Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supporting Information

Neutral Iridium(III) Complexes Bearing BODIPY−Substituted *N*−Heterocyclic Carbene (NHC) Ligands: Synthesis, Photophysics, *in vitro* Theranostic Photodynamic Therapy, and Antimicrobial Activity

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Photobiological Activity Studies.

Cellular Assays. *Metal complex solutions*. Stock solutions (5 mM) of the chloride salts of **Ir3** and **Ir5** were prepared by first dissolving the solids in DMSO (10% of total aqueous solution), using sonication and slight heat, then deionized water (dI) water was added to the appropriate volume. The solutions were vortexed to mixed well and stored in the dark at -20°C when not in use. Working solutions were made by diluting the 5 mM stock solutions in pH 7.4 Dulbecco's phosphate buffered saline (DPBS, no Ca^{2+} or Mg^{2+}), where DMSO in the final assay wells were kept under 0.1% at the highest complex concentration.

Cell Culture. *SKMEL28 cells*. Adherent SKMEL28 human malignant melanoma cells (HTB-72, ATCC) were cultured in complete growth media EMEM plus 10% FBS and were kept incubated at 37°C under 5% CO_2. The complete growth media was prepared in 500 mL portions by combining 50 mL Seradigm FB Essence (VWR Life Science) and 450 mL Eagle's Minimum Essential Medium (MEM, Corning® 10-009-CV) then filtered in a Nalgene™ rapid-flow sterile disposable bottle top filter with PES 0.2 μm membrane (Thermo Scientific 09-741-07). SK-MEL-28 cells were initiated at about 300,000 cells mL^{-1} in 75 cm^2 tissue culture flasks and were subcultured 2-3 times per week under standard aseptic conditions when growth reached approximately 500,000 cells mL^{-1}. Subculturing was done by discarding the spent media, rinsing the cell layer once with cold DPBS, followed with the dissociation of the cell monolayer with cold 1X Trypsin-EDTA solution (VWR Life Science Trypsin, 0.25% EDTA 1X). Complete growth media was added to the cell suspension to dilute and distributed to new cell flasks. Cell assays were performed with cells at no higher than ten cell passages (subcultures).

Cytotoxicity and Photocytoxicity Cell Assays. Cell viability assays were completed in triplicate in 96-well polystyrene flat bottom TC-treated microplates (Corning Costar 3595). The outer

periphery wells were filled with 200 μL DPBS in order to minimize evaporation from inner sample wells. SKMEL28 cells, growing in log phase (~500,000 cells mL^{-1}) with at least 95% viability were transferred in 50 μL aliquots to inner wells containing warm complete growth media (25 μL) and placed in a 37°C, 5% CO_2 water-jacketed incubator (Thermo Electron Corp., FormaSeries II, Model 3110, HEPA Class 100) for 3 h to allow for cell attachment. Serial dilutions of **Ir3** and **Ir5** metal complexes (prepared in DPBS and prewarmed in 37°C incubator) were added in 25 μL volumes to the appropriate microplate wells containing cells. Control wells, with no metal complexes, were included in each microplate with either cells only (25 μL growth media, 50 μL cells, 25 μL DPBS) or no cells (75 μL growth media, 25 μL DPBS). All microplates were kept incubated in the dark at 37°C under 5% CO_2 for a pre-treatment time of 16 h. Control microplates not receiving light treatments were kept in the dark while PS-treated microplates were irradiated under one of the following conditions: visible light (400-700 nm, 33 mW cm^{-2}) from a 190 W BenQ MS 510 overhead projector, or red light (625 nm, 43 mW cm^{-2}) from an LED array (Photodynamic Inc., Halifax, NS). Irradiation times using these two light sources were 51 and 39 min respectively to yield total light doses of 100 J cm^{-2}. After PS treatments, all microplates were returned to the incubator for another 48 h. Cell viability was evaluated using a resazurin fluorescent dye assay according to a standard protocol (reference Hanson 2013 patent and O'Brien 2000 EJB). Briefly, 10 μL aliquots of prewarmed, sterile filtered 0.6 mM resazurin reagent (Sigma Aldrich Canada) were added to all sample wells and subsequently incubated another 3-4 h. Viability was determined based on the ability of the blue resazurin dye to be metabolically reduced (by live cells) to the fluorescent red resorufin. Fluorescence was recorded with a Cytofluor 4000 fluorescence microplate reader (excitation 530 ± 25 nm, emission 620 ± 40 nm). The concentrations of the metal complexes where cell viability was reduced by 50% (EC_{50} values) for cytotoxicity (dark) and

photocytoxicity (light) were calculated from sigmoidal fits of the dose-response curves using GraphPad Prism 6.0 according to Eq. 1, where y_i and y_f are the intial and final fluorescence signal intensities. Generally, cells growing in log phase and of the same passage number have EC_{50} values reproducible to within ± 25% in the submicromolar range, ± 10% below 10 μM, and ± 5% above 10 μM. Phototherapeutic indices (PIs), a measure of the therapeutic window, were represented by the ratio of dark to light EC_{50} values from the dose-response curves.

$$y = y_i + \frac{y_i - y_f}{1 + 10^{(\log EC_{50} - x) \times (Hill\ slope)}} \quad (1)$$

Confocal Microscopy. The influence of the metal complexes **Ir3** and **Ir5** on SKMEL28 cells, in dark or light conditions, was monitored using confocal fluorescence microscopy. Live cells were imaged in DPBS using poly-L-lysine coated sterile glass-bottom Petri dishes (MatTek) with several treatments: (i) cells with no complex in dark, (ii) cells with no complex with light, (iii) cells with complex in dark, and (iv) cells with complex with light. SK-MEL-28 cells (approximately 100,000) were transferred in 1 mL volumes to the dishes and placed in a 37°C, 5% CO_2 water-jacketed incubator for approximately 3 hr to equilibrate. The cells were then washed with warm PBS followed by the addition of 50 μM **Ir3** or **Ir5** (1 mL, prepared in DPBS and warmed in incubator) to the sample dishes containing cells. The dishes were returned to the incubator for 15 min prior to further treatment. Light treated samples were irradiated with visible light for 26 min from a 190 W BenQ MS 510 projector (400–700 nm, power density 33 mW cm^{-2} for a total light dose 50 J cm^{-2}), where dark samples were covered with foil and placed in a dark drawer for the same amount of time. Dishes containing both dark and light samples were imaged 15 min post-treatment using a Carl Zeiss LSM 510 laser scanning confocal microscope with a 60X oil objective lens. Excitation was delivered at 458/488 nm from an argon-krypton laser, and signals were acquired through a 505 nm long-pass filter. Pinhole diameters for all the treatments were at

1 AU (airy unit) equaling 98 μm. The images were collected and analyzed using the Zeiss LSM Image Browser Version 4.2.0.121 software (Carl Zeiss Inc.).

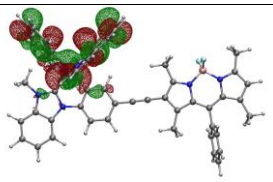
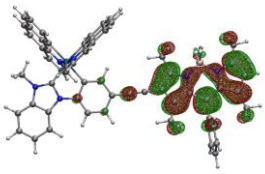
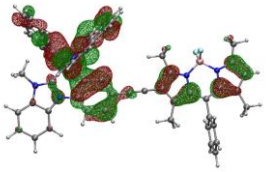
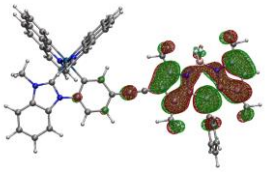
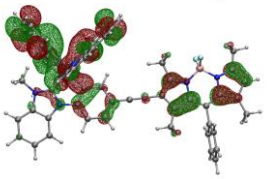
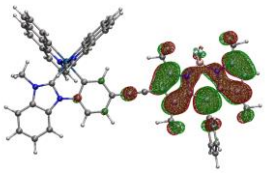
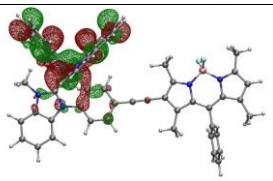
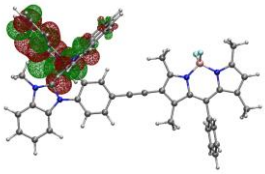
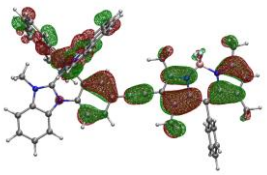
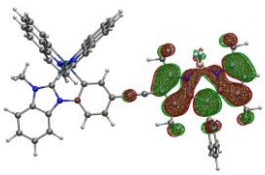
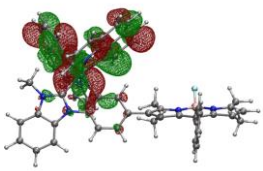
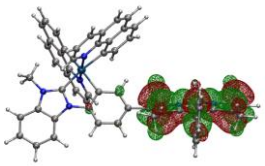
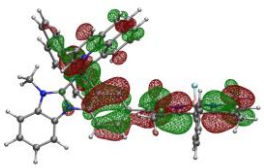
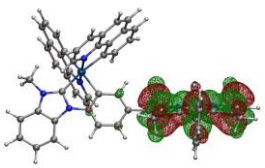
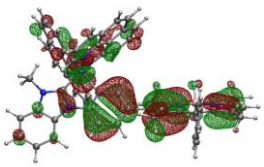
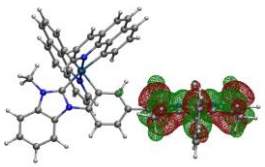
Bacterial Survival Assays. Photodynamic inactivation (PDI) of *Staphylococcus aureus* (ATCC 25923, Cedarlane) growing as planktonic culture, by **Ir3** and I**r5**, was probed using a standard broth microdilution method.[28] In order to standardize the starting bacterial concentration for all experiments, a standard curve of McFarland barium sulfate turbidity standards was generated, representing approximate bacterial concentrations. McFarland barium sulfate standards 0.5, 1, 2, 3, 4, and 5, were prepared, representing approximately 1.5, 3, 6, 9, 12, 15 × 10^8 bacteria mL^{-1} respectively. The absorbance at 562 nm was measured for all McFarland standards, using a BioTek EL800 microplate reader, and a standard curve was generated. An inoculum of *S. aureus* was then prepared by transferring colonies from a room temperature secondary growth plate to a sterile 15 mL conical tube containing 2 mL sterile distilled water, and the contents were mixed well by vortexing. The absorbance at 562 nm was read and the approximate concentration was calculated according to the McFarland barium sulfate standard curve. The solution was further diluted in fresh TSB in order to match a starting bacterial cell concentration of approximately 1 × 10^6 CFU mL^{-1} and was used within 1 hr of preparation. Dark and light experiments were performed in duplicate in 96-well microplates (Corning Costar, Acton, MA), where outer wells along the top and bottom contained 200 μL of sterile distilled water to prevent evaporation. Dilutions of **Ir3** and **Ir5** were prepared in TSB media at 200 μM (twice the desired top concentration of 100 μM) and added to triplicate wells of the microplate and 1:2 serial dilutions were performed in wells containing 50 μL TSB using an electronic multichannel pipettor. (Final concentrations were 100, 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1 μM). To sample wells, 50 μL bacterial inoculum was added (final concentration in wells were ~5 x 10^5 CFU mL^{-1}). Final assay volumes in the wells were 100 μL.

Both plates were incubated for 30 min prior to treatments. Dark treatment microplates were wrapped in foil and placed in a dark drawer, while PDI-treated microplates were irradiated with visible light (400 – 700 nm, 28 ± 0.9 mW cm^{-2}) using a Solla 30W Cree LED light panel. The irradiation time was 60 min to yield a light dose of approximately 100 J cm^{-2} to the microplate wells (lid on). Both dark and PDT-treated microplates were incubated overnight. Sample turbidity was measured as absorbance at 562 nm for all microplates and EC_{50} values (effective concentration at which ≥ 50% of bacterial growth is inhibited) for antibiotic (dark) and antimicrobial PDI (light) activity were calculated from sigmoidal fits of the dose response curves using Graph Pad Prism 6.0 according to Eq 1 (as shown above in cell culture section), where y_i and y_f are the initial and final absorbance intensities. Data was normalized using triplicate control wells containing 50 μL TSB and 50 μL bacterial inoculum and were deemed as 100% growth. Bacteria-free control wells received 100 μL TSB only. Highly colored concentrations of PS were given duplicate control wells containing 50 μL water containing PS and 50 μL TSB (no bacteria). PS controls were prepared due to the color content of high concentration skewing the absorbance values of treated wells. PS control absorbance values were subtracted from the corresponding treated PS absorbance values to show true comparative growth rates.

Measurement of ROS in SKMEL28 cells. The generation of intracellular ROS was measured using the fluorescent stain 2,7′-Dichlorofluorescin diacetate (DCFDA, Sigma D6883), following a simple microplate assay.[29] DCFDA is a cell-permeable probe that measures hydroxyl, peroxyl, and other ROS activity within the cell or the cellular environment. SKMEL28 cells were seeded at approximately 25,000 cells per well on two 96-well ultra-low attachment flat bottom microtiter plates (Corning 3595) and incubated overnight. The spent media was carefully removed and the cells were washed with 1X buffer (Hank's 1X Balanced Balanced Salt Solutions, HBSS, HyClone

SH30268.01), followed by the addition of 100 μL of 25 μM DCFDA prepared in 1X buffer. The two microplates were incubated for 45 min (37°C under 5% CO_2). Compound serial dilutions of **Ir3** and **Ir5** were prepared in 1X supplemented buffer (HBSS + 10% FBS). The supernatant was carefully removed, the cells were washed once with 1X buffer, then 75 μL 1X supplemented buffer was added to each sample well followed by 25 μL aliquots of either **Ir3** or **Ir5** (final 9 concentrations in wells ranged from 4 pM - 120 μM). Control wells in both microplates included: 1) Ir(III) complexes with DCFDA, no cells, 2) cells only, no DCFDA, 3) cells only with DCFDA, 4) Ir(III) complexes with cells, no DCFDA. The microplates were incubated (pre-treatment) for 30 min. The dark-treatment microplate was kept in the dark (foiled and kept in dark place), while the light-treatment microplate was irradiated with visible light (400-700 nm, 33 mW cm^{-2}) from a 190 W BenQ MS 510 overhead projector for 26 min, yielding a light dose of 50 J cm^{-2}. Fluorescence signals were measured at several time periods after irradiation (15 min, 30 min, 60 min, 90 min, 120 min) with a Cytofluor 4000 fluorescence microplate reader (excitation 485 ± 20 nm, emission 580 ± 50 nm). Relative ROS production is represented by plotting arbitrary fluorescence units versus the log concentration of test samples in both dark and light treatments.

Table S1. NTOs of the hole and electron of the moderate energy absorption band transitions of complexes **Ir1-Ir5**, calculated by the TD-DFT method with PBE1PBE functional, LANL2DZ/6-31G* basis and toluene as a solvent.

	S_n	Hole	Electron
Ir1	S_2 477 nm f = 0.0017		
	S_3 429 nm f = 0.1557		
	S_4 412 nm f = 0.1551		
	S_5 411 nm f = 0.0442		
	S_7 396 nm f = 0.3499		
Ir2	S_2 473 nm f = 0.0001		
	S_3 424 nm f = 0.3307		
	S_7 387 nm f = 0.1558		

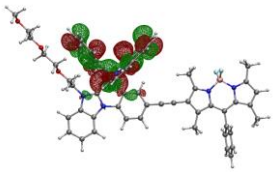
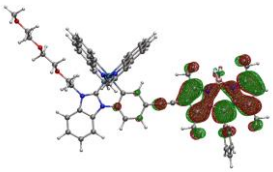
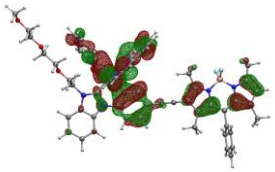
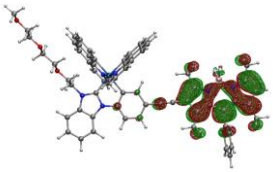
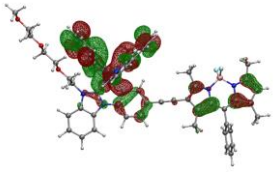
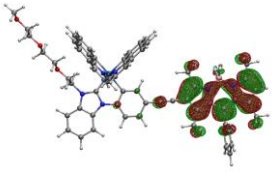
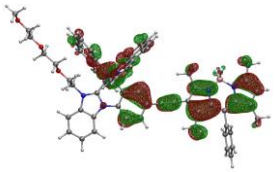
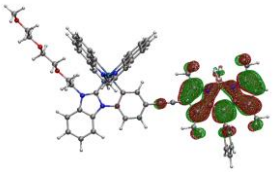
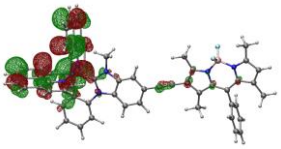
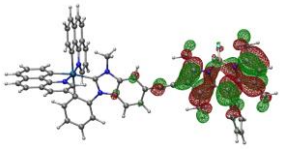
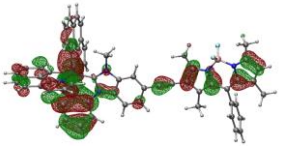
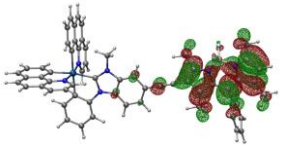
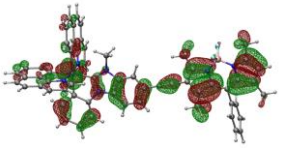
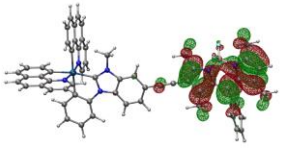
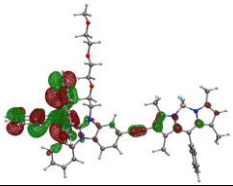
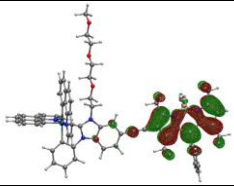
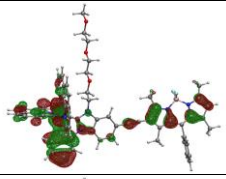
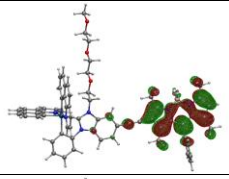
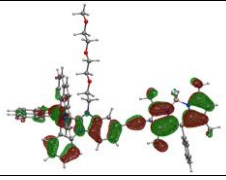
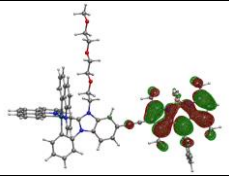
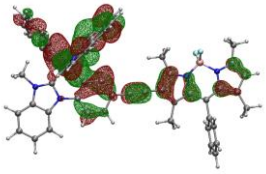
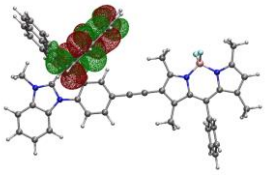
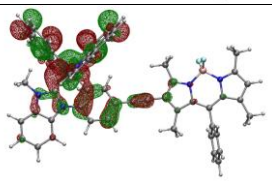
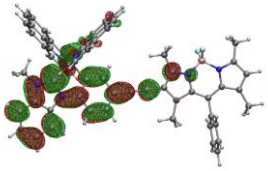
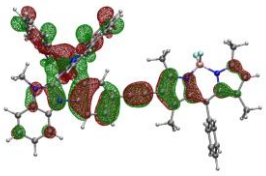
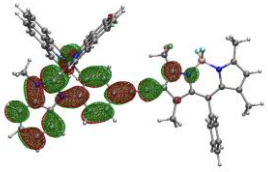
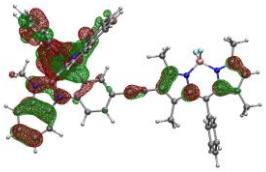
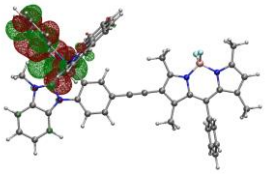
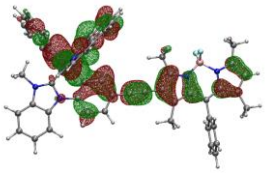
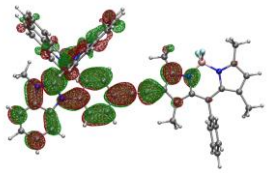
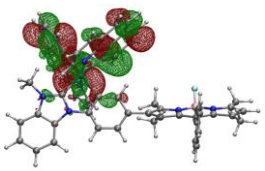
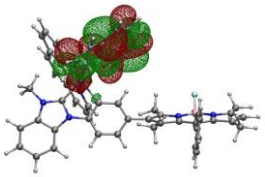
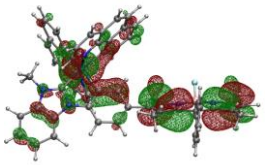
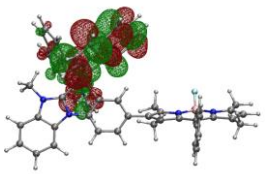
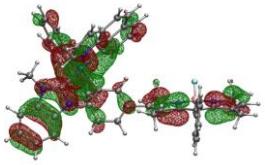
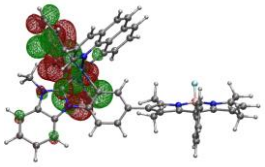
Ir3	S_2 482 nm f = 0.0055		
	S_3 424 nm *f* = 0.1796		
	S_4 413 nm *f* = 0.1221		
	S_7 395 nm *f* = 0.3579		
Ir4	S_2 500 nm *f* = 0.0396		
	S_3 452 nm *f* = 0.1633		
	S_7 401 nm *f* = 0.3959		
Ir5	S_2 496 nm *f* = 0.0862		
	S_3 447 nm *f* = 0.1648		
	S_7 399 nm *f* = 0.3930		

Table S2. NTOs of the hole and electron of the high energy absorption band transitions of complexes **Ir1-Ir5**, calculated by the TD-DFT method with PBE1PBE functional, LANL2DZ/6-31G* basis using toluene as the solvent.

	S_n	Hole	Electron
Ir1	S_{13} 350 nm f = 0.1222		
	S_{18} 340 nm f = 0.0389		
	S_{21} 332 nm f = 0.2753		
	S_{23} 322 nm f = 0.0901		
	S_{30} 309 nm f = 0.3708		
Ir2	S_{18} 334 nm f = 0.1076 (60%)		
	Homo-1/Lumo+1 (33%)		
	S_{22} 325 nm f = 0.1125		

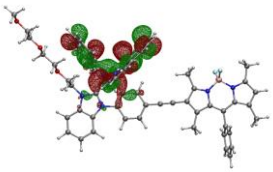
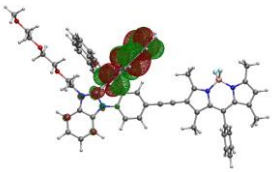
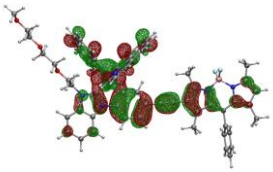
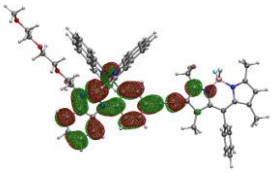
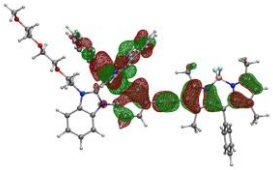
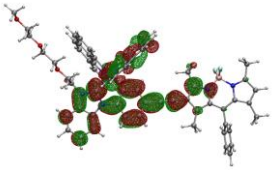
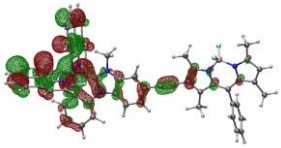
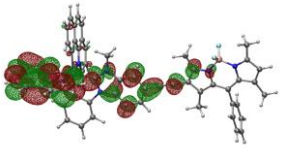
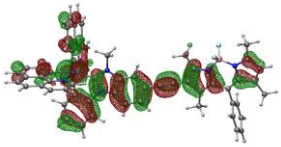
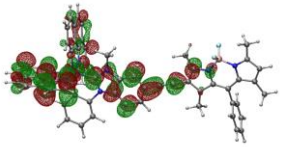
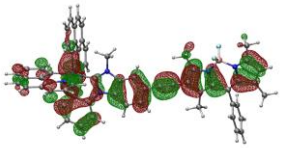
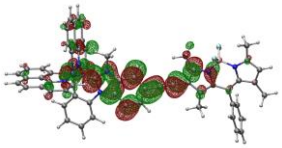
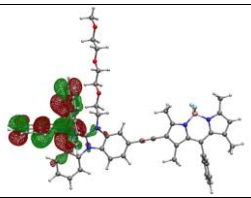
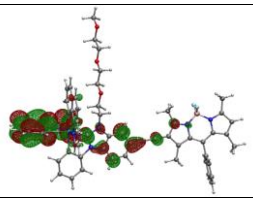
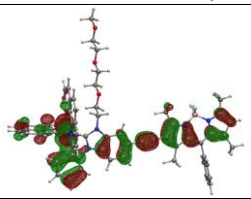
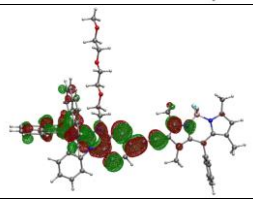
Ir3	S_{20} 333 nm f = 0.1144		
	S_{21} 332 nm f = 0.2474		
	S_{30} 309 nm f = 0.3345		
Ir4	S_{21} 334 nm f = 0.3787 (50%)		
	Homo-1/Lumo+1 (28%)		
	S_{27} 315 nm f = 0.4822		
Ir5	S_{21} 334 nm f = 0.2816		
	S_{27} 316 nm f = 0.3603		

Table S3. NTOs of the optimized T_1 state. All excited state calculations were done with the PBE1 functional with LAN2DZ/6-31G* basis set and toluene as a solvent.

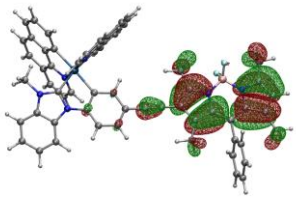
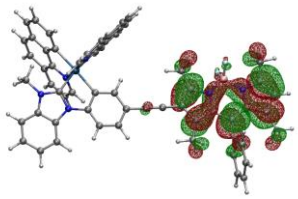
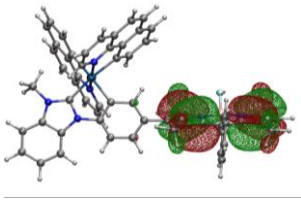
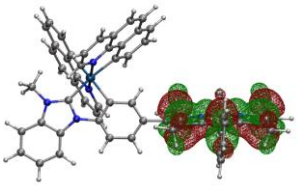
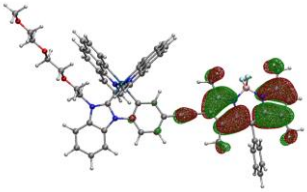
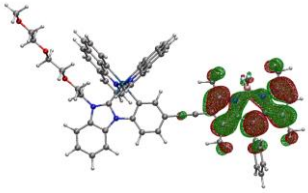
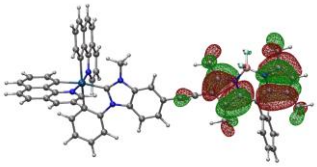
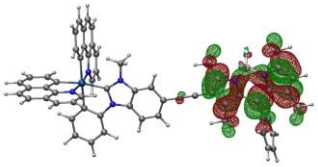
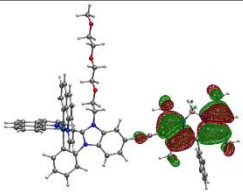
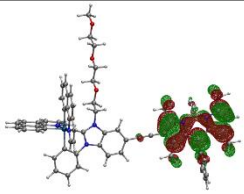
T_1	Hole	Electron
Ir1 1121 nm		
Ir2 1192 nm		
Ir3 1122 nm		
Ir4 1143 nm		
Ir5 1143 nm		

Table S4. Emission characteristics of complexes **Ir1**-**Ir5** in different solvents at room temperature.

	λ_{em} / nm)(τ_{em} / μs); Φ_{em}			
	CH_3CN	THF	CH_2Cl_2	Toluene
Ir1	575 (0.21); 0.31%	608 (0.07); 0.89%	603 (0.05); 0.97%	610 (4.96); 4.11%
Ir2	559 (2.46); 3.01%	561 (4.19); 2.09%	547 (2.83); 5.46%	538 (3.12); 6.32%
Ir3	575 (0.61); 0.74%	560 (3.57); 0.91%	563 (0.06); 0.86%	610 (5.26); 4.69%
Ir4	578 (2.81); 6.61%	560 (3.57); 6.65%	571 (2.71); 5.27%	579 (4.77); 3.36%
Ir5	573 (0.41); 1.27%	561 (3.63); 5.45%	570 (3.61); 11.7%	579 (4.89); 10.1%

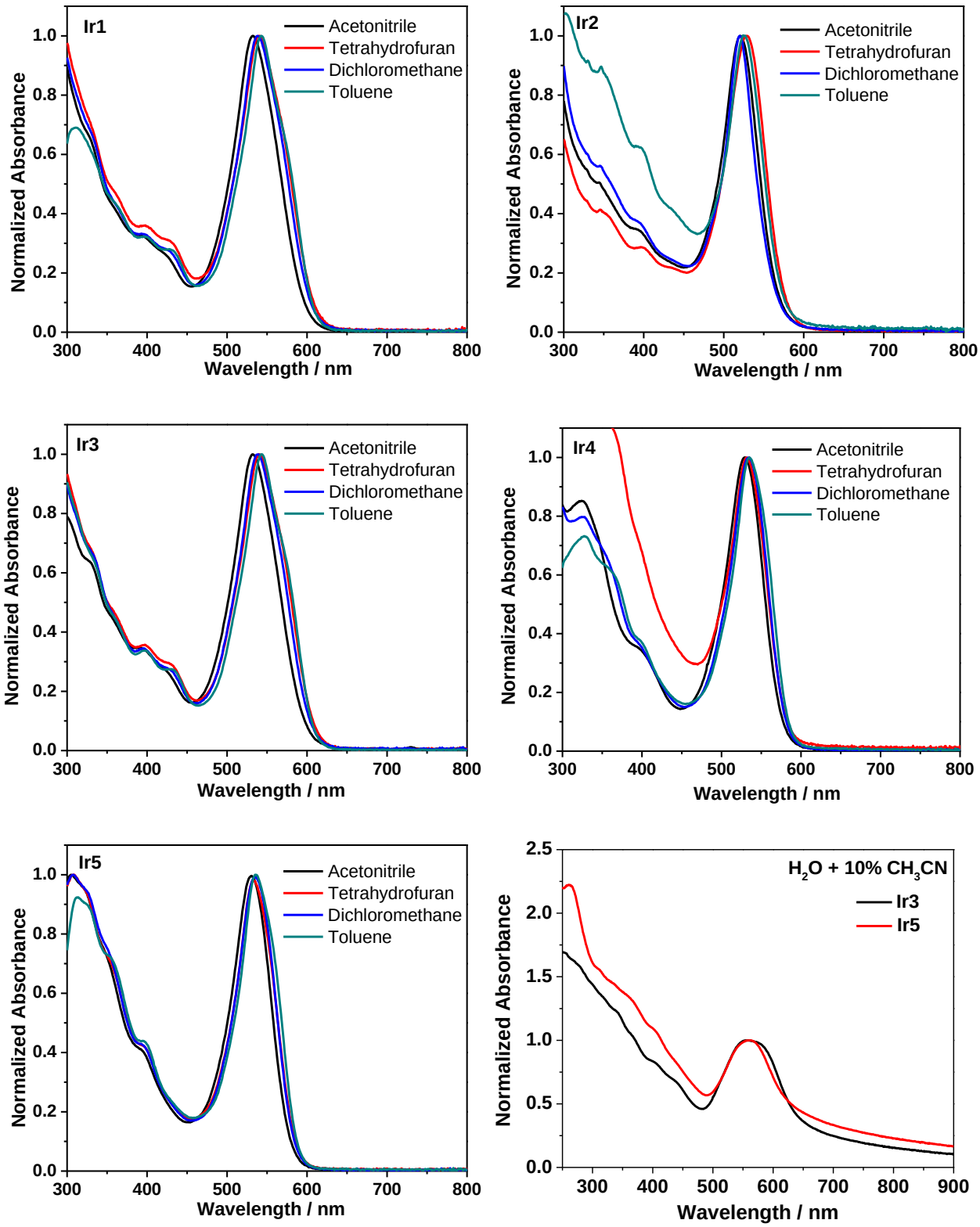


Figure S1. Normalized UV-vis absorption spectra of **Ir1-Ir5** in different solvents. The spectra for **Ir3** and **Ir5** in H_2O + 10% CH_3CN are normalized at 560 nm.

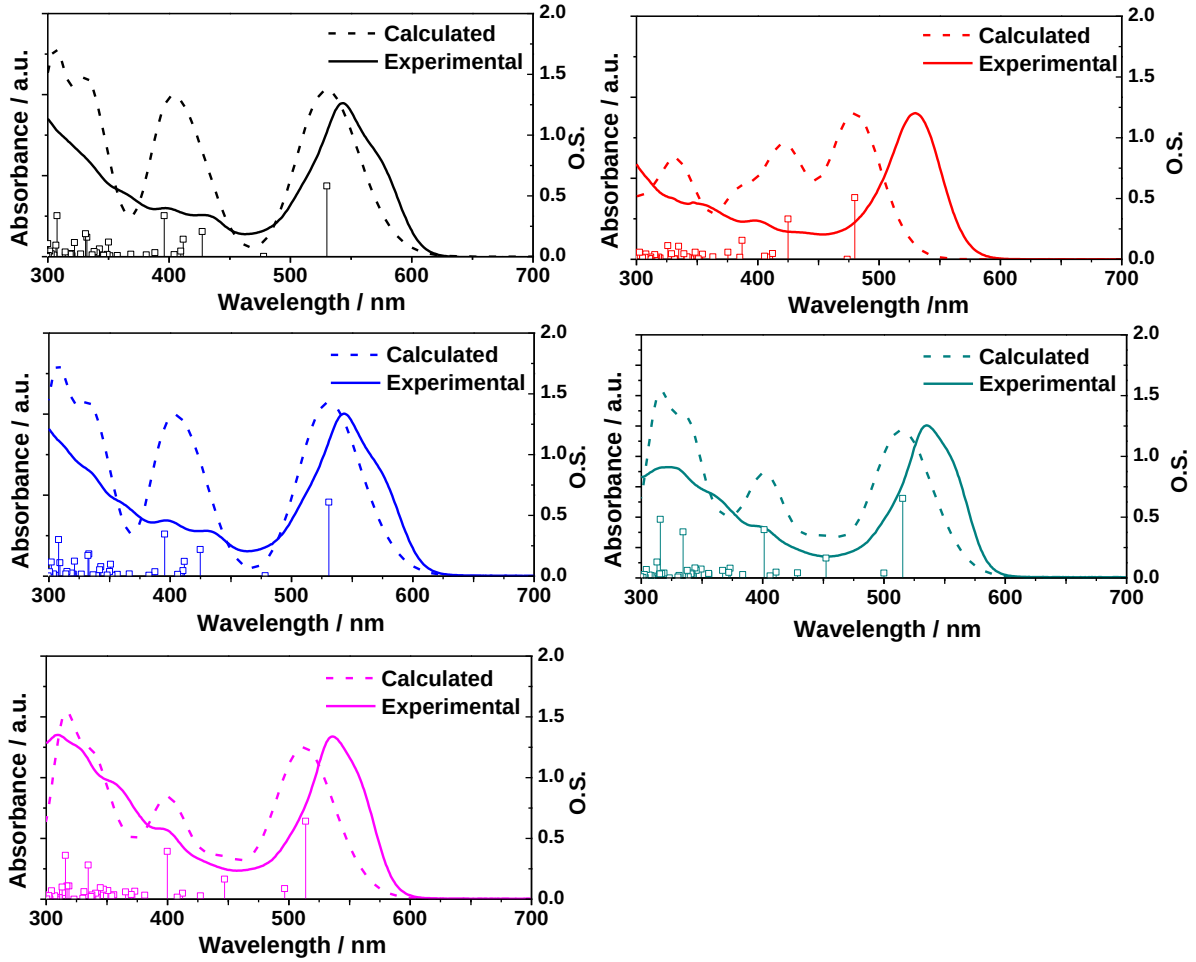


Figure S2. Normalized experimental and calculated absorption spectra with the respective oscillation strength. All absorption spectra are calculated by using the linear response time dependent DFT (TD-DFT) with the PBE1 functional and LAN2DZ/6-31G* basis set. Toluene are used as a solvent. Vertical bar indicates the oscillation strength of the transitions.

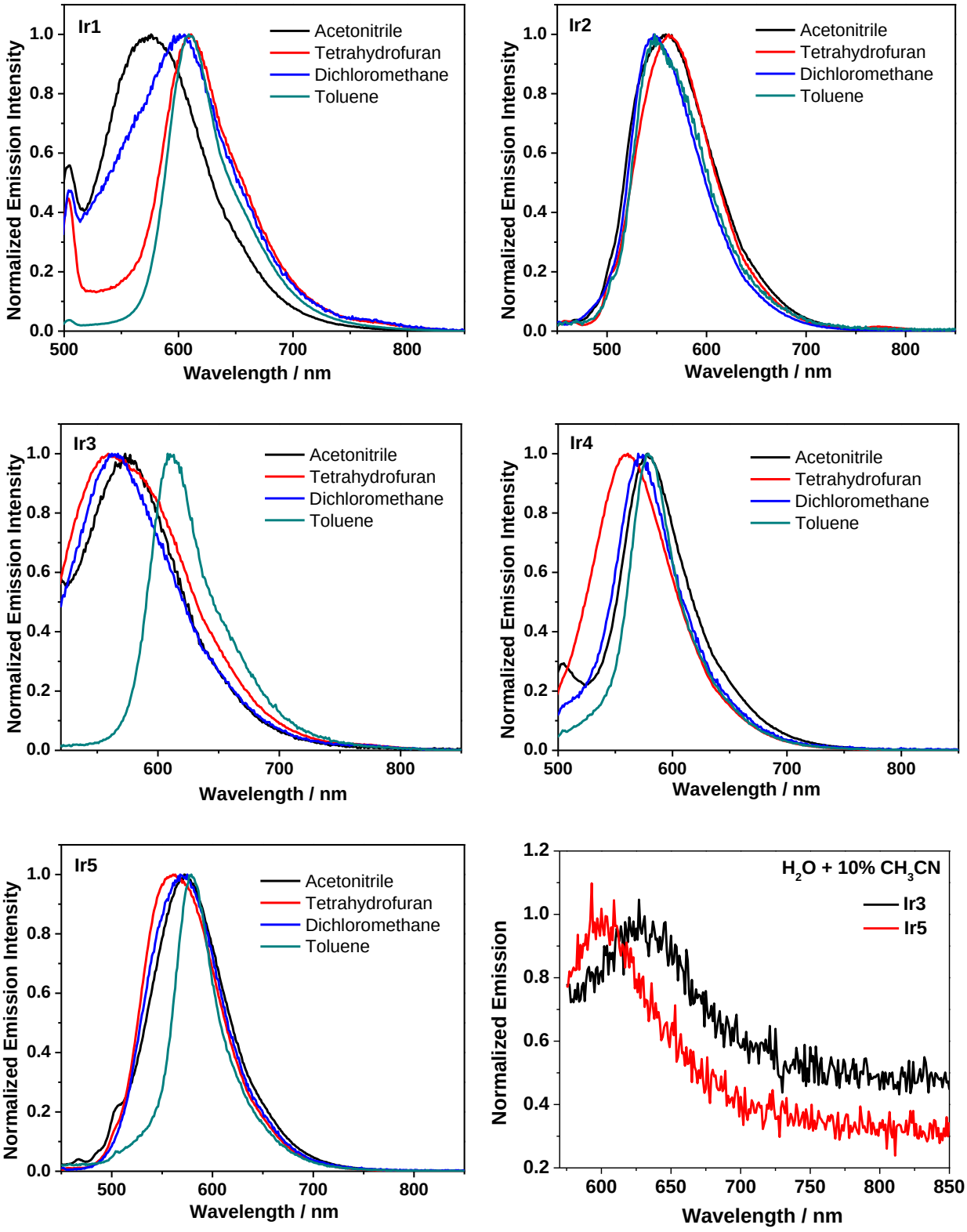


Figure S3. Normalized emission spectra of complexes **Ir1**-**Ir5** in different solvents at r.t. (λ_{ex} = 436 nm). The spectra of **Ir3** and **Ir5** in H_2O + 10% CH_3CN are obtained at λ_{ex} = 550 nm.

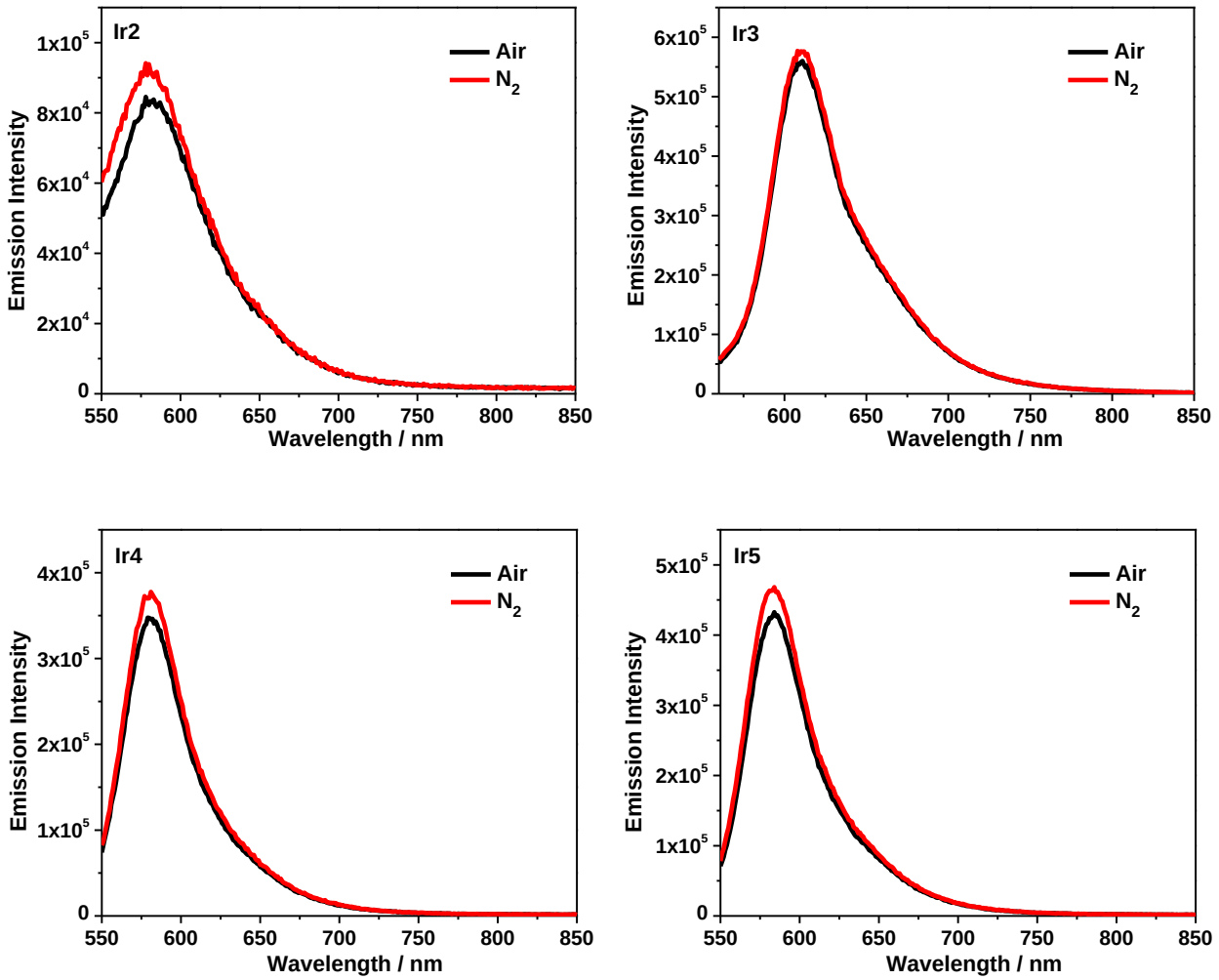


Figure S4. Comparison of the emission spectra in air-saturated and deaerated (degassed with N_2 for 40 min) toluene solution of **Ir2 – Ir5**. λ_{ex} was 530 nm for **Ir2**, 543 nm for **Ir3**, and 535 nm for **Ir4** and **Ir5**. $c = 5\times10^{-6}$ mol.L^{-1}.

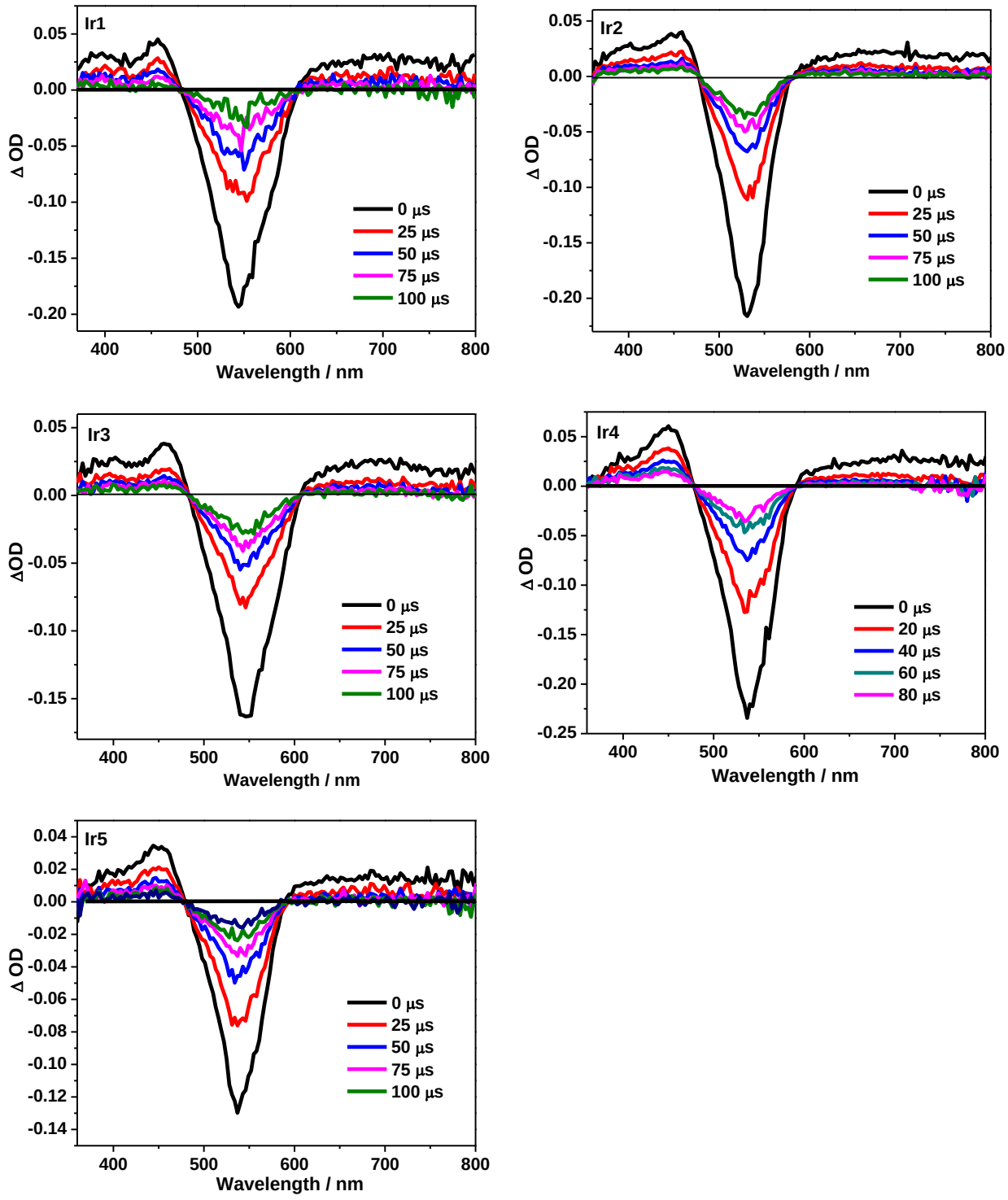


Figure S5. Nanosecond time-resolved transient differential absorption spectra of complexes **Ir1-Ir5** in toluene.