D7** containing an A–T base pair. Further evidence that PTZ intercalates and provides stability is obtained by difference of the T_m of duplex **D4** (PTZ– ϕ) and duplex **D8** (T– ϕ), which shows that PTZ stabilizes the duplex by $+3.4^\circ\text{C}$ when compared to thymine. In general, insertion of a Phen–Phen pair is consistent with our previously obtained results.^[9] One Phen–Phen pair as a replacement for an A–T base pair only slightly destabilizes the duplex ($\Delta T_m = -1.0^\circ\text{C}$, **D1** and **D2**) and three Phen–Phen pairs (duplex **D4**) stabilize the duplex by $+3.8^\circ\text{C}$ compared to duplex **D3** containing three A–T base pairs.

The effect of excess electron transfer in DNAs with A–T or Phen–Phen pairs was investigated by selective photoexcitation of PTZ using UVA light ($\lambda_{\text{max}} = 365 \text{ nm}$, Sylvania 8 W, under N_2). In this assay, when migrating electrons encounter and are captured by ^{Br}U, Br⁻ is released and a uracil-5-yl radical is formed. This radical then abstracts a hydrogen from a 5' adjacent deoxyribose neighbor, and after piperidine treatment strand cleavage occurs giving short DNA fragments.^[10] We prepared the expected fragments containing a 3'-phosphate/5'-fluorescein (Fluo), the tetramer (a: 3'-O4P-ACGC-Fluo) and the pentamer (b: 3'-O4P-TACGC-Fluo), and used them as controls and DNA markers. EET yields are determined by quantifying the formation of fragment a using polyacrylamide gel electrophoresis (PAGE, Figure 2).

Selective strand scission was not detected in duplexes **D5** and **D7** lacking the PTZ electron donor or duplexes **D6** and **D9** lacking the ^{Br}U. A UV independent cleavage at ^{Br}U was observed without irradiation after piperidine treatment and is most likely induced by heat.^[11] Along with various photo-

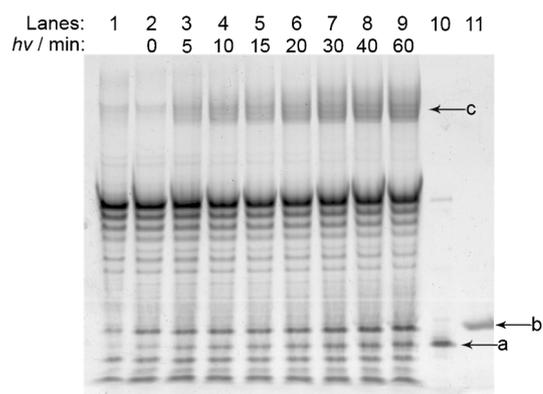


Figure 2. Fluorescence image of a 20% denaturing PAGE showing DNA strand cleavage of duplex **D4** after UV irradiation (365 nm, $\sim 4^\circ\text{C}$). Conditions: $4.0 \mu\text{M}$ duplex in 10 mM NaH_2PO_4 , 0.15 M NaCl, pH 7.0. The DNAs were exposed to UV light for the indicated time and analyzed after subsequent piperidine treatment at 90°C for 30 min. Lane 1: control DNA treated with piperidine at 90°C for 30 min without UV irradiation; lane 10: synthesized DNA fragment a; lane 11: fragment b.

products, which do not accumulate with irradiation time, a low mobility band was observed (Figure 2, fragment c). The product from this band was isolated from the gel and its mass was determined by ESI⁻ and MALDI mass spectrometry (m/z found: 5297.1; see the Supporting Information). It could be attributed to a Phen specific intra-strand photoreaction product (Supporting Information, Scheme S2) that accumulated with irradiation time in duplex DNA and single strands containing Phen residues. This product occurs only if ^{Br}U is present in the sequence and does not accumulate with time to a significant extent, in duplexes **D5** or **D7**, which lack the PTZ donor (see the Supporting Information). Since this reaction results from EET, we propose that it occurs most likely by a radical mechanism between uracil-5-yl radical and an adjacent Phen residue, and less likely by way of a cyclobutane adduct^[12] (Supporting Information, Schemes S3 and S4).

Quantification of the amount of formed cleavage product a versus time for duplexes **D1–D4** is shown in Figure 3, and strand cleavage yields after 60 min of irradiation for duplexes **D1–D4** single strands **2**, **4**, **6** and **8** are shown in Figure 4. Substituting one A–T base pair (duplex **D1**) with a Phen–Phen pair (duplex **D2**) slightly decreased the yield from 7.0 to 5.7%. Increasing the number of A–T base pairs (duplex **D3**) decreased the electron transfer efficiency to 2.4%. However, replacing three A–T base pairs with three Phen–Phen pairs (duplex **D4**) increased the electron transfer efficiency to 7.3%. To test if migrating electrons remain within the DNA π -stack, duplex **D4** was saturated with N_2O .^[10d,13] Irradiation under a N_2O atmosphere maintained a similar cleavage yield (see the Supporting Information), providing evidence that strand cleavage did not occur from solvated electrons present in solution but was due to EET within the DNA π -stack. Single strand **2** (3.4%) and single strand **6** (1.2%) containing A–T base pairs exhibited approximately a 50% decrease in electron transfer efficiency

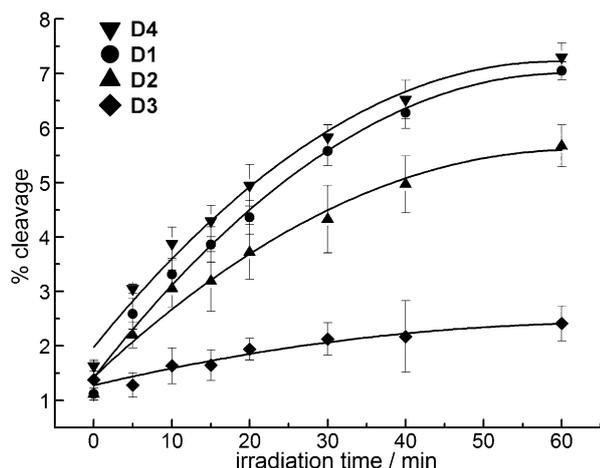


Figure 3. Accumulation of product a, from EET induced DNA strand cleavage, as a function of time. Conditions as given in the legend of Figure 2.

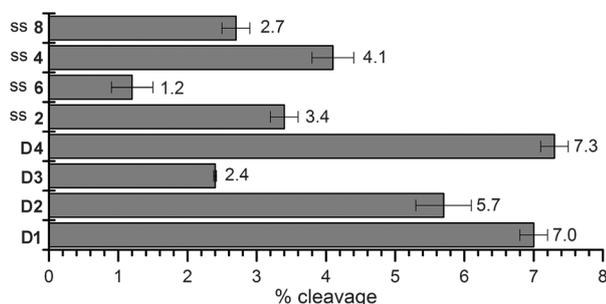


Figure 4. DNA cleavage yields for duplexes **D1–D4** and single strands (ss) **2, 4, 6, and 8** after 60 min of UV irradiation.

when compared to the duplex DNAs (Figure 3). Phen–Phen containing single strands also exhibited a decrease in EET efficiency. However, single strand **4** (4.1%) containing one Phen residue was only 28% less efficient than duplex **D2** (5.7%) containing a Phen–Phen pair, whereas single strand **8** (2.7%) containing three Phen residues was 63% less efficient than duplex **D4** (7.3%) containing three Phen–Phen pairs.

Both the reduction potentials of Phen (−2.62 V vs. NHE)^[14] and thymine (−2.1 V vs. SCE)^[15] are high enough to accept an electron from photoexcited PTZ. Assuming that electron transfer from PTZ to thymine or Phen is an exergonic process (Figure 1b), based on the above-mentioned reduction potentials, electron transfer should occur more efficiently to thymine than to Phen. This was observed in duplex DNAs **D1** and **D2**. However, in duplex **D3** containing three A–T base pairs we observed a decrease in EET efficiency by increasing the distance from one A–T (3.4 Å) to three A–T base pairs (10.2 Å).^[10d,16] On the other hand, three Phen–Phen pairs (**D2**) mediate efficient electron transfer over a distance that is supposed to be twice as long (20.4 Å). This increase in distance is based on interstrand intercalated Phen residues, a structural model derived from biphenyl containing DNA.^[17] This model may also apply here,

although there exists no rigorous experimental proof for it. Although the exact reasons for this efficient EET is not evident from our studies, we can speculate that the six phenanthrenes present in duplex **D4** are interacting/ π -stacking in a way that produces favorable LUMO overlap. Asymmetry in the overlap of the HOMOs of the DNA bases has been proposed as an explanation for hole-transfer preference in the 5'→3' direction.^[18] By analogy, LUMO orbital overlap has also been used to rationalize EET transfer efficiencies.^[10e,19] Less favorable LUMO overlap in single strands could explain why three intrastrand stacked Phen residues are 63% less efficient in EET than duplex **D4** containing three interstrand stacked Phen–Phen pairs.

From these experiments we conclude that EET readily occurs through DNA duplexes containing up to three Phen–Phen pairs with an efficiency that is superior to duplexes containing three A–T base pairs instead, despite of the fact that the distance between injector and acceptor is larger in the former case. We hypothesize that the reduction potential of PTZ is high enough to reduce a Phen unit, permitting EET through a hopping mechanism, much in the same way as it occurs through long stretches of A–T base pairs. We have shown previously that the Phen units can be peripherally modified with electron donating or accepting groups without compromising with the interstrand intercalation recognition motif.^[9] This enables, in the future, the installation of a LUMO gradient within the stack of Phen units in a DNA duplex that may suppress back electron transfer.^[20] This may render EET over longer distances even more efficient. Moreover, such DNA assemblies could essentially be based also on other aromatic units with favorable electronic conducting properties.

Experimental Section

All details are provided in the Supporting Information.

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Keywords: DNA recognition • electron transfer • oligonucleotides • phenanthrene • stacking interactions

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