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Synthesis and characterization of water- soluble long wavelength fluorophores

FACULTY RESEARCH GRANT REPORT
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B. Restatement of problem researched or creative activity

The main goal of the research project was to develop water-soluble long-wavelength emitting fluorophores for potential biological applications. Our hypothesis was if suitable nitrogen donors and acceptors are connected by π -electron conjugation, low-lying molecular energy levels can be generated, and facile excitation of electrons to the new molecular orbitals can be achieved in the far-red to near-infrared region.

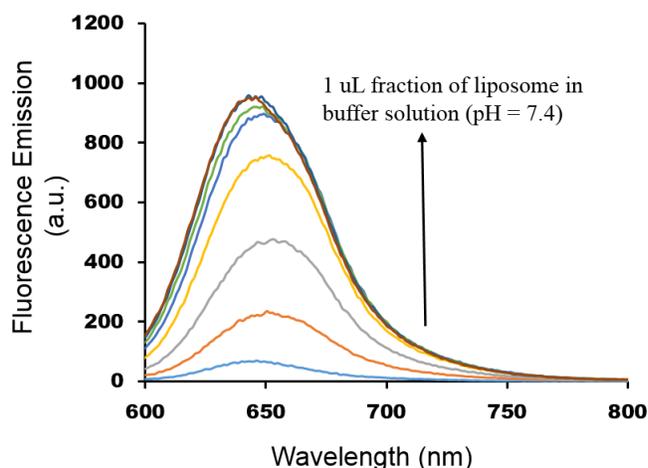
C. Brief review of the research procedure utilized

A water-soluble fluorophore was designed via donor-acceptor (D-A) network of extended conjugation. A nitrogen atom donor and three cyano acceptor groups were connected by Knoevenagel condensation reaction. Fluorophore was purified by chromatography and repeated recrystallization techniques. It was characterized by infrared and NMR spectroscopy. The donor-acceptor interaction, or so-called intramolecular charge-transfer (ICT) interaction, resulted various new opto-electronic properties. Due to the D–A interaction *via* the π electron conjugation, a new low-energy molecular orbital (MO) was formed. Facile excitation of the electrons within the new MO was achieved with visible light irradiation, and therefore the new push–pull molecule was colored. The emission from the low lying MO levels resulted long wavelength light (>650 nm).

D. Summary of findings

Fluorophore was synthesized in two simple steps. In the first step 3-hydroxy-3-methylbutan-2-one was coupled to malononitrile to yield 2-(3-cyano-4,5,5-trimethylfuran-2(5H)-ylidene)malononitrile as off-white powder. Knoevenagel condensation resulted fluorophore in 65% yield. The selectivity of Knoevenagel condensation to all-trans isomer was very high. The presence of vinylic protons was confirmed from NMR coupling constant ($J \approx 16$ Hz). Fluorophore

was highly soluble in polar aprotic solvents such as, acetonitrile, dichloromethane, dimethyl sulfoxide, dimethyl formamide as well as in polar protic solvents such as, ethyl alcohol, methyl alcohol, and isopropyl alcohol. It had significant solubility in water at physiological pH (pH = 7.4).



Fluorophore exhibited different colors in different solvents. For instance, pink in toluene, ethyl acetate, and acetone; blue in acetonitrile, DMF, and DMSO; purple in ethanol, methanol, and water. Solvatochromism was confirmed through changes in absorption spectra of the

fluorophore in solvents of different polarity. Absorption spectra of the fluorophore (10 μM) in ten different solvents including water at pH 7.4 (in water, $\lambda_{\text{max}} = 587 \text{ nm}$; $\epsilon = 28300 \text{ M}^{-1}\text{cm}^{-1}$) were recorded. Absorption maxima (λ_{max}) in nonpolar solvents toluene and ethyl acetate were at 562 nm and 565 nm, respectively. With increased polarity of solvents, λ_{max} bathochromically shifted. Longest absorption maximum was recorded in DMSO ($\lambda_{\text{max}} = 595 \text{ nm}$). All these bands most likely arise from $n-\pi^*$ electronic transition with an intramolecular charge transfer (ICT) from donor aniline moiety to acceptor tri cyano furan moiety. Fluorophore exhibited broad emissions in different solvents, ranging from yellow (in toluene, $\lambda_{\text{max}} = 613 \text{ nm}$) to deep red region (in H_2O , $\lambda_{\text{max}} = 650 \text{ nm}$) of visible spectrum. Bathochromic shifts were observed as polarity of solvents increased due to stabilization of the highly polar excited states by polar solvents, suggesting solvatochromism of the fluorophore. Fluorophore binds to liposome in phosphate buffer. Liposome was prepared as instructed and added to a buffer solution of the fluorophore. Upon addition of liposome the emission maximum hypsochromically shifted, indicating inclusion of the

fluorophore within the nonpolar hydrophobic part of the liposome. Quantum yield also increased significantly (Q.Y. in buffer 0.01 to 0.11 in liposome).

E. Conclusions and recommendations

In conclusion, a long-wavelength emitting fluorophore was designed, synthesized and characterized. Photophysical properties and binding characteristics with a membrane model suggest that the fluorophore can serve as an extrinsic probe for detection and imaging of biomolecules.