Contract No:

This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-08SR22470 with the U.S. Department of Energy (DOE) Office of Environmental Management (EM).

Disclaimer:

LDRD-2020-00256
LDRD External Report Summary
SRNL-STI-2020-00311

Development of FRET Clusters for CBRN Detection

The goal of this project is to develop a novel multifunctional fluorescence based sensors for the simultaneous detection of specific threats with high selectivity, high confidence, and reduced false positives, which will overcome current sensor limitations. The development of miniaturized sensors can be used as visual indicators for exposure or remote sampling of chemical threats, technologies that are pertinent for DoD, first responders, medical surveillance, and more.

Awards and Recognition

None to report for FY20

Intellectual Property Review

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

Signature **Date**

Development of FRET clusters for CBRN Detection

Project Team: Kaitlin Lawrence (Primary), Wendy Kuhne and Ashlee Swindle

Project Type: Seedling

Project Start Date: November 15, 2019
Project End Date: August 21, 2020

Advanced sensor capabilities for the simultaneous on-site detection of specific chemical, biological, and radiological/nuclear (CBRN) threats is important to significantly limit the risk of exposure to personnel and allow the rapid collection of essential scientific data and critical evidence. Commercially available sensor capabilities are generally complex, and/or require highly specific and ultra-sensitive methods that are often power demanding or require offsite post-analysis for positive detection, leaving personnel vulnerable. The goal of this project is to develop a portable sensor capable of simultaneously detecting CBRN signatures using a multiplexed Förster Resonance Energy Transfer (FRET) based sensor. FRET sensors are tailorable, specific, and highly dependent on the donor-acceptor distance. In this project, nanoparticles were functionalized with aptamers that were designed for the detection of methylphosphonate, a sarin metabolite. FRET was measured between quantum dot donors and dye and metal nanoparticle acceptors using optical spectroscopy.

FY2020 Objectives

- Functionalize metal and semiconductor nanoparticles with targeting ligands: Chemically functionalized aptamers and antibodies to target biological warfare agents, chemical warfare surrogate targets, and nuclide resin materials are commercially available and will be coupled to the nanoparticle surfaces through ligand exchange and coupling chemistries.
- Develop individual FRET clusters: A FRET cluster will be developed to target a chemical threat for proof of concept validation. FRET parameters will be measured using optical spectroscopy to determine energy transfer efficiency in the absence and presence of the targeted threat.

Introduction

Development of a multifunctional portable fluorescent sensor with high selectivity for the simultaneous detection of specific chemical, biological, and nuclear threats (CBRN) is extremely valuable for various entities and situations, including emergency responders, forensics, and environmental sampling. Current detection techniques for CBRN are generally complex and require highly specific and ultra-sensitive methods. For example, biological threats, such as anthrax or ricin, are routinely analyzed off-site through the laboratory response network, where samples of interest are first sent to a sentinel lab, followed by a reference laboratory, and then, if the biological threats are not ruled out at the first two laboratories, the samples are sent to a national laboratory. This network uses fixed laboratory equipment with mass spectroscopy (MS) techniques which are highly sensitive (ricin reported detection limits of 0.64 ng/mL[1]); however, they require complex data analysis, especially for biological samples, which have numerous MS fragments and potential matrix effects that can interfere with the determination of the analyte. Alternatively, fluorescence based Förster Resonance Energy Transfer (FRET) sensors for ricin also have reported detection limits below the median lethal dose (LD_{50}=5-10 ng/mL detection limits)[2] with much faster, real-time response time and a simpler signal readout. FRET sensors are ideal for in field detection due to the low detection limits, miniature size and low power requirements.

In FRET, an excited donor (D) molecule transfers energy to an acceptor (A) through nonradiative dipole-dipole interactions.[3-11] For successful FRET, the donor and acceptor should have appropriate spectral overlap, where the emission of the donor overlaps with the absorbance of the acceptor (overlap integral, *J*) and the donor and acceptor must be in close proximity (20-60 Å separation), which can be accomplished by covalently linking the donor and acceptor. The transfer efficiency between a donor and acceptor is very sensitive and is proportional to $1/r^6$ (r=D-A distance) for FRET or $1/r^4$ for nanometal surface energy transfer (NSET) which occurs when a metal nanoparticle is the acceptor. Upon analyte binding (biological, chemical, radionuclide specificity), a decrease in transfer efficiency occurs due to a change in the distance between the covalently bound donor and acceptor (**Figure 1**).

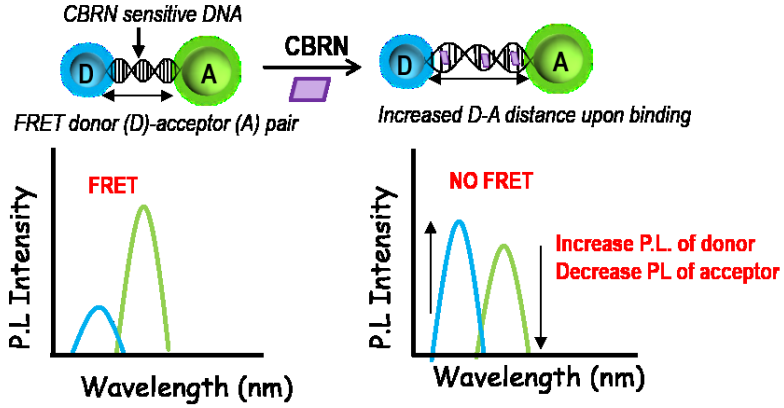

Figure 1. Demonstration of the change in FRET between a donor (D) and acceptor (A) upon analyte binding.

The change in the D/A ratio of fluorescence emission can be used to quantitatively measure the presence of the analytes upon binding, which changes the distance between the donor and acceptor. Similar to absorbance, fluorescence emission follows Beer's Law (A= εbc, where A is absorbance, ε is molar absorptivity, b is cell path length, c is concentration) for quantitative measurements, but fluorescence emission is more sensitive.[11] Most FRET sensors use organic dyes as the fluorescent donors; however, organic dyes are susceptible to photobleaching, have lower quantum yields (QY), and have pH dependent fluorescence. Inorganic fluorophores such as quantum dots (qdots) and quantum rods (qrods) have higher photostability, higher absorption coefficients, narrow emission peaks, and have higher quantum yields compared to organic dyes, therefore they are more robust for deployment applications. Qdot and qrod fluorescence emissions are tailored based on size from around 400-1300 nm and the ligands can be tailored to change solubility and surface functionalization. The narrow emission wavelengths allow for the measurement of multiple qdots simultaneously on one channel with spectral separation that is required for FRET detection.[12-14] Qdots have a large surface area, so multiple types of ligands to be bound to the surface at the same time. Self-assembled and FRET based devices also have applications beyond sensors, including the development of molecular devices,[15] quantum computing,[16] and imaging.[17] **The goal of this project is to develop a FRET sensor for the detection of specific CBRN threats with high selectivity, high confidence, and reduced false positives.** For proof-of-concept validation, a FRET cluster for the detection of a sarin metabolite, methylphosphonic acid, was created. Future work will focus on the creation of multiplexed FRET sensors to detect multiple hazards simultaneously. FRET based sensors can not only provide fast detection capabilities, on the order of minutes rather than hours to weeks for polymerase chain reaction (PCR) and mass spectrometry (MS) techniques, but for some targeted analytes, it can provide detection limits well below what is currently commercially available and with high confidence.[18]

Approach

This seedling focused on the proof of concept validation of inorganic based FRET clusters for chemical warfare detection. For this work, FRET pairs were designed using DNA based aptamers that were designed for the detection of a sarin metabolite, methylphosphonic acid (MePA).[19] These aptamers were chosen against other similar molecules, leading to high selectivity. Qdots were first phase transferred into aqueous solutions using standard ligand exchange techniques and then coupled to the aptamers to create qdot-aptamer conjugates. The energy transfer between qdots and different acceptors, including AuNPs, which which quench fluorescence based on NSET, qdots and dye quenchers, was measured to determine the best acceptor for analyte quantification. FRET was measured using UV-Vis and photoluminescence (PL) spectroscopy.

Results/Discussion

Nanoparticle Functionalization

To create the FRET pairs, qdots and AuNPs were functionalized with DNA aptamers. Qdots with different sizes and emission wavelengths (Figure 2) underwent ligand exchange and dispersed in aqueous buffers where they were then coupled to the aptamers through standard coupling chemistry. After phase transferring, the QY of the qdots were relatively stable, with QY up to 25% after phase transferring. QY preservation is important for the FRET process, as the QY of the donor plays a role in FRET efficiency.[11] AuNPs were functionalized with DNA through ligand exchange with thiol functionalized aptamers. As shown in Figure 3, AuNP-DNA conjugates have an additional absorption peak for the DNA, demonstrating successful functionalization. The AuNP-DNA conjugates that were created with MePA aptamers were calculated to have an average of 113 DNA per AuNP. This high density of targeting ligands is a result of the large surface area, which can lead to more sensitive detection.

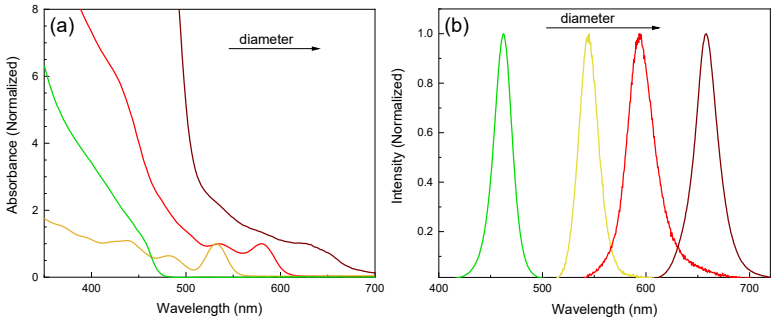

Figure 2. (a) UV-vis of and (b) PL of different sized qdots

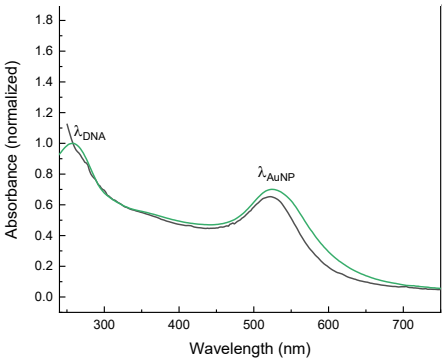

Figure 3. UV-Vis of AuNP-DNA

Qdot donors with quenching acceptors

A quencher was linked to the qdot surface through dsDNA hybridization to study the FRET between the qdot donor and the quenching acceptor. As shown in **Figure 4**a, there is a high degree of spectral overlap between the donor and acceptor. After the addition of the complementary strand to the qdot-ssDNA conjugate, the PL of the qdot decreased as a result of FRET.

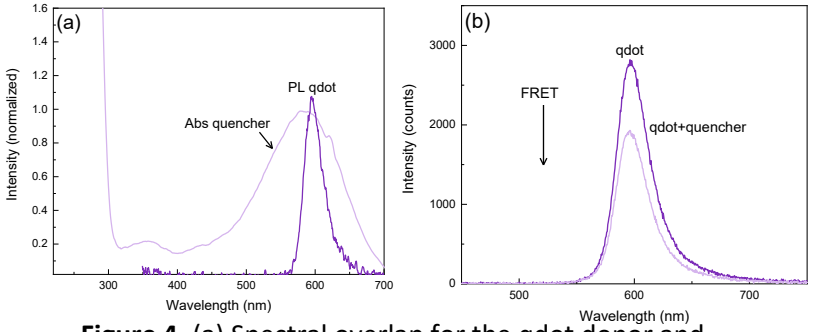

Figure 4. (a) Spectral overlap for the qdot donor and acceptor quencher and (b) decrease in qdot PL after quencher binding

Qdot donors with AuNP acceptors

The energy transfer between qdot donors and AuNP acceptors was measured via PL. As shown in Figure 5a, there is spectral overlap between the UV-vis of the AuNP acceptor and the PL of the qdot, which is required for energy transfer to occur. After addition of AuNPs to the qdots, there was a decrease in PL as a result of energy transfer, as depicted in Figure 5b. This successful demonstration of energy transfer between the qdot and AuNP can be used to measure the concentration of MePA in solution.

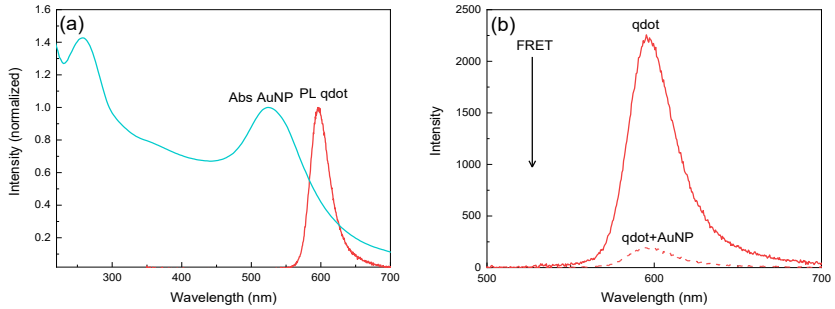

Figure 5. (a) Spectral overlap between the UV-vis of the AuNP acceptor and PL of the qdot donor (b) decrease in qdot PL in the presence of the AuNPs

Qdot donors with qdot acceptors

Qdots have the ability to be both FRET donors and acceptors because of their broad absorption spectra and narrow emission peaks. As shown in Figure 6, there is a high degree of spectral overlap between a qdot with emission at 540 nm (qdot540) and a qdot with emission at 600 nm (qdot600). One challenge to qdot-qdot FRET pairs is spectral cross talk, which results from the broad qdot absorption profiles. Three dimensional fluorescence excitation emission matrix (EEM) spectroscopy was measured for the qdot pair in the absence of FRET (Figure 7). For this FRET pair, there is good spectral separation at all the measured wavelengths. At the higher wavelengths, the acceptor is selectively excited as expected based on the absorbance spectra (Figure 6). EEM spectroscopy allows the measurement of the FRET processes on non-FRET channels, which is above 560 nm for this FRET pair. When the FRET pairs become more complex, such as in three color FRET conjugates, EEM is a powerful technique for quantifying FRET processes. This technique will be applied in higher order FRET pairs for FRET quantification.

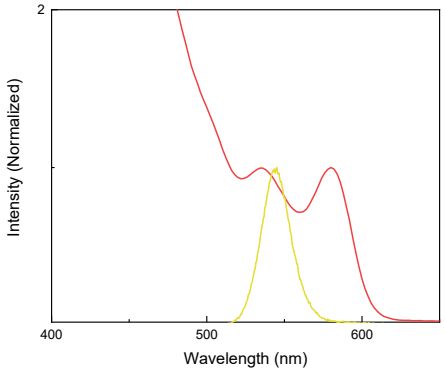

Figure 6. Spectral overlap between the PL of a qdot donor (qdot540) and the absorbance of a qdot acceptor (qdot600)

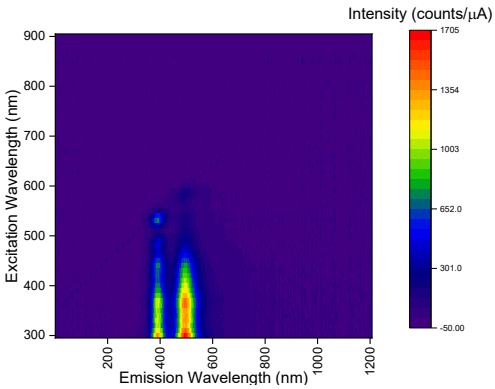

Figure 7. EEM for a qdot donor and acceptor pair

FY2020 Accomplishments

- Created AuNP-DNA and qdot-DNA conjugates
- Successfully measured FRET between qdots with a variety of different acceptors
- Designed FRET pairs for the detection of a sarin metabolite, methylphosphonic acid

Future Directions

Follow up funding in a full LDRD project (LDRD-2021-00250) will build upon the results from this seedling work. Future work will focus on detection quantification using different FRET pairs. The development of biological and radiological/nuclear FRET pairs will be carried out on separate platforms before being combined for the simultaneous CBRN detection. The addition of multiple targeting ligands for the same analyte will also be investigated to decrease the risk of false positives.

FY 2020 Peer-reviewed/Non-peer reviewed Publications

One manuscript has been outlined, with SRNL as the primary research organization. The publication is slated to be completed by early FY21.

Presentations

None to report for FY20

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Acronyms

A: Acceptor
AuNP: Gold nanoparticle
CBRN: Chemical, biological, radiological, nuclear
D: Donor
DNA: Deoxyribonucleic acid
EEM: Excitation Emission Matrix
FRET: Förster Resonance Energy Transfer
LD_{50}: median lethal dose
MePA: Methylphosphonic acid
MS: Mass spectroscopy
NSET: Nanometal surface energy transfer
PCR: Polymerase chain reaction
PL: photoluminescence
Qdot: Quantum dot
QY: Quantum yield
UV-Vis: Ultraviolet-visible spectroscopy

Intellectual Property

None to report for FY20

Total Number of Post-Doctoral Researchers

0

Total Number of Student Researchers

0

External Collaborators (Universities, etc.)

N/A