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Tailoring Oxygen Sensitivity with Halide Substitution in Difluoroboron Dibenzoylmethane Polylactide Materials

Christopher A. DeRosa, Caroline Kerr, Ziyi Fan, Milena Kolpaczynska, Alexander S. Mathew, Ruffin E. Evans, Guoqing Zhang, and Cassandra L. Fraser*

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904

Abstract

The dual-emissive properties of solid-state difluoroboron β -diketonate-poly(lactic acid) (BF₂bdkPLA) materials have been utilized for biological oxygen sensing. In this work, BF₂dbm(X)PLA materials were synthesized, where X = H, F, Cl, Br, and I. The effects of changing the halide substituent and PLA polymer chain length on the optical properties in dilute CH₂Cl₂ solutions and solid-state polymer films were studied. These luminescent materials show fluorescence, phosphorescence, and lifetime tunability on the basis of molecular weight, as well as lifetime modulation via the halide substituent. Short BF₂dbm(Br)PLA (6.0 kDa) and both short and long BF₂dbm(I)PLA polymers (6.0 or 20.3 kDa) have fluorescence and intense phosphorescence ideal for ratiometric oxygen sensing. The lighter halide-dye polymers with hydrogen, fluorine, and chlorine substitution have longer phosphorescence lifetimes and can be utilized as ultrasensitive oxygen sensors. Photostability was also analyzed for the polymer films.

Keywords

Difluoroboron β -diketonate complexes; room-temperature phosphorescence; heavy atom effect; poly(lactic acid); photostability

INTRODUCTION

Developing luminescent materials for *in vivo* biological research can be a daunting task.^{1–3} Brightness and biocompatibility are prerequisites. Dyes must be capable of reporting the concentration of an analyte over a biologically relevant range while retaining essential photophysical properties.^{4,5} Difluoroboron based fluorophores, such as 4,4-difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPY), are common fluorescent reporters because they satisfy these conditions.^{6,7} Other difluoroboron fluorophores, including β -diketonates (OBO),⁸ β -diiminates (NBN),^{9,10} and β -ketoiminates (OBN),^{11,12} have also been utilized

*Corresponding Author: To whom correspondence should be addressed. fraser@virginia.edu.

Supporting Information. ¹H NMR spectra of all new compounds, UV/Vis spectra of boron complex initiators, fluorescence spectra of solution cast films in air, total emission of high molecular weight polymers in air and N₂, and delayed emission of low molecular polymers. This material is available free of charge via internet at <http://pubs.acs.org>.

Author Contributions

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for their unique structural and optical properties. A distinguishing feature of the difluoroboron β -diketonate dyes is that the fluorescence is bright in both solution and the solid state, making them ideal for liquid crystals,^{13,14} ion indicators,¹⁵ and mechanochromic materials.^{16,17}

Difluoroboron β -diketonate (BF₂bdk) dyes combined with poly(lactic acid) (PLA) exhibit short-lived fluorescence and long-lived phosphorescence at room and body temperatures.^{18–20} Thus, these dye-PLA conjugates can serve as single component ratiometric oxygen sensors without the need for fluorophore/phosphor/matrix mixtures. Fluorescence serves as an internal standard while phosphorescence, which is susceptible to oxygen quenching, serves as the sensor.^{21–23} As the dyes are exposed to oxygen, the fluorescence to phosphorescence (F/P) intensity ratio is measured and compared to an oxygen calibration curve to determine the concentration of oxygen present. In addition to fostering dual emissive properties of BF₂bdk dyes—fluorescence and phosphorescence, rather than just fluorescence alone—PLA is a good choice because it is a biocompatible and degradable polymer.^{24,25} Furthermore, processing is well established.^{26,27} For example, boron nanoparticles (BNPs), can be produced from these dye-PLA conjugates, serving as innovative tools in biomedical imaging applications.²⁶

It is widely known that halide substituents can modulate structural and optical properties of materials. Previously, Baronoff *et al.* showed that halide substitution of cyclometalated iridium complexes can alter the ligand geometry and bandgap properties.²⁸ Additionally, Kim *et al.* recently presented benzaldehyde/dibromobenzene co-crystals with tunable phosphorescence by changing alkyl chain length and halide substitution.^{29,30} Mechanochromic luminescence (ML)³¹ and mechanochromic luminescence quenching (MLQ) have also been reported with halide substituted BF₂dbm(X)C₁₂H₂₅ materials, where X = F, Cl, Br, I.³² Previously reported difluoroboron dibenzoylmethane polylactide (BF₂dbmPLA) materials (Figure 1, X = H) had phosphorescence lifetimes in the range of a hundred milliseconds (80–150 ms) depending on the polymer molecular weight,¹⁹ while the iodine substituted derivatives (BF₂dbm(I)PLA) had phosphorescence lifetimes of only 4–5 ms.²² This change in heavy atom substitution drastically affected the oxygen sensitivity of these materials. The BF₂dbmPLA materials are highly sensitive in the hypoxic range (0–1% O₂), while BF₂dbm(I)PLA can effectively sense up to ambient conditions (0–21% O₂). Dyes with different oxygen sensitivities can be adapted for various biological applications.³³ For example, probes with high sensitivities within 0.5–3.0% O₂ are well suited for tumor hypoxia imaging,^{34,35} 2–6% O₂ can monitor brain oxygenation in great detail,³⁶ while less sensitive probes with sensitivities within the 5–15% O₂ range can effectively monitor blood oxygenation (veins versus arteries).³⁷

Here, a systematic study of the effects of halide substitution on BF₂dbmPLA optical and oxygen sensing properties is reported. A series of halide substituted BF₂dbm(X)PLA materials were synthesized, where X = H, F, Cl, Br, and I (Figure 1), to effectively tune the triplet emission of the materials *via* an internal electron withdrawing group and heavy atom effects. Dye-polymer conjugates can be fabricated into nanoparticles²⁶ or nanofibers²⁷ for biomedical imaging, minimizing phase separation, heterogeneity and dye leaching that can occur for comparable dye loadings in dye/PLA blends. The dyes and polymers are analyzed

in dilute CH₂Cl₂ solutions and in solid-state films. In the solid state, phosphorescence lifetime, and thus oxygen sensitivity, can be controlled with the halide substituent and polymer molecular weight. The photostability of the polymers was studied over extended excitation times (~18 hours) with a handheld UV light source.

MATERIALS AND METHODS

3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L- lactide, Sigma Aldrich) was recrystallized twice from ethyl acetate and stored under nitrogen. The ligand precursor 1-(4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)phenyl)ethan-1-one³⁸ ligands dbmOH (**1**)¹⁸ and dbm(I)OH (**5**),²² boron initiators BF₂dbmOH (**6**)¹⁸ and BF₂dbm(I)OH (**9**)²² and polymers **11–15**²³ were prepared as previously described. Tin(II) 2-ethylhexanoate (Sn(oct)₂, Spectrum), boron trifluoride diethyl etherate (Aldrich, purified, redistilled), and all other reagents and solvents were used as received without further purification. Solvents CH₂Cl₂ and THF were dried and purified over 3 Å molecular sieves activated at 300 °C.³⁹ All other chemicals were reagent grade from Sigma-Aldrich and were used without further purification.

¹H NMR spectra were recorded on a Varian Unity Inova 300/51 (300 MHz) or a Varian VMRS/600 (600 MHz) instrument in CDCl₃. ¹H NMR peaks were referenced to the signals for the residual protiochloroform at 7.26 ppm. Coupling constants are given in hertz. Polymer molecular weights were determined by gel permeation chromatography (GPC) (THF, 25 °C, 1.0 mL/min, *dn/dc* = 0.050) using multiangle laser light scattering (SEC-MALS) (λ = 658 nm, 25 °C) and refractive index (RI) (λ = 658 nm, 25 °C) detection. Polymer Laboratories 5 μ m mixed-C columns (guard column plus two columns) along with Wyatt Technology (Optilab T-rEX interferometric refractometer, miniDAWN TREOS multiangle static light scattering (MALS) detector, ASTRA 6.0 software) and Agilent Technologies instrumentation (series 1260 HPLC with diode array (DAD) detector, ChemStation) were used in GPC analysis. Polymer characterization data is provided in the Supporting Information (Figure S7–16; Table 1). UV–vis spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer.

Luminescence Measurements

Steady-state fluorescence emission spectra were recorded on a Horiba Fluorolog-3 Model FL3-22 spectrofluorometer (double-grating excitation and double-grating emission monochromator). A 2 ms delay was used when recording the delayed emission spectra. Time-correlated single-photon counting (TCSPC) fluorescence lifetime measurements were performed with a NanoLED-370 (λ_{ex} = 369 nm) excitation source and a DataStation Hub as the SPC controller. Phosphorescence lifetimes were measured with a 1 ms multichannel scalar (MCS) excited with a flash xenon lamp (λ_{ex} = 369 nm; duration <1 ms). Lifetime data were analyzed with DataStation v2.4 software from Horiba Jobin Yvon. Fluorescence quantum yields (Φ_{F}) of initiator and polymer samples in CH₂Cl₂ were calculated against anthracene as a standard as previously described, using the following values: Φ_{F} (anthracene) = 0.27^{40,41} n_{D}^{20} (EtOH) = 1.360, n_{D}^{20} (CH₂Cl₂) = 1.424⁴² Optically dilute CH₂Cl₂ solutions of the dyes, with absorbances <0.1 au, were prepared in 1 cm path length quartz cuvettes. Thin films were prepared on the inner wall of vials by dissolving polymers

in CH₂Cl₂ (2 mg/mL) and evaporating the solvent by slowly rotating the vial under a low stream of nitrogen. The solution-cast films were then dried *in vacuo* for at least 15 min before measurements. Fluorescence spectra and lifetimes were obtained under ambient conditions (e.g., air, ~21% oxygen). Phosphorescence measurements were performed under a N₂ atmosphere. Phosphorescence measurements were taken under N₂. The vials with the solution-cast films were purged and sealed with a Teflon cap and wrapped in parafilm in a glove box prior to measurements. The glove box was purged for 30 min prior to samples being sealed. For oxygen sensitivity measurements, solution cast films in vials were fitted with a 12 mm PTFE/silicone/PTFE seal (Chromatography Research Supplies), connected by a screw cap. Vials were continuously purged with analytical grade N₂ (Praxair) or 1.0% O₂ (Praxair) during measurements. For 21% O₂ (i.e. air), measurements were taken under ambient conditions (open vial, no cap). Fluorescence and phosphorescence lifetimes were fit to double or triple exponential decays in solid-state films.

Photostability Measurements

Spin-cast films were prepared by dropwise addition of 1 mL of a ~1.0 mg/mg CH₂Cl₂ solution on 18 × 18 mm glass cover slides (Fisher Scientific) using a Laurel Technologies WS-650s spin-coater at 3000 rpm. Films were dried *in vacuo* for ~15 min prior to measurements. Films were placed on a black surface 10 cm below the camera and were irradiated with a 4 W UV lamp. A Watec 902H2 Ultimate monochrome CCD camera, equipped with a Fujinon DV5X3.6R4B-SA2L 3.6–18 mm F/1.8 lens and an Ion Video 2 PC analog to digital converter was used to monitor photostability. Linearity was ensured by a neutral density filter calibration (Tiffen, 58 mm Neutral Density = 0.9) and by disabling automatic gain and gamma control. A custom-made MATLAB program was implemented to record the pixel intensity of each sample every 5 min for 18 h.

1-(4-Fluorophenyl)-3-(4-(2-hydroxyethoxy)phenyl)propane-1,3-dione (dbm(F)OH) (2)

The aromatic ketone, 1-(4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)phenyl)ethan-1-one³⁸ (870 mg, 3.3 mmol), and ethyl 4-fluorobenzoate (674 mg, 3.9 mmol), were dissolved in anhydrous THF (50 mL). A suspension of NaH (120 mg, 5.0 mmol) in THF (10 mL) was transferred to the reaction via cannula. The reaction was refluxed at 60 °C under a nitrogen atmosphere, and monitored by TLC, until consumption of the aromatic ketone was complete (7 h). The reaction mixture was removed from the heat and allowed to cool to RT, before excess NaH was quenched with sat. aqueous NaHCO₂ (20 mL), and the THF was removed via rotary evaporation. The crude reaction mixture was treated with 1M HCl to adjust the pH to ~5. The resulting mixture was extracted with EtOAc (2 × 20 mL), then combined organic layers were washed with H₂O (2 × 20 mL). The organic solvent, EtOAc, was removed *via* rotary evaporation to yield a brown oil. The crude reaction mixture was dissolved in a THF (100 mL)/H₂O (20 mL) solution, a catalytic amount of TsOH (20 mg, 0.12 mmol) was added, then the reaction mixture was refluxed at 60 °C and monitored by TLC for the disappearance of protected diketone (2 h). The THF was removed by rotary evaporation, resulting in an aqueous suspension of the crude organic product. The organics were extracted with CH₂Cl₂ (2 × 20 mL) and washed with H₂O (2 × 20 mL), and brine (2 × 20 mL), and dried over anhydrous Na₂SO₄. The mixture was filtered and the solvent was removed by rotary evaporation to yield a brown solid. The crude solid was purified by

recrystallization (EtOAc/hexanes) to yield a white powder: 649 mg (65 %). ^1H NMR (600 MHz, CDCl_3): δ 17.10 (s, broad, 1H, -OH), 8.03–7.93 (m, broad, 4 H, 2', 6'-ArH, 2'', 6''-ArH), 7.20–7.10 (m, broad, 2H, 3', 5'-ArH), 6.99 (d, J = 9, 2H, 3'', 5''-ArH), 6.74 (s, 1H, -COCHCO), 4.17 (t, J = 6, 2H, Ar-OCH₂CH₂OH), 4.01 (t, J = 6, 2H, Ar-OCH₂CH₂OH), 1.65 (s, broad, 1H, Ar-OCH₂CH₂OH). HRMS (ESI, TOF) m/z calcd for $\text{C}_{17}\text{H}_{16}\text{O}_4\text{F}$, 303.1033 $[\text{M} + \text{H}]^+$; found 303.1031.

1-(4-Chlorophenyl)-3-(4-(2-hydroxyethoxy)phenyl)propane-1,3-dione (dbm(Cl)OH) (3)

The chloro ligand (**3**) was prepared as described for **2**, but using methyl 4-chlorobenzoate as the aromatic ester in the Claisen condensation to yield a tan flaky solid: 210 mg (45%). ^1H NMR (300 MHz, CDCl_3): δ 16.91 (s, broad, 1H, -OH), 7.96 (d, J = 9, 2H, 2', 6'-ArH), 7.89 (d, J = 8.4, 2H, 2'', 6''-ArH), 7.44 (d, J = 8.4, 2H, 3', 5'-ArH), 7.00 (d, J = 9, 2H, 3'', 5''-ArH), 6.74 (s, 1H, -COCHCO-), 4.16 (t, J = 4.2, 2H, -ArOCH₂-), 4.00 (s, broad, 2H, -CH₂OH), 3.50 (s, broad, 1H, -CH₂OH). HRMS (ESI, TOF) m/z calcd for $\text{C}_{17}\text{H}_{16}\text{O}_4\text{Cl}$ 319.0728 $[\text{M} + \text{H}]^+$; found 319.0737.

1-(4-Bromophenyl)-3-(4-(2-hydroxyethoxy)phenyl)propane-1,3-dione (dbm(Br)OH) (4)

The bromo ligand (**4**) was prepared as described for **2**, but using methyl 4-bromobenzoate as the aromatic ester in the Claisen condensation to yield a tan powder: 855 mg (63%). ^1H NMR (300 MHz, CDCl_3): δ 16.90 (s, broad, 1H, -OH), 7.97 (d, J = 9, 2H, 2', 6'-ArH), 7.83 (d, J = 8.4, 2H, 2'', 6''-ArH), 7.62 (d, J = 8.4, 2H, 3', 5'-ArH), 7.00 (d, J = 9, 2H, 3'', 5''-ArH), 6.75 (s, 1H, -COCHCO-), 3.89 (s, 3H, -ArOCH₃), 4.17 (t, J = 4.2, 2H, -ArOCH₂-), 4.02 (s, broad, 2H, -CH₂OH), 1.99 (s, broad, 1H, -CH₂OH). HRMS (ESI, TOF) m/z calcd for $\text{C}_{17}\text{H}_{16}\text{O}_4\text{Br}$ 363.0232 $[\text{M} + \text{H}]^+$; found 363.0237.

BF₂dbm(F)OH (7)

The fluoro initiator **7** was prepared by dissolving dbm(F)OH (**2**) (200 mg, 0.66 mmol) in anhydrous CH_2Cl_2 (50 mL) in a dry 100 mL round bottom flask. Boron trifluoride diethyl etherate (122 μL , 0.99 mmol) was added *via* syringe, whereupon the solution turned light yellow. The reaction mixture was stirred at room temperature and monitored by TLC until the ligand substrate was consumed (4 h). Excess boron trifluoride diethyl etherate was quenched by addition of $\text{K}_2\text{CO}_3(\text{s})$ (100 mg, 0.72 mmol). After stirring for 15 min, the reaction mixture was filtered and the solvent was removed *via* rotary evaporation to yield a dark yellow powder. The product was purified by column chromatography (1:1 hexanes/EtOAc, then EtOAc) to yield a faint yellow powder: 151 mg (69%). ^1H NMR (600 MHz, CDCl_3): δ 8.17–8.14 (m, broad, 4H, 2', 6'-ArH, 2'', 6''-ArH), 7.21 (d, J = 6, 2H, 3', 5'-ArH), 7.07–7.04 (m, 2H, 3'', 5''-ArH), 6.97 (s, 1H, COCHCO), 4.99 (s, 1H, Ar-OCH₂CH₂OH), 4.21 (t, J = 6, 2H, Ar-OCH₂CH₂OH), 4.03 (t, J = 6, 2H, Ar-OCH₂CH₂OH). HRMS (ESI, TOF) m/z calcd for $\text{C}_{17}\text{H}_{14}\text{O}_4\text{BF}_2$ 331.0953 $[\text{M} - \text{F}]^+$; found 331.0949.

BF₂dbm(Cl)OH (8)

The chloro initiator **8** was prepared as described for **7** using dbm(Cl)OH (**3**) instead of dbm(F)OH (**2**). A yellow powder was obtained: 118 mg (76%). ^1H NMR (600 MHz, CDCl_3): δ 8.16 (d, J = 9, 2H, 2', 6'-ArH), 8.06 (d, J = 9, 2H, 2'', 6''-ArH), 7.52 (d, J = 8.4, 2H,

3', 5'-ArH), 7.07–7.05 (m, 3H, 3'',5''-ArH, -COCHCO-), 4.21 (t, J = 3.9, 2H, -ArOCH₂-), 4.04–4.02 (m, 2H, -CH₂OH). HRMS (ESI, TOF) m/z calcd for C₁₇H₁₄O₄ClBF 347.0661 [M – F]⁺; found 347.0658.

BF₂dbm(Br)OH (9)

The bromo initiator was prepared as previously described for **7** using dbm(Br)OH (**4**) instead of dbm(F)OH (**2**). A yellow powder was obtained: 110 mg (49%). ¹H NMR (600 MHz, CDCl₃): δ 8.16 (d, J = 9, 2H, 2', 6'-ArH), 7.97 (d, J = 9, 2H, 2'', 6''-ArH), 7.69 (d, J = 8.4, 2H, 3', 5'-ArH), 7.07–7.05 (m, 3H, 3'',5''-ArH, -COCHCO-), 4.21 (t, J = 3.9, 2H, -ArOCH₂-), 4.07–4.03 (m, 2H, -CH₂OH). HRMS (ESI, TOF) m/z calcd for C₁₇H₁₄O₄BrBF 391.0153 [M – F]⁺; found 391.0156.

RESULTS AND DISCUSSION

Synthesis

The β-diketones **1–5** (Scheme 1) were prepared as previously described.³⁸ Namely, the aromatic ketone 1-(4-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)phenyl)ethan-1-one was combined with the appropriate aromatic ester to generate the diketone via a Claisen condensation, followed by removal of the dihydropyran hydroxyl protecting group, to give compounds **1–5**. By varying the aromatic ester, ligands with different halide substituents were easily produced via the same synthetic methods. The diketones were purified by recrystallization in EtOAc and hexanes.

Boronation was performed by stirring boron trifluoride diethyletherate with the β-diketones, **1–5**, in anhydrous CH₂Cl₂ for 4–10 hours at room temperature. The parent compound, BF₂dbmOH (**6**), and the iodine substituted BF₂dbm(I)OH (**10**) were purified by recrystallization from acetone/hexanes to obtain yellow crystalline powders. The lighter halide derivatives (**7–9**) had very similar solubility to the ligand analogues, and were instead purified by silica column chromatography (EtOAc/hexanes eluent) and were also obtained as yellow powders.

Poly(lactic acid) (PLA) was grown from the primary alcohol sites of boron initiators **6–10** in lactide melts in the presence of a stannous octoate catalyst as previously described.²³ Compared to less soluble dinaphthyl initiators,²³ **6–10** readily dissolved even in low loadings of molten lactide (i.e. 50 equivalents). To assess the effects of molecular weight (MW) on the luminescence properties of dye-PLA conjugates, two polymers were grown for each halide initiator—one with low MW (6.0–7.5 kDa) and one with higher MW (16.5–20.0 kDa) (Table 1). With 50 equivalents of lactide, polymerizations became viscous and were stopped after 1 hour by cooling to room temperature followed by precipitation from CH₂Cl₂/ice cold MeOH then CH₂Cl₂/hexanes.²³ Reactions with 200 equivalents were typically run for 3 hours. Reactions were stopped at ~60–80% conversion to ensure more controlled reaction conditions and polymers with low polydispersities (1.05–1.18). This minimizes thermal depolymerization or transesterification side reactions, as previously described.^{43,44}

Optical Properties in Solution

The dye initiators (**6–10**) and polymers (**11–15**) were analyzed in dilute CH₂Cl₂ solutions in air at room temperature, and the optical properties are summarized in Table 2. The dyes and polymers have near identical properties in CH₂Cl₂ and all absorb light in the UV to violet range (395–410 nm, Figure S17). As the halide substituent becomes heavier, the absorbance is red-shifted (i.e. BF₂dbmOH (**6**) $\lambda_{\text{abs}} = 397$ nm versus BF₂dbm(I)OH (**10**) $\lambda_{\text{abs}} = 407$ nm). The extinction coefficient increased slightly with heavier substituted halides as well (BF₂dbmOH (**6**) $\epsilon = 53000$ M⁻¹ cm⁻¹ versus BF₂dbm(I)OH (**10**) $\epsilon = 58000$ M⁻¹ cm⁻¹). As is commonly observed,¹⁸ extinction coefficients decreased from dyes to polymers with the same halide substituent (i.e. BF₂dbm(F)OH (**7**): $\epsilon = 48000$ M⁻¹ cm⁻¹ versus BF₂dbm(F)PLA (**12a**) $\epsilon = 45000$ M⁻¹ cm⁻¹), perhaps due to minor dye degradation during the polymerization, purification processes, or inherent error in estimating dye content and ascribing an extinction coefficient to polydisperse samples.

The dyes emit blue fluorescence in CH₂Cl₂ solution, and the halide substituents have only minor influences on the color (BF₂dbmOH (**6**); $\lambda_{\text{F}} = 433$ nm, BF₂dbm(I)OH (**10**); $\lambda_{\text{F}} = 441$ nm). However, halide substitution does alter the fluorescence lifetime (τ_{F}) and quantum yield (Φ_{F}) of the boron dyes. Lighter halide dyes and polymers (X = H, F, Cl, and Br) have similar lifetimes of (~1.95 ns), and quantum yields near unity (~0.95). The iodine derivatives showed lower quantum yields and shorter fluorescence lifetimes as a result of the heavy atom effect.²² In solution, enhanced intersystem crossing via the heavy atom effect resulted in non-radiative decay pathways as a result of oxygen quenching or intramolecular motions.

Optical Properties of Films

Boron polymers were also studied as films in the solid state. Optical properties for polymers **11–15** with variable molecular weights are presented in Table 3. Fluorescence spectra and lifetime measurements were obtained under ambient conditions (e.g., air, ~21% oxygen). Phosphorescence measurements were conducted under N₂.

Molecular weight is an effective way to tune the luminescence properties of boron dye-PLA materials.^{19,20,22,23} In previously reported BF₂dbmPLA polymer conjugates, low MW polymers (~4 kDa) showed green fluorescence (500 nm), high MW polymers (~20 kDa) showed blue fluorescence (425 nm), and peak emission could be modulated using the PLA MW or dye concentration in blends.¹⁹ This is attributed to a change in the dye-dye interactions within the PLA matrix; therefore, dye concentration plays an important role. Similarly, this concentration dependent emission can also be seen in other difluoroboron dyes. For example, Venkatesan *et al.* successfully generated β -ketoiminates that exhibited phosphorescence across the visible spectrum at different concentrations in CH₂Cl₂ and poly(methyl methacrylate) (PMMA) environments.¹¹ In the solid-state, dye structure is critical in determining how dye-dye interactions influence material optical properties. Phenyl-phenyl systems (BF₂dbmPLA) reached a maximal blue-shifted fluorescence at a MW of ~20 kDa, which corresponded to about 1% dye by total mass; further dilution did not affect the fluorescence color significantly.¹⁹ With increased conjugation in naphthyl-naphthyl based dyes (BF₂dnmPLA), which corresponded to lower solubility and increased

propensity for dyes to aggregate, a maximal blue-shifted fluorescence was reached with only 0.2% dye loading in PLA blends.²³

Halide interactions play important roles in molecular assemblies and crystal engineering^{22,45,46} and serve as a way to modulate optical properties. In this study two polymers of each halide-substituted dye were prepared to analyze MW effects and dye-dye interactions on the optical properties. (See spectra of dye-polymer films in Figures S18–20). The expected trend in fluorescence lifetimes (τ_F) across the halide series is observed. As the halide becomes heavier, τ_F decreases (H = 6.6 ns, F = 45.4 ns, Cl = 6.9 ns, Br = 5.3 ns, I = 3.4 ns). The fluorescence color sensitivity to dye loading (i.e., M_n) also varied with different halide dyes. For instance, between BF₂dbm(F)PLA, **12a** (6.1 kDa; λ_F = 502 nm) and **12b** (16.5 kDa; λ_F = 428 nm), the emission wavelength shifted by 74 nm. In contrast, emission wavelengths for the bromide analogues BF₂dbm(Br)PLA, **14a** (6.0 kDa; λ_F = 471 nm) and **14b** (17.1 kDa; λ_F = 436 nm), shifted only 35 nm with a comparable shift in molecular weight (Figure 2 and Table 3). The difference in emission maxima between low and high MW polymers decreases for heavier halides. When heavy atoms are present, there is enhanced intersystem crossing (ISC) to the triplet state, siphoning off redshifted fluorescence. The unsubstituted dye, BF₂dbmPLA, showed a smaller range than the chloro- and fluoro- adducts. This could be a result of increased inductive effects due to the electron withdrawing properties of the aromatic halides influencing the donor-acceptor scaffold of the dye.^{47–49} Fluorescence properties can inspire designs for future dye scaffolds, controlling color ranges within the PLA matrix and by modulating the donor-acceptor structure of the boron dyes.

The phosphorescence properties of boron polymers were studied under a N₂ atmosphere at room temperature. All polymers showed delayed emission in the absence of oxygen at room temperature (Figures 3 and S22). Delayed emissions were green and the maxima ranged from 500 to 530 nm. Unlike the fluorescence maxima (λ_F), the phosphorescence maxima (λ_P) did not vary with polymer MW. For example, the fluorescence of BF₂dbmPLA (**11**) blue-shifted from 457 nm (~6 kDa) to 426 nm (~19 kDa), while the phosphorescence changed only slightly, from 516 nm to 510 nm with increasing MW. Heavier halide substituents red-shifted the phosphorescence (**11a–15a**; λ_P : H = 516 nm, F = 512 nm, Cl = 520 nm, Br = 522 nm, I = 528 nm). The emission peak also sharpened with heavier halide substituents, along with a decrease in delayed fluorescence at shorter wavelengths, similar to previously described BF₂bdk-PLA conjugates.²² Because delayed fluorescence (DF) is temperature sensitive, it is important to suppress DF in ratiometric oxygen probes, as the fluorescence internal “standard” would otherwise respond to O₂ quenching and temperature. The addition of heavier halides (Br and I) is an effective way to minimize the DF transitions.

The phosphorescence lifetime (τ_P) is highly dependent on both the molecular weight and the halide substituent. The phosphorescence emission maxima stayed at a constant wavelength with changing MW, while the fluorescence maxima blue-shifted. Increasing the molecular weight expanded the singlet-triplet gap, and decreased the rate of intersystem crossing and thermally activated delayed fluorescence, resulting in longer lifetimes (e.g. τ_P of BF₂dbmPLA: **11a** = 142 ms, **11b** = 207 ms). The halides influenced the τ_P differently. With increased atomic mass of the halide substituents, the phosphorescence intensity increased

(Table 3), at the expense of the fluorescence intensity. Lighter halide substituents (H **11a**, F **12a**, and Cl **13a**) had weak phosphorescence shoulders in their total emission under N₂ but long green afterglows (>100 ms lifetimes) (Figure 4). For heavier halide dyes (Br and I) with short polymers, the emission color of the material changed from blue-green (~470 nm) to yellow (~525 nm), as the phosphorescence dominated the total emission. These types of materials are well suited for ratiometric sensing, where the fluorescence serves as the internal standard, and the phosphorescence intensity relates to the O₂ concentration. Lighter halide substituted dyes with long RTP lifetimes are well suited for lifetime methods for quantifying O₂ concentration.

The fluorescence to phosphorescence intensity ratio (F/P) can be controlled by polymer molecular weight.^{22,23} This is linked to the singlet-triplet energy gap; as the gap becomes larger, the rate of intersystem crossing decreases, lowering the phosphorescence intensity. For short polymers, Br **14a** and I **15a**, the fluorescence and phosphorescence peaks overlap (Figure 4). Higher MW polymers can potentially resolve the two peaks to facilitate detection of the fluorescence and phosphorescence. This strategy has been demonstrated for naphthyl dye polymers to make them useful as ratiometric sensing materials.²³ For some naphthalene-substituted dyes, intense phosphorescence is still present even for dilute dye samples (>20 kDa).^{20,23} But in other cases, as the singlet triplet energy gap gets larger, crossover to the triplet state and phosphorescence intensity are significantly decreased, rendering the material impractical for sensing. In fact, this is what is observed for bromine and iodine substituted BF₂dbm(X)PLA derivatives in this study. The singlet-triplet gap in 20 kDa polymers became so great that the phosphorescence no longer dominated the total emission under N₂ (Figure S21). The phosphorescence peak still resulted in a visible color change for BF₂dbm(I)PLA, but was much weaker than the fluorescence. Thus, while naphthyl dyes work well even at low dye loading, BF₂dbm(X)PLA (X = Br, I) materials are most useful at higher dye loadings for ratiometric sensing.

Oxygen Sensitivity

The two most conventional ways for luminescence O₂ quantification are based on lifetime and intensity based phosphorescence measurements. Lifetime methods do not require internal standards to determine O₂ concentration.³³ In this method, spectrally isolated fluorescence and phosphorescence peaks, or intense phosphorescence, are not needed for O₂ quantification, as long as the lifetime is gated and does not include the fluorescence.³³ Lifetime is directly linked to oxygen sensitivity, as shown in Equations 1 and 2.⁵⁰ Therefore, materials with the longest unquenched phosphorescence lifetimes (τ_0) and fastest diffusion of oxygen through the matrix, will be the most sensitive. Klimant *et al.* recently developed ultrasensitive materials with aluminum or difluoroboron hydroxyphenalenone complexes within polystyrene, Teflon and Hyflon polymer matrices.⁵¹ By combining complexes of variable unquenched lifetimes, with polymer matrices of high O₂ permeabilities (P), such as polystyrene ($P \approx 8.8 \times 10^{-16} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$),⁵² Hyflon ($P \approx 170 \times 10^{-16} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$),⁵³ or Teflon ($P \approx 1200 \times 10^{-16} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$),⁵⁴ the oxygen hypersensitivity could be tuned, and oxygen concentration could be detected in the parts per million range. In contrast, purely organic crystals exhibited long-lived phosphorescence, insensitive to

oxygen. When the phosphorescence is confined to a crystal, the oxygen barrier is too great, and the phosphorescence persists under ambient conditions.^{29,30,55–58}

$$\frac{\tau}{\tau_0} = 1 + K_{SV} [O_2] \quad (1)$$

$$K_{SV} = \tau_0 * k_D \quad (2)$$

This effect has yet to be studied for boron dyes in biodegradable polylactide. Polylactide exhibits permeabilities that are magnitudes lower than that of perfluorinated polymers and polystyrene (PLA: $P \approx 0.86 \times 10^{-16} \text{ mol m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$)⁵⁹ and can change dependent on material fabrication, film treatment (e.g. annealing), polymer microstructure (e.g. stereochemistry) and other factors affecting material microcrystallinity. At first this might seem like a drawback, but in fact, the lower permeability of oxygen in PLA is beneficial, given that it counterbalances the long-lived phosphorescence in BF₂dbkPLA materials. The net effect is biocompatible materials with dynamic ranges that are well matched to biological processes. Another advantage of the long phosphorescence lifetimes in BF₂dbm(X)PLA materials is that they enable detection by convenient and inexpensive imaging techniques, such as with portable high-speed cameras⁶⁰ that can be useful in clinical and field contexts.

Given that phosphorescence lifetimes vary with halide substituent and polymer MW, samples **11–15** provide a convenient model to test O₂ sensitivity versus lifetime. Previously, it was shown that the phosphorescence intensity of BF₂dbm(I)PLA varied linearly with oxygen concentrations between 0–1%.²² Highly sensitive dual-emitters within this O₂ range could assist in detecting tumors with severe hypoxia, and be used in monitoring tumor growth and proliferation during therapy.³⁶ To determine sensitivity within this range, the unquenched phosphorescence lifetime (τ_0) (i.e. 0% oxygen) was compared to the lifetime at 1% oxygen (τ_1) (Table 4). The sensitivity of each polymer was determined by the rate of the change between the two O₂ concentrations (τ_0/τ_1), as is routine within the field for comparison between oxygen probes.³³

Lighter halide substituted dye-polymer conjugates (H, F, and Cl) showed the most dynamic response to oxygen, as the phosphorescence lifetimes became ~14 times shorter at 1% oxygen. However, between these three samples, sensitivity changes are minimal (BF₂dbmPLA, **11a**, $\tau_0/\tau_1 = 13.9$; BF₂dbm(F)PLA, **12a**, $\tau_0/\tau_1 = 13.5$; BF₂dbm(Cl)PLA, **13a**, $\tau_0/\tau_1 = 14.7$). The sensitivities are too similar to conclude definitively that the lighter halides (F, Cl) are altering the oxygen sensitivity, as minor changes in polymer MW may be the defining factor for the observed changes (i.e. BF₂dbm(F)PLA: **12a** (6.1 kDa) and BF₂dbm(Cl)PLA: **13a** (7.5 kDa)). At 1% oxygen, these dyes are approaching the upper limit of detection as well, defined as $\tau_1/\tau_0 = 1\%$ (e.g. BF₂dbmPLA: **11b**, $\tau_1/\tau_0 = 7\%$).⁶¹ Therefore, the detected phosphorescence at 1% oxygen may be the result of a different detectable solid-state species (i.e. aggregates or dimers) within the PLA matrix as BF₂dbm type fluorophores are well known to form in polymer matrices.^{62–64} Different phosphorescence quenching rates, or completely unquenchable species may also influence

the sensitivity of these materials.⁶³ The mere presence of long-lived phosphorescence within the BF₂dbm(X)PLA samples, where X = H, F, and Cl, will indicate a highly deoxygenated environment, useful for numerous applications.⁶⁵

The less dynamically sensitive bromine (**14**) and iodine (**15**) polymers serve as better systems for analyzing trends in phosphorescent lifetimes. The lifetimes of the bromide **14a** and iodide **15a** polymers under nitrogen were 28.9 ms and 6.6 ms, respectively. The resultant change in oxygen sensitivity was dramatic (Br, **14a**, $\tau_0/\tau_1 = 5.0$; I, **15a**, $\tau_0/\tau_1 = 2.1$). In the higher MW polymers, **14b** and **15b**, the τ_0 became longer, and thus, the sensitivity changed (Br, **14b**, $\tau_0/\tau_1 = 6.8$; I, **15b**, $\tau_0/\tau_1 = 2.4$). This suggests that heavy atom substitution and polymer MW can effectively tune the oxygen sensitivity of these BF₂dbm(X)PLA materials.

Oxygen sensitive materials with the unique combination of millisecond long decays and intense phosphorescence are scarce.^{22,51} Polymers with heavier halide substituted dyes, bromine (**14a**) and iodine (**15a–b**), meet these criteria (Table 5). The long phosphorescence lifetimes can be used to detect oxygen by phosphorescence lifetime imaging microscopy (PLIM),⁶⁶ and the phosphorescence is strong enough for intensity-based ratiometric measurements.^{22,23} When analyzing the fluorescence to phosphorescence ratios (F/P) of the low MW polymers, the bromide polymer (**14a**) experienced slightly higher sensitivity in the 0–1% O₂ range than the iodide polymers (BF₂dbm(Br)PLA: **14a**, $P_0/P_1 = 2.09$, BF₂dbm(I)PLA: **15a**, $P_0/P_1 = 1.61$) (Figure 5). More pronounced than sensitivity is the change in range and limits of detection with heavy atom substitution. At 21% oxygen (air), the bromide polymer **14a** has a negligible difference in F/P compared to the ratio at 1% (BF₂dbm(Br)PLA: **14a**, $P_0/P_1 = 2.09$, $P_0/P_{21} = 2.35$). In contrast, iodide polymers, **15a–b**, have more intense phosphorescence that can sense well beyond 1% oxygen. Iodide polymers of different MWs, have similar oxygen sensitivities, but the range of detection changes. The phosphorescence of high MW BF₂dbm(I)PLA (20 kDa) was fully quenched in air (21% O₂), whereas low MW BF₂dbm(I)PLA (6 kDa) exhibited phosphorescence under ambient conditions (i.e. air). This suggests that oxygen levels higher than 21% could be detected with this sensing material. These results demonstrate that the oxygen sensing capability of BF₂dbm(X)PLA-type fluorophore probes can be fine-tuned with halide substitution and polymer molecular weight for a variety of applications in biological and other contexts.

Photostability

It is well known that β -diketones can photobleach with UV exposure.⁶⁷ For example, in sunscreens, the diketone avobenzene is commonly used to absorb short-wave UVA radiation and is known to photodegrade over time. Recently it was reported that halide substitution can create new degradation pathways for diketone compounds.⁶⁸ Furthermore, Zakharova and co-workers demonstrated that both boron detachment and singlet oxygen generation are relevant pathways for BF₂dbm degradation in organic solvents.⁶⁹ Thus, it is important to analyze the degradation and photobleaching of boron dye polymers to assess the material lifetime and accuracy for sensing.

Photostability studies were conducted by exposing spin-cast polymer films to UV light using a handheld 4 W UV lamp ($\lambda_{\text{ex}} = 369 \text{ nm}$) for 18 hours. A monochrome camera was used to monitor the total intensity while the dyes were excited. An image was captured every five minutes during the 18 hour period. This was a simple way to monitor multiple samples at once, and to eliminate environmental variations between samples. The resultant changes in intensity are shown in Figure 6. For lighter halide substituted $\text{BF}_2\text{dbm}(\text{X})\text{PLA}$ ($\text{X} = \text{H}, \text{F}$, and Cl , **11a–13a**), the total intensity decreased but only by $\sim 5\%$ over the 18 hour period, consistent with only modest dye degradation. Compared to many fluorescent probes commonly used in biology,^{1,2} these materials are intense and show excellent photostability. Furthermore, these findings confirm their suitability for lifetime oxygen sensing or as turn on sensors in oxygen-free or very low oxygen environments.

Low molecular weight polymers with bromide and iodide (**14a** and **15a**), in contrast, actually became 9–10% brighter after excitation for 18 hours (Figure 6). At first it might seem surprising that the overall intensity increased even though the number of fluorophores decreased upon photodegradation, but in fact, this is understandable given what is known about dye loading and intersystem crossing effects in BF_2bdkPLA materials.²² When dye concentration decreases in PLA the fluorescence blueshifts and the energy gap between fluorescence and phosphorescence maxima increases. This decreases the rate of intersystem crossing to the oxygen sensitive triplet state, which is quenched in air, and thus, results in more intense total emission. It is interesting to compare this process with mechanochromic luminescence quenching (MLQ) in solid BF_2bdk dye samples, wherein thermally annealed samples have a larger singlet triplet gap and are bright until smearing narrows the singlet triplet gap, thus facilitating intersystem crossing and quenching of the triplet state, causing total emission to decrease.³² Unlike the 7 kDa iodide polymer the 20 kDa sample **15b** showed little change in the fluorescence maximum or total intensity over time (Figure 6). Given that the dye is already primarily in the monomeric state in the high molecular weight polymer, it does not blueshift upon photodegradation and further dilution. Higher molecular weight samples show good photostability however some changes in the F/P intensity ratio are noted for phototreated 20kDa $\text{BF}_2\text{dbm}(\text{I})\text{PLA}$ analyzed under nitrogen (Figure 7, bottom right). It is possible that dye monomers and aggregates could degrade at different rates in PLA samples. To test this, a control was run with initiator $\text{BF}_2\text{dbm}(\text{I})\text{OH}$ (**10**) spin cast films. Results in Figure 6 (right) showed stable emission for this sample. In the solid state however, under air or nitrogen, no phosphorescence is observed for **10**. This suggests involvement of the triplet state for photodegradation pathways through singlet oxygen generation or radical formation in dye-PLA films where phosphorescence is present. In summary, these findings indicate that high molecular weight samples with long phosphorescent lifetimes (i.e. $\text{BF}_2\text{dbm}(\text{X})\text{PLA}$ where $\text{X} = \text{H}, \text{F}, \text{Cl}$) are best for lifetime imaging given greater photostability. But further improvements in dye design are required for reliable quantitative ratiometric sensing. Instead, heavy atom substituted dyes that exhibit strong phosphorescence at low dye loading are desirable.^{20,23}

CONCLUSIONS

In summary, $\text{BF}_2\text{dbm}(\text{X})\text{PLA}$ materials were synthesized for $\text{X} = \text{H}, \text{F}, \text{Cl}, \text{Br}$, and I . The effects of changing the halide substituent as well as the length of the PLA on the optical

properties of the polymers were studied in dilute CH_2Cl_2 solutions and solid state films. All samples show intense fluorescence suitable for use as probes. Several properties ideal for oxygen sensing were also observed, including fluorescence and intense phosphorescence for the short iodine and bromine polymers suitable for ratiometric sensing, and long phosphorescence lifetimes for the lighter halide-dye polymers useful for lifetime sensing and convenient and cost effective camera imaging. The long lifetimes increased the sensitivity of the dye-polymers. The lifetimes of the iodine and bromine polymers were shorter due to the heavy atom effect. Varying the polymer MW affected the ratio of fluorescence to phosphorescence intensity under anoxic conditions. For the long bromine and iodine polymers, increasing the polymer molecular weight to ~20 kDa created a larger singlet-triplet gap but decreased the rate of intersystem crossing. The result is slightly improved fluorescence and phosphorescence peak resolution but with much weaker phosphorescence, demonstrating that low molecular weight $\text{BF}_2\text{dbm(X)PLA}$ polymers are preferable for ratiometric oxygen sensing.

Comparing the phosphorescence lifetimes of films under nitrogen versus a 1% oxygen environment revealed the ability to tune the oxygen sensitivity by changing the halide substituent from bromine to iodine or by changing the polymer molecular weight. Finally, the photostability of the polymers was monitored over an 18 hour continuous UV light excitation period. The total intensity for the lighter halides decreased only 5% over time, indicating good photostability. Bromide and iodide derivatives, on the other hand, display an increase in fluorescence intensity, which counter intuitively, is also ascribed to dye degradation, and thus, lower dye loading. Dye dilution corresponds to a blueshift in fluorescence, an increase in the singlet triplet energy gap, and thus, decreased rates of intersystem crossing, analogous to the molecular weight effect commonly observed for this family of materials.^{21–23,25,26} The longer bromide and iodide substituted polymers did not show an increase in signal intensity upon extended exposure to UV light, given dyes existed in the maximally blueshifted monomeric state both before and after UV illumination. Results from this investigation point to dyes with long lifetimes for lifetime imaging modalities or more conjugated heavy atom substituted dyes with intense, separated and readily detectable fluorescence and phosphorescence peaks for ratiometric sensing. And importantly, sensing materials should be made from high MW polymers (lower dye loading) to ensure photostability and reliable quantitative sensing with BF_2bdkPLA materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wolfbeis O. An Overview of Nanoparticles Commonly Used in Fluorescent Bioimaging. *Chem Soc Rev.* 2015; 44:4743–4768. [PubMed: 25620543]

2. Vollrath A, Schubert S, Schubert US. Fluorescence Imaging of Cancer Tissue Based on Metal-Free Polymeric Nanoparticles – A Review. *J Mater Chem B*. 2013; 1:1993–2007.
3. Zheng Q, Juette MF, Jockusch S, Wasserman MR, Zhou Z, Altman RB, Blanchard SC. Ultra-stable Organic Fluorophores for Single-molecule Research. *Chem Soc Rev*. 2014; 43:1044–1056. [PubMed: 24177677]
4. Feng Y, Cheng J, Zhou L, Zhou X, Xiang H. Ratiometric Optical Oxygen Sensing: A Review in Respect of Material Design. *Analyst*. 2012; 137:4885–4901. [PubMed: 22943050]
5. Yang Y, Zhao Q, Feng W, Li F. Luminescent Chemodosimeters for Bioimaging. *Chem Rev*. 2013; 113:192–270. [PubMed: 22702347]
6. Loudet A, Burgess K. BODIPY Dyes and their Derivatives: Syntheses and Spectroscopic Properties. *Chem Rev*. 2007; 107:4891–4932. [PubMed: 17924696]
7. Boens N, Leen V, Dehaen W. Fluorescent Indicators Based on BODIPY. *Chem Soc Rev*. 2012; 41:1130–1172. [PubMed: 21796324]
8. Ran C, Xu X, Raymond SB, Ferrara BJ, Neal K, Bacskaí BJ, Medarova Z, Moore A. Design, Synthesis, and Testing of Difluoroboron-derivatized Curcumins as Near-infrared Probes for in Vivo Detection of Amyloid- β Deposits. *J Am Chem Soc*. 2009; 131:15257–15261. [PubMed: 19807070]
9. Yoshii R, Hirose A, Tanaka K, Chujo Y. Functionalization of Boron Diimines with Unique Optical Properties: Multicolor Tuning of Crystallization-Induced Emission and Introduction into the Main Chain of Conjugated Polymers. *J Am Chem Soc*. 2014; 136:18131–18139. [PubMed: 25522172]
10. Perumal K, Garg JA, Blacque O, Saiganesh R, Kaliban S, Balasubramanian KK, Venkatesan K. b-Iminoenamine-BF₂ Complexes: Aggregation-Induced Emission and Pronounced Effects of Aliphatic Rings on Radiationless Deactivation. *Chem – Asian J*. 2012; 7:2670–2677. [PubMed: 22945790]
11. Koch M, Perumal K, Blacque O, Garg JA, Saiganesh R, Kabilan S, Balasubramanian KK, Venkatesan K. Metal-Free Triplet Phosphors with High Emission Efficiency and High Tunability. *Angew Chem, Int Ed*. 2014; 126:6496–6500.
12. Yoshii R, Tanaka K, Chujo Y. Conjugated Polymers Based on Tautomeric Units: Regulation of Main-Chain Conjugation and Expression of Aggregation Induced Emission Property via Boron-Complexation. *Macromolecules*. 2014; 47:2268–2278.
13. Sanchez I, Nunez C, Campo JA, Torres MR, Cano M, Lodeiro C. Polycatenar Unsymmetrical β -diketonate Ligands as a Useful Tool to Induce Columnar Mesomorphism on Highly Luminescent Boron Difluoride Complexes. *J Mater Chem C*. 2014; 2:9653–9665.
14. Sanchez I, Mayoral MJ, Ovejero P, Campo JA, Heras JV, Cano M, Lodeiro C. Luminescent Liquid Crystal Materials Based on Unsymmetrical Boron Difluoride β -Diketonate Adducts. *New J Chem*. 2010; 34:2937–2942.
15. Xin L, Chen YZ, Niu LY, Wu LZ, Tung CH, Tong Q, Yang QZ. A Selective Turn-on Fluorescent Probe for Cd²⁺ Based on a Boron Difluoride β -Dibenzoyl Dye and its Application in Living Cells. *Org Biomol Chem*. 2013; 11:3014–3019. [PubMed: 23532210]
16. Zhang G, Lu J, Sabat M, Fraser CL. Polymorphism and Reversible Mechanochromic Luminescence for Solid-state Difluoroboron Avobenzene. *J Am Chem Soc*. 2010; 132:2160–2162. [PubMed: 20108897]
17. Galer P, Korosec RC, Vidmar M, Sket B. Crystal Structures and Emission Properties of the BF₂ Complex 1-Phenyl-3-(3, 5-dimethoxyphenyl)-propane-1, 3-dione: Multiple Chromisms, Aggregation-or Crystallization-Induced Emission, and the Self-Assembly Effect. *J Am Chem Soc*. 2014; 136:7383–7394. [PubMed: 24819115]
18. Zhang G, Chen J, Payne SJ, Kooi SM, Demas JN, Fraser CL. Multi-emissive Difluoroboron Dibenzoylmethane Polylactide Exhibiting Intense Fluorescence and Oxygen-Sensitive Room-Temperature Phosphorescence. *J Am Chem Soc*. 2007; 129:8942–8943. [PubMed: 17608480]
19. Zhang G, Kooi SE, Demas JN, Fraser CL. Emission Color Tuning with Polymer Molecular Weight for Difluoroboron Dibenzoylmethane-Polylactide. *Adv Mater*. 2008; 20:2099–2104.
20. Samonina-Kosicka J, DeRosa CA, Morris WA, Fan Z, Fraser CL. Dual-Emissive Difluoroboron Naphthyl-Phenyl β -Diketonate Polylactide Materials: Effects of Heavy Atom Placement and Polymer Molecular Weight. *Macromolecules*. 2014; 47:3736–3746. [PubMed: 24954954]

21. Samonina-Kosicka J, Weitzel DH, Hofmann CL, Hendargo H, Hanna G, Dewhirst MW, Palmer GM, Fraser CL. Luminescent Difluoroboron β -Diketonate PEG-PLA Oxygen Nanosensors for Tumor Imaging. *Macromol Rapid Commun.* 2015; 36:694–699. [PubMed: 25753154]
22. Zhang G, Palmer GM, Dewhirst MW, Fraser CL. A Dual-Emissive-Materials Design Concept Enables Tumour Hypoxia Imaging. *Nat Mater.* 2009; 8:747–751. [PubMed: 19668206]
23. DeRosa CA, Samonina-Kosicka J, Fan Z, Handargo HC, Weitzel DH, Palmer GM, Fraser CL. Oxygen Sensing Difluoroboron Dinaphthoylethane Poly(lactide). *Macromolecules.* 2015; 48:2967–2977. [PubMed: 26056421]
24. Oh JK. Poly(lactide) (PLA)-Based Amphiphilic Block Copolymers: Synthesis, Self-Assembly, and Biomedical Applications. *Soft Matter.* 2011; 7:5096–5108.
25. Tang L, Tong R, Coyle VJ, Yin Q, Ponden H, Borst LB, Cheng J, Fan TM. Targeting Tumor Vasculature with Aptamer-Functionalized Doxorubicin–Poly(lactide) Nanoconjugates for Enhanced Cancer Therapy. *ACS Nano.* 2015; 9:5072–5081. [PubMed: 25938427]
26. Pfister A, Zhang G, Zareno J, Horwitz AF, Fraser CL. Boron Poly(lactide) Nanoparticles Exhibiting Fluorescence and Phosphorescence in Aqueous Medium. *ACS Nano.* 2008; 2:1252–1258. [PubMed: 19081748]
27. Bowers DT, Tanes ML, Das A, Lin Y, Keane NA, Neal RA, Ogle ME, Brayman KL, Fraser CL, Botchwey EA. Spatiotemporal Oxygen Sensing Using Dual Emissive Boron Dye–Poly(lactide) Nanofibers. *ACS Nano.* 2014; 8:12080–12091. [PubMed: 25426706]
28. Baronoff E, Curchos BF, Monti E, Filippo M, Stelmer F, Accorsi G, Tavernelli I, Rothlisberger U, Scopelliti R, Gratzel M, Nazeeruddin MK. Influence of Halogen Atoms on a Homologous Series of Bis-Cyclometalated Iridium(III) Complexes. *Inorg Chem.* 2012; 51:799–811. [PubMed: 22220696]
29. Bolton O, Lee K, Kim HJ, Lin KY, Kim J. Activating Efficient Phosphorescence from Purely Organic Materials by Crystal Design. *Nat Chem.* 2011; 3:205–210. [PubMed: 21336325]
30. Bolton O, Lee D, Jung J, Kim J. Tuning the Photophysical Properties of Metal-Free Room Temperature Organic Phosphors via Compositional Variations in Bromobenzaldehyde/Dibromobenzene Mixed Crystals. *Chem Mater.* 2014; 26:6644–6649.
31. Yoshii R, Suenaga K, Tanaka K, Chujo Y. Mechanofluorochromic Materials Based on Aggregation-Induced Emission-Active Boron Ketoimines: Regulation of the Direction of the Emission Color Changes. *Chem – Eur J.* 2015; 21:7231–7237. [PubMed: 25820468]
32. Morris WA, Liu T, Fraser CL. Mechanochromic Luminescence of Halide-Substituted Difluoroboron β -Diketonate Dyes. *J Mater Chem C.* 2015; 3:352–363.
33. Wang, X-d; Wolfbeis, O. Optical Methods for Sensing and Imaging Oxygen: Materials, Spectroscopies and Applications. *Chem Soc Rev.* 2014; 43:3666–2761. [PubMed: 24638858]
34. Carreau A, Hafny-Rahbi BE, Matejuk A, Grillon C, Kieda C. Why is the Partial Oxygen Pressure of Human Tissues a Crucial Parameter? Small Molecules and Hypoxia. *J Cell Mol Med.* 2011; 15:1239–1253. [PubMed: 21251211]
35. Tredan O, Galmarini CM, Patel K, Tannock IF. Drug Resistance and the Solid Tumor Microenvironment. *J Natl Cancer Inst.* 2007; 99:1441–1454. [PubMed: 17895480]
36. Vaupel P, Kallinowski F, Okunieff P. Blood Flow, Oxygen and Nutrient Supply, and Metabolic Microenvironment of Human Tumors: A Review. *Cancer Res.* 1989; 49:6449–6465. [PubMed: 2684393]
37. Sinks LE, Roussakis E, Esipova TV, Vinogradov SA. Synthesis and Calibration of Phosphorescent Nanoprobes for Oxygen Imaging in Biological Systems. *J Visualized Exp.* 2010; 37:1731.
38. Bender JL, Corbin PS, Fraser CL, Metcalf DH, Richardson FS, Thomas EL, Urbas AM. Site-Isolated Luminescent Europium Complexes with Polyester Macroligands: Metal-Centered Heteroarm Stars and Nanoscale Assemblies with Labile Block Junctions. *J Am Chem Soc.* 2002; 124:8526–8527. [PubMed: 12121083]
39. Williams DBG, Lawton M. Drying of Organic Solvents: Quantitative Evaluation of the Efficiency of Several Desiccants. *J Org Chem.* 2010; 75:8351–8354. [PubMed: 20945830]
40. Heller CA, Henry RA, McLaughlin B, Bliss DEJ. Fluorescence Spectra and Quantum Yields. Quinine, Uranine, 9, 10-Diphenylanthracene, and 9, 10-bis (Phenylethynyl) anthracenes. *J Chem Eng Data.* 1974; 19:214–219.

41. Melhuish WH. Quantum Efficiencies of Fluorescence of Organic Substances: Effect of Solvent and Concentration of the Fluorescent Solute. *J Phys Chem.* 1961; 65:229–235.
42. Rioboo RJ, Phillip M, Ramos MA, Kruger JK. Concentration and Temperature Dependence of the Refractive Index of Ethanol-Water Mixtures: Influence of Intermolecular Interactions. *Eur Phys J E.* 2009; 30:19–26. [PubMed: 19730906]
43. Johnson RM, Fraser CL. Metalloinitiation Routes to Biocompatible Poly (lactic acid) and Poly (acrylic acid) Stars with Luminescent Ruthenium Tris (bipyridine) Cores. *Biomacromolecules.* 2004; 5:580–588. [PubMed: 15003024]
44. Chen J, Gorczynski J, Zhang G, Fraser CL. Iron Tris(dibenzoylmethane-poly lactide). *Macromolecules.* 2010; 43:4909–4920.
45. Kwon MS, Lee D, Seo S, Jung J, Kim J. Tailoring Intermolecular Interactions for Efficient Room-Temperature Phosphorescence from Purely Organic Materials in Amorphous Polymer Matrices. *Angew Chem, Int Ed.* 2014; 53:11177–11181.
46. Priimagi A, Cavallo G, Metrangolo P, Resnati G. The Halogen Bond in the Design of Functional Supramolecular Materials: Recent Advances. *Acc Chem Res.* 2013; 46:2686–2695. [PubMed: 23805801]
47. Wang D, Kang Y, Fan L, Hu Y, Zheng J. Synthesis and Photoluminescence Behavior of Difluoroboron Complexes with β -Diketone Ligands. *Opt Mater.* 2013; 36:357–361.
48. Ono K, Yamaguchi H, Taga K, Saito K, Yamashita Y. Synthesis and Properties of BF₂ Complexes to Dihydroxydiones of Tetracene and Perylene: Novel Electron Acceptors Showing n-type Semiconducting Behavior. *Org Lett.* 2008; 11:149–152. [PubMed: 19053501]
49. Ono K, Hashizume J, Yamaguchi H, Tomura M, Nishida J, Yamashita Y. Synthesis, Crystal Structure, and Electron-Accepting Property of the BF₂ Complex of a Dihydroxydione with a Perfluorotetracene Skeleton. *Org Lett.* 2009; 11:4326–4329. [PubMed: 19711907]
50. Lackowicz, JR. Principles of Fluorescence Spectroscopy. 3rd. Springer; New York: 2006.
51. Lehner P, Staudinger C, Borisov SM, Klimant I. Ultra-sensitive Optical Oxygen Sensors for Characterization of Nearly Anoxic Systems. *Nat Commun.* 2014; 5:4460. [PubMed: 25042041]
52. Brandrup, J.; Immergut, EH.; Grulke, EA. Polymer Handbook. 4th. Wiley; New York: 2004.
53. Arcella V, Ghielmi A, Tommasi G. High Performance Perfluoropolymer Films and Membranes. *Ann N Y Acad Sci.* 2003; 984:226–244. [PubMed: 12783820]
54. Nemser SM, Roman IC. US Patent. 1991; 5:051–114.
55. Kuno S, Akeno H, Ohtani H, Yuasa H. Visible Room-Temperature Phosphorescence of Pure Organic Crystals via a Radical-ion-pair Mechanism. *Phys Chem Chem Phys.* 2015; 17:15989–15995. [PubMed: 26027521]
56. Gong Y, Zhao L, Peng Q, Fan D, Yuan WZ, Zhang Y, Tang BZ. Crystallization-Induced Dual Emission from Metal-and Heavy Atom-free Aromatic Acids and Esters. *Chem Sci.* 2015; 6:4438–4444.
57. An Z, Zheng C, Tao Y, Chen R, Shi H, Chen T, Wang Z, Li H, Deng R, Liu X, Huang W. Stabilizing Triplet Excited States for Ultralong Organic Phosphorescence. *Nat Mater.* 2015; 14:685–690. [PubMed: 25849370]
58. Zhang X, Xie T, Cui M, Yang L, Sun X, Jiang J, Zhang G. General Design Strategy for Aromatic Ketone-Based Single-Component Dual-Emissive Materials. *ACS Appl Mater Interfaces.* 2014; 6:2279–2284. [PubMed: 24484404]
59. Colomines G, Ducruet V, Courgneau C, Guinault A, Domenek S. Barrier Properties of Poly (lactic acid) and its Morphological Changes Induced by Aroma Compound Sorption. *Polym Int.* 2010; 59:818–826.
60. Meier RJ, Fischer LH, Wolfbeis OS, Schäferling M. Referenced Luminescent Sensing and Imaging with Digital Color Cameras: A Comparative Study. *Sens Actuators, B.* 2013; 177:500–506.
61. Kochman S, Baleizao C, Bereran-Santos MN, Wolfbeis OS. Sensing and Imaging of Oxygen with Parts Per Billion Limits of Detection and Based on the Quenching of the Delayed Fluorescence of 13C70 Fullerene in Polymer Hosts. *Anal Chem.* 2013; 85:1300–1304. [PubMed: 23320578]
62. Payne SJ, Fiore GL, Fraser CL, Demas JN. Luminescence Oxygen Sensor Based on a Ruthenium (II) Star Polymer Complex. *Anal Chem.* 2010; 82:917–921. [PubMed: 20050641]

63. Carraway E, Demas J, DeGraff B. Luminescence Quenching Mechanism for Microheterogeneous Systems. *Anal Chem.* 1991; 63:332–336.
64. Xinyang L, Liu H, Sun X, Bi G, Zhang G. Highly Fluorescent Dye-Aggregate-Enhanced Energy-Transfer Nanoparticles for Neuronal Cell Imaging. *Adv Opt Mater.* 2013; 1:549–553.
65. Zheng X, Tang H, Chen X, Zhang J, Wu W, Jiang X. Tracking Cancer Metastasis In Vivo by Using an Iridium-Based Hypoxia-Activated Optical Oxygen Nanosensor. *Angew Chem, Int Ed.* 2015; 54:8094–8099.
66. Baggaley E, Botchway SW, Haycock JW, Morris H, Sazanovich IV, Williams JAG, Weinstein JA. Long-lived Metal Complexes Open Up Microsecond Lifetime Imaging Microscopy Under Multiphoton Excitation: from FLIM to PLIM and Beyond. *Chem Sci.* 2014; 5:879–886.
67. Sayre RM, Dowdy JC, Gerwig AJ, Shields RV. Unexpected Photolysis of the Sunscreen Octinoxate in the Presence of the Sunscreen Avobenzone. *Photochem Photobiol.* 2005; 81:452–456. [PubMed: 15560736]
68. Yamaji M, Suwa Y, Shimokawa R, Paris C, Miranda MA. Photochemical Reactions of Halogenated Aromatic 1, 3-Diketones in Solution Studied by Steady State, One-and Two-color Laser Flash Photolyses. *Photochem Photobiol Sci.* 2015; 14:1673–1684. [PubMed: 26147877]
69. Zakharova GV, Chibisov AK, Saznikov VA, Kononevich YN, Muzafarov AM, Alfimov MV. Photodegradation of Boron Difluoride Dibenzoylmethanate in Solutions. *High Energy Chem.* 2013; 47:327–330.

Figure 1.
Difluoroboron dibenzoylmethane-poly(lactic acid) and halide-substituted derivatives.

Figure 2. Molecular weight and fluorescence; total emission of $\text{BF}_2\text{dbm(F)PLA}$ (**12**) and $\text{BF}_2\text{dbm(Br)PLA}$ (**14**) in air ($\lambda_{\text{ex}} = 369$ nm).

Figure 3. Phosphorescence of high molecular weight $\text{BF}_2\text{dbm(X)PLA}$ polymer films (**11b–15b**). Left: Images of $\text{BF}_2\text{dbm(X)PLA}$ films under N_2 with UV illumination and after the lamp is turned off (delay). Right: Delayed emission spectra of films (excitation = xenon flash lamp with 2 ms delay).

Figure 4.

Images and total emission spectra of low molecular weight polymer films (**11a–15a**) under air and nitrogen ($\lambda_{\text{ex}} = 369$ nm).

Figure 5.

Oxygen sensitive $\text{BF}_2\text{dbm}(\text{X})\text{PLA}$ polymers suitable for ratiometric sensing. Images: $\text{BF}_2\text{dbm}(\text{Br})\text{PLA}$ (**14a**, 6 kDa) and $\text{BF}_2\text{dbm}(\text{I})\text{PLA}$ (**15b**, 20 kDa) spin cast films with a stream of nitrogen gas blown across the surface. Spectra: excitation monitored at peak fluorescence wavelength (Ex, dashed line) and total emission spectra under 0% O_2 (nitrogen), 1% O_2 and 21% O_2 (air) ($\lambda_{\text{ex}} = 369 \text{ nm}$).

Figure 6.

Influence of the halide substituent on the photostability of BF₂dbm(X)PLA spin cast films.

Total intensity versus UV exposure time ($\lambda_{\text{ex}} = 369$ nm; normalized to initial intensity).

Left: Short polymers **11a–15a**. Right: Comparison of the photostability of **10** (Initiator) and iodide polymers **15a** (7 kDa) and **15b** (20 kDa).

Figure 7. Total emission of BF₂dbm(I)PLA spin cast films under air and nitrogen before (0h) and after 18 hour UV exposure in air (18h) ($\lambda_{\text{ex}} = 369$ nm).

Scheme 1.
Synthesis of Dye-PLA Conjugates

Table 1**Polymer Molecular Weight Characterization**

Polymer	Loading^a	M_n (NMR)^b	M_n (GPC)^c	M_w (GPC)^c	PDI^c
11a	50:1/40	6000	6600	6900	1.05
12a	50:1/40	6200	6100	6600	1.08
13a	50:1/40	7700	7500	8200	1.09
14a	50:1/40	5800	6400	7600	1.18
15a	50:1/40	6700	6600	7100	1.08
11b	200:1/40	24200	19100	20800	1.09
12b	200:1/40	20400	16500	18500	1.12
13b	200:1/40	23400	18700	20600	1.10
14b	200:1/40	18500	17100	18500	1.08
15b	200:1/40	24200	20300	23100	1.14

^a Molar ratio of monomer to Sn(oct)₂ catalyst per equiv. boron initiator.

^b Molecular weights determined by end group analysis (Dye-Ar-OC₂H₂CH₂- vs PLA-CH integration).

^c Polydispersity index (PDI) = M_w/M_n determined by GPC.

Table 2

Optical Properties of Boron Dye Initiators and Polymers in CH₂Cl₂

Sample	λ_{abs}^a (nm)	ϵ^b (M ⁻¹ cm ⁻¹)	λ_{em}^c (nm)	τ_{f}^d (ns)	Φ_{f}^e
BF ₂ dbmOH	6	53 000	433	1.96	0.95
BF ₂ dbmPLA	11a	52 000	425	1.94	0.95
BF ₂ dbm(F)OH	7	48 000	430	3.27	0.99
BF ₂ dbm(F)PLA	12a	45 000	426	1.95	0.98
BF ₂ dbm(Cl)OH	8	56 000	438	3.43	0.99
BF ₂ dbm(Cl)PLA	13a	55 000	435	1.96	0.99
BF ₂ dbm(Br)OH	9	56 000	440	2.24	0.96
BF ₂ dbm(Br)PLA	14a	54 000	438	1.77	0.91
BF ₂ dbm(I)OH	10	58 000	441	1.03 ^g	0.55
BF ₂ dbm(I)PLA	15a	49 000	435	0.95	0.40

^a Absorption maxima.^b Extinction coefficients calculated at the absorption maxima.^c Fluorescence emission maxima excited at 369 nm.^d Fluorescence lifetime excited with a 369 nm light-emitting diode (LED) monitored at the emission maximum. All fluorescence lifetimes are fitted with single-exponential decay.^e Relative quantum yield, versus anthracene in EtOH as a standard.^f Values taken from ref. 18.^g Values taken from ref. 22.

Table 3

Optical Properties of Solution-Cast Films

Sample	Fluorescence			Phosphorescence		
	M_n^a (kDa)	λ_{em}^b (nm)	τ_{pw0}^c (ns)	λ_{em}^d (nm)	τ_{pw0}^e (ms)	
BF ₂ dbmPLA 11a	6.6	457	6.63	516	142.1	
11b	19.1	426	1.53	510	207.4	
BF ₂ dbm(F)PLA 12a	6.1	502	45.47	512	122.8	
12b	16.5	428	2.53	508	195.9	
BF ₂ dbm(Cl)PLA 13a	7.5	466	6.91	520	113.6	
13b	18.7	437	2.57	516	168.0	
BF ₂ dbm(Br)PLA 14a	6.4	471	5.32	522	28.9	
14b	17.1	436	1.79	520	47.1	
BF ₂ dbm(I)PLA 15a	6.6	455	3.46	528	5.6	
15b	20.3	437	0.90	522	5.8	

^a Number-average molecular weight (GPC)^b Steady-state fluorescence spectra emission maximum under air. Excitation source: 369 nm xenon lamp.^c Fluorescence lifetime excited with a 369 nm light-emitting diode (LED) monitored at the emission maximum. All fluorescence lifetimes are fitted with triple-exponential decay.^d Delayed emission spectra maxima under N₂. Excitation source: xenon flash lamp.^e Pre-exponential weighted RTP lifetime. Excitation source: xenon flash lamp; RTP lifetime fit to triple-exponential decay.

Table 4Lifetime Oxygen Sensitivity of BF₂dbm(X)PLA Films

Sample		τ_0^a (ms)	τ_1^b (ms)	Sensitivity ^c
BF ₂ dbmPLA	11a	142.1	10.2	13.9
	11b	207.4	14.7	14.1
BF ₂ dbm(F)PLA	12a	122.8	9.1	13.5
	12b	195.9	10.2	19.2
BF ₂ dbm(Cl)PLA	13a	113.6	7.7	14.7
	13b	168.0	9.7	17.3
BF ₂ dbm(Br)PLA	14a	28.9	5.8	5.0
	14b	47.1	6.9	6.8
BF ₂ dbm(I)PLA	15a	6.6	3.2	2.1
	15b	7.4	3.1	2.4

^aPhosphorescence lifetime in N₂.^bPhosphorescence lifetime in 1% O₂.^c(τ_0/τ_1)

Table 5

Ratiometric Oxygen Sensitivity of BF₂dbm(X)PLA Films

Sample	F/P_0^a	F/P_1^b	F/P_2^c	Sensitivity ^d (1% [O ₂])	Sensitivity ^e (21% [O ₂])
BF ₂ dbm(Br)PLA 14a	0.64	1.34	1.51	2.09	2.35
BF ₂ dbm(I)PLA 15a	0.28	0.45	1.15	1.61	4.10
15b	1.43	2.48	6.43	1.73	4.50

^a Fluorescence/phosphorescence in N₂.^b Fluorescence/phosphorescence in 1% O₂.^c Fluorescence/phosphorescence in air (21% O₂).^d $F/P_1 \div F/P_0 = P_0/P_1$.^e P_0/P_2 .