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Full Color Luminescent Difluoroboron β -Diketonate PLA-PEG Nanoparticle Imaging Agents

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Abstract

Luminescent difluoroboron β -diketonate poly(lactic acid) (BF₂bdkPLA) materials serve as biological imaging agents. In this study, dye structures were modified to achieve emission colors that span the visible region with potential for multiplexing applications. Four dyes with varying π -conjugation (phenyl, naphthyl) and donor groups (-OMe, -NMe₂) were coupled to PLLA-PEG block copolymers (~11 kDa) by a post-polymerization Mitsunobu reaction. The resulting dye-polymer conjugates were fabricated as nanoparticles (~55 nm diameter) to produce nanomaterials with a range of emission colors (420–640 nm). For increased stability, dye-PLLA-PEG conjugates were also blended with dye-free PDLA-PEG to form stereocomplex nanoparticles of smaller size (~45 nm diameter). The decreased dye loading in the stereoblocks blue-shifted the emission, generating a broader range of fluorescence colors (410–620 nm). Tumor accumulation was confirmed in a murine model through biodistribution studies with a red emitting dimethyl amino-substituted dye-polymer analogue. The synthesis, optical properties, oxygen-sensing capabilities, and stability of these block copolymer nanoparticles are presented.

Graphical Abstract

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ASSOCIATED CONTENT

Available in the supporting information, naming procedure used in naming ligands and dyes, images associated with the Mitsunobu reaction, GPC-MALLS data of polymers, tables of polymer molecular weights and optical properties in CH₂Cl₂ solution, DLS histograms of nanoparticle size distributions, UV/Vis spectra of nanoparticles, nanoparticle emission spectra overlays, phosphorescence properties of nanoparticles, degradation data of nanoparticles, associated spectrum suggesting sensitization of phenol red in DMEM solution, epifluorescence data during biodistribution experiments, and ¹H and ¹³C NMR spectra of new ligands, dyes and polymers.

Keywords

Difluoroboron β -diketonate; poly(lactic acid); poly(ethylene glycol); block copolymer; fluorescence; phosphorescence; color; nanoparticle; biodistribution

INTRODUCTION

In vivo fluorescence imaging is an important tool for biology and medicine. Fluorescent markers offer spatial specificity and high resolution to scientists and surgeons.^{1–3} In cancer biology, fluorescence imaging with both small molecule and polymeric nanoparticles, and tumor-specific agents or passive targeting,⁴ are expanding the applications of existing imaging methods.⁵ Polymer nanoparticles often feature hydrophobic and hydrophilic components that self-assemble in water. Although purely hydrophobic polymers can also self-assemble in water, the hydrophobicity of these aggregates results in shorter circulation times.^{6,7} To address this, a water-soluble polymer can be used for stealth properties, resulting in longer circulation times and tumor uptake by the enhanced permeation and retention (EPR) effect⁸ given leaky vasculature and poor lymphatic drainage.^{9–12}

Dye embedded polymer assemblies have been tailored for imaging and drug release applications.^{13–15} For example, Zheng et al. designed iridium-conjugated block copolymer assemblies comprised of poly(ϵ -caprolactone) (PCL; hydrophobic) and poly(*N*-vinylpyrrolidone) (PVP; hydrophilic) that accumulated in tumors after intravenous injection into mice.^{16,17} These agents were used to monitor mouse lung metastases via pO₂ imaging *in vivo* for seven days. Analogous materials designed by Kwon et al., demonstrated the passive uptake from poly(ethylene glycol)-polyester assemblies in tumors.^{18–20} Poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) micelles were used as drug nanocarriers for hydrophobic drugs (i.e. doxorubicin, curcumin), and similarly, more crystalline, and highly stable poly(ethylene glycol)-poly(ϵ -caprolactone) (PEG-PCL) micelles can be used as imaging agents when the lipophilic dye, 1,10-dioctadecyl tetramethyl indotricarbocyanine iodide is embedded in the polymer matrix.²¹ Using these materials in succession (drug nanocarrier then imaging agent) allowed for controlled drug release and imaging.

For biological imaging, a diverse set of agents are important in assessing tumor growth and therapeutic responses and to contrast and sense different regions of interest. Full color range luminescence for *in vivo* imaging has been developed for a variety of materials, such as

inorganic quantum dots (Qdots),²² carbon dots,^{23,24} polymer dots (Pdots),^{25–28} and aggregation induced emission fluorophores (AIE-gens).^{29–33} Qdot luminescence is attractive due to brightness, stability, and size dependent color tunability, however, because the major component of conventional Qdots is Cd(II), there is concern about unintended toxicity.³⁴ Semiconducting Pdots and AIE-gens are purely organic alternatives that offer a bright, color tunable platform for imaging and sensing.

Boron-based fluorophores have emerged as versatile materials, such as boron β -diiminate (NBN),^{35,36} β -ketoiminate (OBN),^{37–39} and β -diketonate (OBO) dye scaffolds.^{40–43} Difluoroboron β -diketonate (BF₂bdk) dyes, for example, have high quantum yields and extinction coefficients. Emission color tuning for multicolored luminescence has been studied extensively.^{44–46} The distinguishing feature of BF₂bdk dyes over similar analogues (i.e. boron β -diiminate and boron β -ketoiminate) is the presence of both fluorescence (F) and room temperature phosphorescence (RTP) when the dyes are confined to a rigid matrix, such as biodegradable PLA.⁴¹ In previous reports, it was suggested that the polymer T_g is linked the presence of RTP.^{47,48} Other common polymers of interest, such as poly(ϵ -caprolactone) (T_g = –60 °C) and poly(ethylene glycol) (T_g = –66 °C) did not support RTP, whereas poly(lactic acid) (T_g = ~60 °C) does show phosphorescence at room temperature and 37 °C. In order to maintain both the F and RTP properties of the dye, PLA (or other polymers with suitable rigidity) are an essential building block, and must be used alone or in combination with other polymers (i.e. block, or graft). New nanomaterials thus constituted can be harnessed for ratiometric sensing of molecular oxygen, where the F acts as an oxygen insensitive internal reference for the oxygen sensitive RTP intensity.^{49–51} Oxygen concentration can be a powerful indicator of health and healing as excesses and deficits of oxygen are associated with cancer, cardiac ischemia, macular degeneration, chronic wounds, seizing brain tissue, strokes and more.^{52–54} These dye-polymer conjugates show potential as a multifunctional platform for imaging, sensing and targeted therapies for cancer biology.

Polymer conjugates of luminescent or therapeutic molecules are of great interest.^{55–59} Preparation of the polymer-conjugated materials requires lengthy, multi-step organic synthesis compared to small molecule/polymer blend formulations. However, increased stability or controlled release of macromolecular products are beneficial for long-term applications, where side effects such as “burst” release are essentially eliminated. Three methods are commonly employed for generating polymer conjugated small molecules; 1) material (e.g. dye or drug) initiated polymerization,^{60–62} 2) polymerizable side chains on the material of interest^{63–65} and 3) post-polymerization modification.^{66–68} In standard protocols, BF₂bdk have been covalently linked to PLA via dye initiation of lactide polymerization to yield BF₂bdkPLA. For PEGylated nanoparticles, our first approach was stereocomplexation through blending BF₂bdkPLLA (poly(L-lactic acid)) and PEG-PDLA (poly(D-lactic acid)) to create stealth nanoparticles for IV injection and tumor accumulation.⁶⁹ Another approach was post polymerization modification to attach a carboxyl-PEG to the BF₂bdkPLA.⁷⁰ In this report, we simplify and considerably streamline the process for generating BF₂bdkPLA-PEG materials by preparing the boron dye with an acidic phenol for the post polymerization modification of a PEG-PLA block copolymer via the Mitsunobu reaction.^{71,72}

As shown in Figure 1, phenolic dyes (e.g. BF₂dbmOH) are prepared in fewer steps than dye initiators (e.g. BF₂dbmCH₂CH₂OH), and a single batch of PEG-PLA copolymer can be used in multiple coupling reactions with different luminescent dyes to yield full color luminescent polymers. In this report, we describe the synthesis and characterization of dual emissive BF₂bdkPLLA-PEG materials with a range of emission colors for future biomedical applications. Stereocomplexes with PDLA-PEG are fabricated to tailor color (i.e. dye loading) and stability in different media.^{73–75} To demonstrate the potential of these new materials, a red luminescent stereocomplexed nanoparticle formulation (BF₂dapvmPLLA-PEG/PDLA-PEG; **4scNP**) is used in biodistribution studies in a mouse flank tumor model.

EXPERIMENTAL SECTION

Materials.

The lactide monomers (L-lactide and D-lactide) were generous gifts from Corbion Purac®. Lactide was recrystallized twice from EtOAc and dried in vacuo overnight (10 h) prior to use. Poly(ethylene glycol) (PEG) was purchased from Sigma Aldrich (2 000 Da, $\eta_{inh} = \sim 1.05$) and dried via azeotropic distillation in toluene according to a previously described protocol.⁷⁶ The PEG was stored under nitrogen in a glovebox prior to use as a macroinitiator. The polymers PEG-PLLA-OH (GPC: $M_n = 10\,300$, $\eta_{inh} = 1.05$, ¹H NMR = 12 600) and PEG-PDLA-OH ($M_n = 12\,500$, $\eta_{inh} = 1.08$, ¹H NMR = 13 800),⁶⁹ the ligand, dbmOH,⁷⁷ and the boron coordinated dye, BF₂dbmOH (**1**),⁷⁸ were prepared as previously described and ¹H NMR spectra are in accord with literature values. In this report, dbmOH and BF₂dbmOH refer to the phenol dibenzoylmethane ligand and difluoroboron dye as shown in Figure 1. In previous reports by our group these abbreviations referred to a primary alcohol ligand and boron dye initiators for lactide polymerization, renamed in this manuscript as dbmOC₂H₄OH BF₂dbmOC₂H₄OH.⁴¹ Solvents CH₂Cl₂ and THF were dried over 3 Å molecular sieves activated at 300 °C, transferred via cannula, and dried a second time over 3 Å molecular sieves activated at 300 °C.⁷⁹ The solvents were stored in a dry pot prior to use. All other chemicals were reagent grade from Sigma-Aldrich and were used without further purification. Phosphate buffered saline (PBS) and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Life Technologies.

Methods.

¹H (600 MHz) and ¹³C (150 MHz) NMR spectra (Figures S15–33) were recorded on a Varian VMRS 600/51 instrument in CDCl₃ or d₆-DMSO. ¹H NMR spectra were referenced to the residual signals for protiochloroform (7.26 ppm), protioDMSO (2.50 ppm), and protioacetone (2.09 ppm). ¹³C NMR spectra were referenced to the residual signals for chloroform (76.97 ppm), DMSO (39.95 ppm). In the ¹H NMR assignments, aromatic positions are defined as follows; phenyl (Ph), vanillinone (v-Ph), naphthyl (Np), trimethyl gallate (g-Ph), and dimethylamino-phenyl (a-Ph). For a guide to the nomenclature of the ligands and boron dyes, refer to Table S1 and Scheme S1. Coupling constants are given in hertz. Number average molecular weights (M_n), weight average molecular weights (M_w) and polydispersity index (M_w/M_n) were determined by gel permeation chromatography (GPC) (THF, 25 °C, 1.0 mL/min) using multiangle laser light scattering (MALLS; $\lambda = 658$ nm, 25 °C) and refractive index ($\lambda = 658$ nm, 25 °C) detection. A Polymer Laboratories 5 μ m mixed-C

guard column and two GPC columns along with Wyatt Technology Corp. (Optilab REX interferometric refractometer, miniDawn TREOS laser photometer) and Agilent Technologies instrumentation (series 1260 HPLC) and Wyatt Technology software (ASTRA 6.0) were used for analysis. The incremental refractive index (dn/dc) was determined by a single-injection method assuming 100% mass recovery from the columns. 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 ($\lambda_{\text{ex}} = 369$ 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 ($\lambda_{\text{ex}} = 369$ 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 CH_2Cl_2 were calculated against anthracene or Rhodamine G6 (Exciton) as a standard as previously described, using the following values: Φ_{F} (anthracene) = 0.27,⁸⁰ Φ_{F} (Rhodamine G6) = 0.97, n_{D}^{20} (EtOH) = 1.360, n_{D}^{20} (CH_2Cl_2) = 1.424. Optically dilute CH_2Cl_2 solutions of the dyes, with absorbances <0.1 au, were prepared in 1 cm path length quartz cuvettes. Fluorescence spectra and lifetimes were obtained under ambient conditions (e.g., air, $\sim 21\%$ oxygen). Phosphorescence measurements were performed under a N_2 atmosphere. Dilute nanoparticle solutions (absorbance = ~ 0.1) were purged with N_2 (Praxair) in a cuvette and sealed with a 12 mm PTFE/silicone/PTFE seal (Chromatography Research Supplies), connected by a screw cap. Vials were continuously purged in the headspace between the solution and the vial cap with analytical grade N_2 (Praxair) during measurements. For 21% O_2 (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 nanoparticles. Fluorescence lifetimes in CH_2Cl_2 were fit to single exponential decays.

Nanoparticle Characterization.

Nanoparticles were fabricated as previously described.⁴⁰ Nanoparticle sizes and polydispersities were analyzed via dynamic light scattering (DLS, Wyatt, DynaPro) and cryo transmission electron microscopy (cryoTEM, 120kV Tecnai Spirit is equipped with a tungsten filament electron source, and a $2\text{k} \times 2\text{k}$ UltraScan CCD camera). For cryoTEM, nanoparticles were concentrated by centrifugation at 4000 rpm for 5 min in a cellulose filter tube (Amicon, Ultra –15, 30,000 Da MW cutoff).

Animal Preparation.

Female mice (BALB/c), 6–8 weeks old, were purchased from Charles River Laboratory. All mice were maintained in pathogen-free barrier facilities at Duke University and were used in accordance with protocols approved by the Division of Laboratory Animal Resources and Institutional Animal Care & Use Committee at Duke University. Before implanting flank tumors, mouse hair was first removed from the dorsal area and right leg by using Nair hair

remover lotion. Mouse breast cancer cells (4T1, 2.5×10^5 cells per mouse) were then subcutaneously injected into the flank of the right leg. Flank tumors typically grow to a measurable size within a week.

IVIS Imaging.

In order to characterize the biodistribution of **4scNP** dye-PEG-PLA nanoparticles, mice were anaesthetized and slowly, intravenously infused with either saline (negative control) or **4-scNP** suspensions (0.05 mg/g). IVIS Kinetic (Perkin-Elmer Corp) was used to study distribution of BNPs in the mice after IV injection. For pharmacokinetic analysis (PKA), a small amount ($\sim 10 \mu\text{l}$) of blood was also collected with a capillary tube with a cheek-pouch in a given time interval after infusion of **4scNP** into bloodstream. The collected blood sample was first centrifuged for three minutes, then immediately imaged with the IVIS Kinetic. One day after **4scNP** injection, mice were sacrificed and organs were collected, rinsed with saline, and then fluorescence images were taken.

Synthesis.

1-(4-Hydroxy-3-methoxyphenyl)-3-(naphthalen-2-yl)propane-1,3-dione (nvmOH).—The naphthyl-vanillin phenolic ligand was prepared as described by Jin et. al.,⁷⁷ except the ketone, acetovanillinone, was used in place acetophenone, and the ester, methyl 2-naphthoate, was used in place of methyl benzoate to yield a tan powder after recrystallization from acetone/hexanes: 720 mg (39%). ¹H NMR: (600 MHz, CDCl₃) δ 17.05 (s, 1H, -OH), 8.52 (s, 1H, 1-Np-H), 7.99 (m, 2H, 3, 8-Np-H), 7.92 (m, 2H, 4, 5-Np-H), 7.62 (m, 2H, 2, 6-Ph-H), 7.59 (m, 2H, 6, 7-Np-H), 7.01 (d, J = 6, 1H, 5-Ph-H) 6.93 (s, 1H, COCHCO), 6.03 (s, 1H, Ph-OH), 4.00 (s, 3H, Ph-OCH₃). ¹³C NMR: (150 MHz, CDCl₃) δ 187.10, 182.53, 153.40, 135.22, 132.82, 132.35, 129.70, 128.75, 128.71, 128.49, 128.12, 127.36, 126.63, 123.76, 122.92, 115.92, 111.44, 93.16, 56.32. HRMS (ESI, TOF) m/z calcd for C₂₀H₁₇O₄: 321.1127 [M + H]⁺; found 321.1125.

1-(4-Hydroxy-3-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)propane-1,3-dione (gvmOH).—The trimethyl-gallate phenolic ligand was prepared as described for nvmOH, but the ester methyl 3,4,5-trimethoxybenzoate was used in place of methyl 2-naphthoate to yield a tan powder after purification by silica column chromatography (3:1 hexanes/EtOAc): 550 mg (16%). ¹H NMR: (600 MHz, DMSO) δ 17.53 (s, broad, 1H, -OH), 10.07 (s, broad, 1H, 4-v-Ph-OH), 7.75 (d, J = 6, 1H, 6-v-Ph-H), 7.58 (s, 1H, 1H, 2-v-Ph-H), 7.35 (s, 2H, 2, 6-g-Ph-H), 7.14 (s, 1H, COCHCO), 6.90 (d, J = 6, 1H, 5-v-Ph-H), 3.86 (s, broad, 6H, 3, 5-g-Ph-OCH₃), 3.84 (s, broad, 3H, 3-v-Ph-OCH₃), 3.72 (s, broad, 3H, 4-g-Ph-OCH₃). ¹³C NMR: (150 MHz, CDCl₃) δ 188.75, 183.65, 153.22, 149.91, 146.71, 130.81, 128.08, 121.78, 114.16, 109.37, 107.00, 104.48, 92.07, 60.98, 56.37, 56.13. HRMS (ESI, TOF) m/z calcd for C₁₉H₂₁O₇: 361.1287 [M + H]⁺; found 361.1287.

1-(4-Hydroxy-3-methoxyphenyl)butane-1,3-dione (mvmOH).—The methyl phenolic ligand was prepared as described for nvmOH, but the ester, ethyl acetate, was used in place of methyl 2-naphthoate to yield a white powder after purification by silica column chromatography (3:1 hexanes/EtOAc): 854 mg (28 %). ¹H NMR: (CDCl₃) δ 16.28 (s, 1H, -OH), 6.94 (d, J = 6, 1H, 5-Ph-H), 7.47 (s, 1H, 2-Ph-H), 7.44 (d, J = 12, 1H, 6-Ph-H), 6.10 (s,

1H, COCHCO), 5.98 (s, 1H, Ph-OH), 3.95 (s, 3H, Ph-OCH₃). ¹³C NMR: (150 MHz, CDCl₃) δ 196.76, 150.36, 146.57, 130.19, 123.97, 113.72, 109.67, 56.04, 26.16. HRMS (ESI, TOF) m/z calcd for C₁₁H₁₁O₄: 207.0658 [M - H]⁺; found 207.0657.

Difluoroboron-1-(4-hydroxy-3-methoxyphenyl)-3-(naphthalen-2-yl)propane-1,3-dione, BF₂nvmOH (2).—The boron dye was prepared according to a method previously

described for BF₂dbmOC₂H₄OH,⁷⁸ except the naphthyl ligand nvmOH was used in place of dbmOC₂H₄OH, to yield a yellow-orange solid after recrystallization from acetone/hexanes: 108 mg (35%). ¹H NMR (600 MHz, CDCl₃): δ 8.74 (s, 1H, 1-Np-H), 8.07 (d, J = 6, 1H, 3-Np-H), 8.01 (d, J = 12, 6-v-Ph-H), 7.96 (d, J = 6, 1H, 4-Np-H), 7.91 (d, J = 6, 1H, 8-Np-H), 7.80 (d, J = 6, 1H, 5-Np-H), 7.75 (s, 1H, 2-v-Ph-H), 7.66 (t, J = 6, 1H, 7-Np-H), 7.60 (t, J = 6, 1H, 6-Np-H), 7.06 (d, J = 12, 1H, 5-v-Ph-H), 6.35 (s, 1H, COCHCO), 4.03 (s, 3H, 3-v-Ph-OCH₃), 3.73 (s, broad, 1H, 4-v-Ph-OH). ¹³C NMR: (150 MHz, DMSO) δ 181.98, 179.63, 155.96, 147.67, 136.22, 132.67, 131.34, 140.31, 130.07, 129.56, 129.28, 128.26, 127.85, 126.46, 124.23, 122.61, 112.66, 94.13, 56.53. HRMS (ESI, TOF) m/z calcd for C₂₀H₁₄O₄BF₂: 367.0953 [M - H]⁺; found 367.0943.

Difluoroboron-1-(4-hydroxy-3-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)propane-1,3-dione BF₂gvmOH (3).—The boron dye was prepared

according to a method previously described for BF₂dbmOC₂H₄OH, except the trimethyl gallate, ligand (gvmOH) was used in place of dbmOC₂H₄OH, to yield a yellow-orange solid after recrystallization from acetone/hexanes: 311 mg (69%). ¹H NMR: (600 MHz, DMSO) δ 10.85 (s, 1H, v-Ph-OH), 8.06 (d, J = 12, 1H, 6-v-Ph-H), 7.69 (m, 2H, 2-v-Ph-H, COCHCO), 7.51 (s, 2H, 2, 4-g-Ph-H), 7.01 (d, J = 12, 1H, 5-v-Ph-H), 3.90 (s, broad, 9H, 3,4,5-g-Ph-OCH₃), 3.79 (s, 3H, 3-v-Ph-OCH₃). ¹³C NMR: (150 MHz, DMSO) δ 181.44, 179.34, 155.71, 153.51, 148.61, 144.14, 127.16, 126.44, 122.63, 116.43, 112.46, 106.90, 93.67, 60.87, 56.90, 56.43. HRMS (ESI, TOF) m/z calcd for C₁₉H₁₈O₇BF₂: 407.1114 [M - H]⁺; found 407.1111.

Difluoroboron-1-(4-hydroxy-3-methoxyphenyl)butane-1,3-dione, BF₂mvmOH.—

The boron dye was prepared according to a method previously described for BF₂dbmOC₂H₄OH, except the methyl-vanilin ligand, mvmOH, was used in place of dbmOC₂H₄OH, to yield a yellow solid after recrystallization from acetone/hexanes: 544 mg (72%). ¹H NMR: (600 MHz, CDCl₃) δ 7.62 (m, 2H, 2, 6-v-Ph-H), 7.00 (d, J = 6, 1H, 5-v-Ph-H), 6.45 (s, 1H, COCHCO), 6.31 (s, 1H, 4-v-Ph-OH), 3.99 (s, 3H, 3-v-Ph-OCH₃), 2.36 (s, 3H, -OC-CH₃). ¹³C NMR: (150 MHz, DMSO) δ 190.49, 181.08, 155.55, 148.56, 125.62, 121.69, 116.51, 112.33, 97.25, 56.31, 24.38. HRMS (ESI, TOF) m/z calcd for C₁₁H₁₀O₄BF₂: 255.0640 [M - H]⁺; found 255.0640.

Difluoroboron-(E)-5-(4-(dimethylamino)phenyl)-1-(4-hydroxy-3-methoxyphenyl)pent-4-ene-1,3-dione, BF₂dapvmOH (4).—The amino substituted

dye was prepared by a method described by Fedorenko et. al,⁸¹ except the boron dye BF₂mvmOH was used in place of 2,2-difluoro-4-(9H-fluorene-2-yl)-6-methyl-1,3,2-dioxaborine to yield a blue solid with metallic luster after precipitation from diethyl ether, filtration, and washing with copious amounts of ice cold diethyl ether (250 mL): 107 mg

(56%). ^1H NMR: (600 MHz, CDCl_3) δ 8.10 (d, $J = 12$, 1H, $-\text{COCH}=\text{CH}-\text{a-Ph}$), 7.68 (s, broad, 1H, 2-v-Ph- H), 7.58 (d, $J = 6$, 1H, 6-v-Ph- H), 7.54 (d, $J = 12$, 2H, 2, 6-a-Ph- H), 7.14 (d, $J = 12$, 1H, 5-v-Ph- H), 6.69 (d, $J = 6$, 2H, 3, 5-a-Ph- H), 6.54 (d, $J = 12$, 1H, $-\text{COCH}=\text{CH}-\text{a-Ph}$), 6.46 (s, 1H, COCHCO), 3.92 (s, 3H, 3-v-Ph- OCH_3), 3.09 (s, 6H, 4-a-Ph- CH_3NCH_3), phenolic hydrogen was not observed. ^{13}C NMR: (150 MHz, DMSO) δ 181.16, 175.70, 168.57, 154.08, 151.78, 150.52, 144.39, 133.21, 131.36, 124.20, 121.84, 121.61, 114.04, 112.64, 111.89, 97.54, 56.62, 21.47, 20.84. HRMS (ESI, TOF) m/z calcd for $\text{C}_{20}\text{H}_{19}\text{BNO}_4\text{F}_2$: 386.1375 $[\text{M} - \text{H}]^+$; found 386.1374.

BF₂dbmPLLA-PEG (1P).—The BF_2dbmOH dye was coupled to the PLLA-PEG block copolymer via a Mitsunobu reaction with diisopropyl azodicarboxylate (DIAD) as the activating reagent, as described by Balog et al.⁷¹ In an oven dried, 100 mL round bottom flask, PEG-PLLA-OH (250 mg, 0.024 mmol), BF_2dbmOH (**1**) (28 mg, 0.097 mmol) and triphenyl phosphine (PPh_3 , 25 mg, 0.097 mmol) were dissolved in anhydrous THF (50 mL). The reaction mixture was chilled to -10°C (acetone/ice bath) for 30 min before the DIAD reagent was added via syringe (10 μL , 0.049 mmol). The reaction was removed from the chilled bath, and allowed to warm to room temperature. After stirring for 3 d, the THF was removed via rotary evaporation. The polymer was dissolved in minimal CH_2Cl_2 and the viscous solution was precipitated (2 $\times$) into cold methanol (50 mL, -20°C), filtered, and washed with cold methanol (-20°C). After drying in vacuo, a white, fluffy solid was collected: 223 mg (81%). M_n (GPC/MALLS) = 11 500, η_{inh} = 1.09 (^1H NMR) = 13 500; ^1H NMR: (600 MHz, CDCl_3) δ 8.12 (m, 4H, 2', 6'-Ph- H , 2'', 6''-Ph- H), 7.65 (t, $J = 6$, 1H, 4'-Ph- H), 7.53 (t, $J = 6$, 2H, 3', 5'-Ph- H), 7.09 (s, 1H, COCHCO), 7.04 (d, $J = 6$, 2H, 3'', 5''-Ph- H), 5.14 (q, $J = 6$, 172H, PLLA- H), 3.62 (s, broad, 152H, PEG- $\text{OC}_2\text{H}_4\text{O}$), 3.36 (s, 3H, PEG- OCH_3), 1.57 (m, broad, 560H, PLLA- CH_3).

BF₂nvmPLLA-PEG (2P).—The naphthyl dye was coupled to PEG-PLLA-OH as previously described for **1P**, but BF_2nvmOH (**2**) was used in place of BF_2dbmOH (**1**) to yield a yellow, fibrous solid: 185 mg (74%). M_n (GPC/MALLS) = 11 700, η_{inh} = 1.07 (^1H NMR) = 15 400; ^1H NMR: (600 MHz, CDCl_3) δ 8.76 (s, 1H, 1-Np- H), 8.08 (d, $J = 6$, 1H, 3-Np- H), 8.02 (d, $J = 6$, 1H, 6-v-Ph- H), 7.97 (d, $J = 6$, 1H, 4-Np- H), 7.91 (d, $J = 6$, 1H, 8-Np- H), 7.79 (d, $J = 6$, 1H, 5-Np- H), 7.73 (s, 1H, 2-v-Ph- H), 7.66 (t, $J = 6$, 1H, 7-Np- H), 7.61 (t, $J = 6$, 1H, 6-Np- H), 7.07 (s, 1H, COCHCO), 6.98 (d, $J = 12$, 1H, 5-v-Ph- H), 5.15 (q, $J = 6$, 188H, PLLA- H), 3.98 (s, 3H, 3-v-Ph- OCH_3), 3.62 (s, broad, 183H, PEG- $\text{OC}_2\text{H}_4\text{O}$), 3.36 (s, 3H, PEG- OCH_3), 1.57 (m, broad, 712H, PLLA- CH_3).

BF₂gvmPLLA-PEG (3P).—The trimethyl gallate dye was coupled to PEG-PLLA-OH as previously described for **1P**, but BF_2gvmOH (**3**) was used in place of BF_2dbmOH (**1**) to yield a yellow solid: 245 mg (64%). M_n (GPC/MALLS) = 12 600, η_{inh} = 1.13 (^1H NMR) = 14 900; ^1H NMR: (600 MHz, CDCl_3) δ 7.70 (s, broad, 2H, 2, 6-v-Ph- H), 7.34 (s, broad, 2H, 2, 6-g-Ph- H), 7.00 (s, 1H, COCHCO), 6.93 (d, $J = 6$, 1H, 5-v-Ph- H), 5.17 (q, $J = 6$, 170H, PLLA- H), 3.94 (m, broad, 12H, 3, 4, 5-g-Ph- OCH_3 , 3-v-Ph- OCH_3), 3.66 (s, broad, 154H, PEG- $\text{OC}_2\text{H}_4\text{O}$), 3.36 (s, 3H, PEG- OCH_3), 1.57 (m, broad, 554H, PLLA- CH_3).

BF₂dapvmPLLA-PEG (4P).—The dimethyl amino dye was coupled to PEG-PLLA-OH as previously described for **1P**, but BF₂dapvmOH (**4**) was used in place of BF₂dbmOH (**1**) to yield a magenta, fibrous solid: 95 mg (29%). *M_n* (GPC/MALLS) = 15 200, *M_w* = 1.15 (¹H NMR) = 16 000; ¹H NMR: (600 MHz, CDCl₃) δ 8.05 (d, *J* = 18, 1H, -COCH=CH-*a*-Ph), 7.59 (m, 2H, 2, 6-*v*-Ph-*H*) 7.53 (d, *J* = 6, 2H, 2, 6-*a*-Ph-*H*) 6.90 (d, *J* = 6, 1H, 5-*v*-Ph-*H*) 6.69 (d, *J* = 6, 2H, 3, 5-*a*-Ph-*H*), 6.53 (d, *J* = 12, 1H, -COCH=CH-*a*-Ph), 6.44 (s, 1H, COCHCO) 5.15 (q, *J* = 6, 197H, PLLA-*H*), 3.93 (s, 3H, 3-*v*-Ph-OCH₃), 3.66 (s, broad, 183H, PEG-OC₂H₄O), 3.36 (s, 3H, PEG-OCH₃), 3.08 (s, 6H, 4-*a*-Ph-CH₃NCH₃), 1.57 (m, broad, 699H, PLLA-CH₃).

RESULTS AND DISCUSSION

Synthesis.

The β-diketones were prepared by a method reported by Jin et al.,⁷⁷ with commercially available ketones and esters, followed by coordination of difluoroboron in anhydrous CH₂Cl₂. To prepare the dimethyl amino derivative, a synthetic protocol reported by Fedorenko et al.⁸¹ was used involving a condensation reaction of the boron dye ketone and an amino-substituted aldehyde (Scheme 1). For dyes **2–4**, methoxy-substituted acetovanillinone was employed as the ketone for the Claisen condensations to increase the donor ability of the dye, potentially red-shifting the luminescence wavelengths (Figure 1). The boron dyes showed excellent solubility in common organic solvents (i.e. acetone, CH₂Cl₂, THF). These phenol dye couplers for post polymerization modification were prepared in moderate yields (35–72%) in two to three steps. This contrasts with dye initiators for lactide polymerization that take 5–7 steps to synthesize, and where issues with dye solubility are common when larger aromatics (e.g. naphthalene) are present in the dye scaffold.

Dye-polymer conjugates were successfully prepared via Mitsunobu reaction conditions (Figure 2).⁷¹ The coupling process could be monitored visually through color changes. Upon addition of the DIAD reagent, the yellow solution of BF₂dapvmOH became red, and the strong green emission was quenched (Figure S1). This is likely due to deprotonation of the phenol, inducing a stronger dipole, also seen in BF₂Curcumin dyes.⁸² Over the course of the reaction, the solution became yellow again, as expected for the ether functionality formed in the coupled product. The reaction was allowed to proceed for three days under N₂ to insure maximum coupling efficiency. After this time, the THF solvent was removed via rotary evaporation, and the reaction mixture was dissolved in minimal CH₂Cl₂ for purification via precipitation. The oxidized triphenylphosphine generated during the reaction, excess boron dye, and DIAD reagents were easily removed from the final product via precipitation into cold methanol (50 mL at 0° C × 2), as dye-PLLA-PEG materials were insoluble in MeOH, whereas the byproducts remained in MeOH solution.

Coupling was confirmed by ¹H NMR spectroscopy and gel permeation chromatography (GPC). The GPC trace of the uncoupled PLLA-PEG has no UV absorbance. However, after dye functionalization, the elution of the polymer coincides with the absorbance of the dye via GPC (Figures 2 and S2–3). Upon dye coupling to the polymer, the MW and PDI increased slightly as shown in Table S2 (i.e. MW: HO-PLLA-PEG = 10 300, *M_w* = 1.05; **2P** =

11 700, $M_w/M_n = 1.07$). This is likely a result of purification, as lower MW polymers are more soluble and less likely to precipitate from solution than the higher MW polymers, causing the overall MW to increase.^{83,84} Furthermore, the coupling is confirmed by the disappearance of the ^1H NMR signal at ~ 2.5 ppm corresponding to the PLLA-*OH* end group (Figures S30–33). Coupling efficiency is estimated to be ~ 80 – 95% based on NMR integration for dye arene and PEG- OCH_3 peaks. Importantly, no degraded dye byproducts were observed in GPC (UV trace) or NMR analysis (e.g. ligand enol peaks), which has been a problem for boron dye-PLA analogues previously with heat and certain base mediated reactions.^{85,86} This is further supported by the optical properties in dilute CH_2Cl_2 solutions, as the polymer conjugates and the uncoupled dye precursors have similar optical properties (Table S3). A small high molecular weight shoulder was present in the GPC trace, possibly due to polymer backbiting⁸⁷ and recombination of chains.⁸⁴ High molecular weight shoulders are a common observation in PEG-polyester materials with functionalized moieties (e.g. bioconjugates, ligands, fluorophores).⁸⁸ Despite this, the PDI values are low, indicating a narrow distribution of products. Therefore, the Mitsunobu reaction method is a reliable means for coupling boron dyes to polymers.

Nanoparticles.

Nanoparticles were fabricated by precipitation of DMF solutions of the polymer into water with stirring.⁴⁰ Two nanoparticle formulations were studied for each dye; namely, nanoparticles were prepared from the dye-PLLA-PEG homopolymer (**1–4NP**), and from a $\sim 1:1$ mixture of dye-PLLA-PEG and PDLA-PEG to form the stereocomplex systems (**1–4scNP**) (Figure 3). In previous reports, stereocomplexation is a simple method to alter stability and size of the nanoparticles.^{89–91} Based on literature precedent, stereocomplexed PLA materials should offer more stable, yet biodegradable nanoparticle systems, more resistant to changes in pH.⁷⁴ During the fabrication process, $\text{BF}_2\text{dbmPLLA-PEG}$ (**1NP**) readily dissolved in DMF and formed nanoparticles as expected. For the stereocomplex nanoparticles, the PDLA-PEG/ $\text{BF}_2\text{dbmPLLA-PEG}$ mixture showed limited solubility in DMF. The addition of other solvents, such as acetone, THF or acetonitrile did not increase solubility. As a result, during precipitation into water, aggregation visibly increased. Thus, precipitated polymer suspensions were passed through filter paper (Whatman, quantitative) before dialysis to produce uniform nanoparticles. Other nanoparticles prepared from **2P–4P** polymers were treated similarly.

Homopolymer and stereocomplex nanoparticles showed expected radius and size distributions. According to DLS measurements, the homopolymer nanoparticles showed slightly larger radii compared to the stereocomplexes (R_h ; **1NP** = 53.6 nm, **1scNP** = 52.0 nm). Greater differences were noted in polydispersities (PD) of **1NP** and **1scNP**. The homopolymer nanoparticles showed a broader distribution of sizes compared to the stereocomplex congener (PD (%); **1NP** = 23.3 nm, **1scNP** = 13.6 nm) (Figure 3). The cryoTEM results are in good agreement with the DLS results. On average, the cryoTEM revealed smaller, somewhat more regular nanoparticles for the **scNP** samples compared to the **NP** samples. However, according to DLS, all dye-polymer and dye-polymer stereocomplex nanoparticles showed similar radii and distribution results. One outlier was the BF_2nvm dye derivative, as the homopolymer nanoparticles were slightly larger than the

scNP counterpart, as shown in Table 1 and Figure S4 (R_H ; **2NP** = 34.3 nm, **2scNP** = 45.5 nm). Differences in size and distribution can vary depending on drop rate and stirring speed from the nanoprecipitation process. Nevertheless, all nanoparticles are well under the size ranges needed for enhanced permeation and retention (EPR) in tumors and for renal clearance.⁹² More in depth analysis to understand ways to further control the nanoparticle size, and influences of filtering, concentration, and stir rate on the resultant nanoparticles, may be performed when this is merited for specific biological investigations.

Fluorescence.

The optical properties of PLLA-PEG and scPLA-PEG nanoparticles are shown in Table 1. Fluorescence attributed to the dye scaffold was elucidated by comparing homopolymer nanoparticles (**1–4NP**). Using BF₂dbmPLLA-PEG (**1**) for comparison, increased π –conjugation and addition of a methoxy group (**1NP** vs **2NP**) resulted in a red-shift ($\lambda_{\max}$ = 77 nm), and emission color change from blue-violet to green (Figure 4). The yellow emission of compound **3NP** is due to the increased number of -OMe electron-donating groups, found to red-shift emissive properties in previous reports ($\lambda_{\max}$ = 127 nm vs **1NP**).^{44,93} Extended conjugation with a vinyl spacer group and the dimethyl amino substitution resulted in further red-shifting for **4NP** ($\lambda_{\max}$ = 213 nm vs **1NP**).^{90,91} This red fluorescence makes compound **4NP** a promising candidate for tissue imaging, given greater tissue penetration of red light; however, all derivatives have useful fluorescence properties. For example, nanoparticles based on **1–3** have high extinction coefficients (ϵ = 28 000 – 61 000 M⁻¹ cm⁻¹) with absorbance wavelengths between 375–420 nm, well aligned with a 405 nm violet laser, commonly used for hydroxycoumarins.⁹⁶ The red fluorescent nanoparticle, **4NP** with redder absorption, can be used in combination with a 515 nm Ar-ion gas laser, typically used for rhodamine dyes.⁹⁷ Boron β -diketonates also typically have good two-photon cross sections in alignment for Ti-sapphire pulsed lasers. Therefore, these materials could find ready application in fluorescence imaging techniques, such as two-photon fluorescence lifetime imaging microscopy (TP-FLIM).⁹⁸

When nanoparticles are formed from polymers **1–4-PLLA-PEG** and PDLA-PEG through stereocomplexation, dye loading is decreased by ~50%. It is well known that optical properties of BF₂dbk materials are affected by dye loading.^{49,99} As expected based on previous results with dye blends and dye-PLA conjugates of variable molecular weights, the fluorescence blue-shifted in stereocomplexes (i.e. λ_F ; **3NP** = 553 nm, **3scNP** = 499 nm), while the absorption is essentially unchanged (Figure S5–6). The blue-shifted fluorescence was accompanied by a decrease in fluorescence lifetime (i.e. τ_F ; **3NP** = 17.63 ns, **3scNP** = 4.34 ns). Stereocomplexation-induced changes in luminescence based on dye loading serve as an additional way to modulate emission wavelength (i.e. color) and lifetimes in dye-PLA materials, and lifetime is related to oxygen sensitivity.

Phosphorescence.

The delayed emission of **1–3** was studied at room temperature (298 K) and in liquid nitrogen (77 K) (Figures 5, S7–8). Phosphorescence was not detected for compound **4**, possibly due to twisting and non-radiative decay due to the vinyl spacer. For compounds **1–3**, the delayed emission at room temperature red-shifted with dye modifications, similar to the fluorescence

(NPs: λ_{RTP} ; **1NP** = 520 nm, **2NP** = 556 nm, **3NP** = 556 nm). Surprisingly, in **3NP** the RT fluorescence and phosphorescence bands completely overlapped. To better understand this phenomenon, low temperature phosphorescence (LTP) measurements at 77 K (liquid N₂) were performed. The LTP of **3** red-shifted to λ_{em} = 596 nm (Figure S8). This suggests that the triplet state of **3** is observable as orange-red phosphorescence at low temperature (596 nm), but at room temperature, thermally activated delayed fluorescence (TADF) is the dominant transition. The TADF is a result of the decrease in the singlet-triplet gap of dye **3**, and reverse intersystem crossing; that is, triplet excited state electrons can back populate the singlet excited state, if the barrier is low enough. Because this is a thermal process, the TADF is absent at low temperature (i.e. 77K; liq. N₂). The TADF of organic materials is a desirable feature for organic light emitting diodes (OLEDs).^{100,101} Nanoparticles **1NP** and **2NP** had large singlet-triplet gaps, decreasing the probability of TADF. The emission spectra at 77 K did not change dramatically, indicating minimal TADF (Figure S7). Stereocomplex nanoparticles (**1–3scNP**) showed similar RTP wavelengths as the homopolymer analogues (i.e. λ_{RTP} ; **1NP** = 520 nm, **1scNP** = 516 nm). However, when the dye loading was decreased via stereocomplexation, the RTP lifetime (τ_{RTP}) increased (i.e. τ_{RTP} ; **1NP** = 104 ms, **1scNP** = 167 ms). With the long RTP lifetimes of these nanoparticles, gated techniques can be utilized for image capture and oxygen concentration imaging.¹⁰² These multi-color dual-emitters are promising starting points for generating oxygen sensors with stronger phosphorescence.^{46,49,103} Ratiometric oxygen sensing may be enabled by introducing halide heavy atoms into these boron dye-PLA-PEG nanoparticle materials.

Stability.

The stability of **3NP** and **3scNP** nanoparticles (0.5 mg/mL) was studied over the course of three days in deionized (DI) water, phosphate buffered saline (PBS), and Dulbecco's modified Eagle's medium (DMEM). Dye **3** nanoparticles were selected for the stability experiments because **3NP** and **3scNP** samples had the largest difference in size and color, suggesting that there might be a greater difference in stability/aggregation that can be easily tracked by DLS and fluorescence. Nanoparticles were prepared in DI water, but this is unsuitable for cells, as application of NPs in DI water would cause cell lysing through differences in osmotic pressure. Thus, PBS and DMEM are commonly used in conjunction with cells and IV injection. The size and polydispersity of the nanoparticles were monitored at 37 °C (body and incubation temperature) by dynamic light scattering (DLS) for each medium. Also, the emission spectra of the NPs were monitored at room temperature and at 37 °C for three days, to account for changes at elevated temperatures. Three days was chosen for analysis as a reasonable timeframe for tumor accumulation via passive targeting and the EPR effect. Nanoparticles appear to be stable on the timescale for imaging the biological process of interest (e.g. tumor uptake), as the EPR effect typically occurs within 24 hours post injection.⁹

In deionized water and PBS, changes in size and luminescence for **3NP** and **3scNP** formulations were similar. Over the course of three days, the radii (R_H) and polydispersity (PD) of the NPs increased slightly. This is a result of the concentration gradients of ions (PBS; Na⁺ and Cl⁻) in the solution and NP matrix causing swelling.¹⁰⁴ This effect can be observed after incubation for 12 hours at 37 °C (Figures S9–12). Although the nanoparticle

size did not remain constant over time, the emission spectra were consistent. Therefore, both PLLA-PEG and scPLA-PEG formulations showed good stability in DI water and PBS for three days at room and body temperatures.

In Dulbeccos modified Eagle's medium (DMEM), a common medium for cell culture, radii and polydispersity increased over time, and significant aggregation was observed (Figure S7), again due to the concentration gradients of ions in DMEM (Ca^{2+} , Na^{+} , K^{+} , Cl^{-} , HCO_3^{-}) causing swelling (**3scNP**: start = 55.8 nm, end = 131.3 nm).¹⁰⁰ In DMEM, two peaks were present in the total emission spectra (Figures S9 and S11). The peak absorbance of the phenol red pH indicator in the DMEM occurs at the same wavelength as the peak emission of **3NP** in DI water. Phenol red has a weak emissive band at 600 nm, but when combined with the boron dye, sensitization occurred, increasing the phenol red emission. Overlaying the absorbance spectra of the DMEM with the emission spectra of the NPs confirmed these results (Figure S13). This overlap is strongest with the **3NP** sample, which is why the energy transfer is not observed as significantly in the emission spectra of the other three samples in DMEM. This is surprising considering that phenol red is virtually non-emissive, however BF_2bdks have been utilized for energy transfer donors in previous reports.⁴⁴ While stereocomplexed nanoparticles offered no advantages in DI water or PBS at room (25 °C) and body temperature (37 °C), in DMEM, the fluorescence properties of **3scNP** showed greater stability (Figure S13). It has been shown in previous reports that DMEM selectively degrades PLLA over the PDLA isomer.¹⁰⁵ Our results suggest that the stereocomplexation of PLLA with the PDLA isomer can overcome this.

The consistency of the emission spectra suggests that these materials could be used as imaging agents in vivo, provided delivery and uptake occur before aggregation and degradation in the three-day time period, given aggregation limits the potential for NP biodistribution and the usefulness of the materials as imaging agents.¹⁰⁶ Aggregation was also more significant in the stereocomplex nanoparticles. Similar aggregation results for tri-block copolymers of PDLA-PEG-PDLA and PLLA-PEG-PLA flower-like micelles in water.⁹⁰ When stereocomplex PLA cores interact, gels can readily form and precipitate from water.^{107,108} However, these PEGylated nanoparticles have superior aqueous stability in media and buffers compared to the pure PLA analogues published previously.^{40,46} In future studies, longer PEG chains will be investigated to tune the stability of boron dye polymers in different media to the specific biomedical application of interest.

Biodistribution.

Biodistribution studies were performed to determine the passive targeting efficacy of the imaging agents. These studies served to elucidate the timescale of accumulation and circulation of the NPs and to assess the NP metabolism after imaging. To characterize the distribution of **4scNPs** in a live mouse, either saline (control or PBS) or **4scNPs** were intravenously infused into mice bearing flank tumors. After infusion, **4scNPs** were detected in the bloodstream for at least eight hours. At 24 hours after IV infusion no **4scNP** signal was detected in the bloodstream (Figure 6 and S14). IVIS live imaging of mice showed good accumulation of **4scNP** in the tumor area ($6.03 \times 10^7 \pm 2.91 \times 10^7$ to $1.73 \times 10^8 \pm 5.89 \times 10^7$ average radiant efficiency [p/s/cm²/sr] / [$\mu\text{W}/\text{cm}^2$]). This result indicated successful targeted

delivery of **4scNP** to tumors and strong efficacy of PEG as a passive targeting agent by the EPR effect. IVIS imaging of the dissected organs from the control mice (saline injected) and a **4scNP** injected mouse further indicated significant accumulation in the tumor and liver (Figure 7). The presence of NPs in the liver suggests its metabolism in that organ. No significant accumulation was detected in heart, skeletal muscle, lung, kidney and spleen.

CONCLUSIONS

Color tuning strategies based on dye structure and loading were employed to achieve full color emissions for BF₂bdk-PLA-PEG nanoparticles. These multicolored nanoparticles show promise for multiplexing in vitro and in vivo. The red emission of the amino derivative (**4**) has potential for greater tissue penetration and reduction in autofluorescence noise. Both the red emission and the full color emission are useful characteristics for in vivo fluorescence imaging for applications such as image-guided surgery. Additionally, the long phosphorescence lifetimes and distinct fluorescence and phosphorescence peaks of **1–3NP** indicate utility of these materials for hypersensitive lifetime-based oxygen sensing. The **4scNP** sample was imaged in vivo and accumulated in a murine flank tumor 24 hours after IV injection, showing the efficacy of PEG for passive targeting. After dye scaffold modification with a heavy atom to increase phosphorescence intensity, materials **1P** and **2P**, with well separated fluorescence and phosphorescence peaks, may be compatible with ratiometric oxygen sensing. The self-assembly of the boron dye-PLLA-PEG conjugates with PDLA-PEG can be extended, in future work, to PDLA-PEG-bioconjugates for active targeting to tumors or other biological targets. This is an important first step to generating modular multicolor nanosensors with specialized surfaces for passive and active targeting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Design of boron dyes for polymer initiation (initiators) or post polymerization modification (couplers) used in this work.

Figure 2. Dye-Polymer Conjugation. (A) Mitsunobu coupling of BF_2dbmOH to PLLA-PEG. (B) 3-D GPC trace of **1P** showing the dye coupled to the PLLA-PEG polymer via UV-Vis detection. Plot shows elution time (min) versus wavelength (nm) versus relative absorbance.

Figure 3. Nanoparticle Fabrication. (A) Schematic of stereocomplex (scPLA) nanoparticle self-assembly. (B) CryoTEM of concentrated **1NP** and **1scNP** nanoparticles (3.0 mg/mL). (C) BNP size and polydispersity determined by DLS (left) and autocorrelation functions and size distribution of nanoparticles (right) (1.0 mg/mL).

Figure 4.

Images and total emission spectra of **1–4NP** (solid lines) and **1–4scNP** (dashed lines) in air. (1 = blue, 2 = green, 3 = yellow, 4 = red). Fluorescence emission maxima excited at 369 nm, except sample 4 ($\lambda_{\text{ex}} = 475$ nm).

Figure 5.

Images and total emission of **1–3NP**. Images: photos taken in air with UV illumination (365 nm; F) and afterglow emission under N_2 after UV lamp is turned off (RTP) Spectra: total emission in air and N_2 ($\lambda_{ex} = 385$ nm, note: the spectra are very similar) and the delayed emission under N_2 (RTP, $\lambda_{ex} = 385$ nm, 2 ms delay).

Figure 6. Biodistribution of **4scNP**. (A) Schematic of **4scNP** injection via tail vein infusion. (B) Pharmacokinetic study of BNPs in mouse bloodstream (units in $[p/s/cm^2/sr]/[\mu W/cm^2] \times 10^7$). (C) Epifluorescence images of **4scNP** accumulation in a flank tumor 24 hours after IV injection. A negative control mouse injected with saline is shown on the left. The color bar on the left indicates the fluorescence intensity (units: $[p/s/cm^2/sr]/[\mu W/cm^2] \times 10^8$). (D) Plots of average epifluorescence intensity from the control and **4scNP** (BNPs) (units: $[p/s/cm^2/sr]/[\mu W/cm^2] \times 10^8$).

Figure 7.

IVIS Imaging of Harvested Organs. (A) Radiant efficiency images of harvested organs from control mice (saline: $n = 3$) and **4scNP** injected mice ($n = 6$, Figure S13). (B) Plotted average of **4scNP** distribution in mouse organs 24 hours after injection (units given in $[\text{p/s/cm}^2/\text{sr}]/[\mu\text{W/cm}^2] \times 10^7$).

Scheme 1.
Synthesis of $\text{BF}_2\text{dapvmOH}$ (**4**).

Table 1.

Nanoparticle Properties.

Boron Dye	Sample	DLS ^a		Abs ^b	F		RTP	
		R _H (nm)	PD (%)	λ _{max} (nm)	λ _F ^c (nm)	τ _F ^d (ns)	λ _{RTP} ^e (nm)	τ _{RTP} ^f (ms)
BF ₂ dbm	1NP	53.6	23.3	380	426	1.98	520	104
	1scNP	52.0	13.6	379	418	1.96	516	167
BF ₂ nvm	2NP	34.3	25.1	417	503	5.01	556	77.4
	2scNP	45.5	23.1	419	479	2.42	550	159
BF ₂ gvm	3NP	68.7	15.5	417	553	17.73	556	36.6
	3scNP	37.7	25.9	419	499	4.42	552	90.7
BF ₂ dapvm	4NP	61.0	15.4	531	639	1.28	<i>g</i>	<i>g</i>
	4scNP	57.2	23.6	533	618	1.52	<i>g</i>	<i>g</i>

^aDynamic light scattering (DLS) of NPs; hydrodynamic radius (R_H) radii and polydispersity (PD) determined by DLS.^bAbsorption maxima.^cFluorescence emission maxima excited at 369 nm, except sample 4 (λ_{ex}= 475 nm).^dFluorescence lifetime excited with a 369 nm light-emitting diode (LED) monitored at the emission maximum. All fluorescence lifetimes are fitted with multiexponential decays.^eDelayed emission spectra maxima under N₂ at 298 K. Excitation source: xenon flash lamp.^fPre-exponential weighted RTP lifetime. Excitation source: xenon flash lamp; RTP lifetime fit to double-exponential decay.^gNo phosphorescence was observed.