

## Enhancing excess electron transport in DNA†

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The efficiency of excess electron transport in duplex DNA can be enhanced by limiting the pathways available for migration and using a donor of moderate strength that suppresses radical recombination through selective electron transfer to distal pyrimidines rather than proximal purines.

Charge transport (CT) in DNA contributes to a range of natural processes and has recently been integrated into nanostructures and sensing devices.<sup>1,2</sup> However, challenges still remain before DNA may be widely applied to nanoelectronics, and much effort has focused on identifying and optimizing parameters that limit CT efficiency between defined donors and acceptors. The intervening nucleotide sequence, helical structure and conformational dynamics are all crucial in optimizing long distance CT.<sup>3</sup> The selection of appropriate donors and acceptors is also equally important for successful CT.<sup>4–6</sup>

The number of pathways available for charge migration in DNA is another variable that is expected to influence the apparent efficiency of transport between a specific donor–acceptor pair, but direct study of this has been minimal. Most model systems are based on DNA with a charge donor appended to the 5'-terminus of a duplex strand. For example, hairpin systems are typically capped with a terminal donor.<sup>7</sup> Likewise, most double-stranded systems using organic and organometallic donors rely on their attachment near to a helical terminus as well.<sup>2,8</sup> Alternative placement of a donor at an internal position within helical DNA has received less attention and offers an intrinsic competition between charge migration in two directions (Fig. 1).<sup>9–11</sup> The efficiency of delivering an electron from a donor in the middle of a duplex to an acceptor (BrdU) has now been compared directly to an equivalent system with the donor at the duplex terminus in order to restrict electron migration.

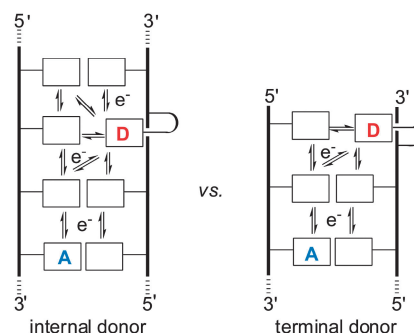


Fig. 1 Freedom of charge migration may influence the efficiency of its transport from a donor (D) to an acceptor (A) in DNA.

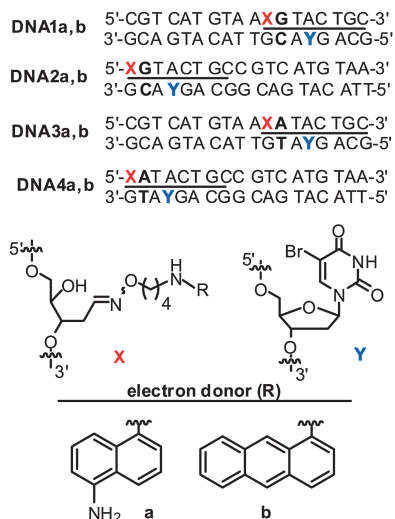
Transport of charge from a donor to acceptor also competes against rapid recombination of the proximal ion pair formed by initial charge injection into DNA.<sup>12</sup> Both experimental and theoretical investigations have demonstrated that increasing the distance between these counter ions will suppress their non-productive recombination.<sup>13</sup> Judicious choice of donors and their surrounding nucleotide sequence may reduce this back electron transfer as illustrated previously with hole transport.<sup>4,14</sup> This same strategy has now been tested as described below for enhancing excess electron transfer (EET) initiated by single electron transfer from a photoexcited donor to DNA.

Our model system for comparing donor placement and electron injection relied on BrdU as an electron acceptor. This is easily incorporated into DNA by standard phosphoramidite chemistry and acts with a trapping efficiency near unity by rapidly eliminating bromide and generating a transient uridyl radical that oxidizes its 5' neighboring deoxyribose and creates a site of alkaline lability.<sup>10,15,16</sup> A methylated derivative of the chosen diaminonaphthyl donor (DN) had originally been synthesized using a norbornyl protection strategy,<sup>10</sup> but the parent donor lacking the methyl groups could only be isolated in low yield under equivalent conditions. Substituting the norbornyl group with a phthalimide group facilitated deprotection and provided high yields of the desired donor (**a**, Chart 1 and ESI†). The donor was easily coupled through its hydroxylamine group

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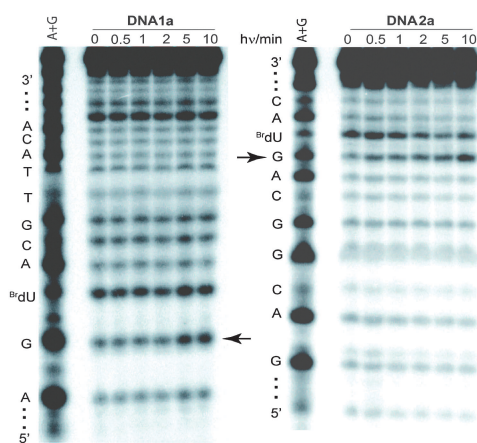


**Chart 1** Duplex DNA alternatively containing donors of (a) diaminonaphthalene and (b) aminoanthracene for EET studies.

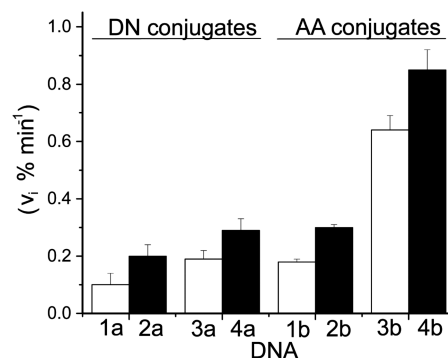
to abasic sites in oligonucleotides that resulted from deglycosylation of the uridine-containing parents (ESI<sup>†</sup>).<sup>17</sup>

Restricting the potential migration of an excess electron in DNA was expected to enhance the efficiency of CT from donor to acceptor. This was confirmed after reaction was initiated by irradiation of the donor DN ( $\lambda > 335$  nm) in duplexes **DNA1a** and **DNA2a** that contained identical nucleotide sequences but alternative placement of the donor DN and acceptor <sup>Br</sup>dU within the duplex as indicated in Chart 1. However, the sequence and the 5' to 3' orientation between these redox active centers remained constant. The characteristic strand scission resulting from electron trapping by <sup>Br</sup>dU was quantified after gel electrophoresis and monitored over time of irradiation (Fig. 2). Initial rates of scission were indeed enhanced 2-fold for the duplex with DN at a terminal *versus* an internal position (Fig. 3). However, the signal-to-noise ratio for this first comparison was only moderate due to the relatively low efficiency of CT.

A second set of duplexes (**DNA3a** and **DNA4a**, Chart 1) was subsequently prepared with an expectation of increasing the



**Fig. 2** Phosphorimager of 20% denaturing polyacrylamide gels showing strand scission of **DNA1a** and **DNA2b** after UV irradiation ( $\lambda > 335$  nm) and piperidine treatment. Arrows indicate the scission sites caused by electron trapping by the <sup>Br</sup>dU residue.



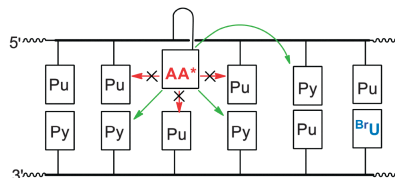
**Fig. 3** EET from donors (DN and AA) to <sup>Br</sup>dU in **DNA1**–**DNA4** as indicated by strand scission after UV irradiation and piperidine treatment. Initial scission rates ( $v_i$ ) were obtained by linear fitting of % scission vs. time (ESI<sup>†</sup>). Each analysis was repeated a minimum of three times and error bars represent their standard deviation.

signal-to-noise for an additional test of donor placement and electron migration. For these duplexes, the G/C base pair adjacent to the donor was replaced with an A/T to avoid the likely suppression of electron migration encountered from an intermediate cytosine radical that is stabilized by proton transfer from its hydrogen bonding partner guanine.<sup>5,11,18</sup> This change of sequence was sufficient to enhance the efficiency of CT to the acceptor <sup>Br</sup>dU by more than 100% (Fig. 3), and the trend of efficiency *versus* donor position remained constant. Each example illustrates the benefit of limiting the migration of the injected electron by placing the donor at a helix terminus.

The reducing potential of the electron donor is another variable that may affect the efficiency of delivering charge to its acceptor within DNA. Initial selection of these donors was guided by the original uncertainty that excess electron transport was even possible in DNA. Thus, a very strong photoexcited donor ( $E_{ox}^*$  below  $-3$  V vs. SCE) was selected at first for injecting an electron indiscriminately into any of the nucleobases.<sup>10</sup> This strategy became unnecessary and unsuitable once back electron transfer from the initial charge-separated species was found to limit long distance transport.<sup>12</sup> Alternative application of a more selective donor provided an opportunity to suppress radical recombination by placing an unreactive nucleobase between the initial pair of charges.<sup>4,14</sup> In order to investigate this strategy, a derivative of 1-aminoanthracene (AA) was chosen as a close analog of DN for comparison. Unlike the excited state of DN, the excited state of AA is capable of only reducing pyrimidines and not purines due to its moderate potential of  $-2.25$  V vs. SCE.<sup>19,20</sup> Consequently, surrounding AA with purines enforced a distance between charged species that should enhance excess electron transport between donor and acceptor in DNA (Fig. 4).

The donor AA was prepared and conjugated to DNA by the same methods used for the DN derivative (ESI<sup>†</sup>). Transport efficiency was then measured within identical nucleotide sequences (Chart 1) to establish direct comparisons between AA, DN and the trends already established by DN. In each example, the AA conjugates supported a greater level of transport than their equivalent DN conjugates (Fig. 3, ESI<sup>†</sup>). The enhancement ranged from a low of approximately 50% for **DNA2** to a high of 250% for **DNA4**. In addition, preferential transport from donors placed at a terminal rather than central region of a duplex was confirmed





**Fig. 4** Photoexcited 1-aminoanthracene (AA\*) conjugates are capable of injecting electrons to pyrimidines (Py) but not purines (Pu).

by comparing AA-containing **DNA1b** and **DNA3b** with **DNA2b** and **DNA4b**, respectively. The sensitivity of excess electron migration through A/T base pairs vs. G/C base pairs is even more pronounced for the selective AA donor as evident from a comparison between **DNA2b** and **DNA4b** (Fig. 3). When all parameters are optimized – donor placement, transport sequence and redox potential of the excited-state donor – the efficiency of CT increases by more than 8-fold as represented by **DNA1a** and **DNA4b** (Fig. 3).

Alternative explanations for this enhanced efficiency of excess electron transport were also considered, but none were consistent with the data or literature precedent. The integrated absorptivities above 335 nm for both donors were nearly identical ( $\text{ESI}^+$ ) and could not account for differences in electron injection into DNA. Conformational differences between AA and DN linked to DNA are also expected to be negligible. Their conjugation to the abasic parent duplexes increased the thermal stability almost equivalently with an average of +5 °C for DN and +6 °C for AA ( $\text{ESI}^+$ ). Typically, aromatic compounds conjugated to the 5'-terminus stack on top of the helix rather than unwind and intercalate into the duplex.<sup>6,21</sup> The similarity in stabilization detected for terminal and internal conjugates indicates similar binding modes, namely stacking rather than intercalation. A solvatochromic derivative of DN also recently confirmed its accommodation within an abasic site.<sup>22</sup> Finally, reduction of  $\text{Br}^{\text{d}}$ U through interduplex transfer of an electron would not have exhibited the sensitivity to the internal nucleobase sequence as evident with **DNA1–DNA4**. Moreover, our original studies with a related DN conjugate were insensitive to conditions that suppress intermolecular processes.<sup>10</sup>

Limiting the available paths for electron migration and suppressing back electron transfer from the initial charge-separated states measurably promote excess electron transport in duplex DNA. These parameters represent only two of the many that affect transport, but their influence is significant enough to support a large increase in its efficiency from the aromatic donor to the  $\text{Br}^{\text{d}}$ U electron trap. Combined with the known preference of excess electron transport for A/T vs. G/C base pairing,<sup>5,11,18</sup> the efficiency of CT can be controlled by judicious choice of donor potential and DNA sequence. Such considerations should be incorporated into future systems that rely on excess electron transport through DNA to convey a signal, trigger an actuator or initiate other related applications of nanosystems based on DNA.

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