Optoelectronics using DNA as a template for dyes

Donna Mamangun[a], Daminda Navarathne[a], Gregory A. Sotzing[a], Jack P. Lombardi[b], Carrie M. Bartsch[b], Emily M. Heckman[b], Kristi M. Singh[b], James G. Grote[b], Thomas R. Nelson, Jr.[b] [a]University of Connecticut, 55 N. Eagleville Road, Storrs, CT, USA 06269-3060; [b]Air Force Research Laboratory, Wright-Patterson Air Force Base, OH

ABSTRACT

Aside from salmon DNA, other DNA sources were explored namely, herring and onion, to prepare DNA-surfactant complex, which will be used as a template for dyes undergoing Forster Resonance Energy Transfer (FRET). Also, salmon DNA of low and high molecular weight were compared. This study aims to assess the effect of using different DNA sources and molecular weight on the efficiency of energy transfer between the dyes, coumarin 480 (Cm 480) and 4-[4-(dimethylamino)styryl]-1-docosyl-pyridinium bromide (Hemi 22 and to understand the fundamental properties of DNA-CTMA as a supporting matrix for optoelectronics applications.

Keywords: DNA-CTMA, FRET, dyes, optoelectronics, solid-state device

1. INTRODUCTION

The molecule of life, deoxyribonucleic acid (DNA), has expanded from its functions in the biological sciences and is now considered a promising material in the material sciences. Similar to other biopolymers, DNA has unique structure and properties that are not easily reproducible in conventional organic or inorganic polymers. Its structure is made up of two intertwined helices of alternating sugar and phosphates connected by hydrogen-bonded base pairs. This unique double helix structure binds small molecules through groove binding or intercalation. Since living organisms produce DNA, it is inherently biodegradable and a renewable resource. Since every living organism has a unique DNA sequence, the number of possible sources is abundant. In addition, DNA can be produced in the laboratory through molecular cloning and polymerase chain reaction. These techniques can provide molecular weight and base sequence control and yield DNA of high purity. However, it is economically impractical to use the said techniques currently, since many applications require a significant amount of material.

DNA by itself is not easily processable. This is a drawback from the materials processing standpoint because aqueous solutions of DNA have poor fiber and film forming properties and cannot be readily incorporated in solid-state devices. To impart processability to DNA, scientists found that when the sodium ions along the phosphate backbone are replaced with surfactants, the resulting material is soluble in many alcohols such as ethanol, methanol, butanol, isopropanol and a mixture of alcohol and chloroform.[1] The most commonly used surfactant is cetyltrimethylammonium (CTMA), which consists of a 16-carbon hydrophobic tail and a positive ammonium head. The C_{16} alkyl chain allows for the recovery of DNA in its native state and imparts good mechanical property to the resulting DNA-CTMA complex.[2]

The processable DNA-lipid complex has many notable properties, making it a good material for optoelectronic applications.[3] Films of DNA-CTMA have been used as an electron-blocking layer in organic light emitting diodes (OLED)[4] and a combined hole-transporting and electron-blocking layer in quantum dots.[5] In organic field effect transistors, it's been used as a dielectric layer.[6] The low optical loss of DNA-CTMA films makes it a good candidate for optical waveguides.[7]

Small molecules can associate to the DNA-CTMA through intercalation, groove-binding and induced dipole – induced dipole interactions within the CTMA phase. We have previously reported that films and fibers of DNA-CTMA can be used as a matrix for dye molecules. Fluorescence enhancement of the dye, 4-[4-(dimethylamino)styryl]-1-docosyl-pyridinium bromide (Hemi 22) in DNA-CTMA thin films and nanofibers was

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observed when compared to conventional host matrix polymethylmethacrylate (PMMA).[8] The double-helix structure of DNA allows for efficient encapsulation of individual dye molecules thus preventing aggregation and self-quenching of dyes. Moreover, the unique interaction of the dye with the DNA also contributes to this amplified emission. We also reported that DNA provided photostability to dye molecules.[9] The bases in the DNA are known to absorb UV radiation and therefore act as a UV filter for the dyes molecules.

Going a step further with this work, we used DNA-CTMA as host for fluorescence resonance energy transfer (FRET) between dyes. With varying ratios between the two dyes, Coumarin 102 (Cm 102) and Hemi 22, the former acting as a donor and the latter an acceptor, we were able to make films that fluoresce from blue to orange going through white.[10] Our next logical step from there is the addition of three dyes into the DNA-lipid complex. The dyes Cm 102, pyrromethene 567 (Pm 567) and sulpharhodamine (SRh) were chosen for this study. Cm 102 was chosen as the donor and SRh as the acceptor while Pm 567 has a dual role, an acceptor for Cm 102 and donor for SRh. From this, we demonstrated the efficient long-range energy transfer between three dyes through cascade FRET in DNA-CTMA.[11] These studies show that DNA is a good candidate for device applications such as sensors, photovoltaics cells, LEDs, lasers and solid-state lighting.

For the applications of DNA-CTMA previously discussed, the most common source of DNA is the salmon milt and roe sacs, which are considered a waste material from the salmon fishing industry. Herein, we report the use of other DNA sources namely, onion and herring, for the preparation of DNA-CTMA. Also, low and high molecular weight salmon DNA will also be used and examined. These DNA complex materials will be evaluated by investigating the energy transfer efficiency between Cm 480 and Hemi 22 across the different DNA sources and molecular weights. The goal is to assess whether the source of DNA, as well as the molecular weight, are factors to consider when using DNA-CTMA for optoelectronic applications.

2. EXPERIMENTAL

2.1 Materials

Salmon and Herring DNA were purchased from Sigma-Aldrich and used as is. Onion DNA was extracted from young onion leaves using a traditional extraction method.[16] Cm 480 and Hemi 22 were bought from Exciton Inc. and Sigma-Aldrich, respectively, and used as is.

2.2 DNA-CTMA Preparation

Aqueous solutions (1% weight by weight, wt/wt) of the DNA samples were put in dialysis membrane with a molecular weight cut-off of 3,500kDa. The dialysate, distilled water reservoir, was changed every 4 hours over a period of 24 hours. To make the DNA-CTMA, the dialyzed DNA solution was added dropwise to an aqueous CTMA solution (1%, wt/wt) (1 DNA bp : 2 mol CTMA) and was left stirring overnight. The resulting precipitate was filtered and put in Soxhlet overnight using distilled water as solvent. The DNA-CTMA complex was then dried in a vacuum oven at 60°C overnight.

2.3 Film Preparation

The DNA-CTMA (5%, wt/wt) stock solution was prepared in ethanol. Cm 480 and Hemi 22 solutions were freshly prepared in ethanol and chloroform, respectively, prior to addition to the DNA-CTMA solution. The molar ratios between the dyes were varied as follows: (donor to acceptor - 1:0, 1:0.01, 1:0.05, 1:0.2, 1:0.5, 1:1, 0:1). The dye loading was kept below 2%, wt/wt with respect to the DNA-CTMA to prevent aggregation. The donor dye was first added to the DNA-CTMA solution followed by the acceptor dye. Lastly, the solutions were diluted with ethanol to achieve a 2% wt/wt, DNA-CTMA to solvent solution. For consistency, the order of addition was kept constant. The dye-doped CTMA solutions were then spin-coated on pre-cleaned glass substrates at 2500 rpm for 60s.

2.4 Optical Characterization

Fluorescence measurements were made using Fluorog-3 using 365nm as excitation wavelength and emission collection was from 400-700nm. UV-Vis measurements were carried out using Varian Cary 5000 UV-Vis-NIR spectrophotometer.

2.5 Energy Transfer Efficiency

The fluorescence energy transfer (FRET) efficiencies were calculated using Equation (1), where I_{DA} and I_D are the fluorescence intensities of the donor with and without acceptor, respectively.[17]

$$\%E = \left(1 - \frac{I_{DA}}{I_D}\right) \times 100. \quad (1)$$

3. RESULTS AND DISCUSSION

The DNAs used in this study, except the low molecular weight salmon DNA, are all genomic DNA. The agarose-gel electrophoresis experiments showed a wide range of molecular weight for all the genomic DNA samples, from 330kDa to greater than 6600kDa. Some high molecular weight genomic DNA gets cut up into smaller molecular weight fragments during the extraction thus giving a smear pattern on the gel. The low molecular weight salmon DNA is 130kDa to 400kDa.

The fluorescence spectra of dye-doped DNA-CTMA films, prepared from salmon, herring and onion, were obtained as shown in Figure 1. The loading of Cm 480 was kept constant while that of Hemi 22 was increased. The behavior of the emission spectra of Cm 480 and Hemi 22 dyes were consistent with what we reported previously.[10] The intensity of Cm 480 emission at ~450nm decreased as the emission of Hemi 22 at ~595nm increased. Also, for the films with 0:1, Cm 480:Hemi 22, there was little to almost no fluorescence detected when excited at 365nm due to the absence of the donor Cm 480. Figure 2 shows a digital image of the DNA-CTMA films made from onion, salmon and herring. With an increasing Hemi 22 loading and constant Cm 480 loading, the color of the films, when viewed under UV light at 365nm, goes from blue to orange.

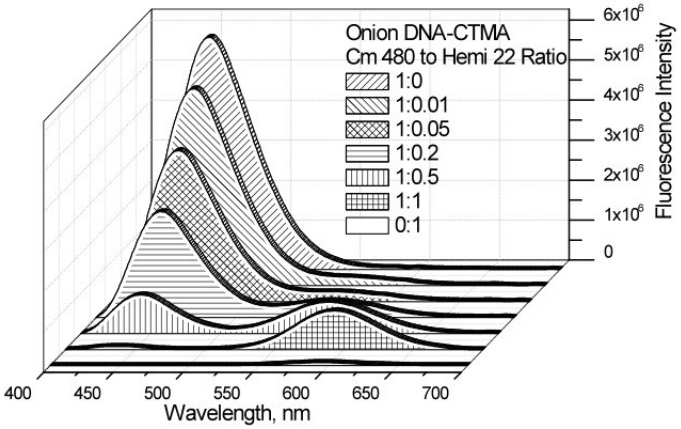

Figure 1. Emission spectra of Onion DNA-CTMA films excited at 365nm.

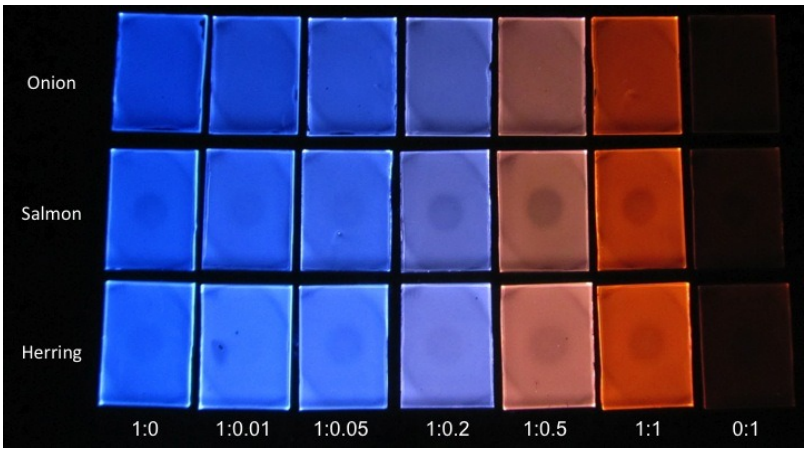

Figure 2. Digital photographs of DNA-CTMA films illuminated under 365 nm UV lamp. The labels indicate the Cm 480 to Hemi 22 molar ratio.

Figure 3 shows the energy transfer efficiency between Cm 480 and Hemi 22 dyes in the DNA-CTMA films. Polymethylmethacrylate (PMMA) was also used as a dye matrix and as a control. All films made of DNA-CTMA gave higher fluorescence yield than PMMA. This result is also consistent with our previous report on DNA being a better template for dye molecules than other conventional polymer matrices.[8] At lower acceptor loading, <0.5, the energy transfer efficiency in onion DNA-CTMA is 2 to 5% higher than the DNA-CTMA made from salmon and herring. However, at higher acceptor loading, ≥0.5, the energy transfer efficiency is similar for all DNA-CTMA films regardless of the DNA source.

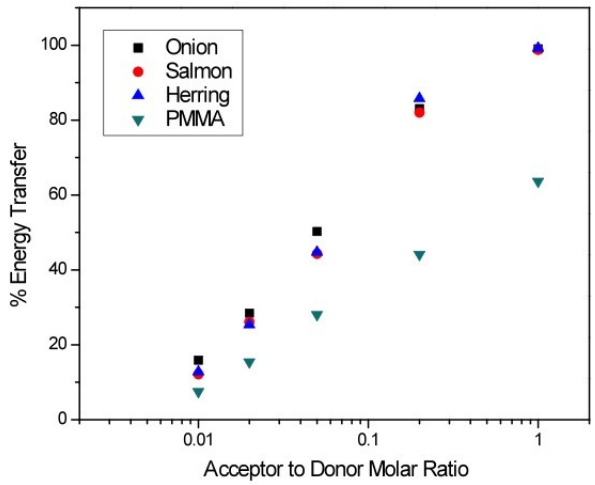

Figure 3. FRET efficiency comparison between different types of DNA-CTMA dye matrix and PMMA.

The %AT of the DNA genome for salmon, herring and onion are summarized in Table 1. Salmon and herring have similar %AT while onion has higher %AT compared to the other two DNAs. This can be one reason why we observed slightly higher energy transfer efficiency in onion DNA-CTMA than salmon and herring. Another

reason can be the binding affinity of dyes towards AT-rich sites versus GC-rich sites in the DNA. Hemi 22 has a similar structure to *Trans*- 4-[4-(dimethylamino)styryl]-1-methylpyrydinium (DMSI), which is a known groove-binder. DMSI was reported to have a higher affinity to bind to AT sequences than GC sequences. On the other hand, intercalators such as Cm 480, have little to no selectivity for binding.[12] At lower Hemi 22 loading, the dye is more dispersed in the DNA grooves, binding to AT-rich minor-grooves first while the Cm 480 having no preference may be scattered equally throughout the matrix. There may be less Hemi 22 dye molecules near the Cm 480 to transfer its energy to. However, at higher Hemi 22 loading, there are more dyes bound to more grooves across the DNA thus making the energy transfer more efficient from Cm 480 to Hemi 22 and so the energy transfer efficiency is similar across all DNA samples.

Table 1. %AT of DNA for salmon, herring and onion.

DNA Source	%AT
Salmon	55.6[13]
Herring	52.6[14]
Onion	64.8[15]

Salmon DNA of low and high molecular weights were also used to prepared dye-doped DNA-CTMA films. As shown in Figure 4, the molecular weight of the DNA also plays a role in the energy transfer between dyes at lower dye loading, <0.5. The energy transfer efficiency is an average of 10% higher, between Cm 480 and Hemi 22, in DNA-CTMA prepared from low molecular weight than high molecular weight salmon DNA. Although, at higher acceptor loading, the energy transfer efficiency becomes similar.

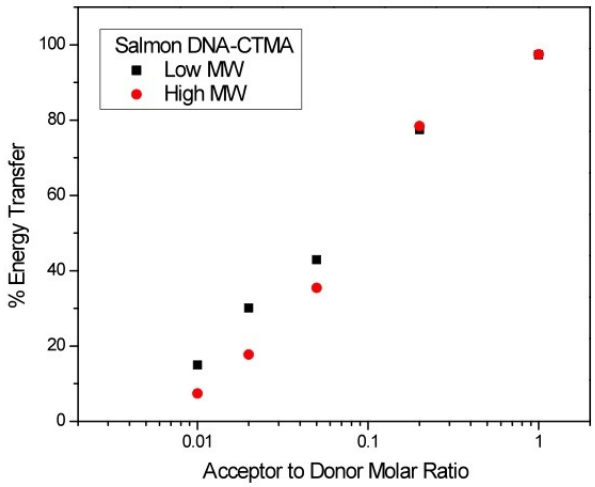

Figure 4. FRET efficiency comparison between high and low molecular weight salmon DNA.

Solutions prepared from low molecular weight salmon DNA-CTMA were less viscous than those made with high molecular weight. The dispersion of individual dye molecules would be more efficient in less viscous solution. If the dyes are not well dispersed, the effective separation distance and dipole orientation of the dyes that is required for FRET to occur could be compromised. This accounts for the higher energy transfer efficiency at lower Hemi 22 loading of low MW than high MW DNA-CTMA films. Higher loading of Hemi 22, similar to what was observed in Figure 3, means more Hemi 22 dyes available to efficiently accept energy transfer from Cm 480.

4. CONCLUSIONS

In conclusion, we have demonstrated the effect of using different DNA sources and molecular weights on the energy transfer between dyes. The energy transfer efficiency between the donor, Cm 480 and the acceptor, Hemi 22 is slightly higher, 2-5%, in DNA-CTMA films prepared from onion than those from salmon and herring at lower Hemi 22 loading. Similarly, DNA-CTMA films prepared from low molecular weight salmon DNA showed an average of 10% higher energy transfer efficiency versus high molecular weight. The energy transfer efficiency is similar for all DNA sources and molecular weight at higher acceptor loading. These observations were attributed to %AT of the genomic DNA samples, binding affinity of dyes to DNA and properties of prepared solutions. This study is part of the effort to understand the fundamental properties of DNA-CTMA and to optimize its use in optoelectronic applications.

REFERENCES

[1] Heckman, E. M., Hagen, J. A., Yaney, P. P., Grote, J. G. and Hopkins, F. K. "Processing techniques for deoxyribonucleic acid: Biopolymer for photonics applications," *Applied Physics Letters* 87, 211115 (2005).

[2] Wang, L., Yoshida, J. and Ogata, N., "Self-Assembled Supramolecular Films Derived from Marine Deoxyribonucleic Acid (DNA) -Cationic Surfactant Complexes□: Large-Scale Preparation and Optical and Thermal Properties," *Society*, 1273–1281 (2001).

[3] Singh, T. B., Sariciftci, N. S. and Grote, J. G., "Bio-Organic Optoelectronic Devices Using DNA," *Advanced Computer Simulation Approaches For Soft Matter Sciences I* (2009).

[4] Hagen J. A, W. Li, W. and Steckl, A. J. "Enhanced emission efficiency in organic light-emitting diodes using deoxyribonucleic acid complex as an electron blocking layer," *Applied Physics Letters*, 3–5 (2006).

[5] Sun, Q., Subramanyam, G., Dai, L., Check, M., Campbell, A., Naik, R., Grote, J. and Wang Y., "Highly efficient quantum-dot light-emitting diodes with DNA-CTMA as a combined hole-transporting and electron-blocking layer," ACS Nano 3(3), 737-743 (2009).

[6] Singh, B., Sariciftci, N. S., Grote, J. G. and Hopkins, F. K., "Bio-organic-semiconductor-field-effect-transistor based on deoxyribonucleic acid gate electric," *Journal of Applied Physics,* 100, 024514 (2006).

[7] Heckman, E. M., Yaney, P. P., Grote, J. G., Hopkins, F. and Tomezak, M. M., "Development of an all-DNA-surfactant electro-optic modulator," Proc. SPIE 6117:61170K (2006).

[8] Ner, Y., Grote, J. G., Stuart, J. A. and Sotzing, G. A., "Enhanced fluorescence in electrospun dye-doped DNA nanofibers," *Soft Matter* 4, 1448-1453 (2008).

[9] Ner, Y., Navarathne, D., Niedzwiedzki, D. M., Grote, J. G., Dobrynin, A. V., Frank, H. A. and Sotzing, G. A., "Stabilization of fluorophore in DNA thin films," *Applied Physics Letters* 95, 263701 (2009).

[10] Ner, Y., Grote, J. G., Stuart, J. A. and Sotzing, G. A., "White luminescence from multiple-dye-doped electrospun DNA nanofibers by fluorescence resonance energy transfer," *Angewandte Chemie (International ed. in English)* 48, 5134–5138 (2009).

[11] Navarathne, D., Ner, Y., Grote, J. G. and Sotzing, G. A., "Three dye energy transfer cascade within DNA thin films," *Chemical communications (Cambridge, England)* 47, 12125–12127 (2011).

[12] C. V. Kumar and R. S. Turner, "Groove binding salt effect of a styrylcyanine dye to the DNA double helix□: the salt effect," *J. Photochem. Photobiol. A: Chem.* 74, 231–238 (1993).

[13] Bucciarelli G, Bernardi G, Bernardi G., "An ultracentrifugation analysis of two hundred fish genomes," Gene 295, 153–162 (2002).

[14] Lavoue, S., Miya, M., Saitoh, K., Ishiguro, N. B. and Nishida, M., "Phylogenetic relationships among anchovies, sardines, herrings and their relatives (Clupeiformes), inferred from whole mitogenomic sequences," Mol. Phylogenet. Evol., 43 (3), 1096-1105 (2007). http://www.ncbi.nlm.nih.gov/bioproject/19869

[15] Kirk, J. T. O., Rees, H. and Evans, G., "Base composition of nuclear DNA with genus *Allium*," Heredity 25, 507-512 (2007).

[16] Richards, E., Reichardt, M. and Rogers, S., [Current Protocols in Molecular Biology], John Wiley & Sons, Inc., 2.3.1-2.3.1, (2004).

[17] S. S. Vogel, C. Thaler, S. V. Koushik, "Fanciful FRET," *Sci. STKE* 331*,* re2 (2006).