nature materials
SUPPLEMENTARY INFORMATION
DOI: 10.1038/NMAT2509

# A Dual-Emissive-Materials Design Concept Enables Tumour Hypoxia Imaging

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**Materials and Methods.** 3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L-lactide, Aldrich) was recrystallized twice from ethyl acetate and stored under nitrogen. Solvents, $CH_2Cl_2$ and THF, were dried and purified by passage through alumina columns. 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. $^1H$ NMR (300 MHz) spectra were recorded on a UnityInova 300/51 instrument and referenced to the signal for residual protio chloroform at 7.26 ppm.

**Dbm(I)OH** A suspension of sodium hydride (36 mg, 1.5 mmol) and THF (~40 mL) was prepared in a 100 mL, dried Schlenk flask. The NaH suspension was transferred to a 100 mL 2-neck round-bottom flask containing 1-{4-[2-(tetrahydropyran-2-yloxy) -ethoxy]-phenyl}-ethanone[1] (200 mg, 0.76 mmol) and methyl p-iodobenzoate (200 mg, 0.76 mmol) via cannula. The resulting reaction mixture was heated at reflux for 4 h, cooled to room temperature, and then further cooled in an ice bath. The reaction was quenched by the dropwise addition of a saturated aqueous solution of sodium bicarbonate (5 mL) and then acidified (pH~3) by the addition of 3M HCl. THF was then removed *in vacuo* and the remaining aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic layer was dried over sodium sulfate, and concentrated *in vacuo* to give a brown, oily residue. The residue was dissolved in THF (50 mL) and water (15 mL) in the presence of 15 mg TsOH as catalyst and was heated at 55 °C for 18 h. The reaction mixture was concentrated *in vacuo* at 30 °C to remove THF, the white solid in aqueous layer was then collected by filtrated and thoroughly washed by water. The crude material was purified by column chromatography (1:1 EtOAc/Hex) to provide the desired product as white/silvery flaky crystals: 142.3 g (46 %) $^1$H NMR δ 16.90 (s, 1H, enol O*H*), 7.98 (d, *J* = 9.0, 2H, 2′,6′-Ar*H*), 7.84 (d, *J* = 8.8, 2H, 2″,6″-Ar*H*), 7.68 (d, *J* = 8.8, 1H, 3′, 5′-Ar*H*), 7.01 (d, *J* = 8.9, 1H, 3″, 5″-Ar*H*), 6.75 (s, 1H, COC*H*CO), 4.17 (t, *J* = 4.4, 2H, $HOCH_2CH_2OAr$), 4.02 (m, 2H, $HOCH_2CH_2OAr$), 1.96 (t, *J* = 6.3, $HOCH_2CH_2OAr$), Anal. Calcd for $C_{17}H_{15}IO_4$: C, 49.78; H, 3.69. Found: C, 49.98; H, 3.60. UV/vis ($CH_2Cl_2$): $\lambda_{max}$ = 360 nm, $\varepsilon_{max}$ = 35,000 $M^{-1}cm^{-1}$.

**$BF_2$dbm(I)OH** (**1**) Dbm(I)OH (125.0 mg, 0.305 mmol) was added to a flame-dried 2-neck round bottom flask under nitrogen, and dissolved in a mixture solvent of THF/$CH_2Cl_2$ (5/15 mL) to give a colorless solution. Boron trifluoride diethyl etherate (72 µL, 0.710 mmol) was added via syringe and the solution turned bright yellow. The flask was equipped with a reflux condenser and heated in an oil bath at 60 °C (15 h). The solution was then cooled to room temperature, and then the solvent was removed *on vacuo*, resulting in a yellow solid. The crude material was purified by column chromatography (1:1 EtOAc/Hex) followed by recrystallization in acetone/hexanes to give $BF_2$dbm(I)OH as dark yellow fine crystals:

106.3 mg (73%). m.p. 262-264 °C. $^1$H NMR ($CDCl_3$) δ 8.17 (d, 2H, $J$ = 8.9, 2′,6′-Ar$H$), 7.92 (d, 2H, $J$ = 8.6, 2″,6″-Ar$H$), 7.82 (d, 2H, $J$ = 8.8, 3″, 5″-Ar$H$), 7.07 (d, $J$ = 8.8, 2H, 3′,5′-Ar$H$), 7.06 (s, 1H, COC$H$CO), 4.23 (t, $J$ = 4.7, 2H, $HOCH_2CH_2$OAr), 4.04 (m, 2H, $HOCH_2CH_2$OAr), 1.94 (t, $J$ = 6.3, $HOCH_2CH_2$OAr). HRMS Calcd for $C_{17}H_{14}BO_4F_2Cl$ (M+Cl) : 492.9692; Found: 492.9707; UV/vis ($CH_2Cl_2$): $\lambda_{max}$ = 407 nm, $\varepsilon_{max}$ = 57,900 $M^{-1}cm^{-1}$.

**$BF_2$dbm(I)PLA**. A representative preparation for **P1** is as follows: $BF_2$dbm(I)OH (**1**) (22.8 mg, 0.05 mmol), lactide (0.360 g, 2.5 mmol) and $Sn(oct)_2$ (0.4 mg, 1.0 μmol) (loading: 1:50:1/50) in hexanes were combined in a sealed Kontes flask under $N_2$. The entire bulb of the flask was submerged in a 130 °C oil bath for 30 min. Crude polymer was purified by precipitation from $CH_2Cl_2$/cold MeOH. The polymer was collected by centrifugation, the filtrate was decanted, and the rubbery solid was washed with additional cold MeOH (2×). The resulting solid was reprecipitated from $CH_2Cl_2$/hexanes, collected by centrifugation, washed with hexanes, and dried *in vacuo* to give a yellow foam: 206 mg (78%, corrected for 64% monomer conversion). $M_n$ (GPC/RI) = 2,700, PDI = 1.11; $M_n$ (NMR) = 4,000. $^1$H NMR ($CDCl_3$) δ $^1$H NMR ($CDCl_3$) 8.16 (d, 2H, $J$ = 8.9, 2′,6′-Ar$H$), 7.92 (d, 2H, $J$ = 8.8, 2″,6″-Ar$H$), 7.82 (d, 2H, $J$ = 8.6, 3″, 5″-Ar$H$), 7.08 (s, 1H, COC$H$CO), 7.04 (d, $J$ = 8.9, 2H, 3′,5′-Ar$H$), 5.12-5.30 (m, broad, 50 H, PLA C$H$), 4.55 (m, 2H, $CH_2CH_2$OAr), 4.32 (m, 2H, $CH_2CH_2$OAr), 2.69 (q, 1H, PLA O$H$), 1.54-1.60 (m, broad, 150 H, PLA $CH_3$). UV/vis ($CH_2Cl_2$): $\lambda_{max}$ = 406 nm, ε = 33,400 $M^{-1}cm^{-1}$, λ = 320 nm, ε = 6280 $M^{-1}cm^{-1}$. Reaction and molecular weight data for **P1-P3** are shown in Table S1.

**Table S1.** Polymerization and Molecular Weight Data for **P1**-**P3**

| | Loading[a] | Time[b] (h) | $M_n$(GPC)/PDI[c] | $M_n$ (NMR) | Conversion[d] (%) | Yield[e] (%) |
|---|---|---|---|---|---|---|
| **P1** | 1:50 | 0.5 | 2,700/1.11 | 4,000 | 64 | 78 |
| **P2** | 1:100 | 1 | 7,300/1.15 | 7,000 | 73 | 84 |
| **P3** | 1:300 | 3 | 17,600/1.17 | 21,700 | 55 | 66 |

a. Molar ratio of initiator to monomer
b. Polymerization was stopped after this time
c. Polydispersity index (PDI) = $M_w/M_n$
d. Percent monomer consumption
e. Polymer product yield corrected for unreacted monomer

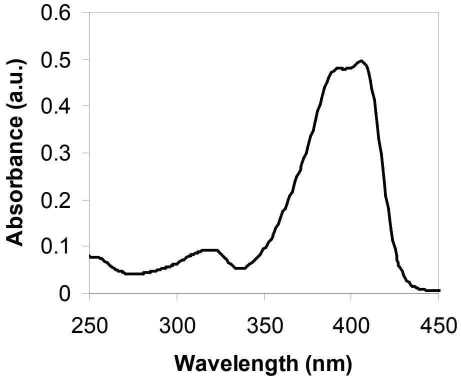

**Figure S1** Absorption spectrum of BF$_2$dbm(I)PLA (**1**) in CH$_2$Cl$_2$.

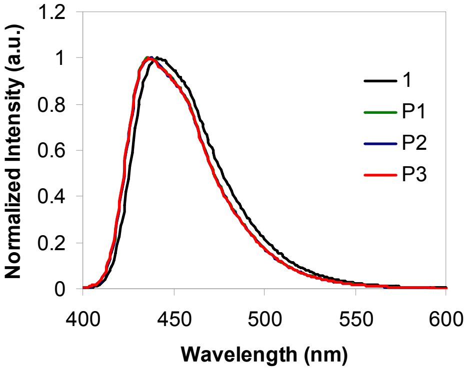

**Figure S2.** Emission spectra of BF$_2$dbm(I)OH (**1**) and BF$_2$bm(I)PLA (**P1**-**P3**) in CH$_2$Cl$_2$.

(Note: Spectra for **P1-P3** overlap.)

**Table S2.** RTP Lifetime Data for Polymer Powders Under Air[a]

| | $M_n$ (Da) | $\tau_{RTP}$(ms)[b] |
|---|---|---|
| **P1** | 2,700 | 0.42 |
| **P2** | 7,300 | 0.55 |
| **P3** | 17,600 | 0.57 |

a. 21% oxygen content

b. Excitation source: xenon flash lamp at 405 nm; pre-exponential weighted lifetime fit to triple exponential decay

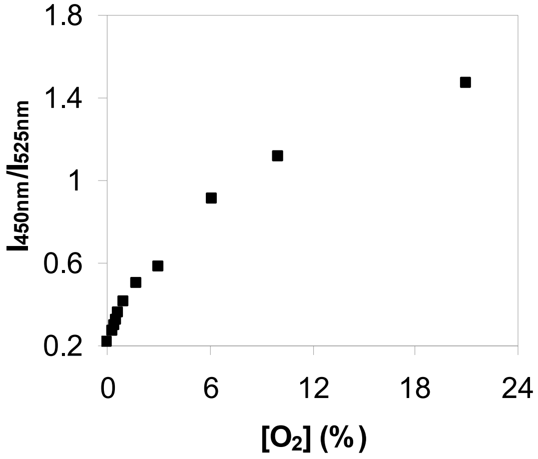

**Figure S3.** Oxygen sensitivity calibration for the **P1** film. Relationship between the $O_2$ level (0-21%) and the F/P emission intensity ratio at two fixed wavelengths (450 nm and 525 nm).

**$BF_2$dbm(I)PLA Nanoparticles.** **P2** nanoparticles were fabricated as previously reported[2]. Briefly, $BF_2$dbm(I)PLA (**P2**, 25 mg) was dissolved in DMF (2.5 mL). The solution was added dropwise to distilled $H_2O$ (25 mL) with stirring. Samples were dialyzed against distilled $H_2O$ with replacement of fresh water every hour for six hours before dialyzing overnight. On the following day, distilled $H_2O$ was replaced again and samples were dialyzed for an additional hour. The suspension was subsequently passed through filter paper (VWR Filter Paper Qualitative Grade: 413), and transferred to vials for storage. Nanoparticle sizes were determined by dynamic light scattering (DLS) (90˚ angle) on the Photocor Complex (Photocor Instruments Inc., USA) equipped with a He–Ne laser (Coherent, USA, Model 31–2082, 632.8 nm, 10 mW). Size and polydispersity analysis were performed using DynaLS software (Alango, Israel). Data for **P2** nanoparticles are given in Table S3.

**Table S3.** Characterization of **P2** nanoparticles.

| $M_n$ [a] (Da) | PDI [b] | Diameter [c] (nm) | PD [c,d] | $\lambda_F$ [e] (nm) | $\tau_F$ [f] (ns) | $\lambda_{RTP}$ [g] (nm) | $\tau_P$ [h] (ms) |
|---|---|---|---|---|---|---|---|
| 7,300 | 1.15 | 98 | 0.04 | 450 | 0.45 | 528 | 4.82 |

a. Determined by GPC in THF vs polysytrene standards
b. GPC PDI = polydispersity index
c. Determined by dynamic light scattering (DLS)
d. PD = polydispersity
e. Excitation source: 369 nm light emitting diode; fluorescence emission maximum for aqueous nanoparticle suspension ($\lambda_{max}$ absorption = 406 nm)
f. Fluorescence lifetime fit to triple exponential decay
g. Room temperature phosphorescence emission maximum for aqueous nanoparticle suspension purged with $N_2$
h. Excitation source: xenon flash lamp at 405 nm; pre-exponential weighted phosphorescence lifetime under $N_2$ fit to triple exponential decay

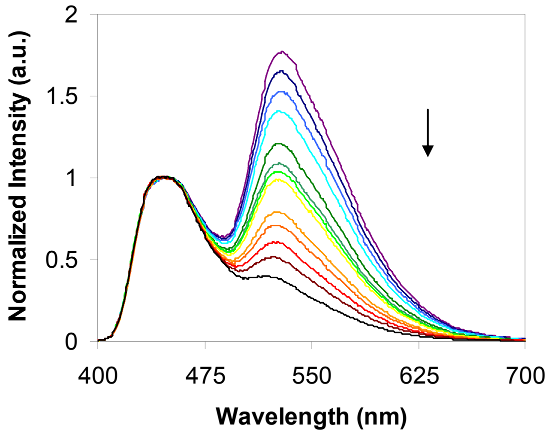

**Figure S4**. Emission spectra of **P2** boron nanoparticles in aqueous suspension under increasing $O_2$ levels (0-21%, indicated by the arrow) normalized to the fluorescence band.

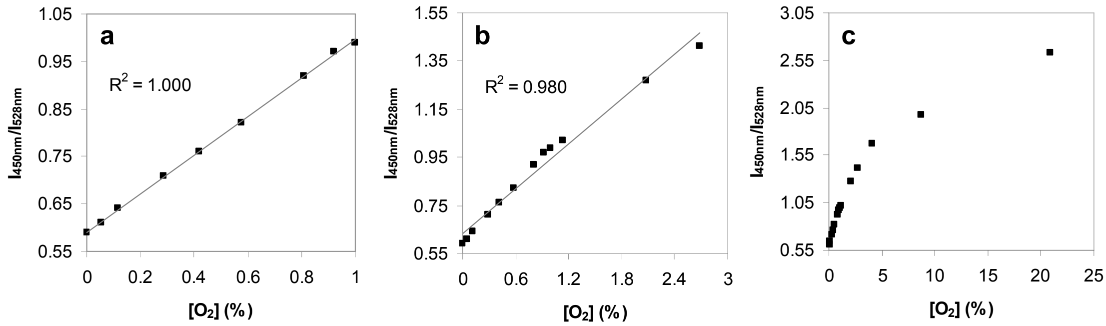

**Figure S5.** Oxygen sensitivity calibration for **P2** nanoparticles. Relationship between the $O_2$ level and the F/P emission intensity ratio at two fixed wavelengths (450 nm and 528 nm). Different oxygen concentration ranges shown for comparison: **a,** linear between 0-1%; **b,** reasonably linear up to 3%; **c,** more significant curvature approaching ambient level (21%).

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1. Bender, J. L. et al. Site-isolated luminescent europium complexes with polyester macroligands: Metal-centered heteroarm stars and nanoscale assemblies with labile block junctions. *J. Am. Chem. Soc.* **124**, 8526-8527 (2002).

2. Pfister, A., Zhang, G., Zareno, J., Horwitz, A. F. & Fraser, C. L. Boron polylactide nanoparticles exhibiting fluorescence and phosphorescence in aqueous medium. *ACS Nano* **2**, 1252-1258 (2008).