Novel Phthalocyanine-Based Near-IR Fluorophores: Development and Bioanalytical Applications

Fluorescence detection in the near-infrared (near-IR) holds great opportunities for providing ultra-high sensitivity, even at the single molecule level, for bioanalyses especially those performed in complex matrices. The technique has been demonstrated to yield overall better detection efficiencies compared to the UV or visible regions of the electromagnetic spectrum (*1-3*). However, the full utilization of near-IR fluorescence in variety of bioanalytical applications has been slow to develop due to the limited number of fluorophores available and the rather poor photophysical properties and stability they offer. Demands placed on readout modalities of bioassays that provide high degrees of multiplexing capabilities as well as high sensitivity require the development of new near-IR fluorophores with a diverse range of photophysical properties and functional groups for labeling a vast range of targets. In collaboration with Professor Robert P. Hammer group we are working on development of water-soluble phthalocyanine (Pc) dyes that possess absorbance and fluorescence maxima in near-IR range. The MPc dyes we designed and synthesized possess favorable photophysical properties (e.g. for Pc depicted in Figure 1: extinction coefficient = 2.9×10^5, fluorescence quantum yield = 0.66, photobleaching quantum yield = 0.5×10^{-6}), are chemically, thermally, and photo stable (Figures 2 and 3), and are highly water-soluble, which was achieved by decorating the

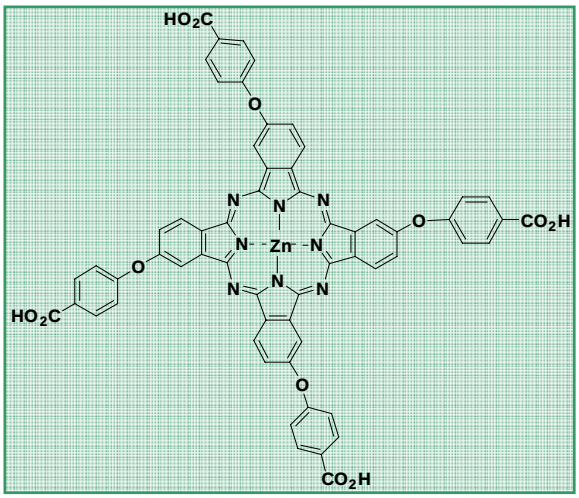

Figure 1. Zn-containing Pc

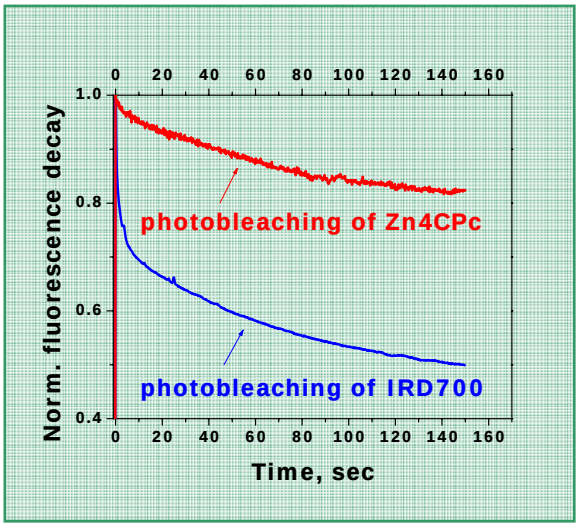

Figure 2. Photobleaching decay curves for ZnPc and IRD 700

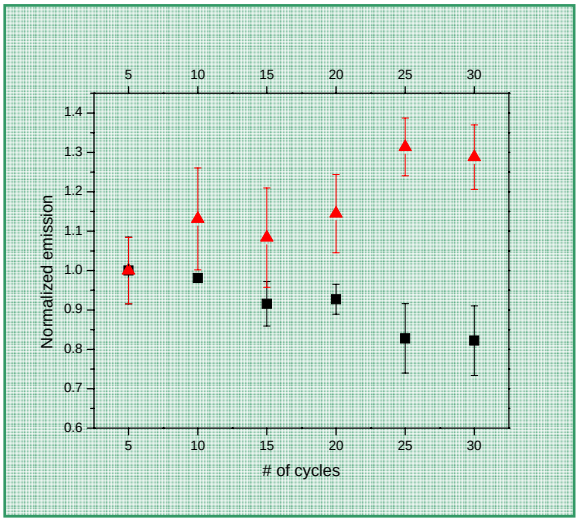

Figure 3. Comparison of stability ZnPc and IRD700 under simulated PCR conditions

macrocycle periphery with polar solubilizing groups. The MPc's also have rather narrow absorption and emission envelopes compared to conventional cyanine-based dyes, making them particularly attractive for multiplexed applications by lowering the amount of color bleed into inappropriate detection channels. Additionally, these dyes contain at least one carboxylic group, a convenient and common functionality used for robust labeling of amino-modified biomolecules, such as oligonucleotides, via NHS ester chemistry. Another appealing feature of Pc's is their photophysical properties can be altered by simply varying peripheral substitution patterns around the macrocylce (*4*) and/or altering the identity of the metal center (*5*).

The dyes can be covalently attached to oligonucleotides or peptides and these Pc dye systems can be used as reporters of molecular association events using such readout formats as resonance energy transfer (RET) or fluorescence resonance energy transfer (FRET). Figure 4 shows FRET studies in the system consisting of Zn- and Sn-containing water soluble Pc molecules.

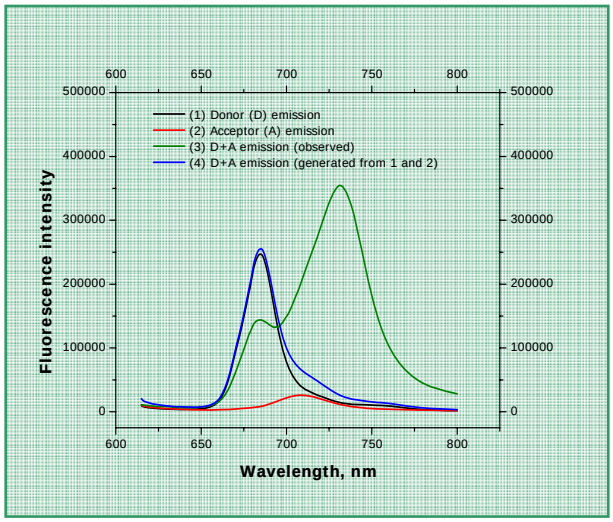

Figure 4. FRET in ZnPc-SnPc system

Thus, intrinsic properties of phthalocyanines make them very suitable for variety of modern molecular biology applications requiring reporter moieties to be chemically, photo, and thermally stable (for long observation times) and fluorescing in near-IR region to overcome autofluorescence from the complex biological milieu.

References

(1) Williams, D. C., and Soper, S. A. (1995) Ultrasensitive near-Ir Fluorescence Detection for Capillary Gel-Electrophoresis and DNA-Sequencing Applications. *Analytical Chemistry 67*, 3427-3432.

(2) Soper, S. A., and Mattingly, Q. L. (1994) Steady-State and Picosecond Laser Fluorescence Studies of Nonradiative Pathways in Tricarbocyanine Dyes - Implications to the Design of near-Ir Fluorochromes with High Fluorescence Efficiencies. *Journal of the American Chemical Society 116*, 3744-3752.

(3) Soper, S. A., Mattingly, Q. L., and Vegunta, P. (1993) Photon Burst Detection of Single near-Infrared Fluorescent Molecules. *Analytical Chemistry 65*, 740-747.

(4) Kobayashi, N., Ogata, H., Nonaka, N., and Luk'yanets, E. A. (2003) Effect of peripheral substitution on the electronic absorption and fluorescence spectra of metal-free and zinc phthalocyanines. *Chemistry-a European Journal 9*, 5123-5134.

(5) Ogunsipe, A., and Nyokong, T. (2005) Effects of central metal on the photophysical and photochemical properties of non-transition metal sulfophthalocyanine. *Journal of Porphyrins and Phthalocyanines 9*, 121-129.