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Development of a water-soluble near infrared (NIR) fluorophore

FACULTY RESEARCH GRANT REPORT

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B. Restatement of problem researched or creative activity

The main goal of the research project is to develop water-soluble near-infrared (NIR) emitting fluorophores for detection of biomarkers in samples. Our hypothesis is if suitable 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 low-lying molecular orbitals can be achieved in the near-infrared region.

C. Brief review of the research procedure utilized

A new water-soluble NIR fluorophore was designed via donor-acceptor (D-A) network of extended conjugation. A nitrogen donor and three nitrile acceptor groups were attached by Knoevenagel condensation. Fluorophore was purified by silica gel chromatography and recrystallization techniques. It was characterized by infrared and NMR spectroscopy. The donor-acceptor interaction, or so-called intramolecular charge-transfer (ICT) interaction, resulted new opto-electronic properties. Due to the D–A interaction *via* the π electron conjugation, a new low-energy molecular orbital (MO) was formed. It was further stabilized in polar solvents such as methanol, DMSO, water, etc. The excited state energy was mostly lost due to the non-radiative decay from the solvent stabilized charge transfer states. It was recovered in highly viscous solvent and with addition of protein. The signal was quantitative to the amount of fluorophore-protein complex in the experimental concentration range. The strong association affinity of the fluorophore toward the protein was attributed to the van der Waals and hydrophobic interactions.

D. Summary of findings

Fluorophore was synthesized in two 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. In the second step, 2-(3-cyano-4,5,5-trimethylfuran-2(5H)-

ylidene)malononitrile was coupled with 4-(bis(2-hydroxyethyl)amino)benzaldehyde to yield the target NIR fluorophore (yield 65%). The selectivity of Knoevenagel condensation to all-trans isomer was very high. The presence of vinylic protons was confirmed from the NMR coupling constant (J ≈ 16 Hz). Fluorophore was soluble in water. It was highly soluble in acetone and in halogenated solvents such as chloroform and dichloromethane, as well as in polar aprotic solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). For all the spectrometric experiments a DMSO stock of the fluorophore was diluted in 0.1 M phosphate buffer (pH = 7.5) solution. The final DMSO content in buffer was 1%, and the concentration of the fluorophore was maintained at 10 μM, unless otherwise stated.

The fluorophore exhibited strong fluorescence in DMSO, DMF, and acetone; moderate emission in nonpolar solvents such as toluene and ethyl acetate and very weak emission in deionized water. All the emission bands were broad and structure-less, which is characteristic of donor-acceptor charge transfer type electronic interactions. Emission intensity and the position of the bands were very sensitive to the polarity of the solvents, indicating strong solvatochromism. As the polarity of the solvents increased the emission maxima shifted toward lower energy, suggesting a positive solvatochromism. The lowest energy charge transfer emission recorded was in DMSO (λ_{max}^{em} ~ 665 nm). In water emission maxima was at ~ 650 nm with lowest intensity, most likely due to loss of excited state energy through additional decay channel facilitated by polar protic water through hydrogen bonds.

Next, to investigate the efficacy of the fluorophore in biomarker detection, fluorimetric titration experiments were carried out in phosphate buffer solutions with 1% DMSO. Emission intensity increased upon addition of biomarker Human serum albumin (HSA). A linear relationship between the concentrations of HSA and the fluorescence intensity of the fluorophore was observed,

suggesting a 1:1 complex formation. From the linear relationship and the Benesi-Hildebrand plot, binding affinity (K_a) was calculated in phosphate buffer. Itwas found to be 5.75×10^4 M^{-1}(ΔG = -6.48 kcal/mol). It indicates strong association of the fluorophore with the HSA guided by multiple weak non-covalent interactions and hydrophobic effect.

E. Conclusions and recommendations

In conclusion, a NIR emitting fluorophore was designed, synthesized and characterized. Excellent photophysical properties and high binding affinity with HSA suggest that the fluorophore can serve as an extrinsic probe for detection of biological macromolecules in samples.

The fluorophore showed positive solvatochromism. The fluorescence intensity was highly sensitive to the nature of the microenvironment. Upon binding with the HSA the fluorescence intensity increased, displaying a "turn-on" fluorescence response. Such a spontaneous supramolecular association and the subsequent turn-on fluorescence response may have potential in medical diagnostic applications.