Electronic Supplementary Information

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Synthesis of DTTPH-PEG$_{24}$

6-(dithieno[3,2-b:2',3'-d]thiophen-2-yl)phenanthridine (dttph)

A mixture of dithieno[3,2-b:2',3'-d]thiophen-2-ylboronic acid (800 mg, 3.3 mmol), 6-chlorophenanthridine (788 mg, 3.7 mmol), tetrakis(triphenylphosphine)palladium(0) (255 mg, 0.22 mmol), THF (30 mL) and an aqueous solution of sodium carbonate (2 moldm^{-3}, 15 mL) was heated at reflux under a nitrogen atmosphere for 6 h. After cooling, the product was extracted with chloroform and the organic layer was washed with water. The organic solution was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by silica-gel column chromatography using chloroform as eluent. The product was obtained as a white solid (987 mg, 2.6 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.73-8.69 (t, 2H), 8.60-8.58 (d, 1H), 8.21-8.19 (d, 1H), 7.93-7.89 (m, 2H), 7.78-7.73 (q, 2H), 7.70-7.66 (t, 1H), 7.45-7.44 (d, 1H), 7.35-7.34 (d, 1H).

Phen-PEG$_{24}$

tert-Butyl 4-(1,10-phenanthrolin-5-yl)piperazine-1-carboxylate (250 mg, 0.69 mmol) was dissolved in 4 M HCl/dioxane solution (15 mL). This solution was kept for 3 h at room temperature and then evaporated to dryness under reduced pressure. The product (5-(piperazin-1-yl)-1,10-phenanthroline hydrochloride, phen) was obtained as a white powder (200 mg, 0.66 mmol, 96%). Phen (200 mg, 0.66 mmol), m-dPEG$^®_{24}$-acid (Quanta BioDesign, 300 mg, 0.29 mmol), and 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 380 mg, 1.0 mmol) were dissolved in anhydrous DMF (6 mL), then *N*,*N*-diisopropylethylamine (DIEPA, 1.36 mL) was added. This solution was stirred for 20 h at room temperature and then evaporated to dryness

under reduced pressure. The crude product was purified by amino-functionalized silica-gel chromatography using chloroform:methanol (98:2 v/v) as eluent. The product (Phen-PEG_{24}) was obtained as a white powder (270 mg, 0.20 mmol, 69%). MALDI-TOF (m/z) of Phen-PEG_{24}: calcd. for $C_{66}H_{114}N_4O_{25}$ $[M+H]^+$: 1363.78, found: 1364.52.

DTTPH-PEG_{24}

A mixture of dttph (1.86 g, 5.0 mmol), $IrCl_3 \cdot 3H_2O$ (0.87 g, 2.47 mmol), 2-ethoxyethanol (120 mL), and distilled water (30 mL) was refluxed for 15 h. After cooling, the solvent was evaporated to give a chloro-bridged dimer. The obtained dimer (300 mg, 0.15 mmol) and Phen-PEG_{24} (250 mg, 0.18 mmol) were dissolved in THF (30 mL) and methanol (20 mL) and the solution was refluxed for 4 h. After cooling, KPF_6 (92 mg, 0.50 mmol) was added, the solution was stirred for 1 h, then dried under reduced pressure. The crude product was purified by diol-functionalized silica-gel column chromatography using chloroform:methanol (98:2 v/v) as eluent. The product (DTTPH-PEG_{24}) was obtained as a red oil (300 mg, 0.12 mmol, 67%). MALDI-TOF (m/z) of DTTPH-PEG_{24}: calcd. for $C_{108}H_{134}IrN_6O_{25}S_6$ $[M-PF_6^-]^+$: 2299.74, found: 2298.67.

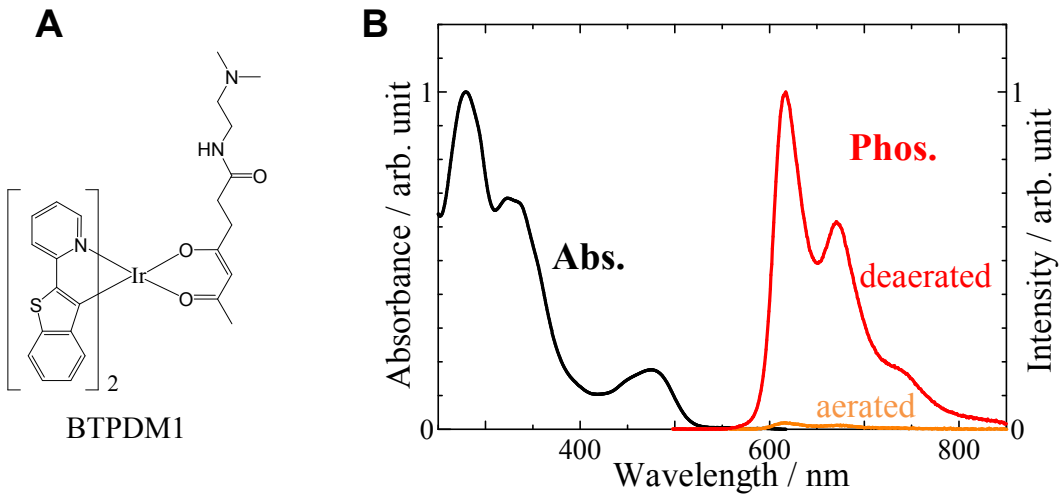

Fig. S1 Structure of BTPDM1, and its absorption and phosphorescence spectra in aerated and deaerated THF at room temperature.

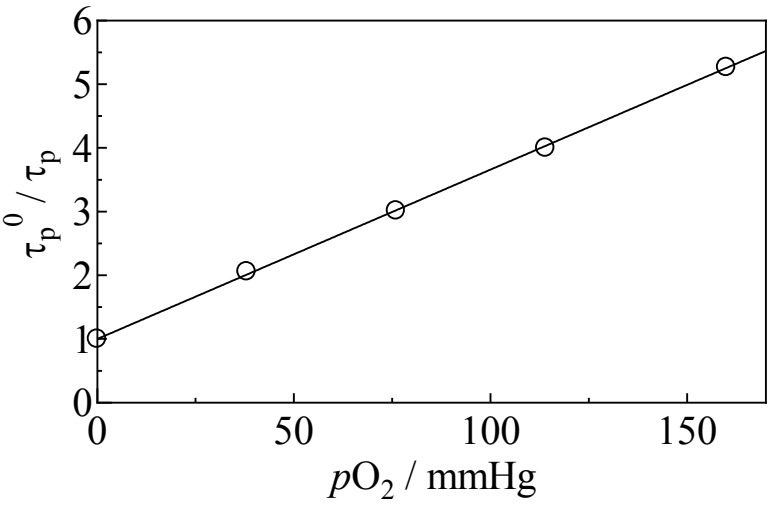

Fig. S2 Stern-Volmer plot of τ_p^0/τ_p vs. oxygen partial pressure in an incubator while culturing HT-29 cells in the presence of BTPDM1.

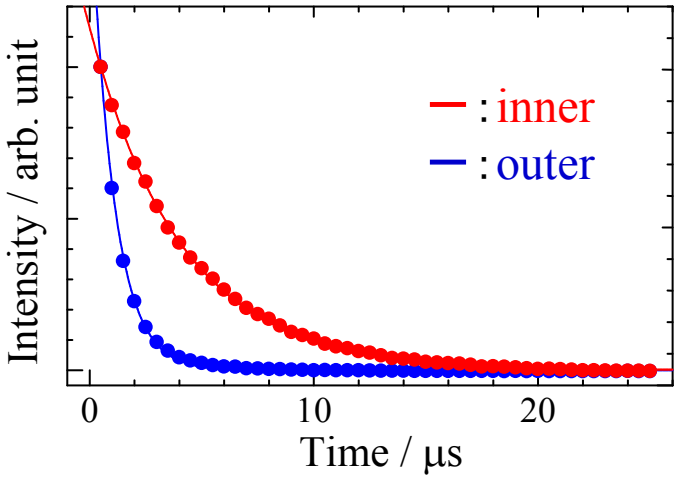

Fig. S3 Phosphorescence decay curves measured for cells at the inner and outer regions of the area covered with a coverslip (see Fig. 2B right).

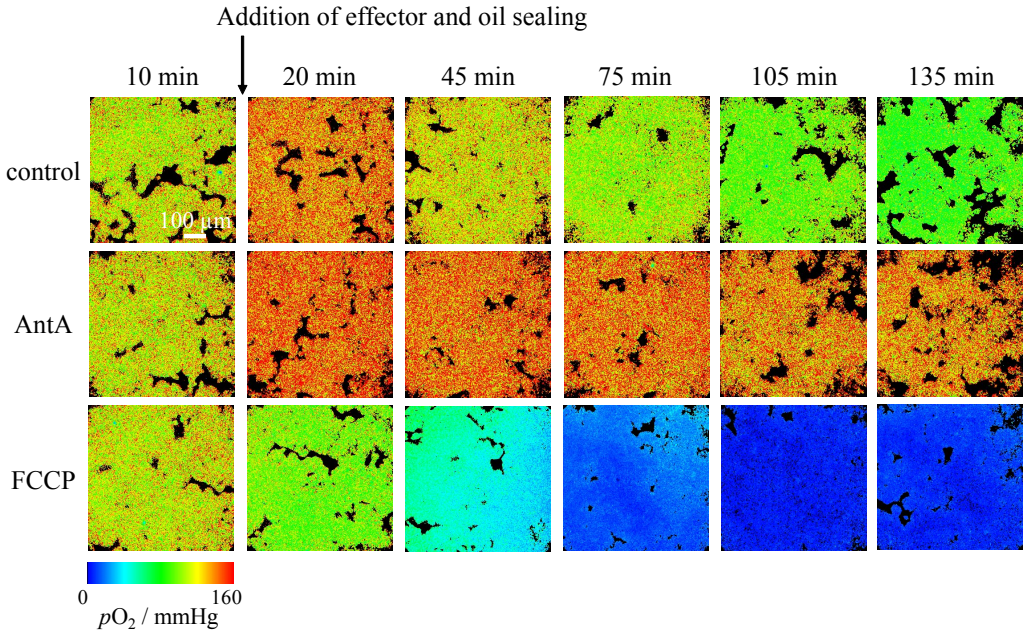

Fig. S4 Variation in O_2 distribution in HT-29 cells upon metabolic stimulation.

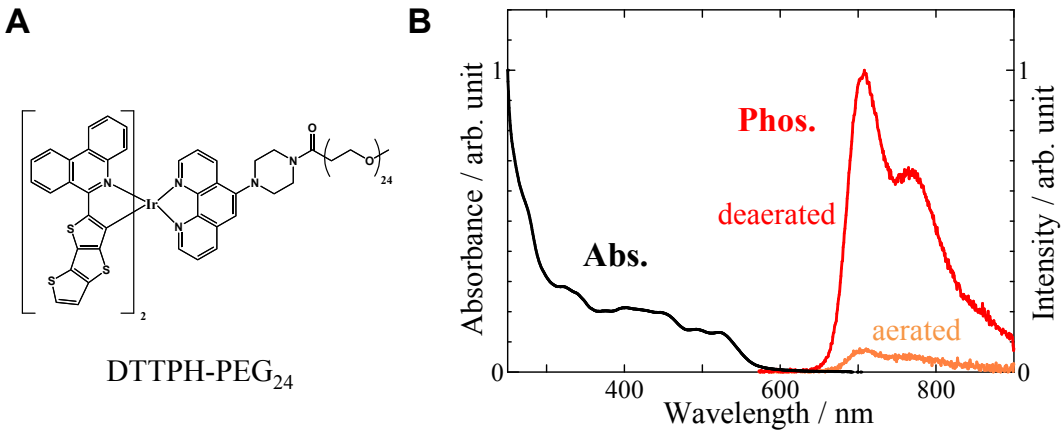

Fig. S5 Structure of DTTPH-PEG_{24}, and its absorption and phosphorescence spectra in aerated and deaerated THF at room temperature.

Table S1 Maximum absorption (λ_{Abs}^{max}) and phosphorescence (λ_{Phos}^{max}) wavelengths, phosphorescence quantum yield (Φ_p^0) and lifetime (τ_p^0) in degassed THF at room temperature.

Compound	λ_{Abs}^{max} / nm	λ_{Phos}^{max} / nm	Φ_p^0	τ_p^0 / μs
BTPDM1	485	616	0.29	5.62

Table S2 Phosphorescence lifetimes of BTPDM1 partitioned into HT-29 cells under different oxygen partial pressures (pO_2).

pO_2 / mmHg	τ_p / μs	SD
0	4.16	±0.02
38	2.02	±0.04
76	1.38	±0.01
114	1.04	±0.01
160	0.79	±0.01

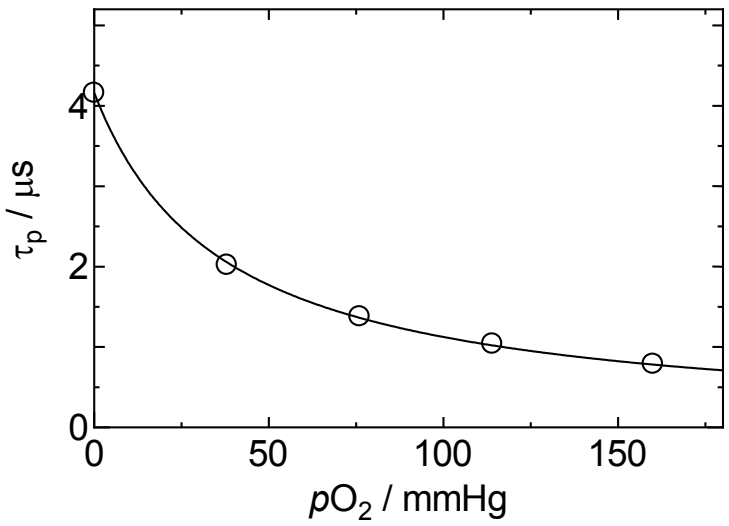

Fig. S6 pO_2 dependence of the phosphorescence lifetime of BTPDM1 partitioned into HT-29 cells.

Table S3 Phosphorescence lifetimes of DTTPH-PEG_{24} in different media at room temperature.

Solvent	$<\tau_1>$ / μs	$<\tau_2>$ / μs	$<\tau_3>$ / μs	$<<\tau>_{int}>$ / μs	$<<\tau>_{amp}>$ / μs
THF	0.538/6.58[2)]	-	-	-	-
H_2O (1 μM)	0.017/0.063	0.173/0.417	1.68/3.24	1.40/1.77	0.069/0.125
H_2O (10 μM)	0.038/0.045	0.225/0.321	1.65/2.53	1.06/1.29	0.087/0.100
FluoroBrite[1)]	0.030/0.038	0.182/0.253	1.53/2.40	0.72/1.39	0.092/0.078
FBS10%	1.80/2.10	4.43/6.07	-	3.63/5.66	3.09/5.08
FBS100%	2.04/2.45	4.73/6.32	-	4.19/5.93	3.76/5.47

1) DMEM without phenol red

2) aerated/N_2 saturated solutions

$<<\tau>_{int}>$ and $<<\tau>_{amp}>$ denote intensity-averaged lifetime and amplitude-averaged lifetime, respectively.