

USING PHOTOINDUCED ELECTRON TRANSFER TO DYNAMICALLY CONTROL RET NETWORKS

Nathan Li

Advisor: Professor Chris Dwyer

Department of Electrical and Computer Engineering
Duke University

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1 Abstract

DNA resonance energy transfer networks are a promising framework for future computer architecture applications. With this approach, a computer could be self-assembled, more compact, biological, and capable of a wider range of calculations. However with the relative novelty of this field, many issues still remain unsolved before a functioning computer can be achieved. One many issue is multiplexing and ability to take multiple inputs.

The purpose of this research is to explore a new method that could potentially solve the issue of multiplexing. By taking advantage of photoinduced electron transfer, a new technique has been developed to dynamically change the energy transfer interactions. With this procedure, light inputs can produce radicals which can route light through the desired pathway. This paper sets up an experiment for a proof of concept and illustrates initial results.

2 Introduction

Computers have brought significant change to society and ushered in the Information Age. The importance of computers has led to the rapid growth of processors. For the past few decades, the progression of semiconductor hardware has followed Moore's Law, and the number of transistors in processors has doubled every two years [1]. However, as research approaches the physical constraints of semiconductor transistors [2], there is an increased demand to develop a new framework.

DNA resonance energy transfer (RET) networks have emerged as a promising new foundation for future processors. Modern processors take electrical energy and route this energy through a network of transistors to create a measurable electrical output, but with RET networks, light energy passes through a network of chromophores to fluoresce a light output. These RET networks will potentially be able to maintain computation power with advantages in size, ease of assembly, cost, and *in vivo* expansion [3].

These benefits have not yet been realized due to the relative recent novelty of this field. This technology is a relatively new field of research and still has many issues to be solved. One major problem is the lack of proper pathway control and multiplexing. This thesis describes a new approach to this problem using photoinduced electron transfer to dynamically change the rate constants of a RET network.

3 Background

DNA RET Networks

DNA is an attractive framework due to its size, self-assembly, and controllability. Due to its strong binding properties, DNA has been shown to reliably self-assemble into various motifs. By controlling the sequence of the nucleotides, the DNA can be engineered to form the desired structures. One structure of particular interest is a grid structure. This design has been successfully demonstrated and is especially useful due to its ability to combine into larger motifs. By utilizing sticky ends, small grids can attach to each other and reach a desired size constraint [4].

These unique properties enable DNA tiles to be the ideal "breadboards" for chromophores. Chromophores, molecules that emit and absorb light, are responsible for the logic in this field of molecular computing, and they can be attached to the DNA through various chemical reactions

[5]. Once linked to the DNA grid, the chromophores are locked in place and the functionality of the network can be predicted and engineered. Thus, the challenges behind fabricating RET networks are the proper selection of chromophores and determination of suitable positions.

One major interaction between chromophores is resonance energy transfer (RET), and this phenomenon has been well-characterized and understood. This energy transfer relies upon a dipole-dipole coupling and enables nonradiative exciton transfer between chromophores in close proximity. To calculate the transfer efficiency between two chromophores, the main parameters are the distance of separation, the orientation of the dipoles of each chromophore, the spectral overlap between the donor's emission spectrum and the acceptor's excitation spectrum, extinction coefficients, and the buffer [6].

However, because RET depends primary on these factors, there is a strong need to be able to dynamically control one parameter and adjust RET interactions. Previously proposed methods include the saturation effect. The saturation effect closes RET connections by taking advantage of quantum states and the inability of excited chromophores to receive an excitons. This effect has been exploited to innovate new devices such as C-DEV, but there are still issues that remain prevalent even with this technique. For example, C-DEV functions similarly to a transistor by controlling the flow of light; however, the amplification of light current is not large enough to produce reliable results. Thus, pathway control in RET networks continues to be an active field of research with a high demand for a better solution [7].

Photoinduced Electron Transfer

Photoinduced electron transfer (PET) is another interaction between chromophores, though not quite as well-characterized and predictable. Unlike RET, PET involves an electron physically jumping from one donor to an acceptor instead of an exciton. For such an event to occur, PET requires the two chromophores to be at a significantly closer distance that is acceptable for quantum tunneling at a sufficient probability. Once this condition is satisfied, an electron can jump between molecules and form radical charged molecules, and slowly over time the electron will return and cause the radical population to decay [8].

Despite the difficulty and lack of understanding behind PET, there are major advantages that RET alone cannot realize. The generation of radicals and potential controllability of these radicals is especially important. By changing the chromophores into their radical forms, this alters the spectroscopic properties [10] and, in turn, can potentially modify the RET interactions. Therefore, PET can lead to new systems that can dynamically adjust RET and enable better pathway control. In addition, the possible control over the longevity of these radicals also facilitates the range of applications this technology can encompass.

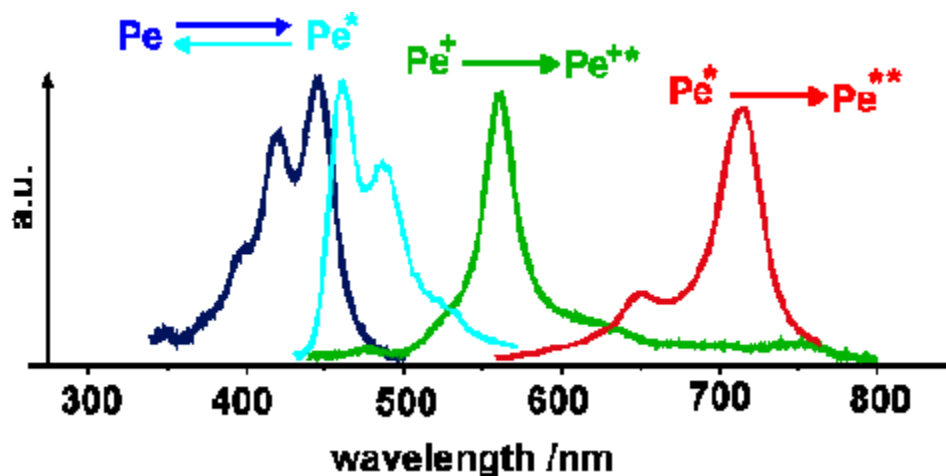


Figure 1: Change in absorption spectra due to radical

4 Theory and Experimental Design

It was hypothesized that a radical's change in absorption spectrum would alter the RET interactions with its surrounding partners. Potentially, this could instantaneously change a RET transfer efficiency from 0% to 100%. This outcome would have immediate benefits in RET network pathway control, as it enables one to dynamically and reversibly change the properties of a RET network using only light.

The goal of this project was to show that the radicals' change in spectroscopic properties will alter RET pathways at the most basic level. To accomplish this, a simple experiment with three chromophores was designed to demonstrate this effect. Two molecules, a PET donor and PET acceptor, would be placed proximally and induce electron transfer once excited. Another molecule, a RET donor, would be placed further away. This RET donor would be excited but unable to transfer its energy until the PET donor has changed into its radical state.

Without any radicals in the system, the RET molecule should ideally not exhibit any RET to the other two chromophores. Only once the radicals are created, there will be strong energy transfer between the RET molecule and the PET donor radical, so the RET molecule will transfer its energy instead of fluorescing. Thus, the predicted outcome is a decrease in fluoresce of the RET molecule when both the PET and RET molecules are excited compared to if only the RET molecule was excited.

For such an experiment, it is crucial to select molecules that maximize this change in RET to obtain the clearest change in fluorescence. The PET electron donor was selected first to be perylene because of the plethora of information on perylene's cation radical [11-13]. Previous experiments have shown perylene to be easily oxidized with an oxidation potential of 1.04eV [14], and literature exists characterizing the absorption spectrum and possible emission spectrum of the cation [13]. Candidate PET acceptors were then chosen by analyzing reduction potentials, and then a MATLAB program iterated through all the possible combinations of networks to determine which network would demonstrate the largest change in transfer efficiency. The final network consisted of Alexa Fluor 488 as the PET electron acceptor, Alexa Fluor 546 as the RET energy donor, and perylene as the PET electron donor. This network minimizes undesired energy transfer before the PET chromophores reach their radical state, and the radical perylene has strong absorbance at Alexa Fluor 546's emission wavelengths. The distances were chosen based

on past experiments [15] of PET in DNA and expected rate constants. The overall predicted rate constants of the system are shown below in Figure 2.

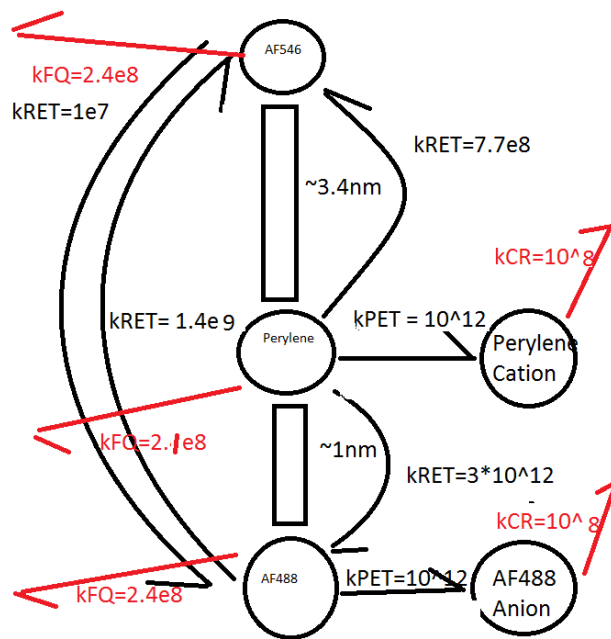


Figure 2: Rate constants of every interaction in the proposed system

The spectral overlap of the chromophores and radicals are shown below in Figure 3. There is a clear separation between all three non-radical absorption spectra, allowing for separate wavelengths to selectively excite each chromophore. In addition, the emission spectrum of the AF546 has minimal overlap with non-radical absorption spectra while having a large degree of intersection with the radical's absorption spectra.

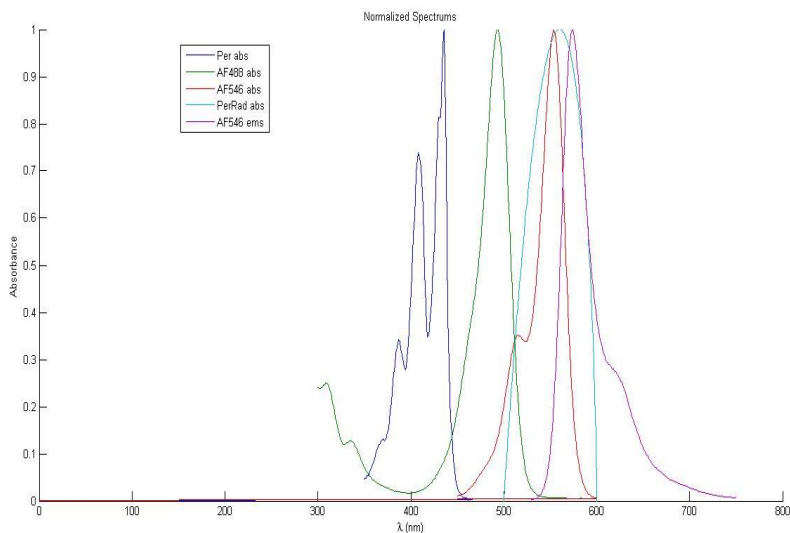


Figure 3: Absorption and emission spectra for the proposed system

The AF488 radical was not shown due to lack of data and understanding of the radical's properties. There is a possibility that the AF488 radical's spectrum also coincides with emission spectrum of AF546, which would cause an even greater decrease in fluorescence. This lack of information could be problematic for future networks, but due to the scope of the experiment, this should not have a dramatic impact.

5 Simulation Results

Using SCIMM, the RET interactions of the pre-radical chromophore system and radical system were simulated to understand the expected change in fluorescence. The overall RET network was split into two simulations due to SCIMM's current inability to properly model PET radical generation.

The results of the non-radical network are shown in Figures 4-6. Of the 100 excitation events, less than 10% of AF546's energy is transferred to the PET acceptor molecule. This energy could potentially lead to generation of radicals, which would not be ideal but still relatively low.

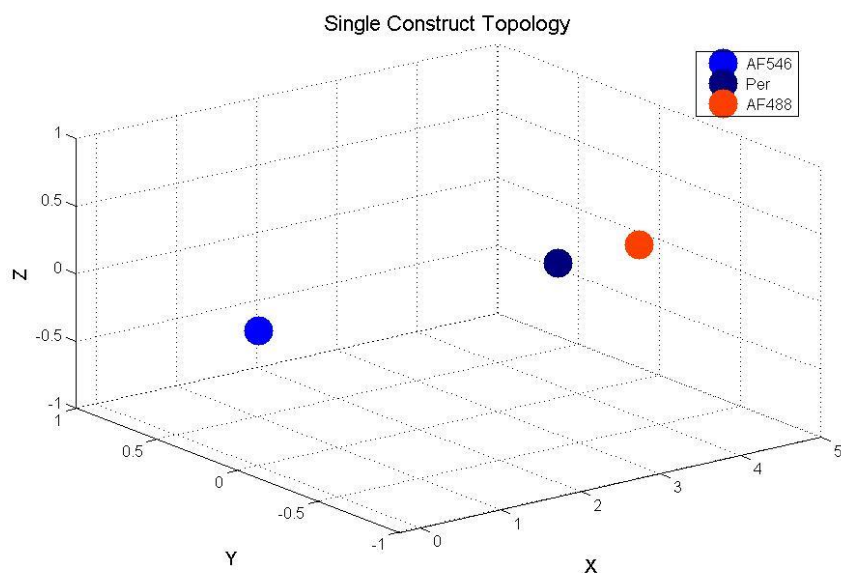


Figure 4: Topography of simulation in 3D space

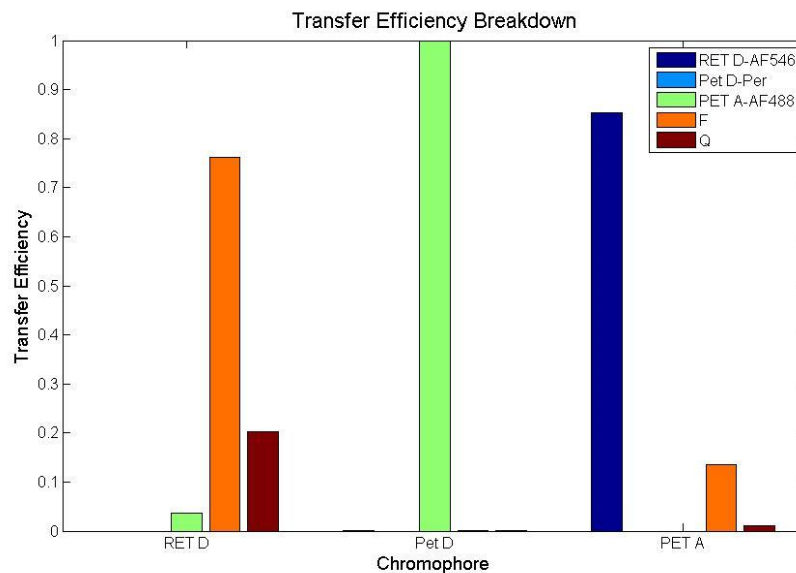


Figure 5: Transfer efficiencies of the network

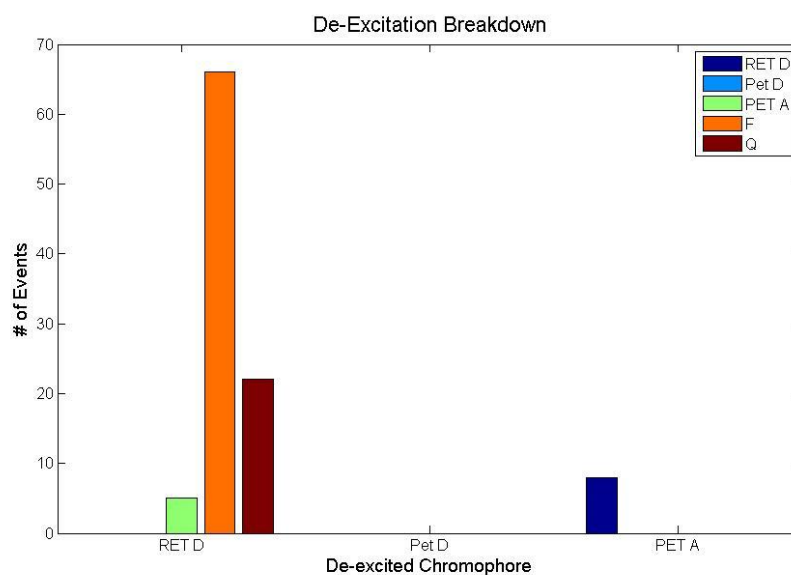


Figure 6: Simulated de-excitation events for a laser input at 500nm

The results of the radical molecule interaction can be shown in Figures 7-9. In this simulation, there is a clear increase in RET from the RET donor to the radical molecule, and about 70% of the excitons transfer from the RET donor molecule to the perylene radical. Again, the AF488 radical was not shown due to lack of information about its radical state.

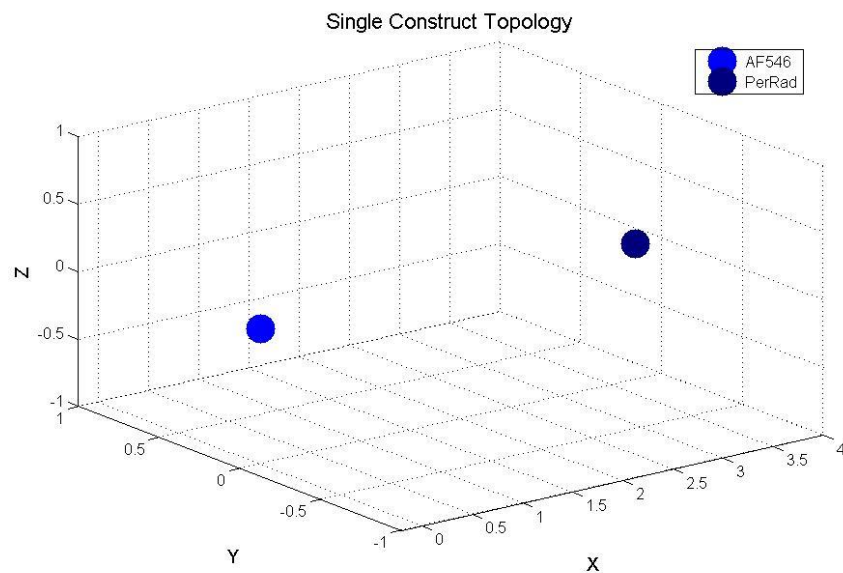


Figure 7: Topography of radical simulation in 3D space

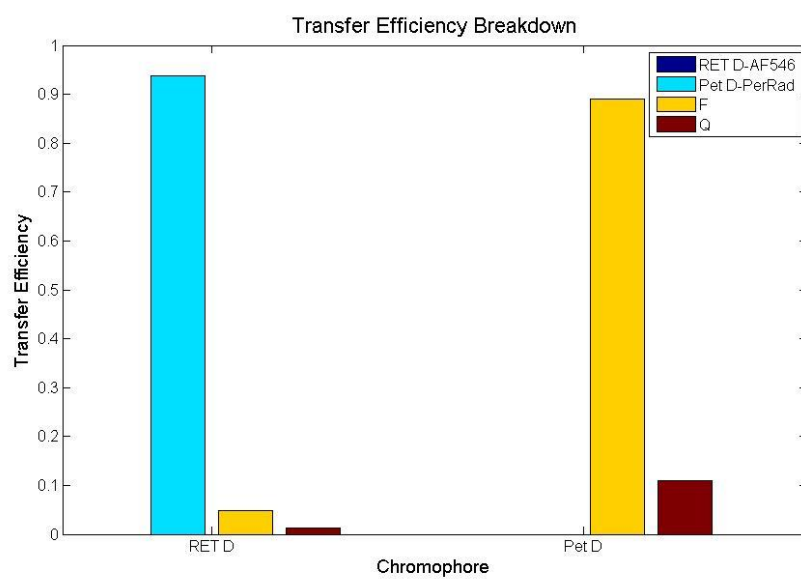


Figure 8: Transfer efficiencies of the radical network

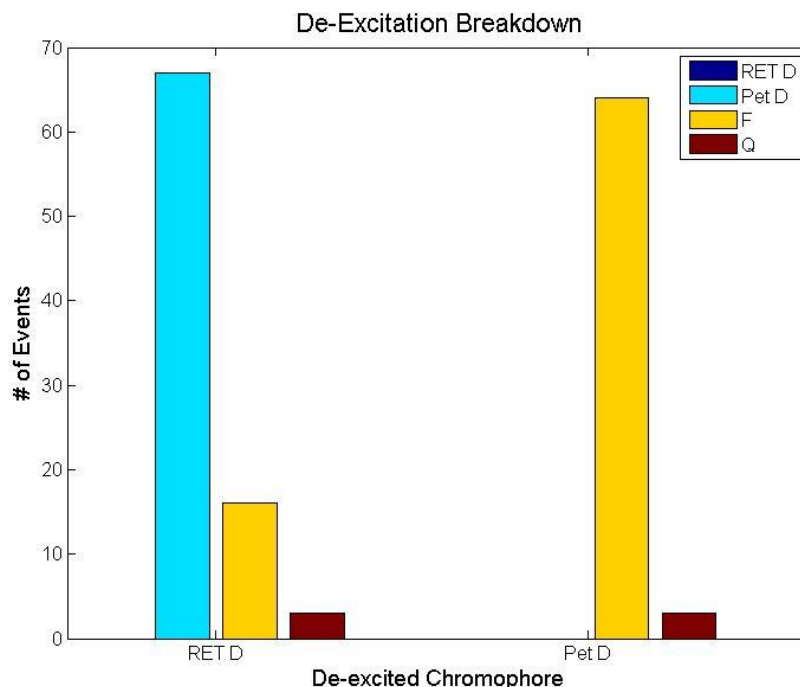


Figure 9: Simulated de-excitation events for a laser input at 500nm in the radical network

6 Construction and Verification of DNA Structure

The RET network was fabricated on a custom DNA duplex. This decision was made to minimize surrounding guanine bases, which are known to have strong oxidation properties and potentially undergo electron transfer with excited chromophores. The absence of guanine molecules within a four base radius ensures minimization of this undesired possibility. The strand and chromophore positions are shown below in Figure 10. To construct the final duplex, three different single strands were used, each attached to one chromophore. The bottom half of the duplex shown in the figure is split into two single strands as indicated by the line of separation. The strands were chosen with a sequence such that the formation of unwanted hybridizations rarely occurred.

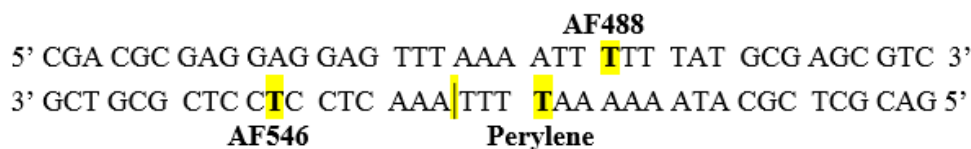


Figure 10: Custom DNA duplex used in PET experiment

The chromophores were each attached through a series of conjugations reactions. The AF546 and AF488 chromophores were attached by reacting an amino-modified oligonucleotide with amine-reactive succinimidyl ester dye. The reaction was performed with a dye to DNA ratio of 30:1, and the reaction ran overnight in a shaker and subsequently spin-filtered to remove unattached dye.

The perylene was attached by a click chemistry reaction. Alkyne-modified perylene at a concentration of 600 μ M was reacted with azide-modified 100 μ M DNA by following an online click chemistry protocol [16].

The yields of the conjugation reactions were found using capillary electrophoresis and are shown below in Table 1. In the CE results, the peaks were distinguished by running CE on non-conjugated DNA as a control. To determine the percent conjugation, the areas of each peak were determined, and the area of the conjugated DNA was compared to the area of the non-conjugated DNA. The percent conjugation for each strand is important to derive the overall structure yield.

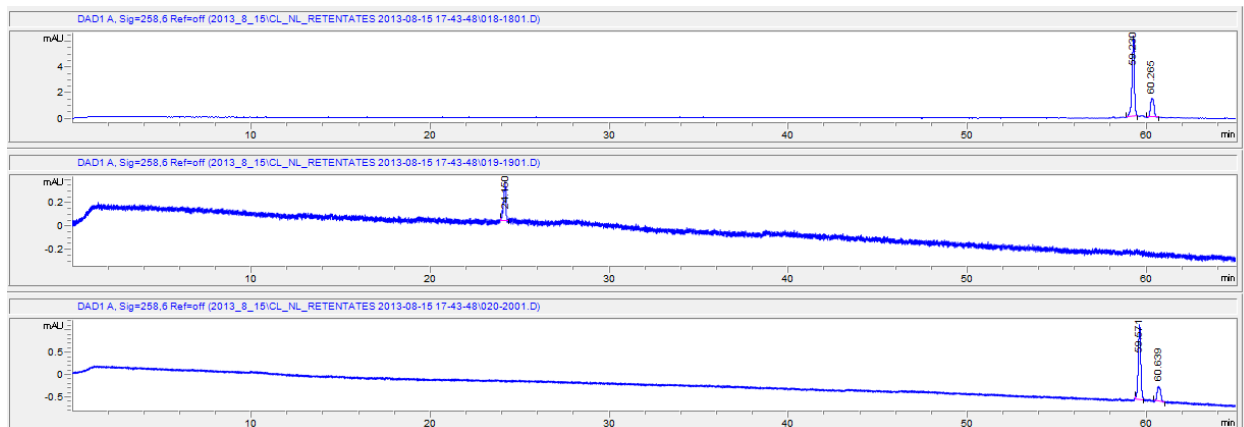


Figure 11: CE results for AF546 conjugate. Retentate (top), non-conjugated DNA (middle), retentate+DNA (bottom)

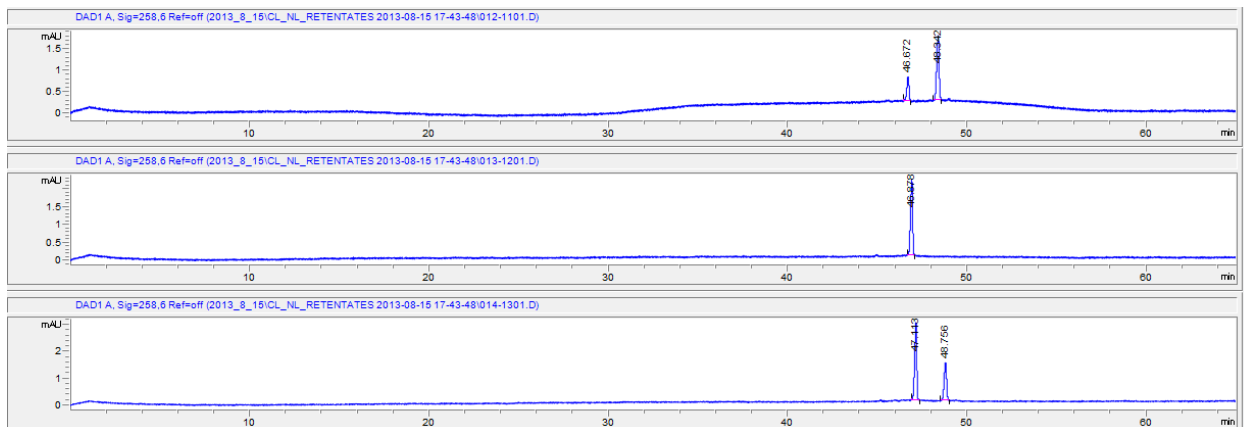


Figure 12: CE results for AF488 conjugate. Retentate (top), non-conjugated DNA (middle), retentate+DNA (bottom)

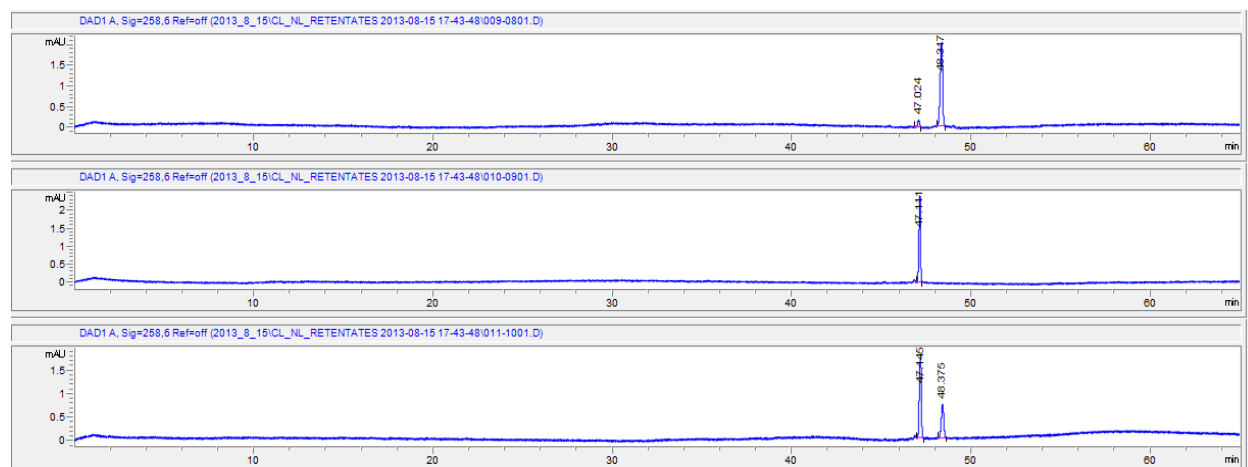


Figure 13: CE results for perylene conjugate. Retentate (top), non-conjugated DNA (middle), retentate+DNA (bottom)

| DNA strand attachment | Percent Conjugation |
|-----------------------|---------------------|
| AF546 | 25% |
| AF488 | 75.3% |
| Perylene | 100% |

Table 1: Conjugation Yields

7 Laser Experiment Results

First, the characteristics of each chromophore were determined and compared to literature results. This step was important to control for any discrepancies with the buffers and molecules from the literature. This information also determined which wavelengths to use to selectively excite specific chromophores without exciting the others. The excitation and emission spectra of each chromophore was found by exciting the dyes in the 12.5mM NaTB buffer. The excitation curve was derived by exciting each chromophore over a span of wavelengths and measuring the output fluorescence at the peak emission wavelength. The fluorescence was adjusted for the power output of the laser to obtain a normalized value. Because the laser does not output wavelengths below 470nm, the measurements for the absorption curves did not include any wavelengths below 470. For this reason, perylene's absorption spectrum could not be obtained, as its entire spectrum is below 470nm. The emission spectrum was determined using a 409nm laser diode. The peak emission wavelengths were 519nm for AF488 and 555nm for AF546.

The time constants of AF488 and AF546 were determined by observing the transient fluorescence of both chromophores and fitting this curve to an exponential. This was measured to demonstrate the changes in the time constants after RET.

| Chromophore | Calculated τ (ns) | Literature τ (ns) |
|-------------|------------------------|------------------------|
| AF488 | 4.2662 | 4.1 |
| AF546 | 4.1454 | 4.1 |

Table 2: Time constants for AF488 and AF546

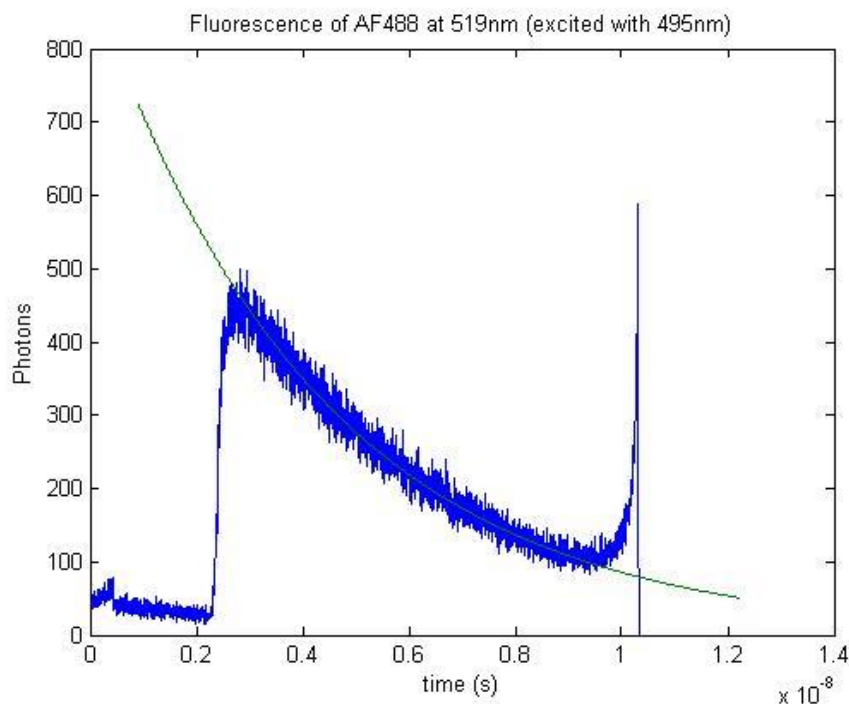


Figure 14: Transient fluorescence of AF488 and the exponential fit

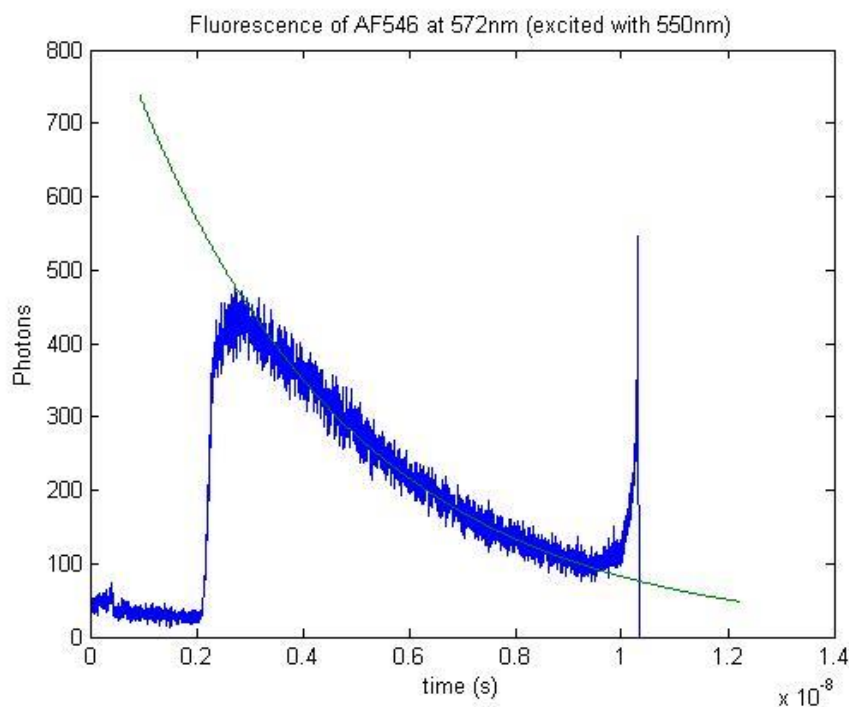


Figure 15: Transient fluorescence of AF546 and the exponential fit

The simulation results indicated there should be a small degree of RET between the AF546 RET donor molecule and the AF488 PET acceptor. As a control measurement, the transfer efficiency was measured by creating a RET network consisting of only AF488 and AF546. The perylene DNA strand was replaced with an inert DNA strand. To determine the amount of RET, the

chromophores were selectively excited, 555nm to excite only AF546 and 490nm to excite only AF488. The fluorescence of each chromophore was also selectively measured at 572nm for AF546 and 519nm for AF488. The expected structure yield is 18.8%. The time constants and RET transfer efficiency have not yet been determined.

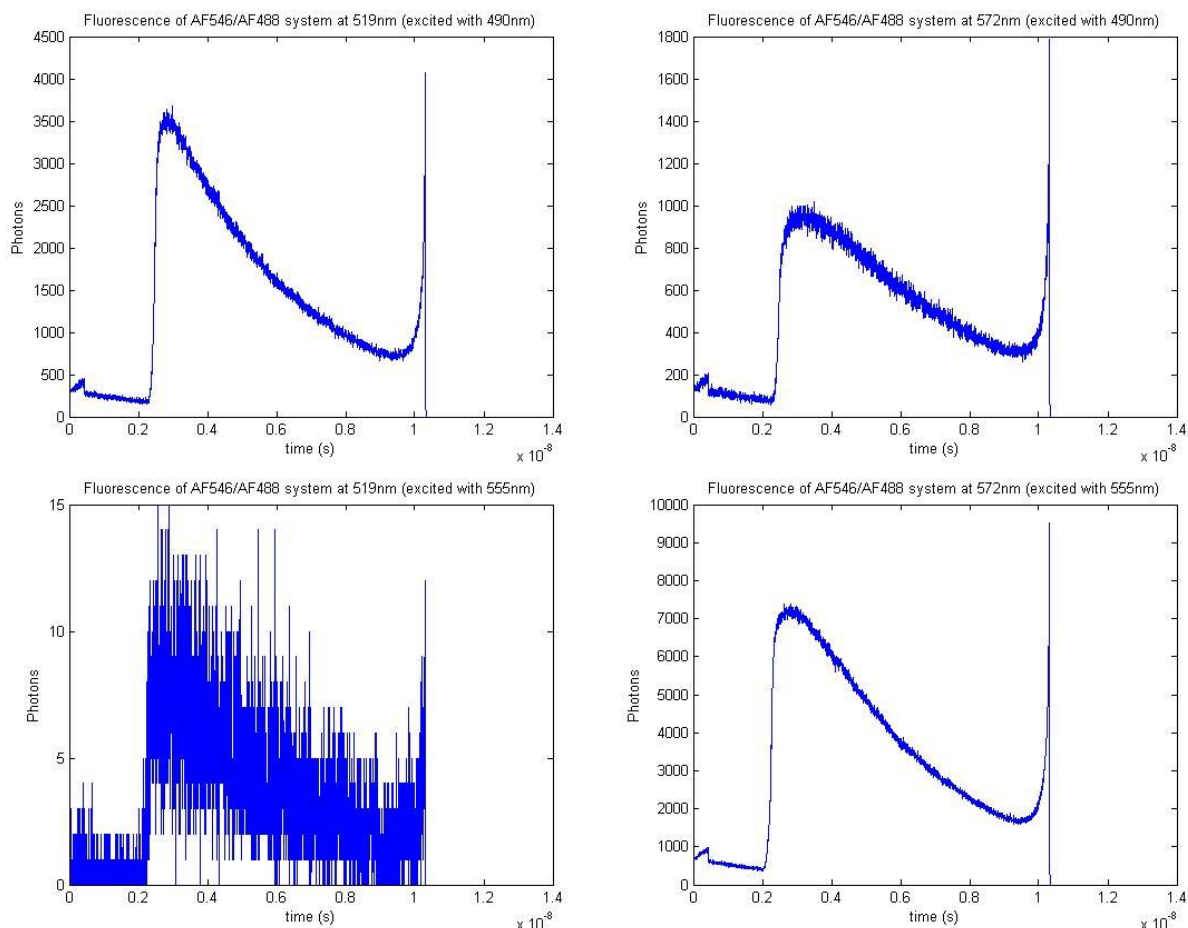


Figure 16: Fluorescence measurements of AF488/AF546 system. Excitation of AF488/emission of AF488 (top left), excitation of AF488/emission of AF546 (top right), excitation of AF546/emission of AF488 (top left), excitation of AF546/emission of AF546 (top left)

Next, the full RET system was excited. The RET donor molecule (AF546) was excited and its emission was monitored. First, only the RET molecule was excited and the fluorescence was demonstrated to remain constant in each trial.

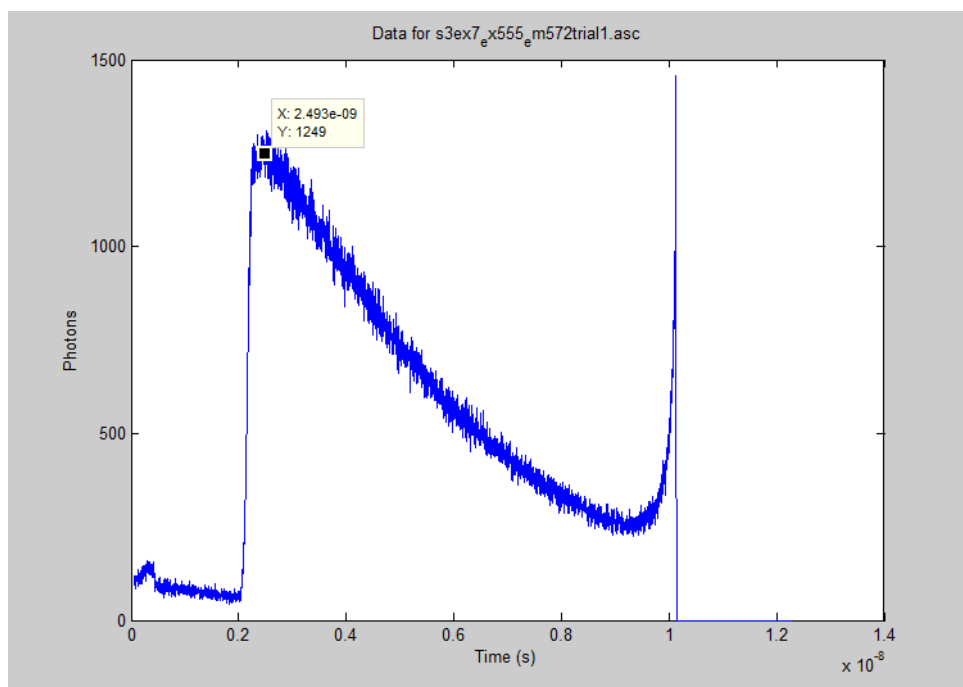


Figure 17: Transient fluorescence of AF546 in the full system when only AF546 is excited

| Trial | Peak Fluorescence (photons) |
|-------|-----------------------------|
| 1 | 1249 |
| 2 | 1262 |
| 3 | 1250 |

Table 3: Peak fluorescence of AF546. Measured for 60s at a wavelength only AF546 emits.
Excited at a wavelength only AF546 absorbs

This information indicates the lack of change between trials, indicating the sample concentration and laser intensity are sufficient to ensure negligible photo-bleaching. Photo-bleaching would cause the absorption of photons to decrease with every usage of a network which would reduce the overall fluorescence.

Next, the laser diode was turned on for a period of time to allow radicals to form. After waiting, the RET donor was again excited and its fluorescence was observed. Once the trial was over, the system was allowed to recover to its initial state with a fluorescence around 1300 photons and then the next trial proceeded. This was done for various time trials.

| Trial | Peak Fluorescence (photons) | Time since diode turned on |
|-------|-----------------------------|----------------------------|
| 1 | 1302 | 0mins |
| 2 | 1116 | 1.5mins |
| 3 | 1036 | 3mins |

Table 4: Peak fluorescence of AF546. Measured for 60s at a wavelength only AF546 emits.
Excited at a wavelength only AF546 absorbs and at 409nm for radical generation

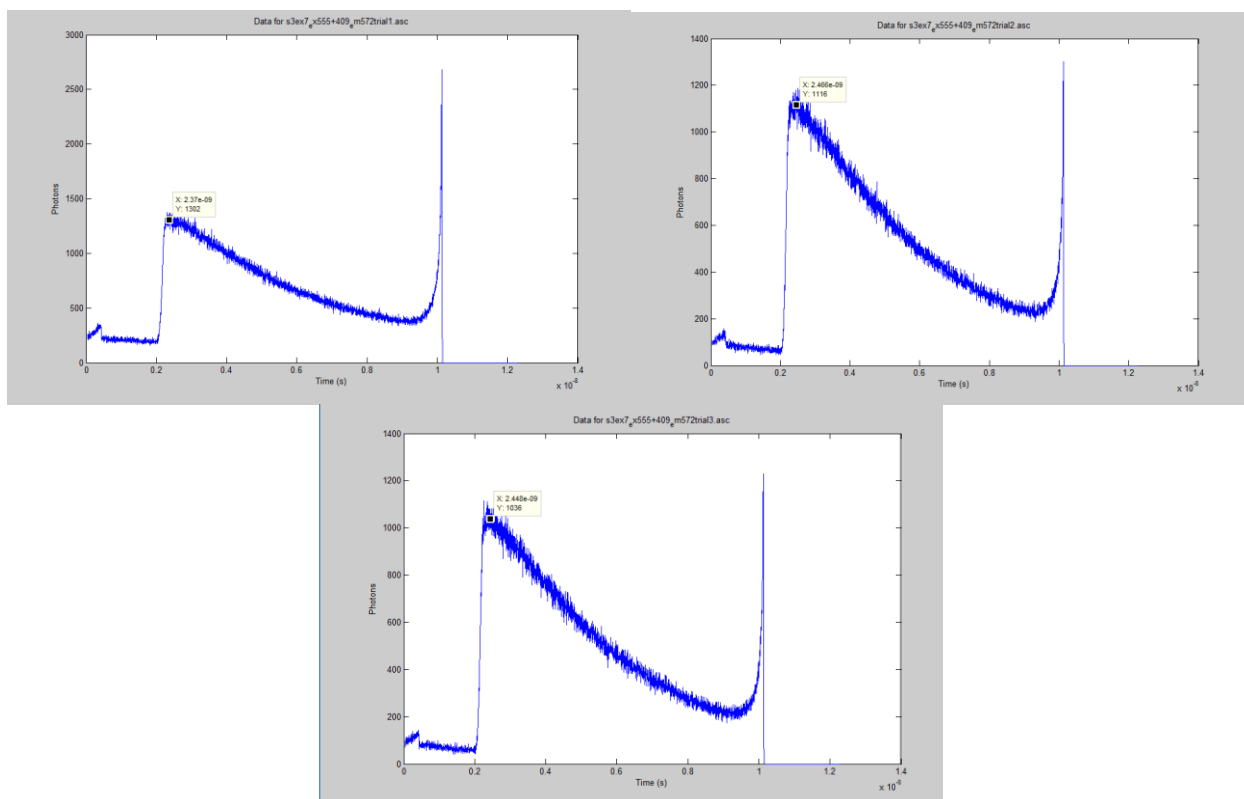


Figure 18: Transient fluorescence measurements for the three trials

The overall fluorescence of the AF546 was shown to decrease with a longer duration of PET excitation. Because the photodiode's output wavelength has no spectral overlap with AF546, it should not have a direct effect on the excitation or emission of AF546. The only molecule that interacts with 409nm light is shown to be perylene. Thus, there exists an interaction between the excited perylene and AF546 molecule. Another possibility is that the perylene radical or AF488 radical absorbs the AF546 excitation photons. Theoretically, the perylene radical's absorption spectrum does not include this wavelength; however, this should be confirmed experimentally. The AF488 radical properties should also be investigated to confirm the theory.

8 Conclusion

The initial results are promising and point towards to possibility of using PET in the future to control RET networks. The results have yet to be replicated or confirmed to be due to the generation of radicals, but this is a starting point to understanding and developing hybrid PET and RET DNA networks. There is still much to be explored and confirmed, especially with the generation of radicals and their interactions. This can be done with high-resolution transient absorption spectroscopy, which can determine the absorption spectra of the radicals, the relative population of radicals, and the lifetime of the radicals. A library of radicals would be especially useful for choosing optimal PET pairs, as there are hundreds of possible pairs to be explored.

Overall, this idea of using radicals to control RET interactions has a wide range of benefits, including the large change in transfer efficiency, controllability of the radical lifetime, and the reversibility of the effects. This tool can be the next step towards realizing a fully functional RET network computer.

9 Acknowledgements

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