

# Heteroatom Substitution for the Development of Near-Infrared Lumiphores

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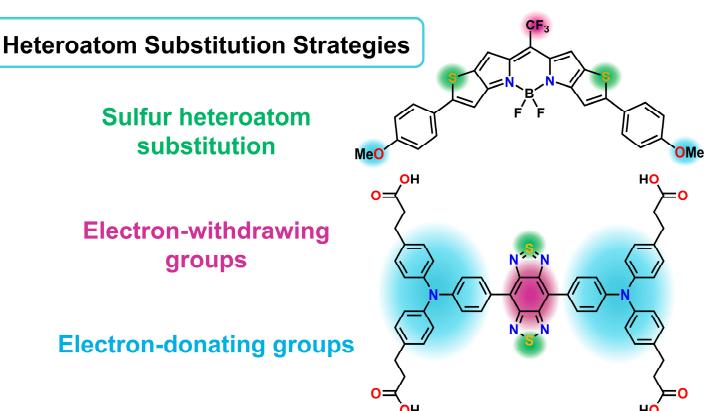
**Abstract:** Near-infrared lumiphores are advantageous for biological applications, as their emission falls within the tissue-transparent region. However, these dyes suffer from low quantum yields as a consequence of their low transition energy. Heteroatom substitution has recently been successfully employed to bathochromically shift emission in various dye scaffolds while maintaining bright emission.

**Keywords:** Near-IR, Tissue Transparent, Imaging, Lumiphore

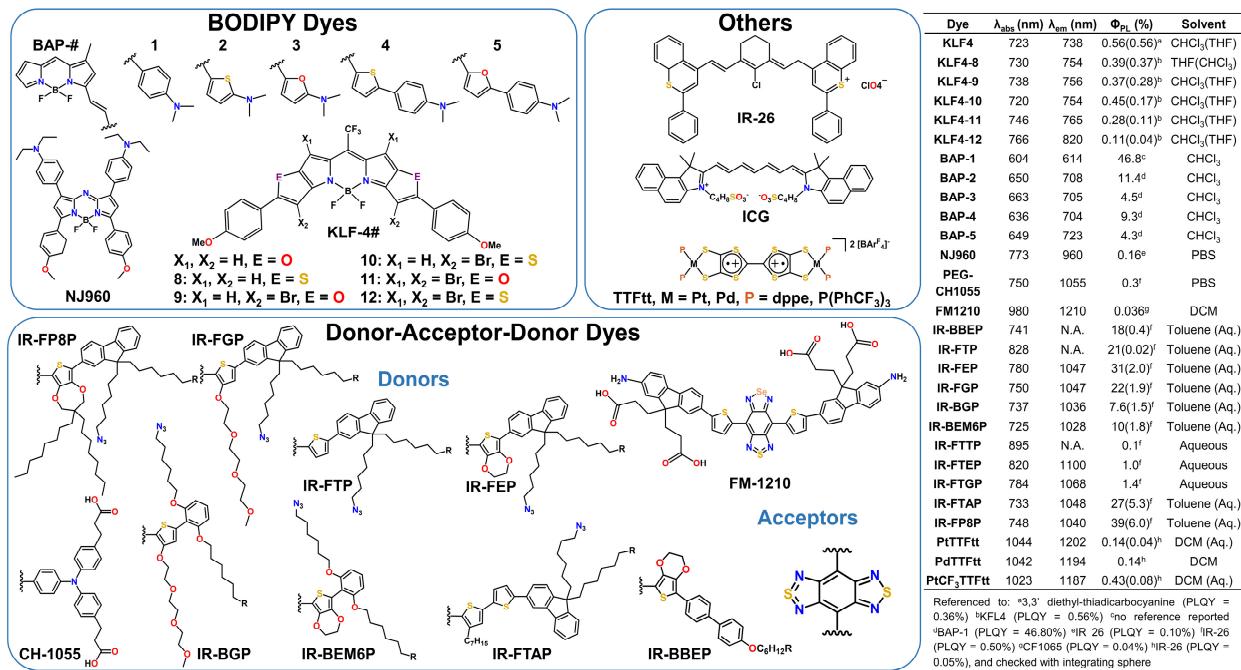
There has been significant interest in novel near-infrared (NIR, ~700-1700 nm) lumiphores due to potential applications in biological imaging and sensing.<sup>1</sup> NIR light is tissue transparent, offering opportunities in noninvasive imaging. Molecular lumiphores are small, tunable, and comparatively non-toxic, but organic systems typically require extended conjugation for NIR absorption/emission and also suffer from low photoluminescence quantum yields (PLQYs).<sup>2</sup> Low PLQYs are a consequence of the empirical energy gap law where PLQYs exponentially decrease with decreasing transition energy.<sup>3</sup> This phenomenon has largely been attributed to C–H stretches, the highest energy vibrations in many dye molecules, promoting nonradiative decay through coupling to low energy transitions.<sup>3</sup>

Polymethine dyes are the prevalent lumiphores in this range, with IR-26 serving as a common PLQY reference.<sup>4</sup> Furthermore, indocyanine green (ICG) and methylene blue (MB) are the only FDA approved imaging agents in the NIR region.<sup>5,6</sup> ICG and MB emit at the very blue end of the NIR region (<850 nm) while IR-26 absorbs and emits in the NIR-II window around 1130 nm.<sup>4</sup> Outside of these polymethines, one promising strategy to red-shift emission while preserving PLQY is heteroatom substitution into dye molecule scaffolds. Heteroatom substitution facilitates NIR emission through comparatively weaker heavy-atom bonding as well as orbital tuning through electron withdrawing/donating properties. Furthermore, it also should limit nonradiative decay through the elimination or reduction of C–H modes (Figure 1). This forum article focuses on recent examples of this strategy in NIR dye design.

BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) scaffolds have favorable photophysical properties for fluorescence imaging and are thus widely employed in probes, sensors, and other applications (Figure 2, top left).<sup>7</sup> While BODIPY dyes frequently



**Figure 1.** Heteroatom Substitution Strategies for NIR Lumiphores.



**Figure 2.** Structures and Properties of NIR Lumiphores.

emit in the visible range, introduction of heavier substituents, such as sulfur or bromine, red-shifts emission.<sup>8</sup> For example, substitution of bromine or thiophene units into the BODIPY core of the dye KLF4 generates a series of red shifted analogues (KLF4-8 through KLF4-12, Figure 2). This substitution enhances intersystem crossing (ISC) and singlet oxygen generation. These substituted BODIPYs show intense absorption and emission at 720–766 nm and 738–820 nm respectively, which are bathochromically shifted compared to KLF4. They also exhibit large molar extinction coefficients and moderate quantum yields, albeit near the visible region.

Similarly, modifications to a  $\beta$ -amyloid (A $\beta$ ) targeting BODIPY scaffold (BAP-1) produced analogues with red-shifted absorption and emission (BAP2-5, Figure 2).<sup>7</sup> BAP-1 absorbs and emits at 614 and 648 nm. The dimethylamino group enables A $\beta$  targeting in BAP-1, so heteroatom substitutions that preserved this group were introduced. Dimethylaminothiophenyl, dimethylaminophenylthiophenyl, dimethylaminophenylfuranyl, and dimethylaminophenylfuranyl groups induce bathochromic shifts, good PLQYs (4.3–11.4% in dichloromethane (DCM)), and maintain sensitivity for A $\beta$  plaques, albeit with emission close to the visible range.

In addition to BODIPY dyes, borane difluoride azadipyrromethene cores (aza-BODIPY) form a donor-acceptor-donor (D-A-D) framework with low energy absorption/emission, phototunability, and reliably high PLQYs.<sup>9</sup> A family of these dyes, NJ960, NJ1030, and NJ1060, was synthesized with strongly electron-donating diethylaminophenyl groups in the 3,5 positions of previously reported NIR-I aza-BODIPY fluorophores. Further 1,2-anisoyl substitution forms a D-A-D' motif with emission into the NIR-II region. D-A-D fluorophores often outperform clinically approved polymethine dyes, showing larger Stokes shifts and signal:noise. This series also displays relatively high aqueous PLQYs, ranging from 0.16% to 1.0% (Figure 2). Solvent plays a notable role in the photophysical character of these dyes; emission red-shifts with increasing polarity of the solvent.

Several other D-A-D cores feature NIR-II emission with high PLQYs (Figure 2).<sup>2</sup> Integration of four carboxylic acid groups to a benzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) (BBTD) framework

generates a D-A-D dye, CH1055, with absorbance and emission farther into the NIR. Subsequent PEGylation enables high water solubility, ~750 nm absorbance, and ~1,055 nm emission which trails into the NIR-IIa region (1,300–1,400 nm). Photophysical characterization showed high photostability in water, PBS, and serum, with a PLQY of 0.3%. The compact structure of CH1055 permits tuning for tumor targeting and uptake, as well as relatively facile renal excretion, an essential property for bioimaging. CH1055 outperforms ICG in image clarity, resolution, and penetration depth for blood and lymphatic vasculature. This performance was attributed to its red-shifted emission leading to lower tissue scattering and superior image quality.

Another BBTD-based lumiphore, FM1210, uses substitution of amino groups and selenium to shift emission even further into the NIR-II region than CH1055.<sup>10</sup> FM1210 absorbs at 980 nm and emits at 1210 nm with a PLQY of 0.036% in DCM. Comparison to structural analogues reveals that both the amino and selenium moieties contribute to the NIR-II emission. The dye's fluorescence weakened in PBS, but its image contrast, blood circulation, and urinary excretion as a blood imaging agent matched or surpassed a control compound CF1065.

Inclusion of 'electron shielding' groups containing heavier heteroatoms can protect the conjugated backbones of lumiphores and increase PLQY.<sup>11</sup> D-A-D lumiphores risk interaction with surrounding molecules, such as water, that have high energy O–H bonds which can drive down PLQY. Inclusion of donor groups and shielding groups allows for the synergistic protection of the fluorophore core from water while strengthening intramolecular charge transfer. This facilitates large PLQYs, Stokes shifts, and extinction coefficients. The addition of groups which are too large, however, can induce a molecular twist which interferes with intramolecular charge transfer. Thus, both donor and shielding groups must be strategically selected. Experimentation with balancing molecular twist, hydrophobic effects, and steric hindrance has produced a range of fluorophores including IR-BEMP6, IR-BGP, IR-FEP, IR-FGP, IR-FTAP, and IR-FP8P (Figure 2). This group exhibits aqueous PLQYs ranging from 1.8% to 6.0% (7.6% to 39% in toluene) when nitrogen and bromine were used in the shielding units. The strength of pi-pi conjugation in these dyes, supported by heteroatom substitution, results in NIR-II absorption and emission. For instance, IR-FP8P emits at 1040 nm.

In a recent development, scaffolds with a high degree of heavy heteroatom substitution in the lumiphore core have also shown luminescence in the NIR-II region. Tetrathiafulvalene tetrathiolate (TTF<sup>2-</sup>) dications form a new family of bright, photostable, and redox-switchable fluorophores.<sup>12</sup> The high degree of sulfur substitution in these compounds compresses their pi manifolds and induces very low-energy ligand centered pi-pi\* transitions. This series of bimetallic bridged TTF<sup>2-</sup> dications exhibits ~1030 nm absorbance and ~1200 nm emission across palladium and platinum analogues. PLQY measurements on this series show values from 0.14% to 0.43% (DCM, 0.04% to 0.08% in aqueous mixtures), comparing favorably to IR-26. The redox active nature of the bridging TTF<sup>2-</sup> ligand also enables redox-switching; fluorescence can be turned on or off over several chemical redox cycles. Furthermore, these compounds exhibit stability in both aerobic and aqueous environments. The compact and modular design of these dyes makes them valuable cores for bioimaging agents going forward.<sup>12</sup>

These examples illustrate that heteroatom substitution shifts emission into the NIR region while preserving good PLQYs. Such substitution modulates conjugated systems through electron donor/acceptor properties, and removal of high energy vibrational modes (i.e. E–H bonds) with heteroatom substitutions is proposed to decrease nonradiative decay. An outstanding question is the effect of heavy atom substitution on ISC. Heavy atoms enhance ISC in the KLF series of dyes for singlet oxygen generation, but sufficiently fast ISC may also reduce PLQYs. Thus, the photophysics of dyes must be carefully managed to optimize NIR emission and PLQYs.

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