

Supporting information

Materials and General Instruments

Doubly purified water used in all experiments is from Milli-Q systems. Other solvents and reagents were of analytical grade and used without further purification. Stock of ct-DNA, chymotrysin, lysozyme, human serum albumin (HSA), protease and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (USA). RNA (*S. cerevisiae* RNA) was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. Hoechst 33258, SYTO RNA-select were purchased from Molecular Probes. DNase and RNase were purchased from Sigma.

^1H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm. Mass spectrometric data were obtained on a Q-ToF MS spectrometer (Micromass, Manchester, England). Absorption spectra were measured on a Perkin-Elmer Lambda35 UV-Vis spectrophotometer. Luminescence measurements were performed on a VARIAN CARY Eclipse Fluorescence Spectrophotometer.

General Methods

All reactions were carried out under a nitrogen atmosphere. Silica gel (200-300 mesh) was used for flash column chromatography. Phosphate buffered saline (PBS) (pH = 7.5, 20 mM) was prepared using doubly purified water. Stock of ct-DNA, chymotrysin, lysozyme, human serum albumin (HSA), protease and bovine serum albumin (BSA) are prepared by dissolving them in doubly purified water. The concentration of ct-DNA is determined spectrophotometrically using the molar absorption coefficients of $\epsilon_{260\text{ nm}} = 6600\text{ M}^{-1}\text{ cm}^{-1}$. 1 mM stock solutions of **RuIr**, **RuRu** and **IrIr** were prepared in DMSO for the absorption and emission spectral measurements. In the absorption and emission titration studies, the solutions of the compounds (2 μM) are titrated with aliquots of RNA solutions to reach the different molar ratio to the dyes. Concentration of RNA in working solutions was 1 mM.

Photostability of RuIr in solution

RuIr and SYTO RNA-select were dissolved in DMSO at a concentration of 10.0 μM , respectively. The solutions were irradiated under a 500W iodine-tungsten lamp for 25 min at a distance of 250 mm away. An aqueous solution of sodium nitrite (50.0 g/L) as heat filter was placed between the samples and the lamp as a light filter (to cut off the light shorter than 400 nm). The photostabilities were expressed in the terms of remaining absorption (%) calculated from the changes of emission at the emission maximum before and after irradiation by iodine-tungsten lamp. The emission was determined on VARIAN CARY Eclipse Fluorescence Spectrophotometer.

Interaction of RuIr with Biomolecules

The interaction of **RuIr** with RNA, DNA Chymotrysin, Lysozyme, BSA, HSA and Protease was carried out in a phosphate buffered saline (PBS) (pH=7.5, 20 mM) using luminescence detection. The emission was determined on VARIAN CARY Eclipse Fluorescence Spectrophotometer.

Cell culture and staining

MCF-7 and Hella and LO2 cells were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). One day before imaging, cells were seeded in 24-well flat-bottomed plates. The next day, the live cells were incubated with 20 μM **RuIr**, **RuRu** and **IrIr** for 2 h at 37 °C under 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times. Fluorescence imaging was then carried out with a TCS-SP2 confocal laser scanning microscope (Leica, Germany), using a 100 \times objective lens. Before DNase and RNase digest and colocalization experiments, cultured cells grown on special confocal microscope dish were fixed by precooled methanol (-20 °C) for 15 min, washed with PBS for 5 min twice. For DNase and RNase digest test, three sets of pretreated MCF-7 cells were stained with 20 μM **RuIr** for 10 min. A total of 100 μL clean PBS (as control experiment), 30 $\mu\text{g/mL}$ DNase (Sigma), or 25 $\mu\text{g/mL}$ DNase-Free RNase (GE) was added into the three adjacent wells and incubated at 37 °C in 5% CO_2 for 1 h. Cells were rinsed by clean PBS twice more before imaging. For each dye test, the fluorescent imaging pictures were obtained with an equal parameter for control. For cells colocalization experiment: fixed MCF-7 cells were stained with 20 μM **RuIr** for 10 min. After rinsing with PBS twice, the same sample was stained with 2 μM Hoechst 33258 and SYTO RNA-select for 10 min and then imaged.

Synthetic procedures

Synthesis of metal complexes

[Ru(bpy)₃-(CH₂)₁₀-Ru(bpy)₃]⁴⁺ and **[Ir(F₂ppy)₂-(CH₂)₁₀-Ir(F₂ppy)₂]²⁺**. The dimetallic complexes **[Ru(bpy)₃-(CH₂)₁₀-Ru(bpy)₃]⁴⁺** and **[Ir(F₂ppy)₂-(CH₂)₁₀-Ir(F₂ppy)₂]²⁺** were synthesized using a published method^{S1, 27, 28} with suitable modification.

[Ru(bpy)₃-(CH₂)₁₀-Ir(F₂ppy)₂]³⁺. The syntheses of **[Ru(bpy)₃-(CH₂)₁₀-Ir(F₂ppy)₂]³⁺** were accomplished as described in Scheme S1. Under nitrogen atmosphere, **[Ru(bpy)₃]2** (231 mg, 0.44 mmol) and bpy-C₁₀-bpy (200 mg, 0.44 mmol) were refluxed in a mixture ethanol-H₂O (8:1, 45 mL) for 24 h. After cooled and solvent removal, purification by preparative tin layer chromatography using as eluent the mixture CH₃COCH₃/H₂O/saturated KNO₃ solution (15/1/1) gave Ru-C₁₀-bpy (386 mg, 70%). Then a mixture of (F₂ppy)₂Ir(m-Cl)₂Ir(F₂ppy)₂ (300 mg, 0.24 mmol) and Ru-C₁₀-bpy (554 mg, 0.48 mmol) were heated to reflux in a dichloromethane-ethanol mixture (3:1, 25 mL) for 4 h. After the solution was cooled to room temperature and filtered, a solution of NH₄PF₆ (3.2 g, 19.6

mmol) in water (ca. 200 mL) was added slowly to the filtrate. The resulting precipitate was collected by filtration. The solid was washed with CHCl_3 and diethyl ether. The solid was dried at 80 °C for 24 h to afford the desired compound as a red solid (826 mg, 92%). ^1H -NMR (400 MHz, δ ppm, Acetone- d_6): 1.25-1.45 (m, 12H), 1.62-1.82 (m, 4H), 2.83-2.94 (m, 4H), 5.79 (dd, $J = 8.5, 2.3$ Hz, 2H), 6.67-6.85 (m, 2H), 7.24 (m, 2H), 7.45 (dd, $J = 5.9, 1.6$ Hz, 1H), 7.53-7.64 (m, 6H), 7.68-7.78 (m, 1H), 7.84-7.96 (m, 3H), 7.98-8.11 (m, 8H), 8.14-8.25 (m, 6H), 8.30-8.42 (m, 3H), 8.65-8.94 (m, 8H). ^{13}C NMR (100 MHz, Acetone- d_6): δ 29.99, 30.06, 34.92, 35.11, 98.66, 113.63, 113.79, 123.52, 123.72, 124.16, 124.31, 124.40, 124.55, 125.02, 125.21, 125.82, 127.73, 127.78, 127.84, 127.92, 128.78, 128.83, 137.95, 139.74, 140.09, 149.64, 149.75, 150.31, 150.95, 151.05, 151.68, 151.74, 151.79, 154.95, 155.51, 155.97, 156.76, 157.13, 157.24, 157.27, 157.31, 157.39, 163.87. HRMS (ESI, m/z): $1/2[(\text{M}-2\text{PF}_6)^+]$ calculated for $\text{C}_{72}\text{H}_{62}\text{N}_{10}\text{F}_{10}\text{PRuIr}$ 791.1705, found 791.1732.

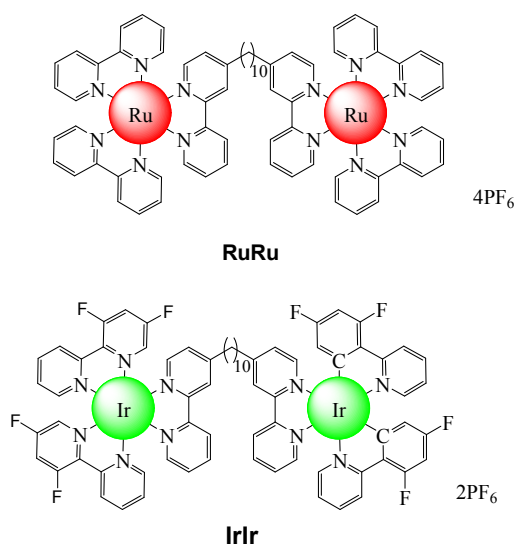
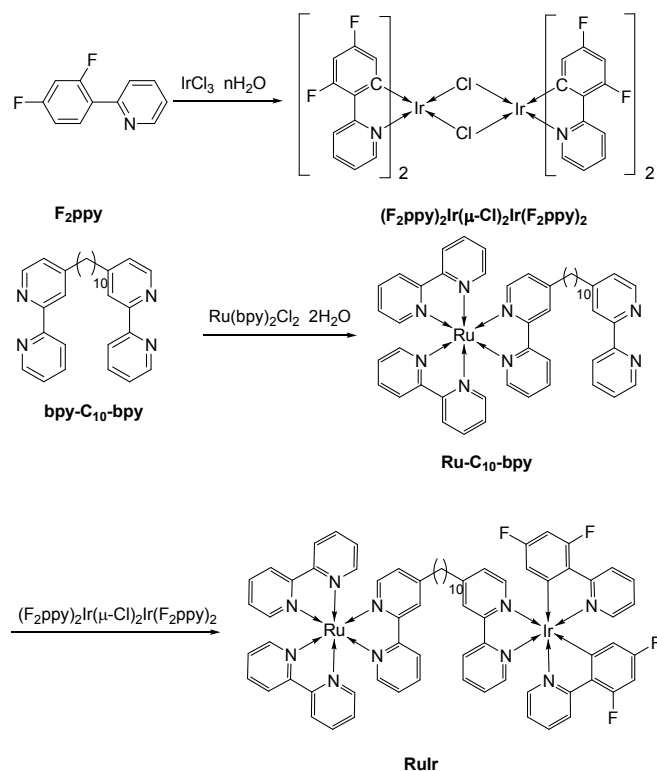


Fig. S1 The chemical structures of **RuRu** and **IrIr**.

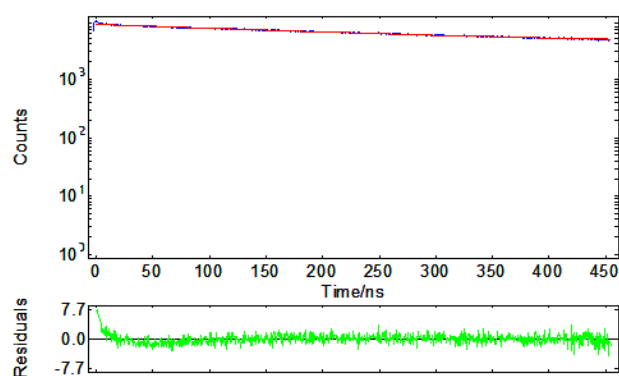


Fig. S2 Emission intensity decay for **RuIr** (10.0 μM) in acetonitrile at room temperature.

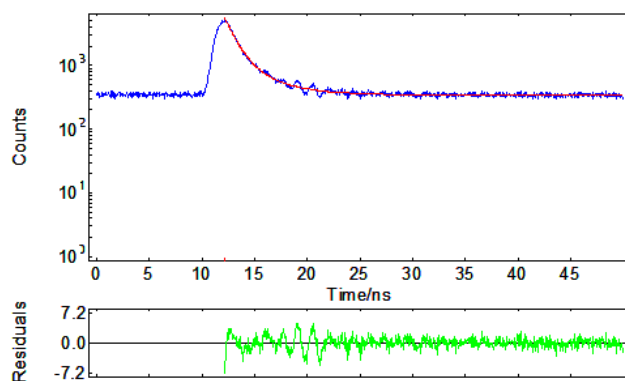


Fig. S3 Emission intensity decay for SYTO RNA-select (10.0 μM) in acetonitrile at room temperature.

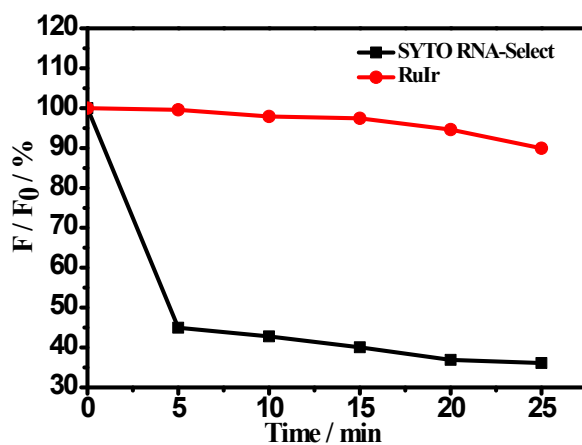


Fig. S4 Photostabilities of **RuIr** and SYTO RNA-select in solution.

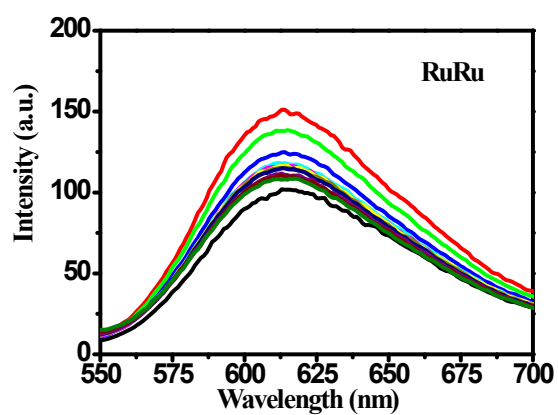


Fig. S5 The luminescent spectra of **RuRu** (2.0 μM) in phosphate buffered saline (PBS) (pH=7.5, 20 mM) at 298 K with the addition of RNA.

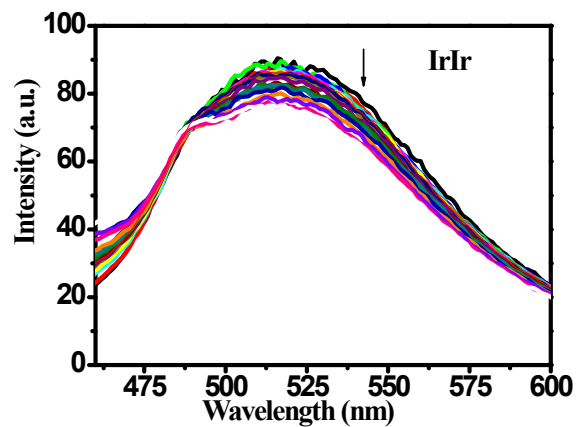


Fig. S6 The luminescent spectra of **IrIr** (2.0 μM) in phosphate buffered saline (PBS) (pH=7.5, 20 mM) at 298 K with the addition of RNA.

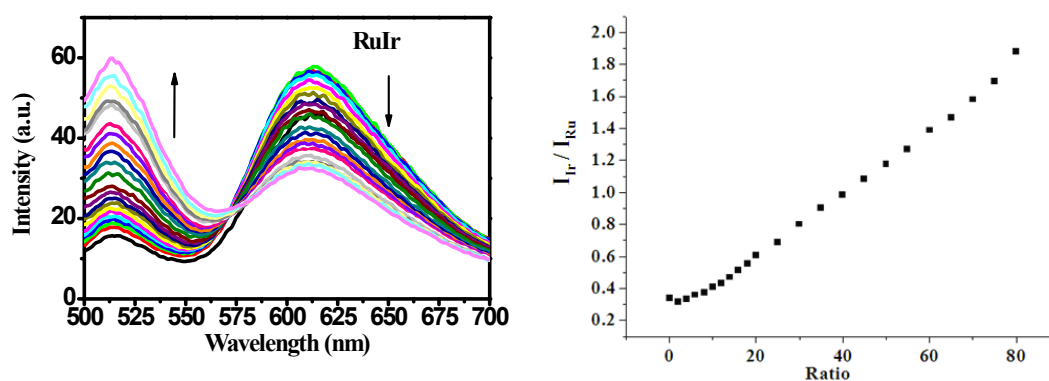


Fig. S7 The luminescent spectra of **RuIr** (2.0 μM) in phosphate buffered saline (PBS) (pH=7.5, 20 mM) at 298 K with the addition of RNA and the ratiometric luminescence response between **RuIr** (2.0 μM) to RNA, “Ratio” means $[RNA]/[RuIr]$, I_{Ir}/I_{Ru} stands for the ratio of the luminescence intensity of iridium moiety (523 nm) and ruthenium moiety (615 nm)..

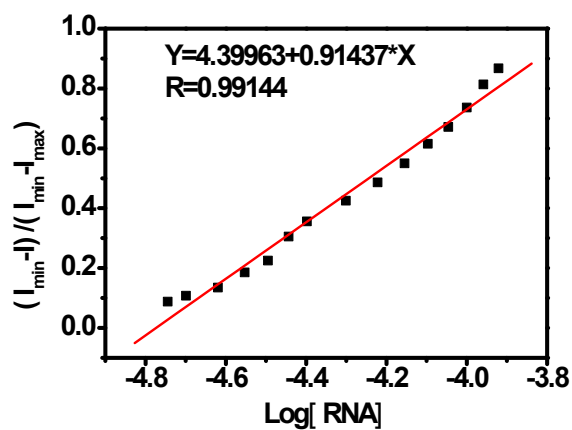


Fig. S8 A linear calibration curve between the I_{Ir}/I_{Ru} ratio and RNA amount, “I” refers to I_{Ir}/I_{Ru} .

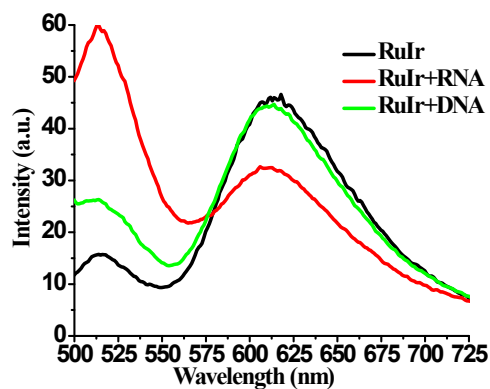


Fig. S9 Emission spectra of **RuIr** (2.0 μM) in the presence and absence of 80-fold excess RNA and DNA.

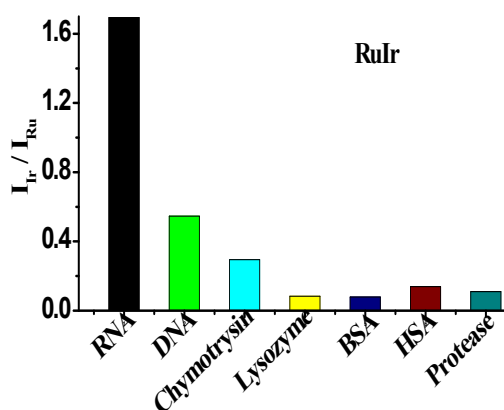


Fig. S10 Emission spectra of **RuIr** (2.0 μM) in phosphate buffered saline (PBS) (pH=7.5, 20 mM) at 298 K in the presence and absence of 80-fold excess RNA, DNA, chymotrysin, lysozyme, BSA, HSA and protease. $I_{\text{Ir}}/I_{\text{Ru}}$ stands for the ratio of the luminescence intensity of iridium moiety (523 nm) and ruthenium moiety (615 nm).

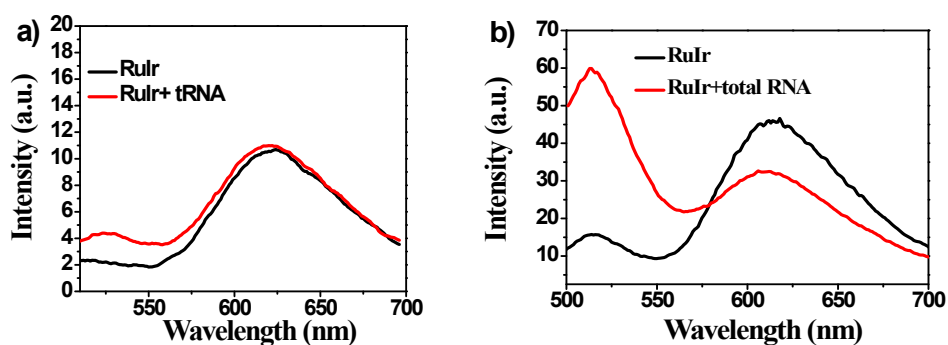


Fig. S11 a) Emission spectra of **RuIr** (0.33 μM) in phosphate-buffered saline (PBS; pH 7.5, 20 mM) in the presence or absence of 80-fold excess tRNA. b) Emission spectra of **RuIr** (2.0 μM) in phosphate-buffered saline (PBS; pH 7.5, 20 mM) in the presence or absence of 80-fold excess total RNA.

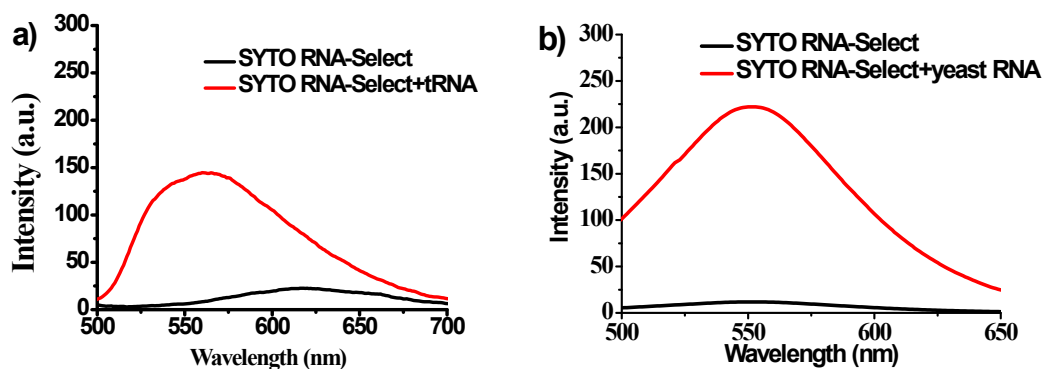


Fig. S12 a) Emission spectra of SYTO RNA-Select (5.0 μM) in phosphate-buffered saline (PBS; pH 7.5, 20 mM) in the presence or absence of 2-fold excess tRNA. b) Emission spectra of SYTO RNA-Select (5.0 μM) in phosphate-buffered saline (PBS; pH 7.5, 20 mM) in the presence or absence of 2-fold excess yeast RNA.

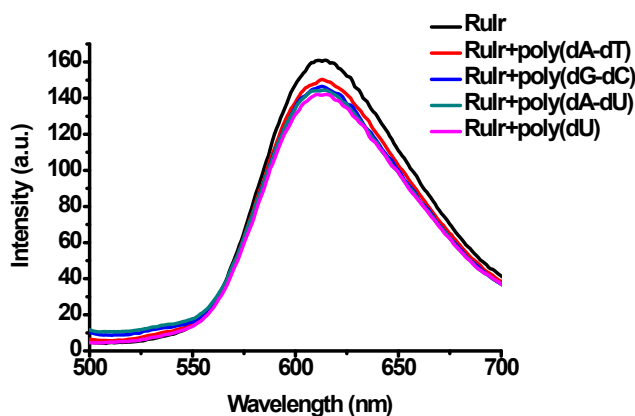


Fig. S13 Emission spectral of complex **RuIr** (2 μM) in PBS (pH=7.5, 20 mM) at 298 K in the presence of 20-fold excess poly(dA-dT), poly(dG-dC), poly(dA-dU) and poly(dU).

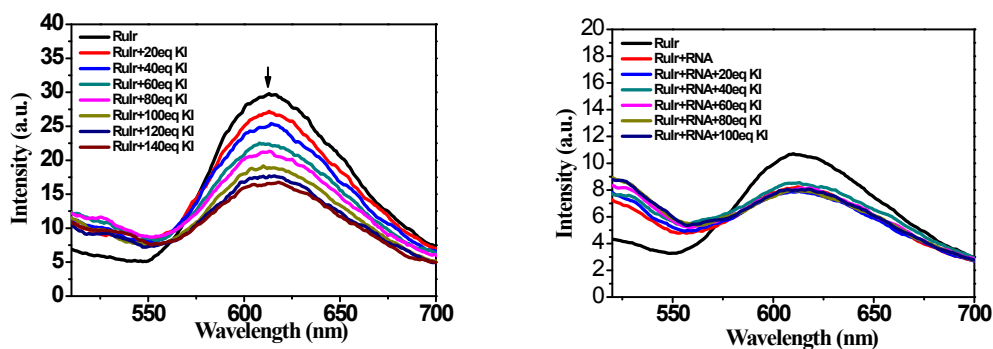


Fig. S14 Emission spectra of complex **RuIr** (0.33 μM) alone and with the presence of 60 eq RNA in phosphate buffered saline (PBS) (pH=7.5, 20 mM) with the addition of KI.

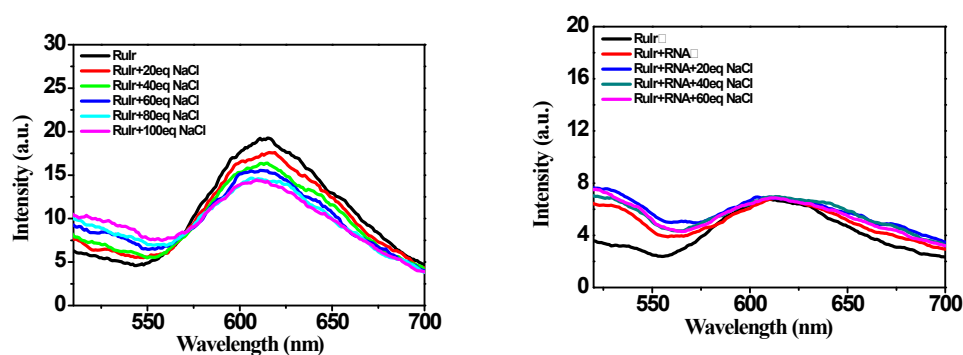


Fig. S15 Emission spectra of complex **RuIr** (0.33 μ M) alone and with the presence of 60 eq RNA in phosphate buffered saline (PBS) (pH=7.5, 20 mM) with the addition of NaCl.

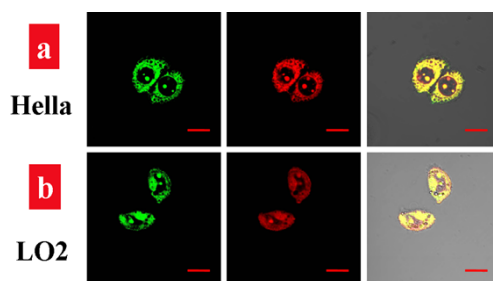


Fig. S16 Luminescence microscope images of live a) Hella cells b) and LO2 cells incubated with 20 μ M **RuIr** for 2h at 37 $^{\circ}$ C. Scale bar: 20 μ m.

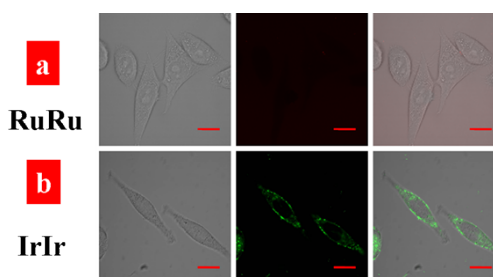


Fig. S17 a) Incubation of live MCF-7 cells with **RuRu** (20 μ M, 2 h) shows no staining of live cells. The red channel was stained with **RuRu** with excitation at 458 nm and a scan range of 590–640 nm. b) Live MCF-7 breast cancer cells incubated with **IrIr** (20 μ M, 2 h). The green channel was stained with **IrIr** with excitation at 405 nm and a scan range of 510–560 nm. From left to right: Optical, luminescence, and overlay images. Scale bar : 20 μ m.

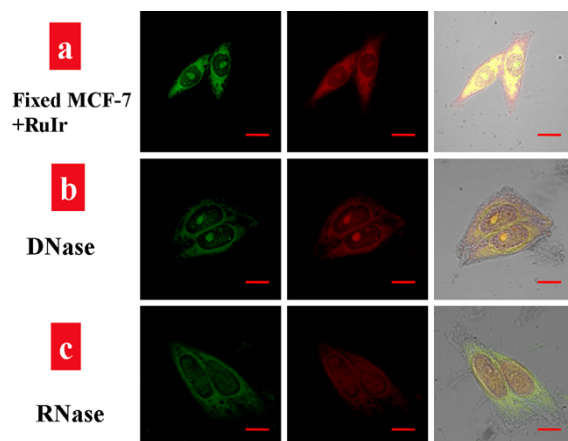


Fig. S18 Treatment of fixed MCF-7 cells with DNase and RNase. **RuIr** was used at a concentration of 20 μM . From left to right: green channel (500–560 nm), red channel (590–640 nm), and overlay images. Scale bar: 20 μm .

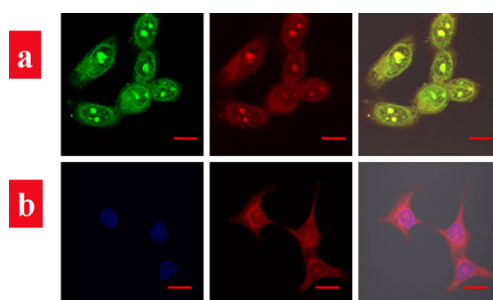


Fig. S19 Colocalization of **RuIr** (20 μM) and a) SYTO RNA-Select dye (5 μM) and b) Hoechst33258 (5 μM), images by fixed MCF-7 cell loading. a) Co-staining of **RuIr** with SYTO RNA-Select dye. Left, RNA-Select dye (green channel: 485–510 nm; excitation at 405 nm); middle, **RuIr** luminescence (red channel: 590–640 nm; excitation at 458 nm); right, merged image. b) Colocalization of **RuIr** and Hoechst33258. Left, Hoechst33258 (blue channel: 429–470 nm; excitation at 405 nm); middle, **RuIr** luminescence (red channel: 590–640 nm; excitation at 458 nm); right, merged image. Scale bar: 20 μm .