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Functionalization of Luminescent Cyclometalated Iridium(III) Polypyridine Complexes with a Fluorous Moiety: Photophysics, Protein-Binding, Bioconjugation, and Cellular Uptake Properties

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Electronic Supplementary Information

Materials, Synthesis, and Instrumentation. All reactions were performed under nitrogen. Solvents were of analytical grade and purified according to standard procedures.[1] Commercially available reagents were used without further purification unless otherwise stated. The dimers $[Ir_2(ppy)_4Cl_2]$[2] and $[Ir_2(pba)_4Cl_2]$,[3] 4'-succinimidyl-carboxy-4'-methyl-2,2'-bipyridine (bpy-NHS),[4] *N*-biotinyl-1,6-diaminohexane,[5] and the complexes $[Ir(pba)_2(bpy\text{-}CONH\text{-}C6\text{-}NH\text{-}biotin)](PF_6)$ and $[Ir(pba)_2(bpy\text{-}CONH\text{-}C_2H_5)](PF_6)$[6] were synthesized according to literature procedures. The instruments for characterization and photophysical studies have been described previously.[3] The methods by which determination of luminescence quantum yields[7] and lipophilicity,[8] HABA assays,[9] labeling of BSA with a pba complex,[3] cellular uptake studies,[9] and MTT assays[10] were undertaken have also been reported previously.

Bpy-CONH-Rf. A mixture of 3-(perfluorooctyl)propylamine (238 mg, 0.50 mmol), bpy-NHS (211 mg, 0.68 mmol), and triethylamine (0.3 mL) in DMF (30 mL) was stirred under nitrogen at room temperature for 12 h. The yellow solution was evaporated to dryness under vacuum to give a yellow solid. The product was purified by column chromatography on silica gel. The product was eluted using CH_2Cl_2/MeOH (15:1, v/v). The eluent was evaporated to dryness and the product was isolated as a white solid. Yield: 232 mg (69%). MS (ESI): *m/z* 674 $[M + H^+]^+$.

$[Ir(ppy)_2(bpy\text{-}CONH\text{-}Rf)](PF_6)$ (1). A mixture of $[Ir_2(ppy)_4Cl_2]$ (107 mg, 0.10 mmol) and bpy-CONH-Rf (135 mg, 0.20 mmol) in CH_2Cl_2/MeOH (1:1, v/v) (50 mL) was refluxed under an inert atmosphere of nitrogen in the dark for 4 h. The orange-yellow solution was then cooled to room temperature and KPF_6 (147 mg, 0.80 mmol) was added to the solution. The mixture was then evaporated to dryness. The solid was dissolved in CH_2Cl_2 and the solution was purified by column chromatography on silica gel. Subsequent recrystallization of the product from a CH_2Cl_2/diethyl ether mixture gave an orange crystal. Yield: 129 mg (49%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.02 (s, 1H, H3 of bpy), 8.63 (s, 1H, H3' of bpy), 8.14 – 8.12 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 7.88 – 7.82 (m, 6H, H6' and H5 of bpy, H3 of phenyl ring of ppy, and H3 of pyridyl ring of ppy), 7.62 (t, 2H, J = 6.6 Hz, H5 of pyridyl ring of ppy), 7.42 (d, 1H, J = 6.6 Hz, H5' of bpy), 7.07 – 7.03 (m, 4H, H5 of phenyl ring of ppy and H4 of pyridyl ring of ppy), 6.92 – 6.86 (m, 2H, H4 of phenyl ring of ppy), 6.31 – 6.26 (m, 2H, H6 of phenyl ring of ppy), 3.53 (t, 2H, J = 6.1 Hz, $CH_2CH_2CH_2C_8F_{17}$), 2.60 (s, 3H, CH_3 on C4' of bpy), 2.40 – 2.20 (m, 2H, $CH_2C_8F_{17}$), 2.00 – 1.90 ppm (m, 2H, $CH_2CH_2C_8F_{17}$); IR

(KBr): ν = 3430 (br, NH), 1627 (s, C=O), 845 cm^{-1} (s, PF_6^-); UV/Vis (CH_2Cl_2, 298 K): λ_{abs} (ε) = 256 (71145), 271 sh (64090), 322 sh (22875), 385 sh (10720), 414 nm sh (5490 dm^3 mol^{-1} cm^{-1}); UV/Vis (CH_3CN, 298 K): λ_{abs} (ε) = 253 (62335), 268 sh (56470), 320 sh (20455), 380 sh (9080), 418 nm sh (3670 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 254 (43970), 270 sh (39305), 321 sh (14430), 385 sh (6540), 418 nm sh (2850 dm^3 mol^{-1} cm^{-1}); MS (ESI): *m*/*z* 1175 [M – PF_6^-]$^+$; elemental analysis calcd (%) for $IrC_{45}H_{32}N_5O_1PF_{23}$: C 40.98, H 2.45, N 5.31; found: C 41.13, H 2.72, N 5.50.

[Ir(pba)$_2$(bpy-CONH-Rf)](PF$_6$) (2). The synthetic procedure was similar to that for the complex [Ir(ppy)$_2$(bpy-CONH-Rf)](PF$_6$) except that [Ir$_2$(pba)$_4$Cl$_2$] was used instead of [Ir$_2$(ppy)$_4$Cl$_2$]. The product was isolated as an orange crystal. Yield: 179 mg (65%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ = 9.74 (s, 2H, CHO of pba), 9.08 (s, 1H, H3 of bpy), 8.83 (s, 1H, H3' of bpy), 8.43 (d, 2H, J = 8.4 Hz, H3 of phenyl ring of pba), 8.38 – 8.36 (m, 1H, H6 of bpy), 8.24 (d, 1H, J = 5.7 Hz, H6' of bpy), 8.15 – 7.96 (m, 9H, H5, H5', and bpy-4-CON*H* of bpy and H3, H5, and H6 of pyridyl ring of pba), 7.56 (d, 2H, J = 3.0 Hz, H3 of phenyl ring of pba), 7.34 – 7.28 (m, 2H, H4 of pyridyl ring of pba), 6.85 (d, 2H, J = 6.3 Hz, H6 of phenyl ring of pba), 3.62 – 3.58 (m, 2H, NHCH_2CH$_2$CH$_2$C$_8$F$_{17}$), 2.62 (s, 1H, CH$_3$ on C4' of bpy), 2.48 – 2.23 (m, 2H, CH_2C$_8$F$_{17}$), 2.00 – 1.85 ppm (m, 2H, CH_2CH$_2$C$_8$F$_{17}$); IR (KBr): ν = 3436 (br, NH), 1680 (s, CHO), 842 cm^{-1} (s, PF_6^-); UV/Vis (CH_2Cl_2, 298 K): λ_{abs} (ε) = 251 sh (35545), 275 (44370), 303 sh (36285), 319 sh (27765), 373 sh (6030), 420 (3890), 445 sh (3385 dm^3 mol^{-1} cm^{-1}); UV/Vis (CH_3CN, 298 K): λ_{abs} (ε) = 248 sh (33050), 273 (42810), 301 sh (34805), 318 sh (26810), 368 sh (6475), 418 (4010), 449 sh (3010 dm^3 mol^{-1} cm^{-1}); MS (ESI): *m*/*z* 1231 [M – PF_6^-]$^+$; elemental analysis calcd (%) for $IrC_{47}H_{32}N_5O_3PF_{23}\cdot H_2O$: C 40.79, H 2.40, N 5.06; found: C 40.75, H 2.48, N 5.21.

[Ir(pba)$_2$(bpy-COOH)](PF$_6$). The synthetic procedure was similar to that for the complex [Ir(pba)$_2$(bpy-CONH-Rf)](PF$_6$) except that bpy-COOH was used instead of bpy-CONH-Rf. The product was isolated as an orange crystal. Yield: 110 mg (60%). ^{1}H NMR (300 MHz, acetone-d_6, 298 K, TMS): δ 9.74 (s, 2H, CHO of pba), 9.26 (s, 1H, COOH on C4 of bpy), 8.85 (s, 1H, H3 of bpy), 8.43 – 8.35 (m, 2H, J = 8.4 Hz, H6 of pyridyl ring of pba), 8.14 – 7.94 (m, 6H, H3', H5, H6, and H6' of bpy and H3 of phenyl ring of pba), 7.89 – 7.85(m, 4H, H5 and H3 of pyridyl ring of pba), 7.55 (dd, 2H, J = 8.2 and 1.5 Hz, H4 of phenyl ring of pba), 7.40 (d, J = 5.1 Hz, 1H, H5' of bpy), 7.23 – 7.21 (m, 1H, H4 of pyridyl ring of pba), 6.82 (d, 2H, J = 8.6 Hz, H6 of phenyl ring of pba), 2.39 ppm (s, 1H, CH$_3$ on C4' of bpy); IR (KBr): ν = 3432 (br, NH and OH), 1684 (s, CHO), 849 cm^{-1} (s, PF_6^-); MS (ESI): *m*/*z* 772 [M – PF_6^-]$^+$.

[Ir(ppy-CH$_2$NH-C6-NH-biotin)$_2$(bpy-CONH-Rf)](PF$_6$) (2a). A mixture of complex **2** (150 mg, 0.11 mmol), *N*-biotinyl-1,6-diaminohexane (75 mg, 0.22 mmol), and triethylamine (0.5 mL) in MeOH (40 mL) was refluxed under an inert atmosphere of nitrogen in the dark 12 h. After the orange solution was cooled to room temperature, NaBH$_4$ (34 mg, 0.88 mmol) was added to the solution. The solution was stirred under an inert atmosphere of nitrogen for 4 h and then evaporated to dryness. The residual orange solid was redissolved in CH$_2$Cl$_2$ (50 mL) and the solution was washed with distilled water (30 mL × 3). The CH$_2$Cl$_2$ layer was then dried over anhydrous MgSO$_4$, filtered, and carefully evaporated to dryness to give an

orange solid. Subsequent recrystallization of the product from a MeOH/diethyl ether mixture afforded complex **2a** as an orange crystal. Yield: 110 mg (49%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.11 (s, 1H, H3 of bpy), 8.73 (s, 1H, H3' of bpy), 8.19 – 8.12 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 8.01 (d, 1H, J = 6.0 Hz, H6' of bpy), 7.93 – 7.91 (m, 4H, H3 of phenyl ring of ppy and H5 of pyridyl ring of ppy), 7.83 (d, 1H, J = 5.7 Hz, H5 of bpy), 7.72 (d, 1H, J = 5.4 Hz, H3 of pyridyl ring of ppy), 7.65 (d, 1H, J = 5.4 Hz, H3 of pyridyl ring of ppy), 7.44 (d, 1H, J = 6.0 Hz, H5' of bpy), 7.29 (t, 2H, J = 5.4 Hz, H4 of pyridyl ring of ppy), 7.16 – 7.10 (m, 2H, H4 of phenyl ring of ppy), 6.42 (s, 1H, H6 of phenyl ring of ppy), 6.32 (s, 1H, H6 of phenyl ring of ppy), 4.50 – 4.46 (m, 2H, NCH of biotin), 4.31 – 4.27 (m, 2H, NCH of biotin), 4.01 – 3.85 (m, 4H, ppy-CH_2NH), 3.53 (t, 2H, J = 7.0 Hz, CH_2CH$_2$CH$_2$C$_8$F$_{17}$), 3.29 – 3.15 (m, 6H, SCH of biotin and CH_2NH-biotin), 2.93 – 2.61 (m, 11H, CH$_3$ on C4' of bpy, SCH$_2$ of biotin, and NHCH_2C$_4$H$_8$CH$_2$NHCO), 2.30 – 2.18 (m, 6H, CH_2C$_8$F$_{17}$ and COCH_2C$_3$H$_6$ of biotin), 2.00 – 1.94 (m, 2H, CH_2CH$_2$C$_8$F$_{17}$), 1.65 – 1.29 ppm (m, 28H, NHCH$_2$C$_4$$H_8CH_2$NHCO and COCH$_2C_3$$H_6$ of biotin); IR (KBr): ν = 3433 (br, NH), 1685 (m, C=O), 850 cm^{-1} (s, PF$_6^-$); UV/Vis (CH$_3$CN, 298 K): λ_{abs} (ε) = 255 (44370), 268 sh (40375), 316 sh (16620), 341 sh (8425), 380 sh (5555), 411 nm sh (3120 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 255 (32690), 268 sh (29980), 317 sh (12605), 382 sh (4295), 413 nm sh (2285 dm^3 mol^{-1} cm^{-1}); MS (ESI): m/z 1884 [M – PF$_6^-$]$^+$; elemental analysis calcd (%) for IrC$_{79}$H$_{92}$N$_{13}$O$_5$PF$_{23}$·CH$_3$OH: C 46.64, H 4.67, N 8.84; found: C 46.35, H 4.39, N 8.57.

[Ir(ppy-CH$_2$NH-C$_4$H$_9$)$_2$(bpy-CONH-Rf)](PF$_6$) (2b). The synthetic procedure was similar to that for complex **2a** except that *n*-butylamine was used instead of *N*-biotinyl-1,6-diaminohexane. The product was isolated as an orange crystal. Yield: 72 mg (44%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.03 (s, 1H, H3 of bpy), 8.64 (s, 1H, H3' of bpy), 8.14 – 8.11 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 7.89 – 7.81 (m, 6H, H5 and H6' of bpy, H3 of phenyl ring of ppy, and H5 of pyridyl ring of ppy), 7.63 (t, 2H, J = 6.0 Hz, H3 of pyridyl ring of ppy), 7.42 (d, 1H, J = 5.7 Hz, H5' of bpy), 7.07 – 7.02 (m, 4H, H4 of phenyl ring of ppy and H4 of pyridyl ring of ppy), 6.26 (s, 1H, H6 of phenyl ring of ppy), 6.23 (s, 1H, H6 of phenyl ring of ppy), 3.56 – 3.44 (m, 6H, CH_2CH$_2$CH$_2$C$_8$F$_{17}$ and ppy-CH_2NH and), 2.60 (s, 3H, CH$_3$ on C4' of bpy), 2.40 (t, 4H, J = 7.5 Hz, NHCH_2CH$_2$CH$_2$CH$_3$), 2.35 – 2.20 (m, 2H, CH$_2$C$_8$F$_{17}$), 1.76 – 1.55 (m, 2H, CH_2CH$_2$C$_8$F$_{17}$), 1.40 – 1.10 (m, 8H, NHCH$_2$$CH_2$$CH_2CH_3$), 0.87 ppm (t, 6H, J = 6.9 Hz, NHC$_3$H$_6$CH_3); IR (KBr): ν = 3425 (br, NH), 1627 (m, C=O), 847 cm^{-1} (s, PF$_6^-$); UV/Vis (CH$_2$Cl$_2$, 298 K): λ_{abs} (ε) = 259 (64060), 273 sh (62315), 321 sh (27495), 386 sh (9840), 416 nm sh (5630 dm^3 mol^{-1} cm^{-1}); UV/Vis (CH$_3$CN, 298 K): λ_{abs} (ε) = 257 (55140), 273 sh (52340), 319 sh (23325), 383 sh (8130), 414 nm sh (4475 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 256 (50885), 271 sh (47705), 319 sh (20435), 383 sh (7190), 413 nm sh (3990 dm^3 mol^{-1} cm^{-1}); MS (ESI): m/z 1345 [M – PF$_6^-$]$^+$; elemental analysis calcd (%) for IrC$_{55}$H$_{54}$N$_7$O$_1$PF$_{23}$·(C$_2$H$_5$)$_2$O: C 45.33, H 4.13, N 6.27; found: C 45.19, H 4.20, N 6.52.

[Ir(ppy-CH$_2$NH-Rf)$_2$(bpy-CONH-C6-NH-biotin)](PF$_6$) (3a). The synthetic procedure was similar to that for complex **2a** except that 3-(perfluorooctyl)propylamine and [Ir(pba)$_2$(bpy-CONH-C6-NH-biotin)](PF$_6$) were

used instead of *N*-biotinyl-1,6-diaminohexane and [Ir(pba)$_2$(bpy-CONH-Rf)](PF$_6$). The product was isolated as an orange crystal. Yield: 76 mg (32%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.07 (s, 1H, H3 of bpy), 8.69 (s, 1H, H3' of bpy), 8.16 – 8.11 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 7.93 – 7.84 (m, 6H, H5 and H6' of bpy, H3 of phenyl ring of ppy, and H5 of pyridyl ring of ppy), 7.68 (d, 1H, J = 5.7 Hz, H3 of pyridyl ring of ppy), 7.63 (d, 1H, J = 5.7 Hz, H3 of pyridyl ring of ppy), 7.42 (d, 1H, J = 5.7 Hz, H5' of bpy), 7.18 (d, 2H, J = 7.8 Hz, H4 of pyridyl ring of ppy), 7.12 – 7.06 (m, 2H, H4 of phenyl ring of ppy), 6.37 (s, 1H, H6 of phenyl ring of ppy), 6.28 (s, 1H, H6 of phenyl ring of ppy), 4.49 – 4.45 (m, 1H, NCH of biotin), 4.30 – 4.26 (m, 1H, NCH of biotin), 3.79 – 3.64 (m, 4H, ppy-CH_2NH), 3.43 (t, 2H, J = 6.9 Hz, NHCH_2C$_4$H$_8$CH$_2$NHCO), 3.21 – 3.14 (m, 3H, SCH of biotin and CH_2NH-biotin), 2.93 – 2.60 (m, 9H, CH$_3$ on C4' of bpy, SCH$_2$ of biotin, and CH_2CH$_2$CH$_2$C$_8$F$_{17}$), 2.20 – 2.11 (m, 6H, COCH_2C$_3$H$_6$ of biotin and CH_2C$_8$F$_{17}$), 1.88 – 1.78 (m, 4H, CH_2CH$_2$C$_8$F$_{17}$), 1.75 – 1.17 ppm (m, 14H, NHCH$_2$C$_4$$H_8CH_2$NHCO and COCH$_2C_3$$H_6$ of biotin); IR (KBr): ν = 3433 (br, NH), 1685 (m, C=O), 846 cm^{-1} (s, PF$_6^-$); UV/Vis (CH$_3$CN, 298 K): λ_{abs} (ε) = 255 (48430), 271 sh (43925), 317 sh (18775), 379 sh (6190), 413 nm sh (3355 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 256 (39075), 271 sh (36370), 312 sh (17825), 381 sh (5340), 412 nm sh (2875 dm^3 mol^{-1} cm^{-1}); MS (ESI): *m*/*z* 2019 [M – PF$_6^-$]$^+$; elemental analysis calcd (%) for IrC$_{74}$H$_{70}$N$_{10}$O$_3$PF$_{40}$·(C$_2$H$_5$)$_2$O: C 41.88, H 3.61, N 6.26; found: C 41.59, H 3.74, N 6.13.

[Ir(ppy-CH$_2$NH-Rf)$_2$(bpy-CONH-C$_2$H$_5$)](PF$_6$) (3b). The synthetic procedure was similar to that for complex **3a** except that [Ir(pba)$_2$(bpy-CONH-C$_2$H$_5$)](PF$_6$) was used instead of [Ir(pba)$_2$(bpy-CONH-C6-NH-biotin)](PF$_6$). The product was isolated as an orange crystal. Yield: 96 mg (47%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 9.00 (s, 1H, H3 of bpy), 8.63 (s, 1H, H3' of bpy), 8.13 – 8.09 (m, 3H, H6 of bpy and H6 of pyridyl ring of ppy), 7.88 – 7.80 (m, 6H, H5 and H6' of bpy, H3 of phenyl ring of ppy, and H5 of pyridyl ring of ppy), 7.62 (t, 2H, J = 5.1 Hz, H3 of pyridyl ring of ppy), 7.41 (d, 1H, J = 5.7 Hz, H5' of bpy), 7.06 – 7.04 (m, 4H, H4 of phenyl ring of ppy and H4 of pyridyl ring of ppy), 6.27 (s, 1H, H6 of phenyl ring of ppy), 6.24 (s, 1H, H6 of phenyl ring of ppy), 3.56 – 3.43 (m, 6H, ppy-CH_2NH and CONHCH_2CH$_3$), 2.60 (s, 3H, CH$_3$ on C4' of bpy), 2.48 (t, 4H, J = 6.9 Hz, CH_2CH$_2$CH$_2$C$_8$F$_{17}$), 2.18 – 2.03 (m, 4H, CH_2C$_8$F$_{17}$), 1.72 – 1.62 (m, 4H, CH_2CH$_2$C$_8$F$_{17}$), 1.25 ppm (t, 3H, J = 7.4 Hz, NHCH$_2$CH_3); IR (KBr): ν = 3444 (br, NH), 1666 (m, C=O), 842 cm^{-1} (s, PF$_6^-$); UV/Vis (CH$_2$Cl$_2$, 298 K): λ_{abs} (ε) = 259 (50145), 273 sh (48020), 318 sh (21710), 382 sh (7805), 413 nm sh (4395 dm^3 mol^{-1} cm^{-1}); UV/Vis (CH$_3$CN, 298 K): λ_{abs} (ε) = 255 (48250), 272 sh (45615), 317 sh (20535), 380 sh (7230), 413 nm sh (3740 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 255 (53595), 272 sh (50235), 318 sh (21430), 381 sh (7850), 414 nm sh (3960 dm^3 mol^{-1} cm^{-1}); MS (ESI) *m*/*z* 1721 [M – PF$_6^-$]$^+$; elemental analysis calcd (%) for IrC$_{60}$H$_{47}$N$_7$O$_1$PF$_{40}$: C 38.64, H 2.54, N 5.26; found: C 38.91, H 2.58, N 5.55.

[Ir(ppy-CH$_2$NH-Rf)$_2$(bpy-COOH)](PF$_6$) (4). The synthetic procedure was similar to that for complex **3a** except that [Ir(pba)$_2$(bpy-COOH)](PF$_6$) was used instead of [Ir(pba)$_2$(bpy-CONH-C6-NH-biotin)](PF$_6$). The product was isolated as an orange crystal. Yield: 105 mg (52%). ^{1}H NMR (300 MHz, methanol-d_4, 298 K, TMS): δ 8.95 (s, 1H, H3 of bpy), 8.55 (s, 1H, H3' of bpy), 8.09 (d, J = 8.4 Hz, 2H, H6 of

pyridyl ring of ppy), 8.02 (d, J = 5.7 Hz, 1H, H6 of bpy), 7.85 – 7.78 (m, 6H, H5 and H6' of bpy, H3 of phenyl ring of ppy, and H5 of pyridyl ring of ppy), 7.62 (d, J = 6.0 Hz, 2H, H3 of pyridyl ring of ppy), 7.36 (d, J = 5.7 Hz, 1H, H5' of bpy), 7.05 – 7.01 (m, 4H, H5 of phenyl ring of ppy and H3 of pyridyl ring of ppy), 6.26 (s, 2H, H6 of phenyl ring of ppy), 3.56 – 3.44 (m, 4H, ppy-CH_2NH), 2.58 (s, 3H, CH_3 on C4' of bpy), 2.49 – 2.44 (m, 4H, CH_2CH$_2$CH$_2$C$_8$F$_{17}$), 2.20 – 2.02 (m, 4H, CH_2C$_8$F$_{17}$), 1.71 – 1.61 ppm (m, 4H, CH_2CH$_2$C$_8$F$_{17}$); IR (KBr): ν = 3445 (br, NH and OH), 1623 (m, C=O), 854 cm^{-1} (m, PF_6^-); UV/Vis (CH_2Cl_2, 298 K): λ_{abs} (ε) = 260 (48985), 274 sh (46880), 315 sh (23435), 373 sh (6500), 415 nm sh (3950 dm^3 mol^{-1} cm^{-1}); UV/Vis (CH_3CN, 298 K): λ_{abs} (ε) = 258 (52030), 273 sh (48950), 314 sh (23740), 345 sh (10225), 417 nm sh (3595 dm^3 mol^{-1} cm^{-1}); UV/Vis (MeOH, 298 K): λ_{abs} (ε) = 256 (48180), 271 sh (46065), 315 sh (22090), 380 sh (6360), 413 nm sh (3650 dm^3 mol^{-1} cm^{-1}); MS (ESI) m/z 1694 [M – PF_6^-]$^+$; elemental analysis Calcd (%) for $IrC_{58}H_{42}N_6O_2PF_{40}$: C 37.90, H 2.30, N 4.57; found: C 38.19, H 2.57, N 4.85.

Preparation of the Adduct 2a-Avidin. The bis-biotin complex **2a** (250 nmol) in MeOH (30 μL) was added to avidin (2 mg, 30 nmol) dissolved in 50 mM phosphate buffer pH 7.4 (600 μL). The suspension was stirred for 24 h in the dark at room temperature. The orange-yellow solid residue was removed by centrifugation. The yellow supernatant was then loaded onto a PD-10 column. The first band with intense orange-yellow photoluminescence was collected. The product was concentrated with a YM-30 centricon to a volume of 250 μL and stored at 4°C before use.

Labeling of BSA with Complex 4. Complex **4** (1.2 mg, 0.65 μmol) in DMSO (100 μL) was added to a mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (1.2 mg, 6.26 μmol) and BSA (5 mg, 76 nmol) dissolved in 50 mM phosphate buffer pH 7.4 (1 mL). The suspension was stirred for 24 h in the dark at room temperature. The orange-yellow solid residue was removed by centrifugation. The orange-yellow supernatant was then loaded onto a PD-10 column. The first band with intense orange-yellow photoluminescence was collected. The product was concentrated with a YM-30 centricon to a volume of 250 μL and stored at 4°C before use.

Labeling of N_α-(*tert*-butoxycarbonyl)-L-lysine (boc-K) with Complex 2 and Subsequent Purification using FSPE. A mixture of boc-K (11 mg, 44.72 μmol), [Ir(pba)$_2$(bpy-CONH-Rf)](PF_6) (30 mg, 22.30 μmol), and triethylamine (20 μL) in MeOH (10 mL) was stirred at room temperature for 12 h. $NaBH_4$ (0.44 mg, 11.6 μmol) was then added to the mixture. After the solution was stirred at room temperature for 12 h, the solution was evaporated to dryness to give an orange solid. The solid was dissolved in 50% aqueous MeOH (1 mL). The solution was then loaded onto an FSPE cartridge (2 g fluorous silica gel) that had been equilibrated with water. The column was washed with deionized water (10 mL × 2). The desired product were eluted with MeOH (10 mL × 2). The eluents were analyzed by ESI-mass spectrometry.

Table S1 Photophysical Data of the Iridium(III) Complexes and Bioconjugates[a]

complex or bioconjugate	medium (*T*/K)	λ_{em}/nm	τ_o/μs	Φ_{em}
1	CH_2Cl_2 (298)	608	0.36	0.11
	CH_3CN (298)	611	0.21	0.047
	MeOH (298)	618	0.063	0.012
	buffer[b] (298)	628	0.039	0.005
	glass[c] (77)	534	4.89	
2	CH_2Cl_2 (298)	537 (max), 567	2.51	0.40
	CH_3CN (298)	536 (max), 567	2.42	0.24
	glass[c] (77)	521 (max), 564, 613 sh	6.92	
2a	CH_2Cl_2 (298)	584	0.53	[d]
	CH_3CN (298)	492 sh,	1.81	0.15
		588	0.47	
	MeOH (298)	490 sh,	2.53	0.055
		592	0.24	
	buffer[b] (298)	502 sh,	2.28	0.013
		606	0.040	
	glass[c] (77)	472 sh, 523	5.20	
2b	CH_2Cl_2 (298)	504 sh,	2.13	0.063
		600	0.29	
	CH_3CN (298)	492 sh, 528 sh,	2.44	0.037
		604	0.17	
	MeOH (298)	499 sh, 526,	2.59	0.026
		600 (max)	0.13	
	buffer[b] (298)	496 sh, 527,	2.73,	0.019
		599 sh	0.046	
	glass[c] (77)	476 sh, 498 sh, 524	6.41	
3a	CH_2Cl_2 (298)	490 sh,	2.41	[d]
		579	0.67	
	CH_3CN (298)	491 sh,	2.70	0.14
		581	0.33	
	MeOH (298)	491, 523 sh,	2.97	0.066
		590 (max)	0.13	
	buffer[b] (298)	491 (max), 519 sh,	2.45	0.021
		607	0.042	
	glass[c] (77)	477 sh, 520	5.22	
3b	CH_2Cl_2 (298)	604	0.38	0.097
	CH_3CN (298)	488 sh,	1.76	0.033
		609	0.19	
	MeOH (298)	490 sh,	1.98	0.021
		609	0.07	
	buffer[b] (298)	489 sh, 527 sh,	2.20	0.014
		606	0.044	
	glass[c] (77)	474 sh, 527	4.85	
4	CH_2Cl_2 (298)	549	0.89	0.24
	CH_3CN (298)	567	0.55	0.15
	MeOH (298)	583	0.23	0.067
	buffer[b] (298)	588	0.12	0.038

	glass[c] (77)	474 sh, 516	4.89	
2-BSA	buffer[e] (298)	491 (max), 525	3.64	0.033
		570	0.47	
2-(boc-K)$_2$	MeOH (298)	493,	2.80	0.019
		598 (max)	0.24	
4-BSA	buffer[e] (298)	566	1.25 (45%),	0.059
			0.30 (55%)	

[a] Excitation wavelength = 350 nm. [b] 50 mM Potassium phosphate buffer pH 7.4/MeOH (35:65, v/v). [c] EtOH/MeOH (4:1, v/v). [d] The solubility of the iridium(III) complex in CH_2Cl_2 was insufficient for accurate determination of emission quantum yields. [e] 50 mM Potassium phosphate buffer pH 7.4.

Fig. S1 Emission spectra of (a) complex **1** and (b) complex **4** in degassed CH_2Cl_2 (solid line) and 50 mM potassium phosphate buffer pH 7.4/MeOH (35:65, v/v) (dashed line) at 298 K and in EtOH/MeOH (4:1, v/v) at 77 K (dotted line).

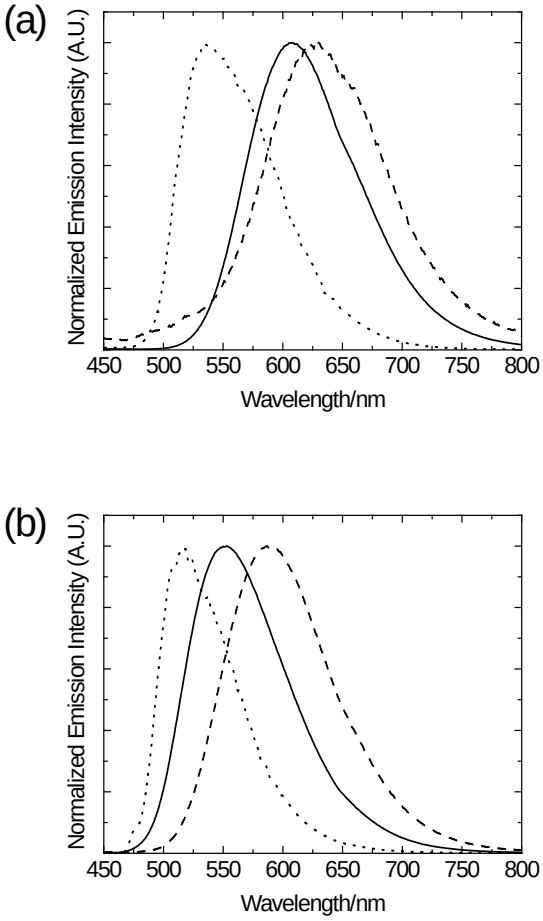


Fig. S2 Emission spectra of complex **2** in degassed CH_2Cl_2 (solid line), CH_3CN (dashed line) at 298 K, and in EtOH/MeOH (4:1, v/v) at 77 K (dotted line).

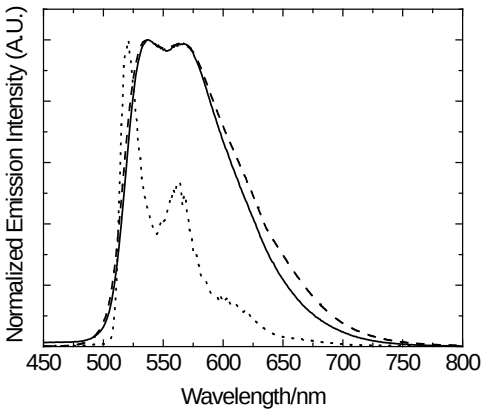


Fig. S3 Results of absorption titrations of avidin-$(HABA)_4$ with complexes **2a** (solid triangles) and **3a** (solid circles) and unmodified biotin (open squares).

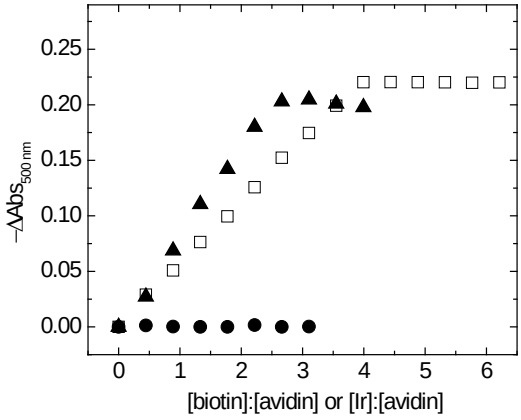


Fig. S4 Emission spectrum of adduct **2a-avidin** in aerated 50 mM potassium phosphate buffer pH 7.4 at 298 K.

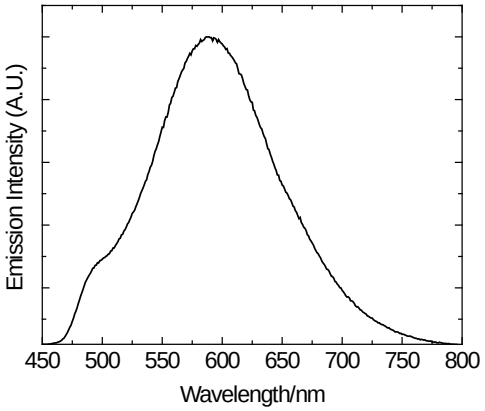


Fig. S5 Emission spectra of (a) bioconjugate **2-BSA** and (b) bioconjugate **4-BSA** in degassed 50 mM potassium phosphate buffer pH 7.4 at 298 K.

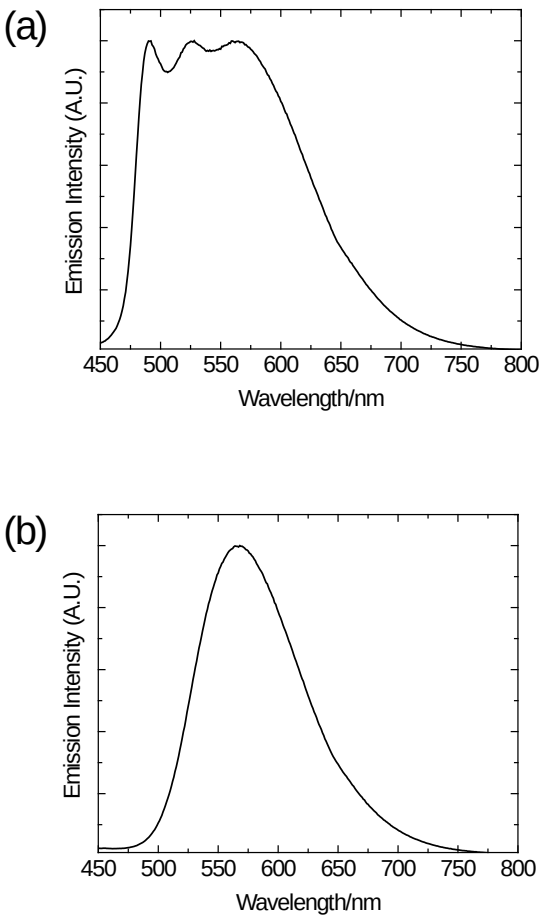


Fig. S6 Laser-scanning confocal microscopy images of HeLa cells incubated with complex **1** (5 μM) at 37°C (left) and 4°C (right) for 90 min.

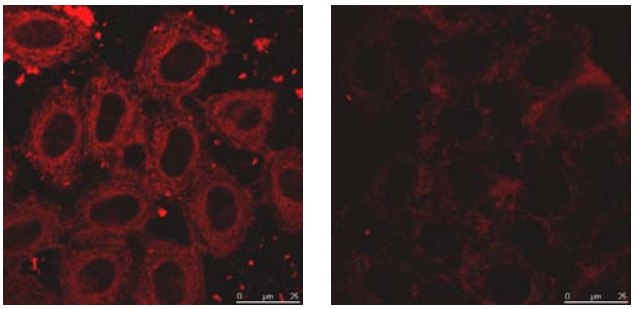
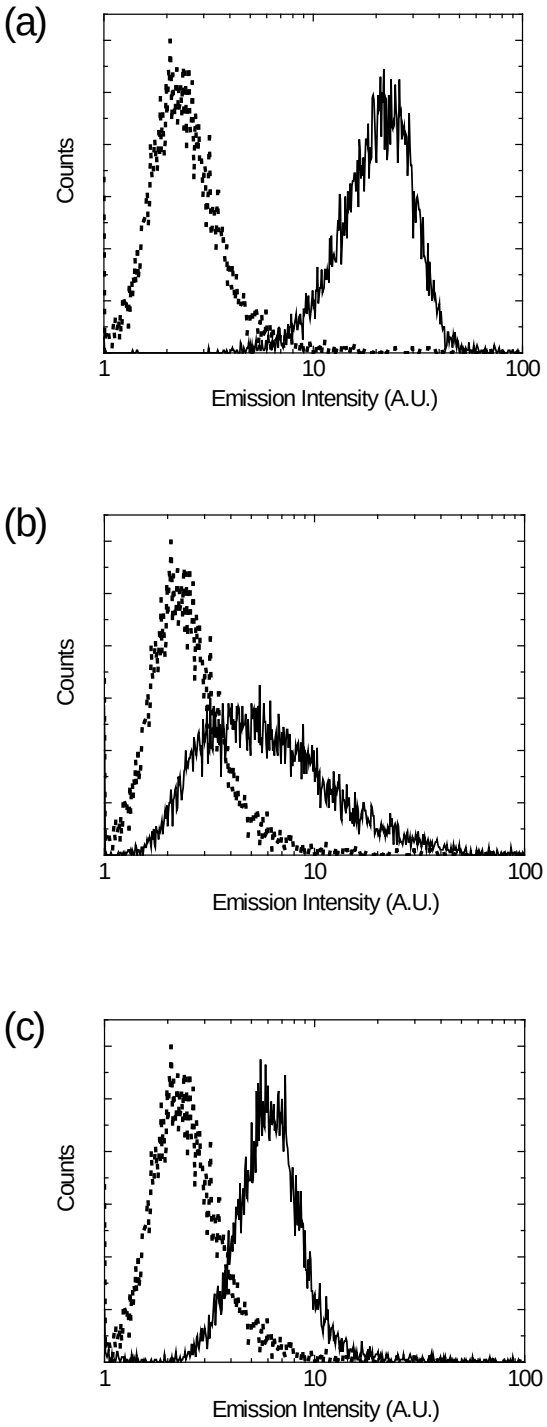


Fig. S7 Flow cytometric results of HeLa cells incubated with blank medium (dotted line) and (a) complex **1** (5 μM) at 37°C for 90 min, (b) complex **1** (5 μM) at 4°C for 90 min, and (c) complex **1** (5 μM) at 37°C for 90 min after the cells were preincuated with CCCP (20 μM) at 37°C for 30 min.



References

1 D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, Pergamon: Oxford, 1997.
2 A. P. Wilde and R. J. Watts, *J. Phys. Chem.*, 1991, **95**, 622.
3 K. K.-W. Lo, C.-K. Chung and N. Zhu, *Chem. Eur. J.*, 2003, **9**, 475.
4 B. M. Peek, G. T. Ross, S. W. Edwards, G. J. Meyer, T. J. Meyer and B. W. Erickson, *Int. J. Pept. Prot. Res.*, 1991, **38**, 114.
5 G. Sabatino, M. Chinol, G. Paganelli, S. Papi, M. Chelli, G. Leone, A. M. Papini, A. De Luca and M. Ginanneschi, *J. Med. Chem.*, 2003, **46**, 3170.
6 S.-K. Leung, K. Y. Kwok, K. Y. Zhang and K. K.-W. Lo, *Inorg. Chem.*, 2010, **49**, 4984.
7 (a) J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991; (b) K. Nakamura, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2697.
8 D. J. Minick, J. H. Frenz, M. A. Patrick and D. A. Brent, *J. Med. Chem.*, 1988, **31**, 1923.
9 K. Y. Zhang and K. K.-W. Lo, *Inorg. Chem.*, 2009, **48**, 6011.
10 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.