Supplementary Information

Addressing Multidrug-resistant Bacterial Infections with Iridium-based Theragnostic Agents

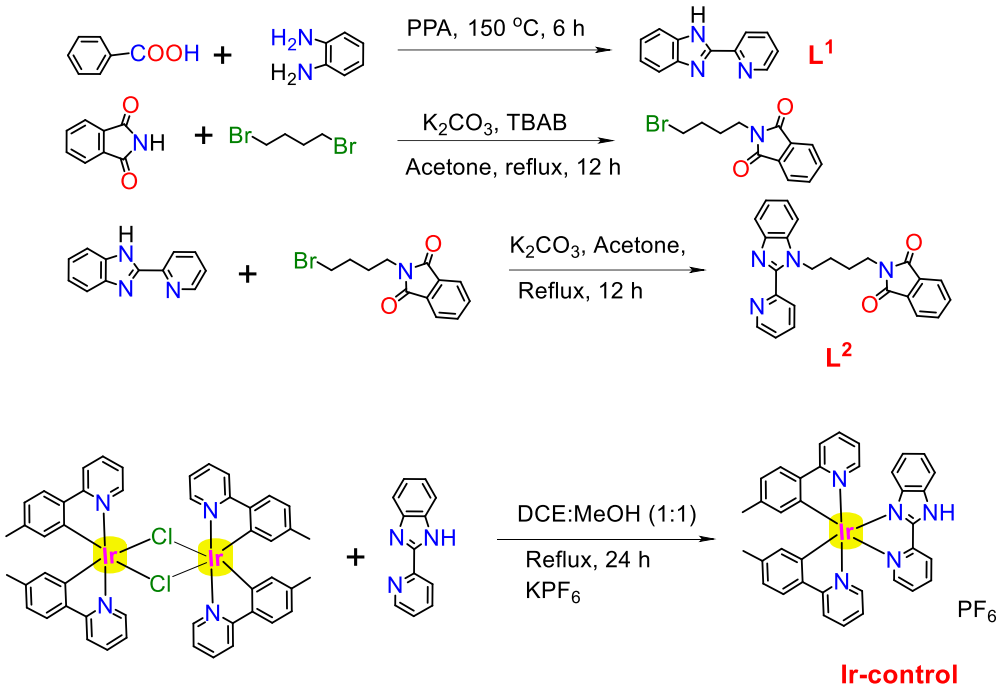
Rama Karn,[a] *Mahesmita Sahoo,*[†b] *Sayantani Biswas,*[†c] *Suravi Chauhan,*[a] *Ajay Kumar Nag,*[b] *Soham Basu,*[c] *Srikanta Patra,*[*b] *Debasis Manna*[*a,d]

[a]Centre for Environment, Indian Institute of Technology Guwahati, Assam 781039, India
[b]School of Basic Sciences, Indian Institute of Technology Bhubaneswar, Orissa 752050, India
[c]Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Assam 781039, India
[d]Department of Chemistry, Indian Institute of Technology Guwahati, Assam 781039, India

EXPERIMENTAL SECTION

The chemicals were received from commercial sources and used as received. All reactions were carried out under a dinitrogen environment using the standard Schlenk technique.

Synthesis of ligands and metal complexes — The benzimidazole-based ligand and its derivatization were carried out as described below (Scheme S1).[1, 2]



Scheme S1. Scheme for the synthesis of ligands and **Ir-control** complex.

Synthesis of ligand L^1 — The ligand L^1 was prepared as per the reported procedure with slight modifications.[2] The *o*-phenylenediamine (2.0 g, 18.4 mmol) and picolinic acid (1.88 g, 15 mmol) were taken in a 100 mL round-bottomed flask. To this, 20 g of polyphosphoric acid was added, and the reaction mixture was heated at 150 °C for 6 h. After that, the reaction mixture was poured into ice-cold water. The pH of the solution was adjusted to 7.0 by adding NaOH solution. The precipitate thus obtained was filtered, washed with distilled water, and dried under vacuum to yield a white solid of 2-phenyl-1H-benzimidazole (L^1). Yield: 2.1 g (72%).

Synthesis of ligand L^2 — The ligand L^2 was prepared in two steps as follows. First, phthalimide (1.0 g, 6.7 mmol) and potassium carbonate (7.0 g, 27 mmol) were combined in 20 mL of acetone and refluxed for 24 h to deprotonate. To this, tetrabutylammonium bromide (TBAB) (5.8 g, 27 mmol) and 1,4-dibromobutane (3.21 mL, 27 mmol) were added, and the reaction was continued for an additional 12 h. After the reaction was complete, the solvent

was removed, and the desired product was extracted with ethyl acetate. Evaporation of ethyl acetate followed by crystallization in ethanol led to the desired crystallized intermediate product N-(2-bromoethyl)phthalimide [(yield: 1.25 g (65%)].

In the second step, the ligand L^1 (500 mg, 2.55 mmol) and K_2CO_3 (1.4 g, 10 mmol) were dissolved in 20 mL of acetone, and the mixture was refluxed for 4 h to ensure complete deprotonation of benzimidazole nitrogen. Subsequently, the intermediate N-(2-bromoethyl)phthalimide (930 mg, 3.31 mmol) was added to the reaction mixture, which was then refluxed for an additional 24 h. Upon completion, the reaction mixture was passed through a celite pad to remove the base and insoluble impurities. The solvent was then evaporated under reduced pressure. The crude residue obtained was purified by column chromatography using hexane: ethyl acetate (70:30) as the eluent, affording the desired L^2 in pure form. Yield: 770 mg (78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J = 4.8 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H), 7.84 (ddd, J = 5.6, 4.8, 2.5 Hz, 4H), 7.75 – 7.69 (m, 2H), 7.50 – 7.46 (m, 1H), 7.33 (ddd, J = 11.0, 7.7, 3.5 Hz, 3H), 4.93 – 4.86 (m, 2H), 3.74 (t, J = 7.1 Hz, 2H), 2.03 – 1.95 (m, 2H), 1.85 – 1.73 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.39, 150.59, 149.74, 148.66, 142.60, 136.79, 136.56, 133.97, 132.06, 124.65, 123.73, 123.37, 123.25, 122.58, 120.12, 110.15, 44.96, 37.53, 27.50, 25.94.

Synthesis of complex — The dimeric $[(Me\text{-}ppy)_2IrCl]_2$ precursor was prepared following the reported procedure.[2]

Synthesis of Ir-control complex — In a round-bottomed flask, the dimeric $[(Me\text{-}ppy)_2Ir(\mu\text{-}Cl)]_2$ precursor (60 mg; 0.052 mmol) and L^1 (22 mg; 0.11 mmol) were taken in a 15 mL 1:1 dichloroethane/methanol (DCE/MeOH) mixture and kept at reflux for 24 h. After completion, the solvent was evaporated, the residue redissolved in 5 mL MeOH, and excess KPF6 was added, stirred for 30 minutes. To this, 10 mL of water was added, and the mixture was kept in the freezer overnight. The yellow precipitate was filtered, washed with a copious amount of cold water, and dried. The solid obtained was chromatographed on an alumina column using CH2Cl2:CH3CN (4:1) as the eluent. Evaporation of the solvent under reduced pressure resulted in a pure **Ir-control** complex. Yield 51 mg (55%). Molar conductivity $[\Lambda_M/(\Omega^{-1}cm^2M^{-1})]$ in CH_3OH: 80. HRMS ((+)-ESI): m/z 724.2028 corresponding to $\{[Ir\text{-}control] - PF_6\}^+$ (calculated molecular mass for 724.2052). : ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.50 (d, *J* = 8.0 Hz, 1H), 8.05 (t, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.82 (dd, *J* = 16.3, 8.6 Hz, 3H), 7.67 (dd, *J* = 15.7, 7.7 Hz, 2H), 7.62 – 7.53 (m, 3H), 7.45 (d, *J* = 5.8 Hz, 1H), 6.96 (dd, *J* = 14.2, 6.8 Hz, 2H), 6.92 – 6.81 (m, 5H), 6.25 – 6.14 (m, 3H), 2.16 (d, *J* = 3.1 Hz, 6H).

Synthesis of Ir-NH_2 complex — The **Ir-NH_2** complex was prepared in two steps:

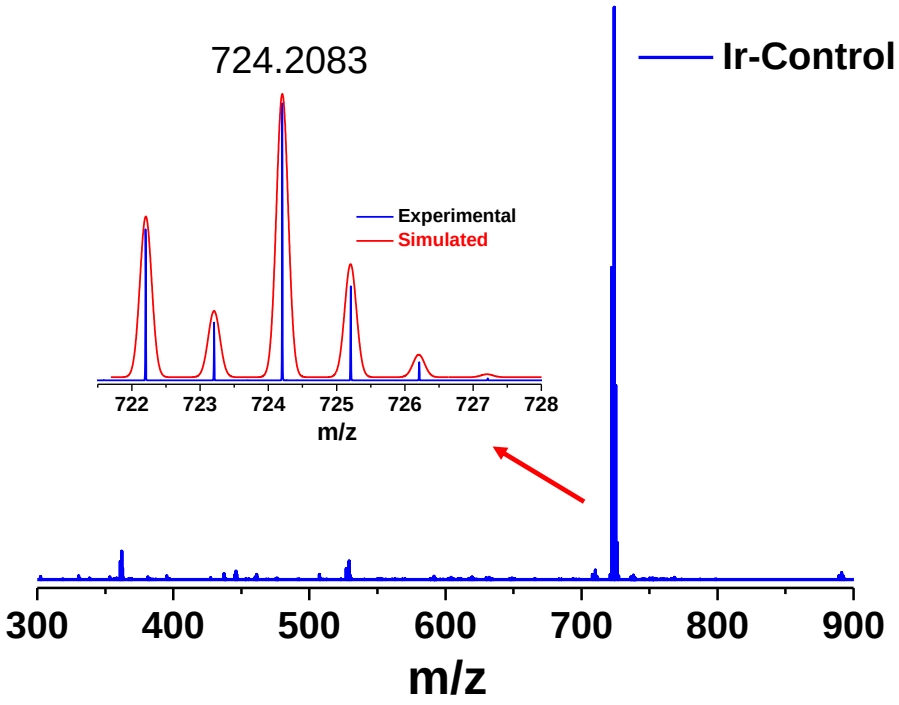
Step-1: In a round-bottomed flask, the dimeric $[(Me\text{-}ppy)_2Ir(\mu\text{-}Cl)]_2$ precursor (50 mg; 0.044 mmol) and L^2 (38 mg; 0.097 mmol) were taken in a 15 mL 1:1 dichloroethane/methanol (DCE/MeOH) mixture and kept at reflux for 24 h. After completion, the solvent was evaporated, the residue redissolved in 5 mL MeOH, and excess KPF_6 was added, stirred for 30 minutes. To this, 10 mL of water was added, and a colored precipitate was observed. The solution was kept in the freezer overnight. The precipitate was then filtered, washed with cold water, and dried. The precipitate was then purified by alumina column chromatography using CH_2Cl_2:CH_3CN (80:20) as the eluent. The solvent was then removed under reduced pressure, yielding an intermediate complex. Yield 80 mg (78 %).

Step-2: In a reaction vessel, the intermediate complex (50 mg, 0.046 mmol) from step-1 was dissolved in 5 mL CH_3CN, and to this, 10 mL hydrazine hydrate in 15 mL water was added. The reaction mixture was stirred for 12 h at 90 °C, yielding a yellow precipitate that was filtered, washed with copious amounts of water, and dried in air. The precipitate was chromatographed using an alumina column. The unreacted intermediate compound was eluted using CH_3CN, and the desired **Ir-NH_2** complex was eluted with methanolic KPF_6 solution. It was then dried, and the excess KPF_6 was removed by washing with water. Yield 30 mg (66%). Molar conductivity $[\Lambda_M/(\Omega^{-1}cm^2M^{-1})]$ in CH_3OH: 120. HRMS ((+)-ESI): m/z 795.2747 corresponding to $\{[Ir\text{-}NH_2] - PF_6\}^+$ (calculated molecular mass for 795.2787). ^{1}H NMR (400 MHz, DMSO) δ 8.70 (d, J = 8.2 Hz, 1H), 8.35 (t, J = 7.0 Hz, 3H), 8.21 (d, J = 8.2 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 5.1 Hz, 1H), 7.89 (dd, J = 14.0, 6.3 Hz, 1H), 7.82 (dd, J = 10.9, 5.6 Hz, 3H), 7.76 – 7.70 (m, 1H), 7.66 (d, J = 5.4 Hz, 2H), 7.43 (t, J = 7.7 Hz, 1H), 7.12 (dt, J = 13.1, 6.5 Hz, 2H), 7.05 (t, J = 7.7 Hz, 1H), 6.88 (dd, J = 15.8, 7.8 Hz, 2H), 6.25 (d, J = 8.3 Hz, 1H), 6.10 (s, 1H), 6.01 (s, 1H), 4.97 (s, 2H), 2.81 (s, 2H), 2.09 (s, 6H), 2.05 – 1.97 (m, 2H), 1.76 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 167.40 (s, J = 15.4 Hz), 152.36 (s), 151.47 (s), 149.75 (s), 149.49 (s), 148.40 (s), 146.79 (s), 142.35 (s), 141.98 (s), 140.56 (s), 139.91 – 139.60 (m), 139.34 (s), 138.90 (s), 138.75 (s), 136.76 (s), 132.82 (s), 132.04 (s), 129.03 – 128.78 (m), 126.56 (s), 126.13 (s), 125.25 (d, J = 15.7 Hz), 123.94 (s), 123.58 (d, J = 21.9 Hz), 120.02 – 119.91 (m), 119.64 (s), 117.48 (s), 113.30 (s), 45.38 (s), 38.58 (s), 26.97 (s), 24.47 (s), 22.03 (s).

HPLC analyses — The HPLC analyses were performed using a silica-based C18 column with a particle size of 5 µm. A water/acetonitrile mobile phase (1:1 v/v) was utilised with a flow

rate of 0.7 mL/min. Detection was carried out using 265 nm light. All compounds are >95% pure by HPLC.

FTIR analyses — All complexes and their polymeric conjugates were characterized using Fourier Transform Infrared (FTIR) spectroscopy. For each measurement, approximately 1 mg of the dried sample was placed directly on the attenuated total reflectance (ATR) crystal. The percent transmittance spectra were recorded over the range of 4000 to 400 cm^{-1}.



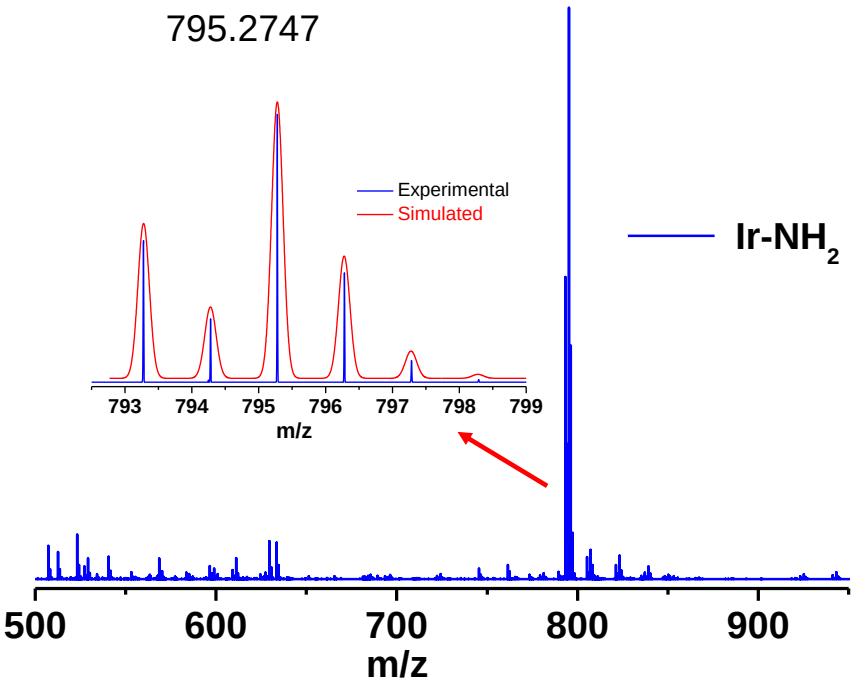


Fig. S1. HRMS of **Ir-NH$_2$** and **Ir-control** in CH_3OH.

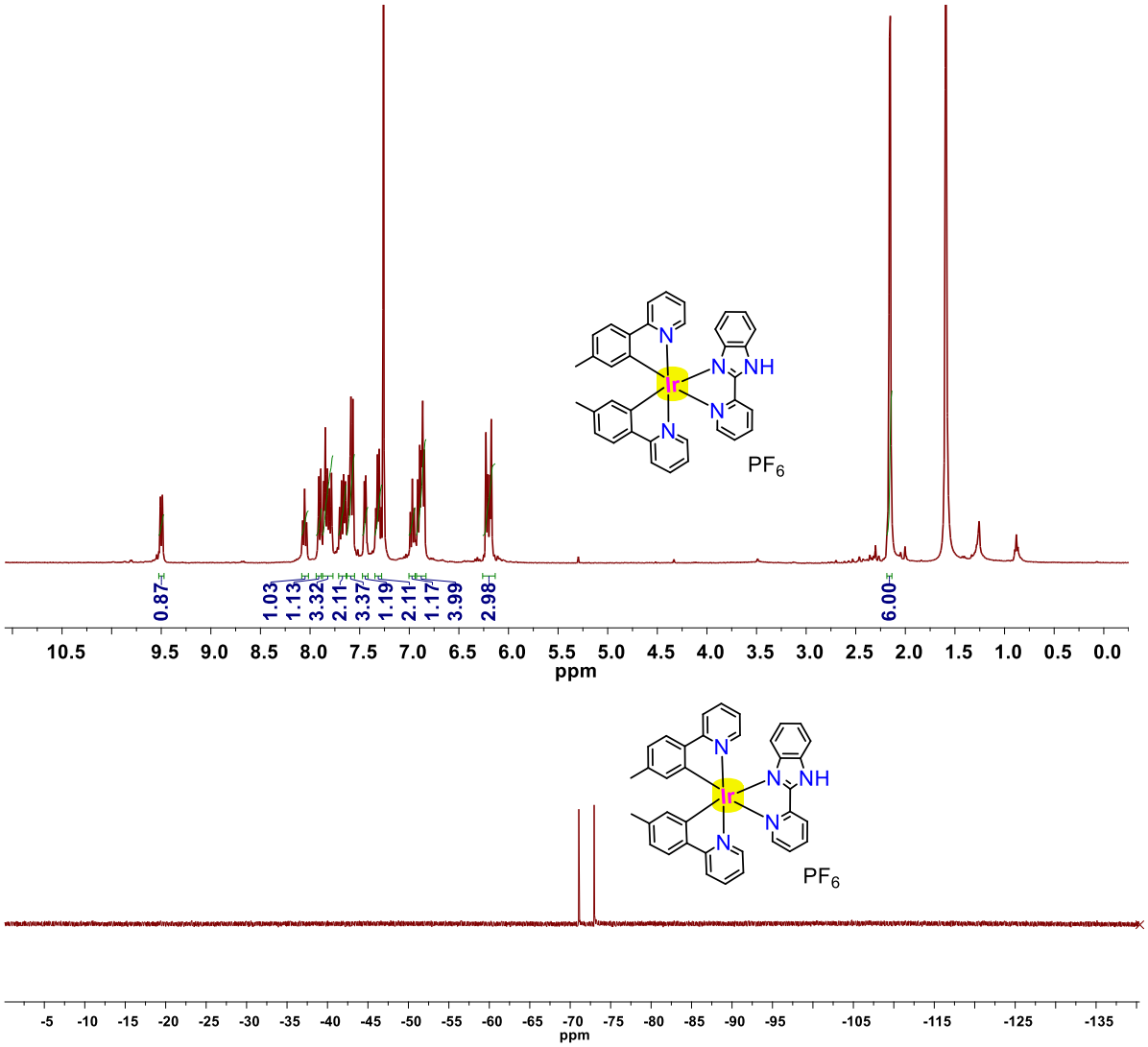


Fig. S2. ^{1}H and ^{19}F NMR spectra of **Ir-control** in $CDCl_3$.

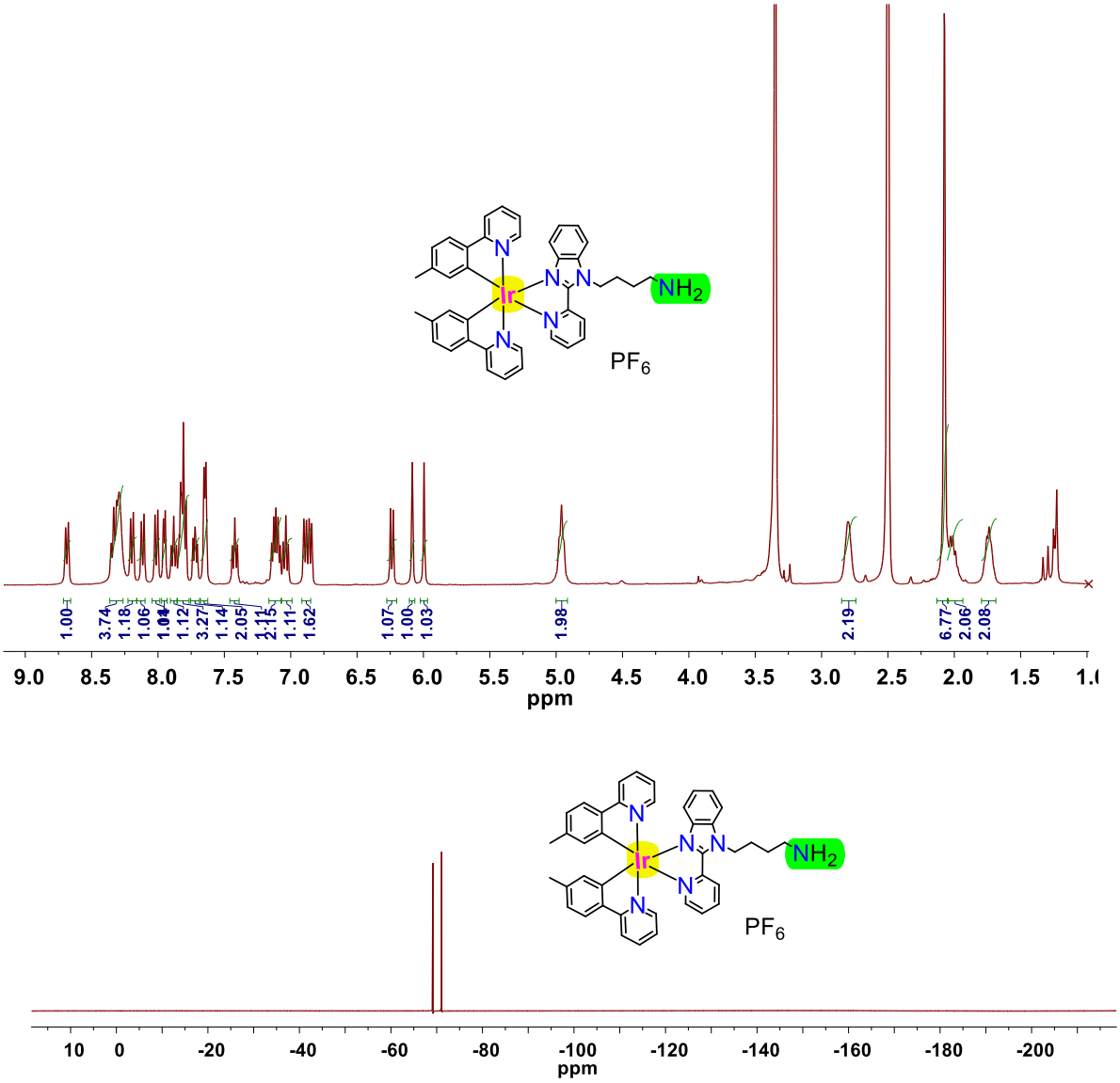


Fig. S3. ^{1}H and ^{19}F NMR spectra of **Ir-NH$_2$** in DMSO-d_6.

UV-Vis and fluorescence spectroscopic analyses — The photophysical properties of the complexes and their polymeric conjugates were evaluated using UV-Visible and fluorescence spectroscopic analyses. For the UV-Visible measurements, 20 µM solutions of each compound were prepared in 700 µL of Milli-Q water and transferred to a quartz cuvette. The absorbance spectra were then recorded over the wavelength range of 200 to 700 nm. For fluorescence measurements, 20 µM solutions of the compounds were prepared in 700 µL of Milli-Q water and placed in a quartz cuvette. The samples were excited at 400 nm, and the corresponding emission spectra were recorded.

Note: The measured quantum yield of the complexes Ir-control and Ir-NH_2 were 4.6% and 4.2% with respect to $Ru(bpy)_3^{2+}$ in an aqueous environment. The observed quantum yields of these complexes were consistent with those reported for iridium complexes.[3]

Determination of critical aggregation concentration — To determine the critical aggregation concentration (CAC) of **Ir-NH_2**, the fluorescence intensities with varying concentrations of the compound were recorded. The aqueous solution of the compound was excited at 400 nm, and its emission spectrum was recorded. The fluorescence intensity at λ_{max} = 562 nm was plotted against the compound concentration and fitted with a linear segment. The inflection point, indicated by a sudden change in slope, denoted the concentration after which nano-aggregation commenced and was delineated as the CAC.

DLS and Zeta potential measurements — The hydrodynamic diameter of the nanoaggregates was measured by dispersing the compounds in Milli-Q water and assessing their size at varying concentrations. The surface zeta potential was also analyzed using the same sample preparation method.

Field-emission scanning electron microscopy — The field-emission scanning electron microscopy (FESEM) technique was used to examine the morphology of the compounds. The samples were first diluted in Milli-Q water and then drop-cast onto a glass grid covered with aluminum foil. After drying overnight at 37 °C, the samples were sputter-coated with a gold solution, mounted on an FESEM stub, and analyzed using a Gemini 300 electron microscope (Zeiss).

Field-emission transmission electron microscopic analysis — For Field-Emission Transmission Electron Microscopy (FETEM) analysis, the **Ir-NH_2** complex was diluted in Milli-Q water. A 10 µL solution was then deposited onto a carbon-coated copper grid and allowed to stand for 5 minutes. Excess liquid was carefully removed using filter paper, and the grid was air-dried at room temperature for 10 minutes. Following this, 10 µL of a 2% (w/v) uranyl acetate solution (in water) was added to enhance image contrast, and the mixture was incubated for 1 minute at room temperature. The excess stain was absorbed with tissue paper, and the grid was left to dry overnight at room temperature. TEM images were acquired using a JEOL JEM-2100 transmission electron microscope. In addition to the FETEM images, Energy-Dispersive X-ray Spectroscopy (EDX) mapping was conducted to analyze the elemental composition and distribution of the **Ir-NH_2** nanostructures.

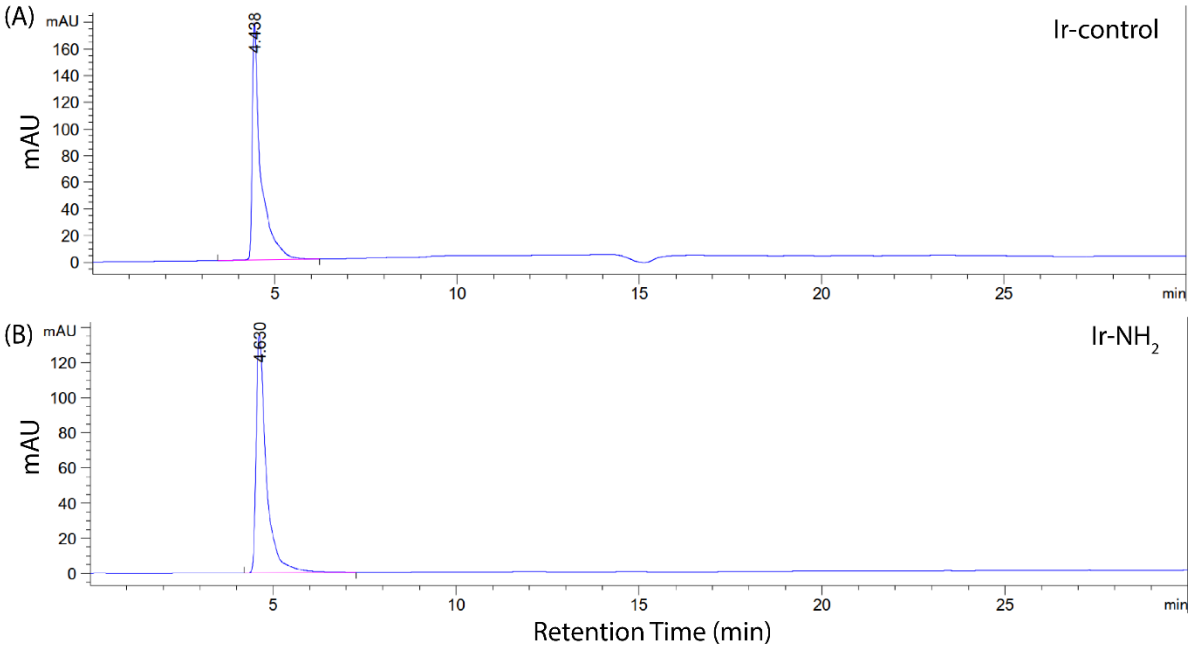


Fig. S4. HPLC traces of **Ir-control** (A) and **Ir-NH2** (B) complexes.

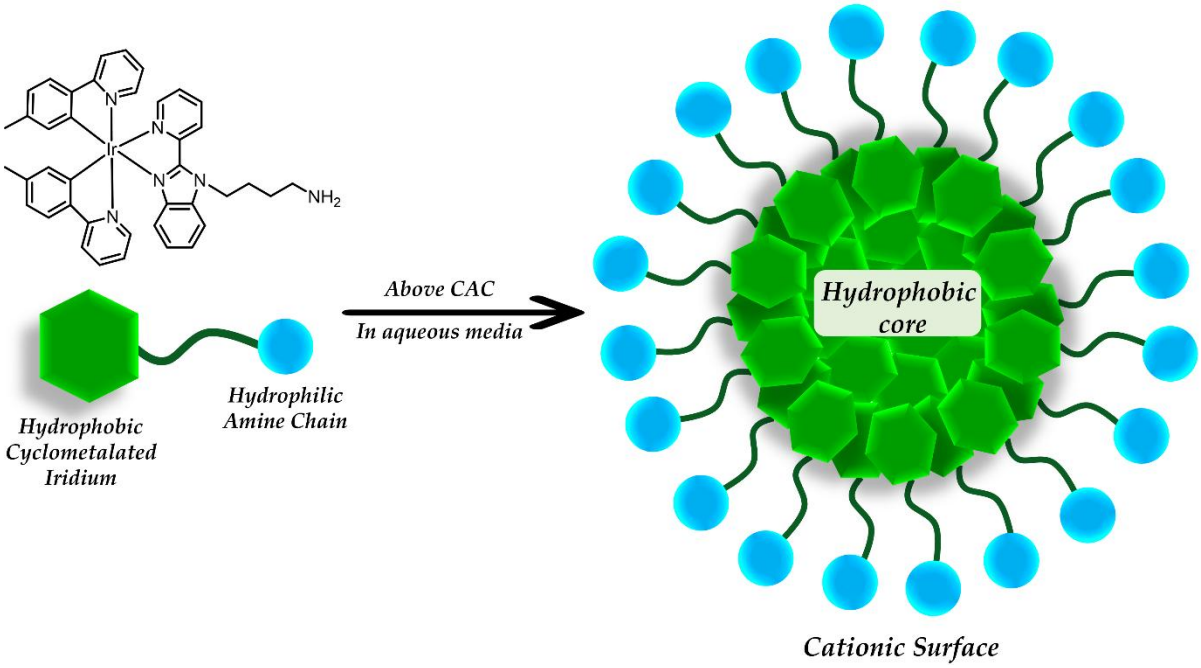


Fig. S5. Schematic representing self-assembly of **Ir-NH2**.

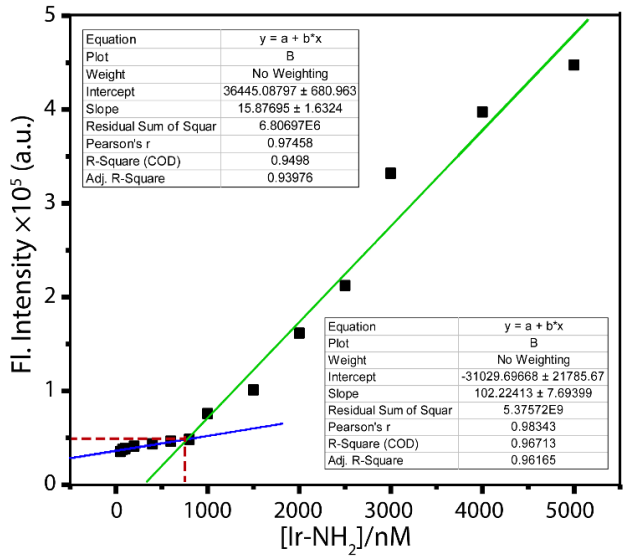


Fig. S6. Concentration-dependent self-assembly pattern of the **Ir-NH2** complex in aqueous medium.

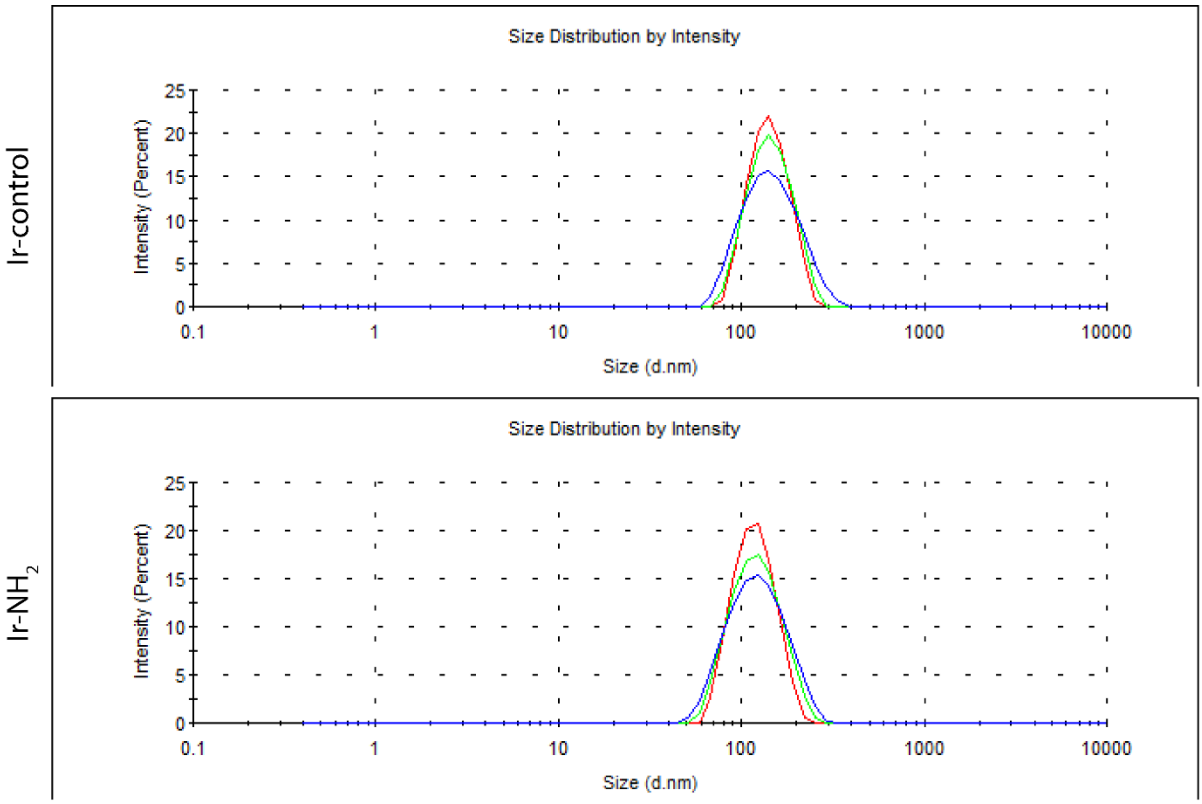


Fig. S7. Hydrodynamic diameter of **Ir-NH2** and **Ir-control** in aqueous medium.

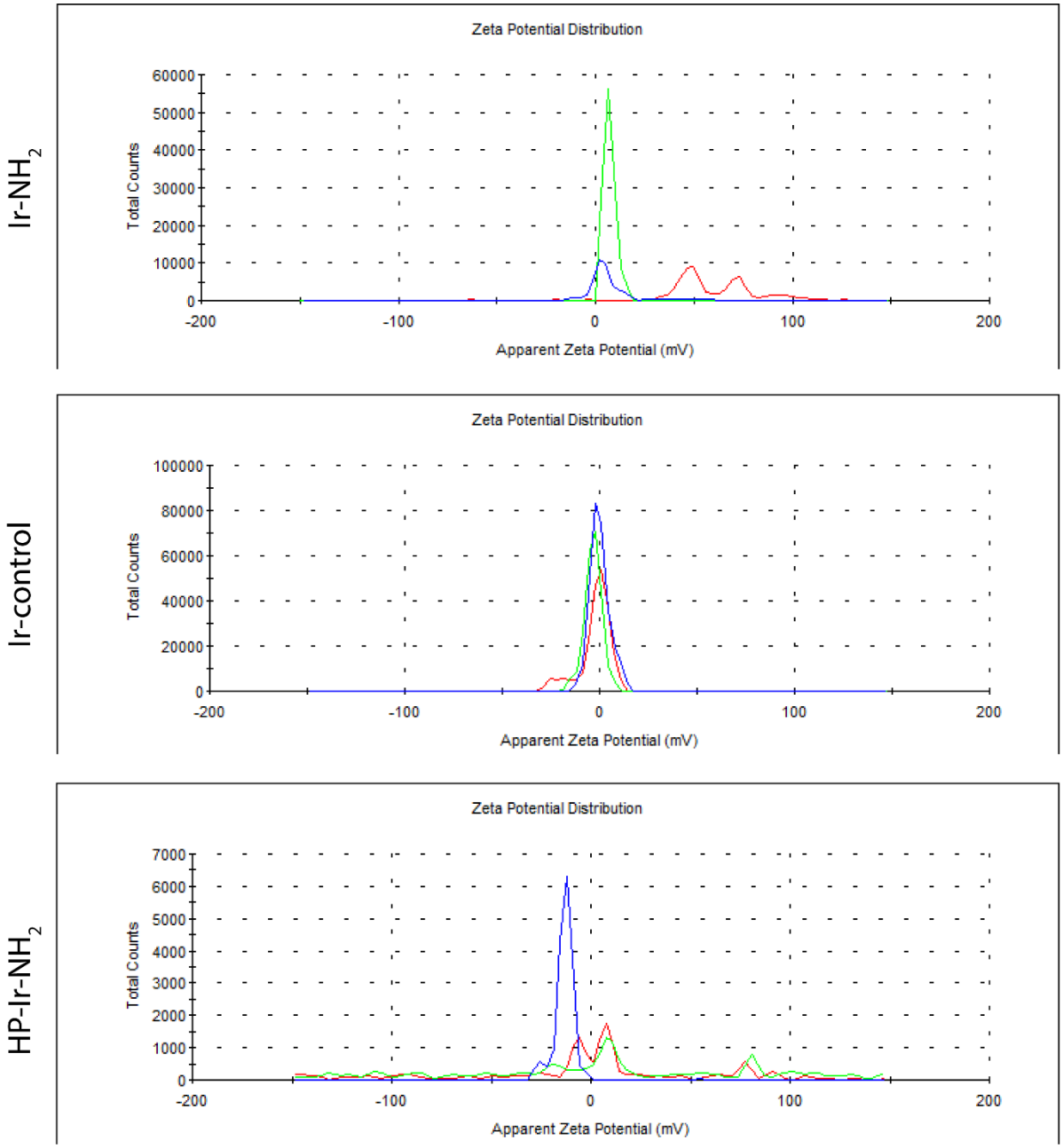


Fig. S8. Surface charge measurements of **Ir-NH$_2$** and **Ir-control** complexes, and the **HP-Ir-NH$_2$** conjugate, in an aqueous medium.

Antibacterial activity analyses — The microbroth dilution assay was used to investigate the antibacterial efficacy of the compounds. *S. aureus* (MTCC 96), gentamicin-resistant MRSA (GR-MRSA; ATCC 33592), *E. coli* (MTCC 1687), and *P. aeruginosa* (MTCC 2488) were cultured in Luria–Bertani (LB) broth at 37 °C until they reached the mid-logarithmic growth phase. The compounds under investigation were serially diluted in Milli-Q water and added to a 96-well plate. After achieving the desired optical density in the bacterial cultures, the cells were collected by centrifugation (5000 rpm for 3 minutes), washed with Milli-Q water, and

then adjusted to a final concentration of 10^6 CFU/mL. This bacterial suspension was then added to the wells of 96-well plates containing serially diluted compounds, and the plates were incubated at 37 °C for 14–16 h. Following incubation, bacterial growth inhibition was first assessed visually and then quantified by measuring absorbance at 600 nm using a Thermo Fisher Multiskan SkyHigh microplate reader. All measurements were performed in triplicate to confirm reproducibility.

Minimum bactericidal concentration analysis — To determine the minimum bactericidal concentration (MBC), 10 µL of inoculum was taken from the wells showing growth inhibition in the **Ir-NH$_2$** antibacterial assay. The inoculum was transferred into 100 µL of fresh medium in adjacent wells, and a portion was also spread onto agar plates. Both the plates and wells were incubated for 24 h, after which the bacterial growth was visually inspected and evaluated.

Zone of inhibition — To assess the effectiveness of **Ir-NH$_2$** on solid media, 1×10^4 CFU/mL *S. aureus* cells were inoculated onto an agar plate. Sterile discs soaked in Milli-Q, ciprofloxacin, **Ir-control**, and **Ir-NH$_2$** were set on pre-inoculated agar plates and incubated at 37 ℃ for 12-16 h. Following incubation, the zone of inhibition was quantified using a measuring scale.

Time-kill kinetics measurements — Time-kill assay was performed to test the time-dependent effect of **Ir-NH$_2$** on *S. aureus*. Mid-logarithmic bacterial culture was adjusted to 106 CFU/mL. Both **Ir-NH$_2$**-treated and untreated cells were kept on a shaker at 37 ℃, and at different time points (0, 1, 2, 4, and 8 h), aliquots were collected and stored at -20 ℃. Afterwards, samples were centrifuged at 5000 rpm for 3 min, then diluted 20-fold. The diluted samples were spread on LB agar plates and incubated at 37 ℃ for 14-16 h to monitor the surviving bacterial colonies.

Assessment of membrane integrity with propidium iodide uptake assay — The propidium iodide (PI) staining assay was performed to evaluate bacterial viability as an indicator of membrane integrity. The *S. aureus* cells were cultured and collected as previously described. Bacterial suspensions in PBS were incubated for 1 h at 37 ℃ with the **Ir-NH$_2$** complex (at MIC value), along with equivalent concentrations of the **Ir-control** and **HP-Ir-NH$_2$** polymer. Buffer alone was used as a negative control. Following this, PI was added to achieve a final concentration of 100 µM, and the samples were incubated for an additional 30 minutes. The cells were then centrifuged at 5000 rpm for 3 minutes and washed twice with PBS to remove any unbound dye. Fluorescence was measured at 535 nm excitation and 550 nm emission.

Dual staining of *S. aureus* cells for live/dead assessment — After being cultivated, *S. aureus* cells were exposed to a 2 × MIC dose of **Ir-NH$_2$** for 2 h. Bacterial cells in buffer alone were

maintained as a control. After treatment, the cells were rinsed to remove excess compound. The live cells were stained with cFDA-SE, and the dead cells were stained with PI for 30 mins. The excess dye was removed, and the cells were drop-cast onto a glass slide after fixation with 2% formaldehyde. The images were captured using an Olympus IX83 fluorescent microscope.

Membrane leakage assay — To produce a thin lipid film, a solution of 92.4 µL of EYPC (Egg Yolk Phosphatidylcholine, 50 mg/mL stock in chloroform) and 23.4 µL CHOL (Cholesterol, 25 mg/mL stock in chloroform) was evaporated under vacuum for 6 h. Afterward, the lipid film was hydrated with 300 µL of buffer (10 mM HEPES, 10 mM NaCl, 25 mM carboxyfluorescein, pH 7.2) for 1 h, with intermittent vortexing (4-5 times). The film was subsequently subjected to a freeze-thaw cycle (≥15 cycles). Vesicles with a mean diameter of approximately 200 nm were obtained by extruding the vesicle solution through a polycarbonate membrane with 200 nm perforations 19 times (note that the number must be odd). Using size-exclusion chromatography (Sephadex G-50), the dye encapsulated vesicles were separated from the extracellular dye employing10 mM HEPES buffer (100 mM NaCl, pH 7.2). Final concentration: approximately 25 mM EYPC-CHOL lipid; intravesicular solution: 10 mM HEPES, 10 mM NaCl, 25 mM CF, pH 7.2; extravesicular solution: 10 mM HEPES, 100 mM NaCl, pH 7.2.[4]

In a clean 3 mL fluorescence cuvette, 1950 µL outside buffer (10 mM HEPES, 100 mM NaCl, pH 7.2) along with 40 µL of aforementioned vesicle solution were added in a slow stirring state by a magnetic stirrer mounted on the fluorimeter (at t = 0 s). The fluorescence emission intensity time course of carboxyfluorescein dye, F_t, was measured with excitation at 492 nm and emission at 517 nm. At t = 50 s, **Ir-NH$_2$** (2µM) was added to the solution, and after the kinetic run, the vesicles were lysed with 20% Triton X-100 (20 µL) to achieve complete dye release.

Evaluation of membrane depolarization with DiSC$_3$(5) dye — Membrane depolarization was evaluated using the potentiometric probe, DiSC$_3$(5) (3,3′-dipropylthiadicarbocyanine iodide) to investigate the membrane-targeting activity of the compounds. *S. aureus* cells were cultured as described above, and the cell pellet was resuspended in HEPES buffer (10 mM HEPES, 50 mM glucose, pH 7.2). DiSC$_3$(5) was added to a final concentration of 0.1 µM, and the suspension was incubated at 37 °C for 1 h. Subsequently, KCl was added to a final concentration of 100 mM and incubated for 10 min. The MIC of **Ir-NH$_2$** and equivalent concentrations of **Ir-control** and **HP-Ir-NH$_2$** were then introduced. Buffer alone and 30 µM valinomycin served as negative and positive controls, respectively. Fluorescence was recorded with excitation and emission wavelengths of 620 nm and 650 nm, respectively.

Intracellular ROS determination — The *S. aureus* cells were cultured as described above. Bacterial suspensions were adjusted to 10^6 CFU/mL and incubated with the MIC of **Ir-NH$_2$**, as well as equivalent concentrations of **Ir-control** and **HP-Ir-NH$_2$**. Buffer alone and 2% v/v H_2O_2 were used as negative and positive controls, respectively. Subsequently, 100 μM 2′,7′-dichlorofluorescein diacetate (H_2DCF-DA) was added, and the samples were incubated in the dark for 30 min. Fluorescence spectra were then recorded with an excitation wavelength of 485 nm.

Morphological analysis of compound-treated bacterial cells — Morphological changes in *S. aureus* following **Ir-NH$_2$** treatment were examined by FESEM analysis. Bacterial cells were cultured and incubated with **Ir-NH$_2$** in a vial for 2 h; untreated cells were processed in parallel as controls. After incubation, cells were centrifuged to remove the medium, washed with buffer, and centrifuged again. Pellets were fixed with 2% formaldehyde for 30 min, washed with Milli-Q water, and resuspended in Milli-Q water. Cell suspensions were drop-cast onto aluminum foil and allowed to dry. Before imaging, the dried samples were sputter-coated with gold, mounted on FESEM stubs, and imaged using a Gemini 300 (Zeiss) FESEM.

DNA binding analysis — For the DNA binding analysis, 10 μM Ir-NH$_2$ solutions were prepared in 700 μL of phosphate-buffered saline (PBS) and transferred to a quartz cuvette. The absorbance spectrum was then recorded over the wavelength range of 200 to 700 nm. Further, various concentration of calf thymus DNA (ctDNA) was added to the solution, and the spectra were recorded.

Antibiofilm Assay — The crystal violet staining technique was used to quantify biofilm biomass and evaluate the efficacy of **Ir-NH$_2$** in preventing biofilm development.[5] GR-MRSA was cultivated in LB medium. After a microbroth dilution assay, the 96-well plate was incubated for 24 h at 37 ℃ to facilitate biofilm formation. Only the media served as the negative control, whereas only the bacteria functioned as the positive control. After the incubation period, the planktonic cells were removed from the wells, and the biomass was rinsed with PBS buffer. Crystal violet (5% w/v) was added to each well, and the mixture was incubated for 30 minutes. The excess dye was removed upon incubation, and the sample was rinsed with Milli-Q water. The plate was allowed to dry before the biomass was solubilized in 95% ethanol. The biofilm biomass was assessed at 550 nm.

Drug resistance study — To analyze possible resistance emergence, *S. aureus* cells were cultured as described previously. Following determination of MIC values for the **Ir-NH$_2$** complex and ciprofloxacin, bacterial cultures were grown at 37 °C with shaking (180 rpm) at sub-inhibitory concentrations, then passed into fresh growth medium for an additional 30 days.

Every other day, a microbroth dilution assay was performed to assess antibacterial susceptibility.

Bacterial cell interaction and imaging with HP-Ir-NH_2 in PBS — *S. aureus* cells were cultivated using the procedure described in the preceding section. Additionally, cell concentrations ranging from 0.25×10^4 to 1.28×10^6 CFU/mL were obtained by serial dilution in PBS buffer (pH 7.2). All bacterial cultures were treated with **HP-Ir-NH_2** (10 µg/mL) and incubated for 30 minutes at 37 °C and 180 rpm shaking. Post-incubation, the cells were centrifuged and rinsed with PBS buffer. Fluorescence spectra were acquired at excitation and emission wavelengths of 400 and 565 nm, respectively.

For imaging, mid-logarithmic *S. aureus* cells were treated with **HP-Ir-NH_2** as indicated above, then fixed with 2% formaldehyde. The agarose pad technique was utilized to image the bacterial cells. A clean glass slide was covered with a thin layer of 2% agarose. Further, the treated bacterial culture was drop cast onto the agarose sheet and covered with a cover slip. Fluorescence microscopic images was captured using Olympus IX83 Fluorescence microscope.

HP-Ir-NH_2 interaction with bacterial cells in artificial urine (AU) — AU was prepared in accordance with the procedure mentioned in the literature.[6] In 100 mL of Milli-Q water, all components of AU were dissolved and stirred overnight at room temperature. The pH of the AU was calibrated to 6.5 and autoclaved prior to use. Various concentrations of *S. aureus* cells were added to the AU along with **HP-Ir-NH_2** (10 µg/mL) conjugate and incubated for 30 mins at 37 ℃ and 180 rpm. After incubation, the cells were washed and fluorescence spectra was obtained at 400 nm wavelength excitation and 565 nm wavelength emission. Additionally, *S. aureus* cells treated with **HP-Ir-NH_2** conjugate in AU were imaged using agarose pad technique in Olympus IX83 fluorescent microscope.

Fluorescence imaging of *S. aureus* cells treated with HP-Ir-NH_2 in bacterial-infected blood model — Informed assent was obtained from all human participants, and all procedures were conducted in accordance with the guidelines of the Ethics Committee at the Indian Institute of Technology Guwahati. Blood was obtained from healthy human donors after the requisite clearance and authorization were obtained. Erythrocytes were separated from the newly obtained whole blood by centrifugation at 1500 rpm for 3 mins. The resultant erythrocyte pellet was rinsed with PBS buffer (pH 7.2) to eliminate remaining blood components. Subsequently, mid-logarithmic-phase bacterial cells were cultured as previously described. Following this, the isolated erythrocytes were added to the bacterial cells to mimic bacterial-

infected blood and subjected to **HP-Ir-NH$_2$** (10 µg/mL) treatment for 30 mins at 37°C and 180 rpm. The microcentrifuge vials were centrifuged at 5000 rpm for 3 minutes to pellet the cells following incubation. To eliminate any unbound conjugate, the cells were subsequently rinsed with PBS (pH 7.2). Following fixation with 2% formaldehyde, the sample was drop-cast onto a glass slide having 2% agarose thin sheet. The fluorescence images were taken with an Olympus IX83 microscope.

Table S1. Antibacterial activity of Ir-NH$_2$ in different bacterial strains

Compound	**MIC (µM)**				
	***S. aureus* (MTCC 96)**	***S. epidermis* (MTCC 435)**	**GR-MRSA (ATCC 33592)**	***E. coli* (MTCC 1687)**	***P. aeruginosa* (MTCC 2488)**
Ir-NH$_2$	0.98 ± 0.01	1.03 ± 0.02	1.96 ± 0.62	31.45 ± 0.62	31.45 ± 0.62
Bovine Albumin Serum (BSA)	<100	-	-	-	-
Ir-NH$_2$ + 400 µg/mL BSA	0.98 ± 0.01	-	-	-	-

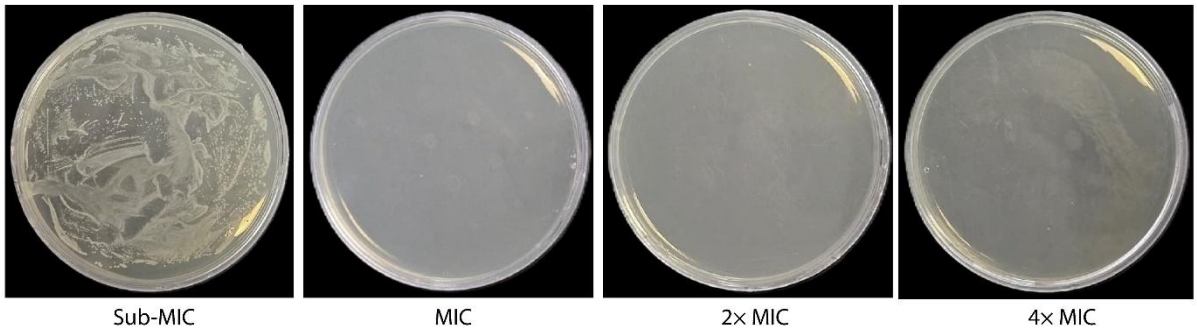


Fig. S9. Minimum bactericidal concentration (MBC) assessment of the **Ir-NH$_2$**-treated *S. aureus* cells using agar plates.

Fig. S10. Zone of inhibition test using sterile discs.

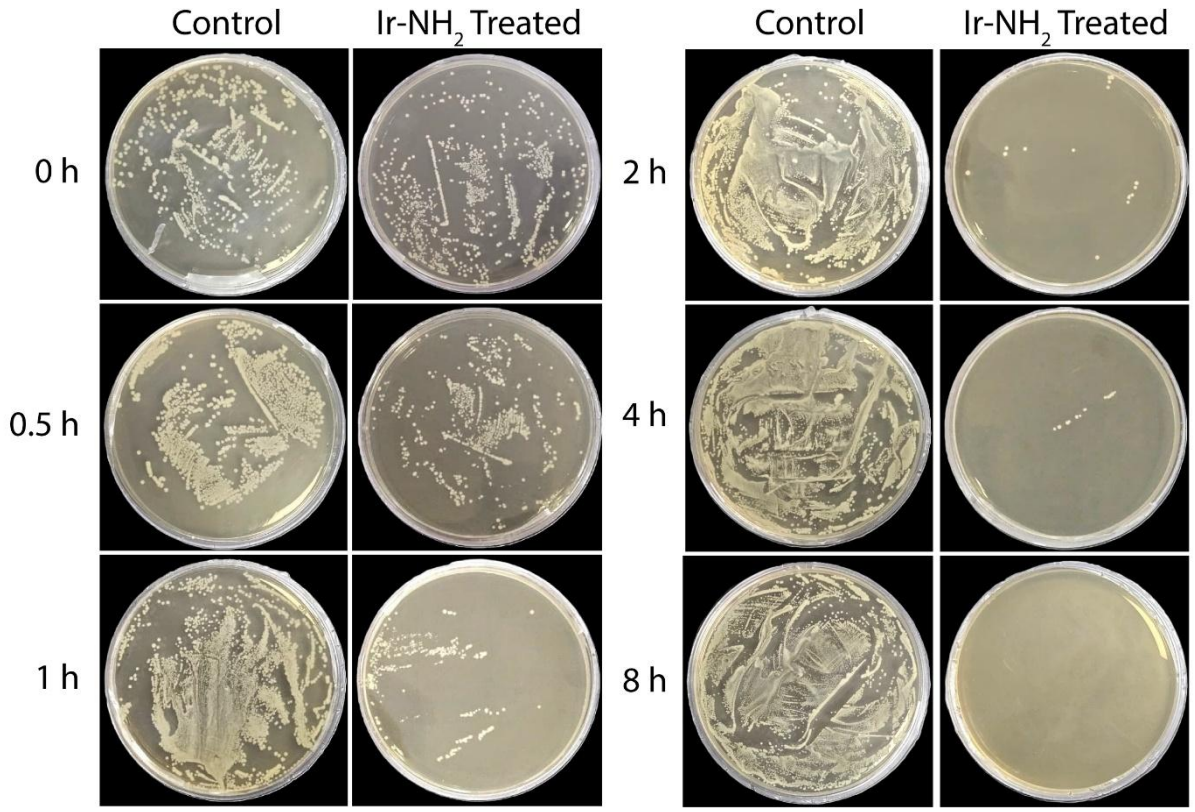


Fig. S11. Representative images of agar plates demonstrating the growth of bacterial cells at different time intervals in the presence and absence of **Ir-NH$_2$** (1 µM) complex.

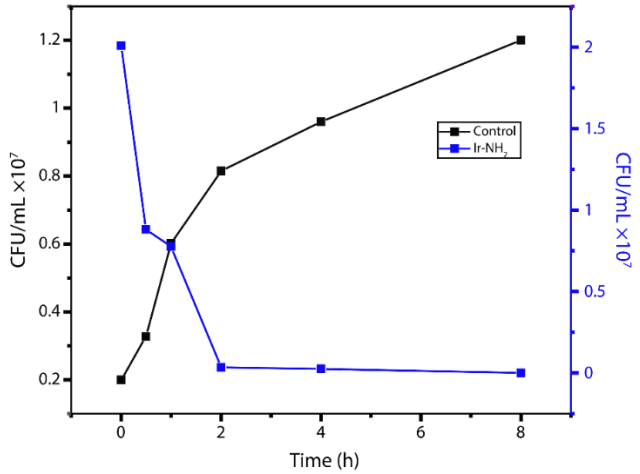


Fig. S12. Time-kill curve showing changes in CFU/mL of control and **Ir-NH$_2$**-treated bacterial culture over time.

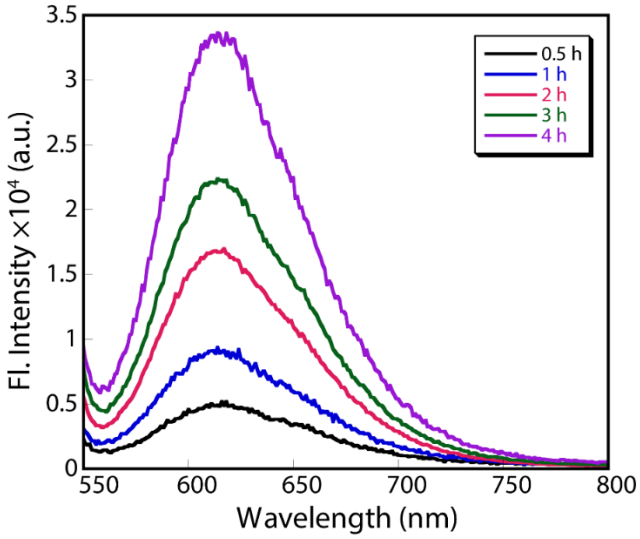


Fig. S13. Time-dependent fluorescence intensity of PI against **Ir-NH$_2$** complex-treated *S. aureus* cells.

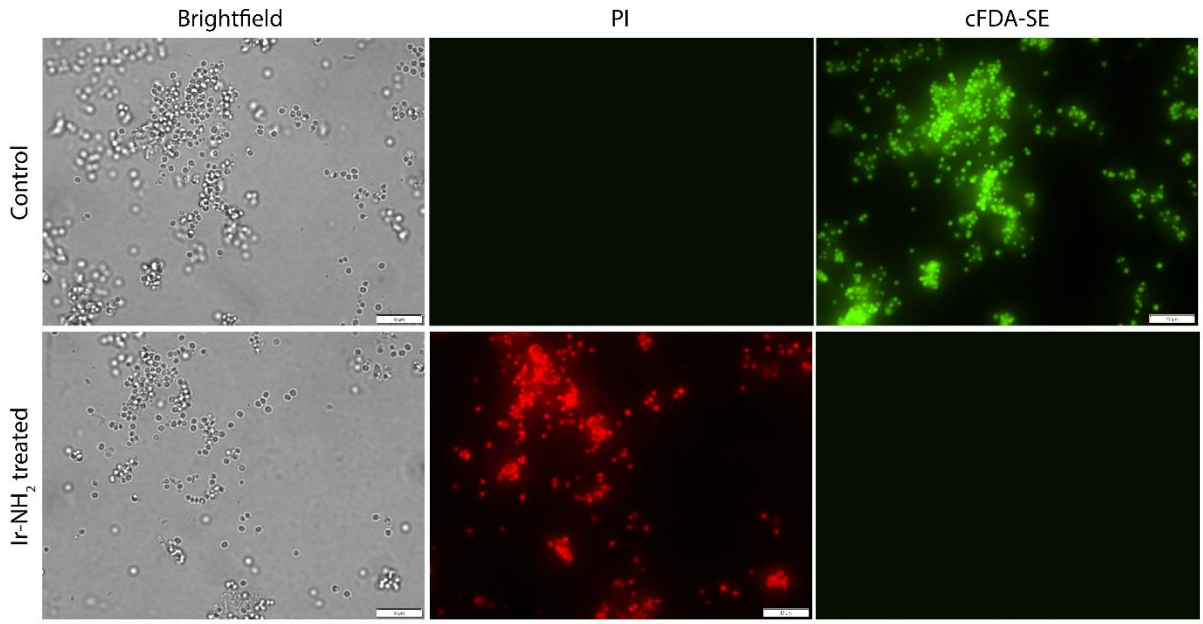


Fig. S14. Representative microscopic images of control and **Ir-NH$_2$** complex-treated *S. aureus* cells stained with PI and cFDA-SE dyes.

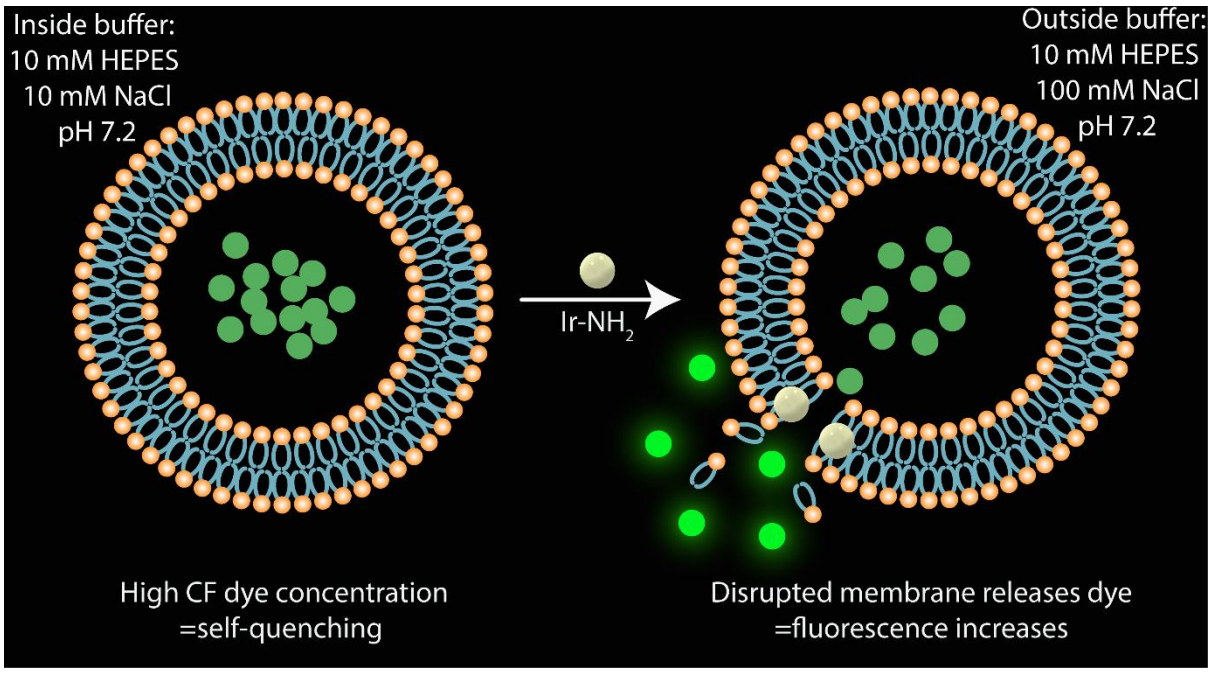


Fig. S15. Schematic representation demonstrating the vesicle leakage activity of **Ir-NH$_2$** complex in carboxyfluorescein (CF)-encapsulated synthetic liposomes.

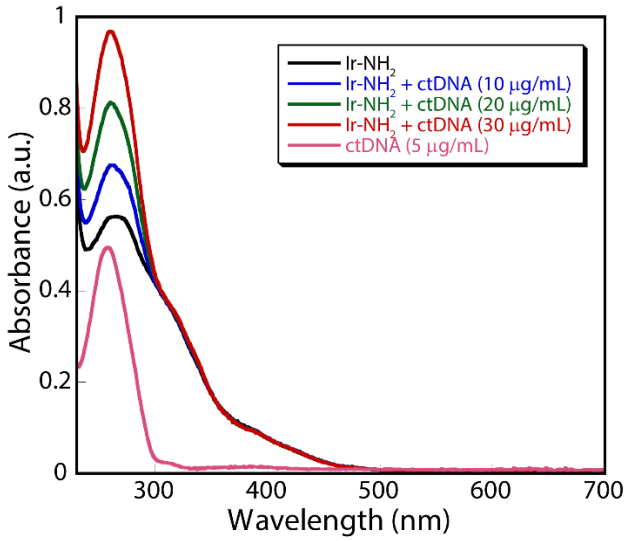


Fig. S16. Interaction of **Ir-NH2** with various concentrations of calf thymus DNA (ctDNA).

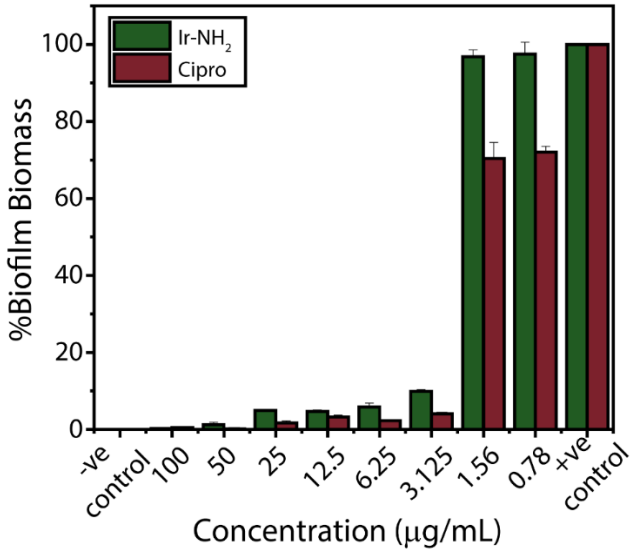


Fig. S17. Ciprofloxacin and **Ir-NH2** complex-mediated eradication of MRSA biofilm.

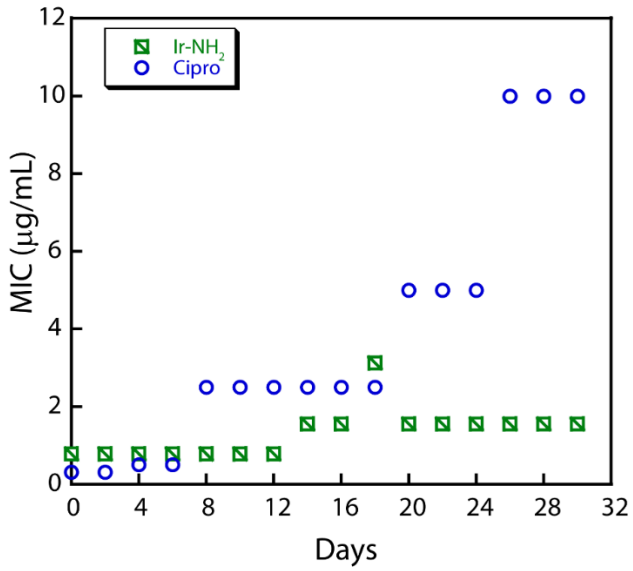


Fig. S18. Development of resistance in *S. aureus* cells following treatment with ciprofloxacin and **Ir-NH2** complex during a 30-day period.

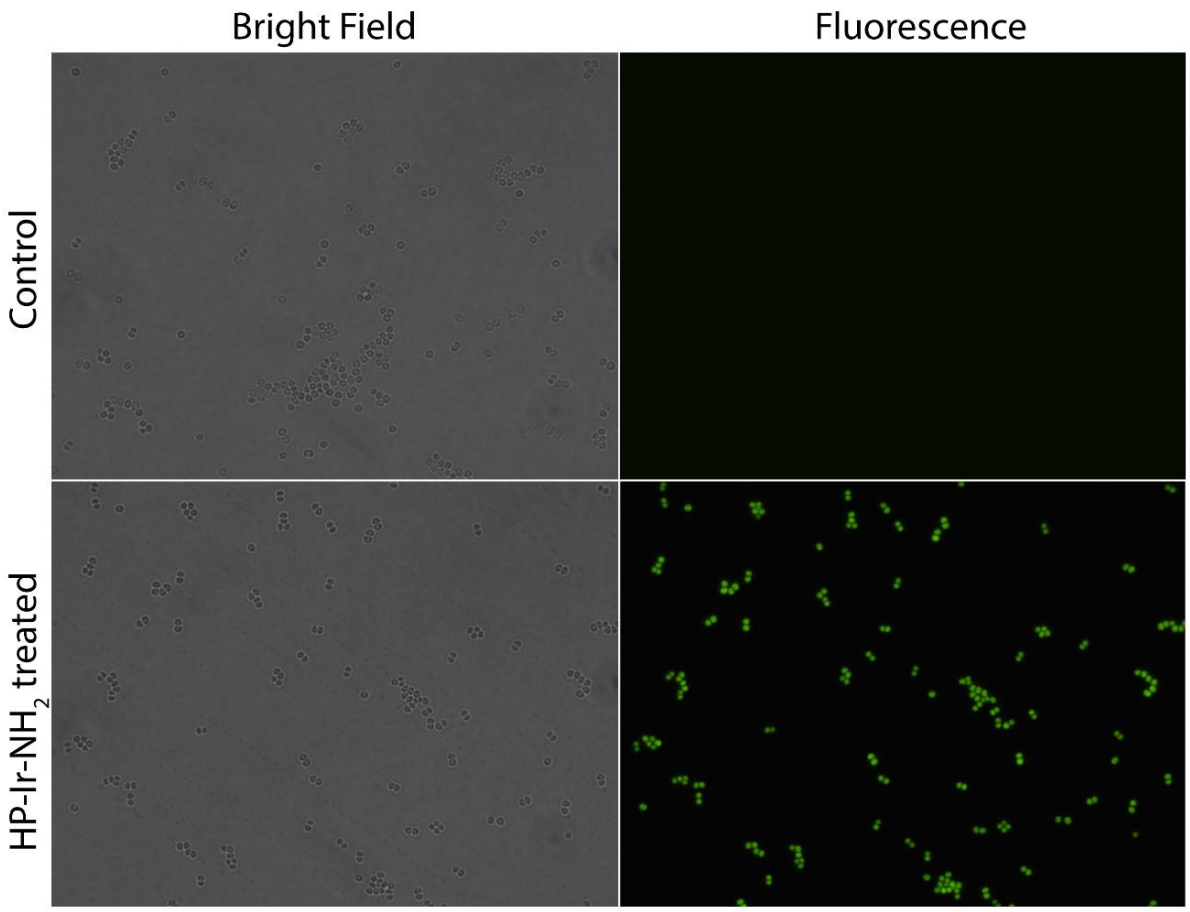


Fig. S19. Fluorescence microscopic imaging of *S. aureus* cells present in PBS buffer using **HP-Ir-NH2** conjugate.

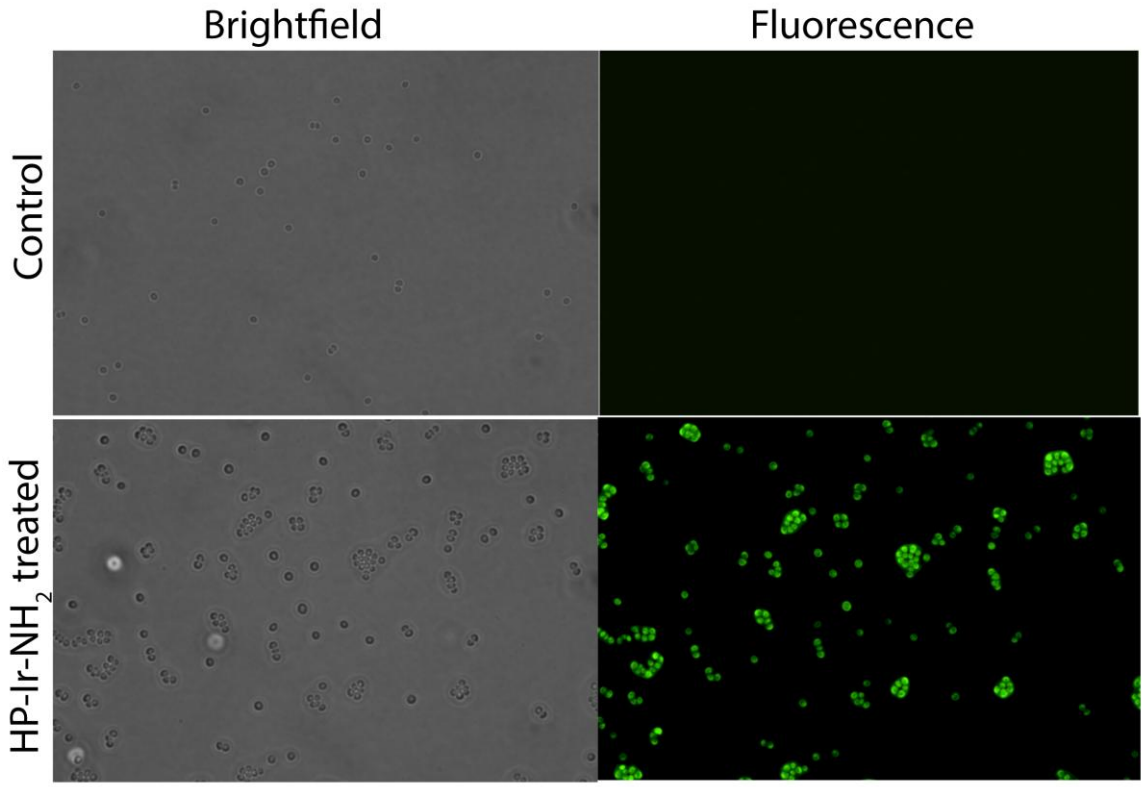


Fig. S20. Fluorescence microscopic imaging of *S. aureus* cells present in artificial urine using **HP-Ir-NH$_2$** conjugate.

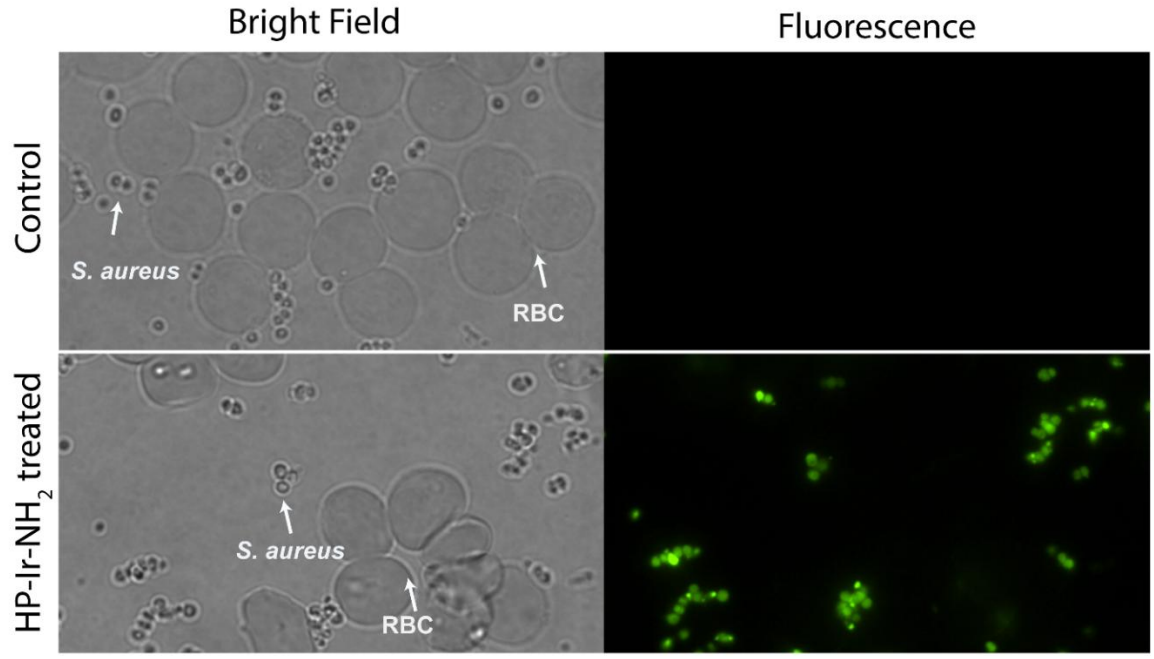


Fig. S21. Selective fluorescence microscopic imaging of *S. aureus* cells in the presence of blood (erythrocytes) cells using **HP-Ir-NH$_2$** conjugate.

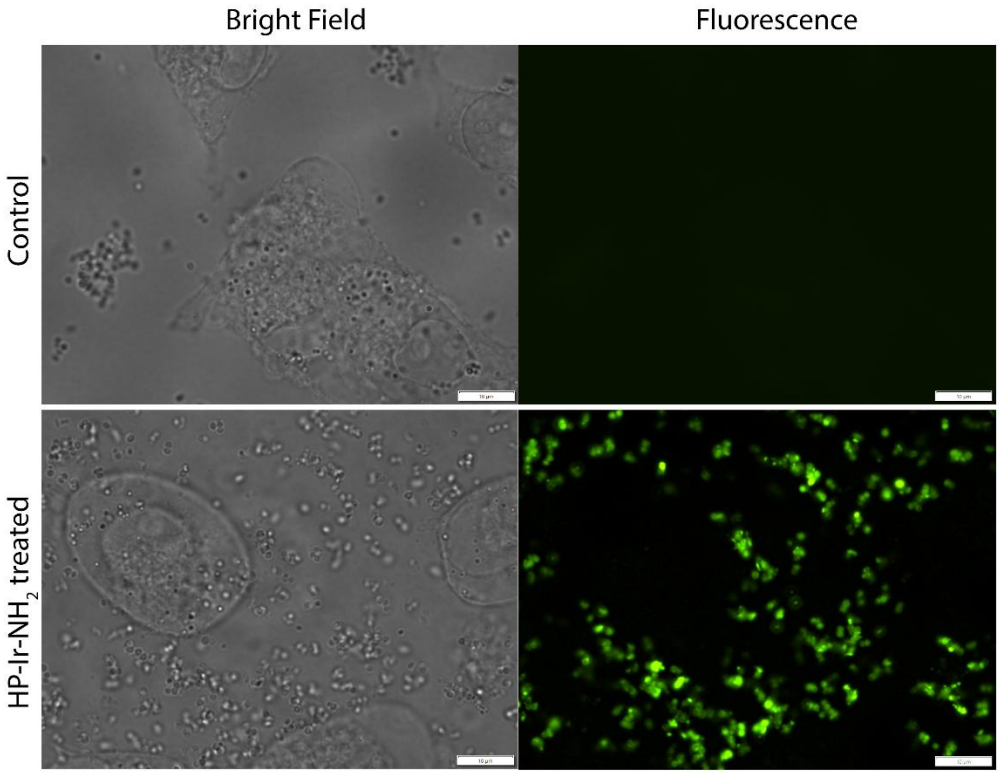


Fig. S22. Selective fluorescence microscopic imaging of *S. aureus* cells in the presence of HeLa cells using **HP-Ir-NH2** conjugate.

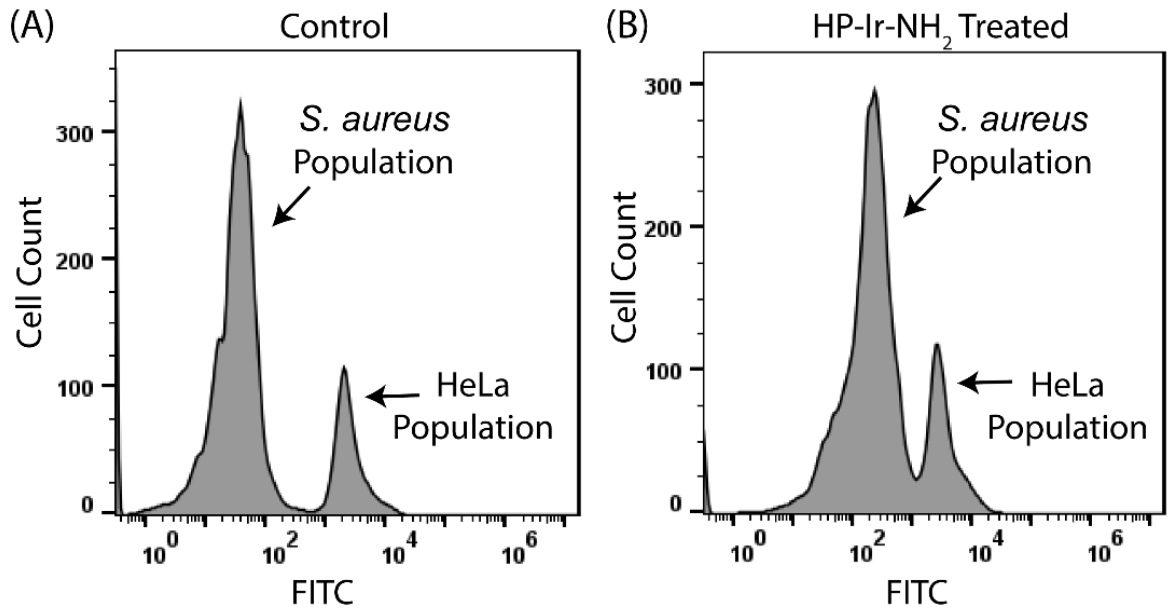


Fig. S23. Cellular uptake study in control and **HP-Ir-NH2**-treated *S. aureus* cells in combination with HeLa cells using flow cytometry.

Cell line maintenance — Human cervical carcinoma (HeLa) cells and human embryonic kidney (HEK 293) cells were procured from the National Centre for Cell Science (NCCS), Pune, India, and used in this study. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), sodium bicarbonate, and 1% antibiotic–antimycotic solution, and maintained at 37 °C in a humidified incubator with 5% CO_2. Cells were routinely passaged using trypsin–EDTA for sub culturing and maintenance.

Investigating the effects of HP-Ir-NH_2 treatment on *S. aureus* infected HeLa cells — HeLa cells (7×10^4) were seeded in a 35 mm glass-bottom dish, and were allowed to adhere. *S. aureus* cells were grown as mentioned in the previous section. The bacterial culture containing 10^6 CFU/mL was precipitated, resuspended in PBS, and added to the mammalian culture. The co-culture was further incubated with **HP-Ir-NH_2** for 6 h at 10 µg/mL concentration. After incubation, the co-culture was visualized in a 35-mm glass-bottom dish using an Olympus IX83 Fluorescence microscope.

Cellular uptake study via flow cytometry — To study and quantify the cellular uptake of **HP-Ir-NH_2** in both the HeLa cells and bacterial cells, flow cytometry analysis was performed. 1.5×10^5 HeLa cells were seeded in 35 mm cell round bottom culture plate. Then the cells were treated with 10µg/mL **HP-Ir-NH_2** for 24 h. In the last 6 h of the treatment 1×10^6 CFU/mL of *S. aureus* cells were added into the cell culture dish for co-culturing. Treated bacterial cells were collected and centrifuged, while the treated HeLa cells were trypsinized and washed with PBS. Trypsinized HeLa cell and the centrifuged bacterial cell were mixed again and were analysed using flow cytometry (BD FACS Accuri) to observe the fluorescence shift in the green channel. FlowJo v10 was used to examine the data.

Haemolytic assessment — Following ethical committee clearance at the Indian Institute of Technology Guwahati, the blood sample was collected and further centrifuged to isolate erythrocytes from the whole blood. The obtained erythrocytes were rinsed with PBS buffer (pH 7.2), and a 3% haematocrit was prepared. Concurrently, the **Ir-NH_2** and **Ir-control** complexes, along with the **HP-Ir-NH_2** conjugate were serially diluted in PBS buffer. Only buffer and Triton X-100 were designated as the negative and positive controls, respectively. Following the incorporation of 3% haematocrit into the serially diluted compounds, the vials were incubated at 37 ℃ for 1 h. Following incubation, the vials were centrifuged, and the supernatant was transferred to a 96-well plate, where absorbance was measured at 410 nm. The evaluation was conducted in triplicate, and the haemolytic percentage was computed as follows.

$$\%\ Haemolysis = \frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}} \times 100$$

Cell viability assay — For the cell viability assay, 5×10^3 cells of each cell line were seeded into 96-well plates and treated for 48 h with **HP-Ir-NH$_2$**, **Ir-NH$_2$**, and **Ir-control** at increasing concentrations. Cell viability was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Following treatment, cells were incubated with MTT for 3 h to allow reduction of the dye by mitochondrial dehydrogenases, which reflects the number of metabolically active, viable cells. After incubation, the MTT-containing medium was replaced with dimethyl sulfoxide (DMSO) to solubilize the formazan crystals, and absorbance was measured at 560 nm with a reference wavelength of 600 nm using a microplate reader (GloMax® Discover). Data were analyzed using GraphPad Prism 8 software, and half-maximal inhibitory concentration (IC_{50}) values were calculated. Cell viability was determined using the formula below.

$$\%\ Cell\ viability = \frac{(Abs_{560} - Abs_{600})\ of\ Treated\ cells}{(Abs_{560} - Abs_{600})\ of\ Untreated\ cells}\ X\ 100$$

Selectivity Index (SI) was calculated using the following equation.

$$SI = \frac{IC_{50}}{MIC}$$

Cellular internalization/co-localization study — HeLa cells (1×10^5) were seeded in a 35 mm glass-bottom dish and incubated for 24h to achieve cell adhesion. Further, the cells were incubated with **Ir-NH$_2$** at 10 µM for 6 h. MitoTracker Red was used to visualize the mitochondria (200 nM concentration). Incubated cells were washed 3 times with 1× PBS, then fixed with 4% formaldehyde, followed by a PBS wash. Cells were visualized using a confocal microscope (Zeiss LSM 880).

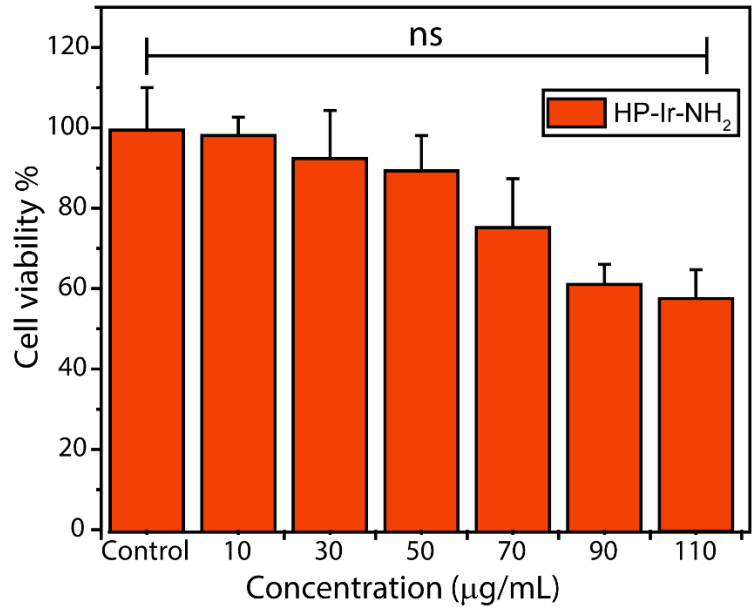


Fig. S24. Viability of HEK 293 cells in the presence of different concentrations of **HP-Ir-NH$_2$** conjugate. "ns" represents nonsignificant for the one-way ANOVA test.

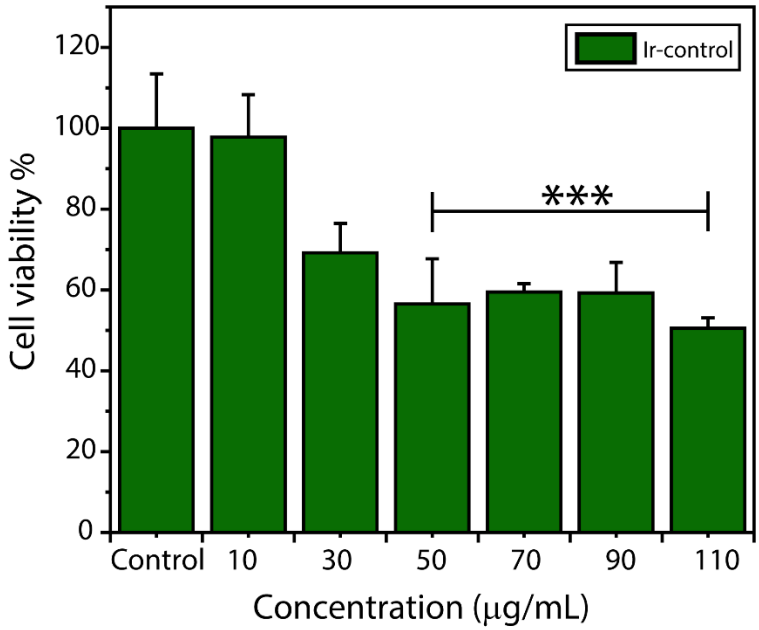


Fig. S25. Viability of HEK 293 cells in the presence of different concentrations of **Ir-control** complex. Statistical significance was indicated by *** for $p < 0.001$.

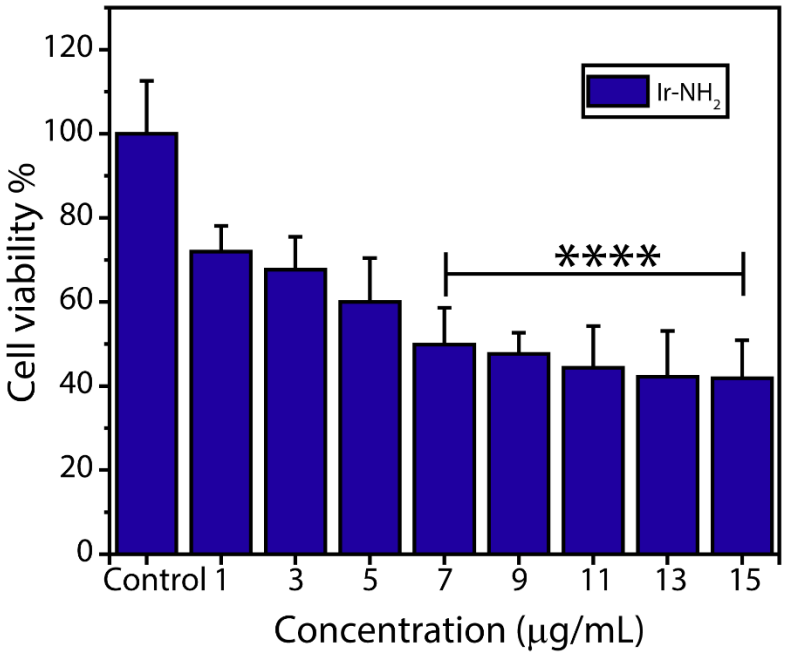


Fig. S26. Viability of HEK 293 cells in the presence of different concentrations of **Ir-NH2** complex. Statistical significance was represented as **** for p <0.0001.

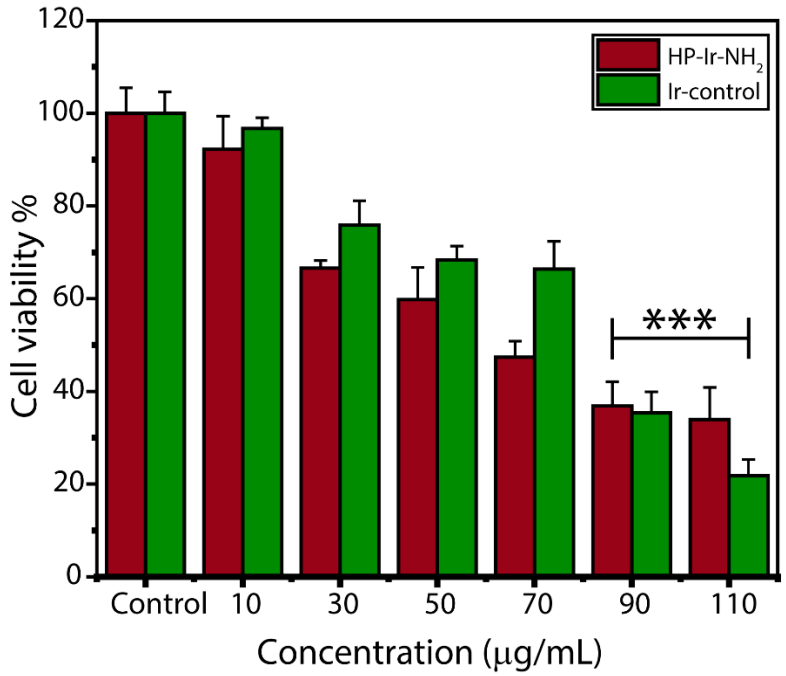


Fig. S27. Viability of HeLa cells in the presence of different concentrations of **HP-Ir-NH2** conjugate and **Ir-control** complex. Statistical significance was indicated by *** for p < 0.001.

Table S2. Selectivity Index of Ir-NH_2 against HEK 293 and HeLa cell lines for different bacterial strains

Compound	**Selectivity Index**				
	***S. aureus* (MTCC 96)**	***S. epidermis* (MTCC 435)**	**GR-MRSA (ATCC 33592)**	***E. coli* (MTCC 1687)**	***P. aeruginosa* (MTCC 2488)**
HEK 293	15.38	14.63	7.69	0.48	0.48
HeLa	10.25	9.75	5.12	0.32	0.32

References

1. M. Nonoyama, *Bull. Chem. Soc. Jpn*, 1974, **47**, 767-768.
2. S. Mishra and S. Patra, *Dalton Trans.*, 2024, **53**, 8214-8222.
3. M. E. G. do Carmo, P. A. de Matos, P. I. S. Maia, A. E. H. Machado, M. E. Beletti, T. M. Tsubone and A. O. T. Patrocinio, *J. Photoch. Photobio. A*, 2024, **448**.
4. S. Srimayee, B. M. Prusty, M. K. Kar, M. Winterhalter and D. Manna, *Angew. Chem. Int. Ed.*, 2025, DOI: 10.1002/anie.202501634, e202501634.
5. A. Patel, S. Paul, N. Akhtar, S. Das, S. Kar, S. Bhattacharjee and D. Manna, *ACS Appl. Nano Mater.*, 2022, **5**, 16602-16611.
6. N. Sarigul, F. Korkmaz and İ. Kurultak, *Sci. Rep.*, 2019, **9**, 20159.