Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Experimental Section

Materials

PIC Ligand (4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline), $[Ru(bpy)_2Cl_2]$, $[Ru(dpp)_2Cl_2]$ and $Ruthenium^{2+}$(bis-2,2-bipyridyl)-2(4-carboxylphenyl)imidazo[4,5-f][1,10]phenanthroline . $[Ru(bpy)_2(picH_2)]^{2+}$ were all prepared according to previously reported methods.[i,ii,iii]

Protected amino acids and HBTU were supplied by Merck. Rink Amide MBHA resin (0.56 mmol/g) and solvents for peptide synthesis were obtained from Applied Biosystems. HOBt was obtained from Iris. Other chemicals were obtained from Aldrich and were the highest commercially available purity. All commercial reagents and solvents were used as received. Analytical RP-HPLC was performed using Varian chromatography systems with reverse-phase C18 column. Semi-preparative RP-HPLC was performed on a BioCAD chromatography system using C18 columns. Compounds were detected by UV using absorption at 214nm. Mass spectra were recorded on a MALDI Laser TOF spectrometer. α-cyano-4-hydroxy cinnamic acid was used as the matrix and was purchased from Aldrich. Synthesizer filters (in-line and reactor) and solvents (DCM, NMP) were sourced from Applied Biosystems and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) / Resazurin reagents were acquired from PromoKine. Cell culture media, serum and penicilin/streptomycin were purchased from Biosera.

Chinese hamster ovarian (CHO) (CHO-K1, ATCC no. CCL-61) were purchased from ATCC Cell Biology Collection (UK) and were grown in DMEM/Hams F-12 supplemented with 10% foetal calf serum at 37 oC with 5% CO2. Cells were harvested or split when they reached 90% confluency. CHO cells were harvested after trypsinisation (0.25% trypsin for 5 mins at 37oC).

Methods

Solid Phase Peptide Synthesis

The synthesis of the peptides for this work employed a Fmoc/tBu solid phase strategy on Rink Amide MBHA Resin. Synthesis was carried out by automated Standard Solid Phase Peptide Synthesis (SPPS), preformed on an Applied Biosystem ABI 433A Synthesizer.

Metal Complexes and Conjugates

$[Ru(dpp)_2(picH_2)]^{2+}$

The compound was synthesized according to a method modified from the literature.[25] $Ru(dpp)_2Cl_2$ (0.20 g, 0.23 mmol) and PIC ligand (0.095 g (0.30 mmol) were refluxed in 1:2 ethanol:water, 30 mL for 48 hours, during which time the solution turned a dark orange color. Lithium perchlorate solution was added to aid precipitation. The product was purified by column chromatography on silica using methanol-dichloromethane (1:9 v/v) as eluent. The material was lyophilized to yield an orange solid (0.15 g, 59%).

^{1}H NMR (DMSO-d_6): δ (ppm) 9.12 (d, 2H, J = 8.4 Hz), 8.44 (d, 2H, J = 8.4 Hz), 8.32 (d, 2H, J = 5.6 Hz), 8.24 (m , 3H), 8.15 (dd, 2H J = 8 Hz), 7.87 (t, 2H, J = 5.6 Hz), 7.79 (d, 2H, J = 5.2 Hz), 7.74 (d, 2H, J = 5.6 Hz), 7.61 (m, 24H). HRMS (MALDI-TOF): 1105.3 *m/z* (M^{2+}) observed, 1105.3 *m/z* (M^{2+}) calculated.

Synthesis of $Ru(dpp)_2$PIC- βA- NF-κB (-VQRKRQKLMP-NH_2) conjugate.

Synthesis of the peptide took place on the AB 433A synthesizer by standard SPPS using 0.1mmol of Rink Amide Resin (substitution 0.7mmol/g). Manual coupling of [$Ru(dpp)_2$PIC] ClO_4 took place on the resin using PyBOP/HOBt (3eq) / DIEA (6eq) coupling chemistry over night. The peptide is cleaved by standard methods and then purified as usual by RP-HPLC. Fractions collected by semi-preparative HPLC were analysed by mass spectrometry and analytical HPLC. Purity 97% (Figure S1). $C_{126}H_{150}N_{30}O_{14}RuS$ [m/z=2441.07 m+H^+= 2440.46] (Figure S2).

Synthesis of $Ru(bpy)_2$PIC-βA-NF-κB (-VQRKRQKLMP-NH_2) conjugate.

The peptide was assembled using 0.1mmol of Rink Amide Resin (substitution 0.7mmol/g) and took place on the AB 433A synthesizer. Manual coupling of [$Ru(bpy)_2$PIC] ClO_4 took place on the resin over night using PyBOP/HOBt (3eq) / DIEA (6eq) coupling chemistry. The peptide is cleaved by standard methods and then purified as usual by RP-HPLC. Fractions collected by semi-preparative HPLC were analysed by mass spectrometry and analytical HPLC. Purity 98% (Figure S3). $C_{98}H_{134}N_{30}O_{14}RuS$ [m/z= 2088.95 m+H^+= ½ mass 1044.64 1/3 mass 696.87] (Figure S4).

Lifetime Measurements

Luminescent lifetimes were obtained using a Picoquant Fluotime 100 TCSPC (Time Correlated Single Photon Counting) system exciting at 450 nm and using a 510 nm narrow band pass dielectric filter for ruthenium complexes. 5,000 counts were collected for each lifetime measurement and all measurements were performed in triplicate using Nanoharp software to confirm results. Typical pulse rates of the excitation source were $1x10^5$ s^{-1} with typical pulse widths of 300 ps. Degassed samples were degassed with nitrogen for 20 minutes prior to analysis.

The calculation of the luminescent lifetimes was performed by fitting an exponential decay function to each decay plot to extract the lifetime information using FluoFit software. Due to the inherently long lifetimes of inorganic complexes all data were fitted to mono exponential decay functions to the baseline of the decay curve using tail-fit with an x2 value of between 0.9-1.1.

Cell culture

Chinese hamster ovarian (CHO) (ATCC no. CCL-61) and Hela cells (ATCC no. CCL-2) were grown in DMEM/Hams F-12 and RPMI respectively both supplemented with 10% foetal calf serum (Biosera) at 37 ^oC with 5% CO_2. Cells were harvested or split (with 0.25% trypsin for 5 mins at 37^oC) when they reached 90% confluency. For confocal measurements, 1 x 10^5 cells in 2 mls media, were cultured on 35mm glass bottom culture dishes for 48 hours before addition of 0.5 ml of parent complex (40 μM) or dye-peptide (40 μM) in PBS (pH 7.4, supplemented with 1.1 mM $MgCl_2$ and 0.9 mM $CaCl_2$). Images were acquired immediately for uptake studies or after an overnight incubation with the dyes. Confocal measurements were performed on live cells at 37 ^oC using a heated stage and a Zeiss LSM510 Confocal microscope with a 63x oil immersion lens. DAPI (300nM) nuclear counter stain was added.

Cytotoxicity assay

Cells were seeded in a 96-well plates in 100µl of media containing 1 x 10^4 cells per well for 24 hours at 37 ^oC with 5% CO_2 before addition of compounds. Compounds were added to a final concentration of 140, 70, 35 and 17.5 µM, DMSO and ethanol were added at final concentrations of between 20 to 0.3 % and left for 16 hours at 37 ^oC in a 5% CO_2 incubator. 10µl of Resazurin reagent (PromoKine) was added and incubated for 7 hours at 37 ^oC. The resazurin converted to resorufin in viable cells was detected at absorbance 570 nm with background subtracted at 600 nm. Absorbance readings were made using a Tecan 96-well plate reader.

Instrumentation

UV/vis absorption spectra were recorded on a Varian Cary 50 spectrometer. The background was corrected for the absorption of the buffer. Fluorescence spectra were obtained on a Varian Cary Eclipse spectrometer. Emission lifetime data were determined using Picoquant Timeharp/Nanoharp Time-Correlated Single Photon Counting (TCSPC) and fitted using FluoFit/SigmaPlot software.

Confocal luminescence measurements were performed on live cells at 37 ^oC using a heated stage and a Zeiss LSM510 Confocal microscope with a 63x oil immersion lens objective lens (NA 1.4). The 458 nm laser excitation was used for ruthenium samples and emission was collected using a long pass 560 nm filter set. DAPI (300nM) nuclear counter stain was added and detected using 375 nm laser excitation and 420-480 band pass filter.

Results

HPLC and Mass Spectrometry of Peptide Conjugates

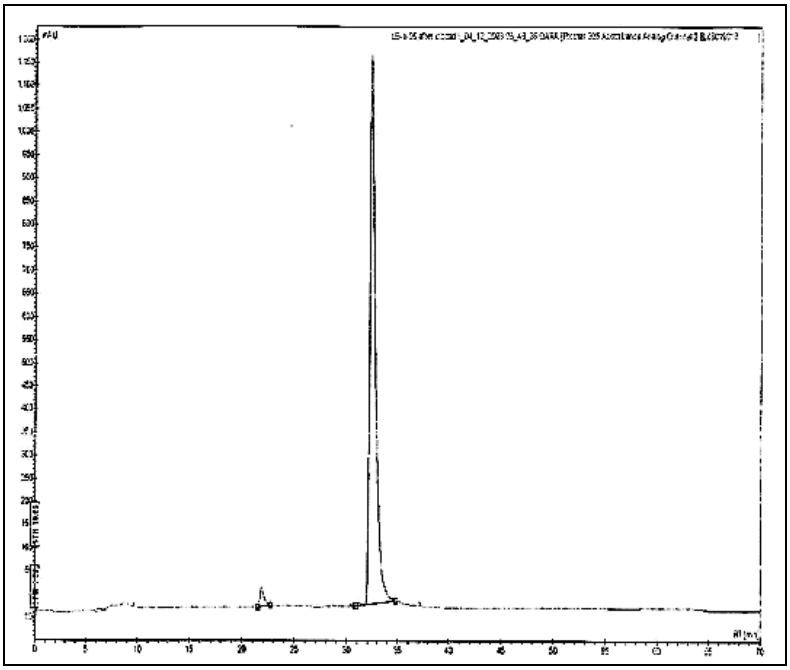

Figure S1 HPLC Trace for $[Ru(dpp)_2PIC\text{-}\beta Ala\text{-}NF\kappa B]^{6+}$ conjugate run on a Varian chromatography systems with reverse-phase C18 column.

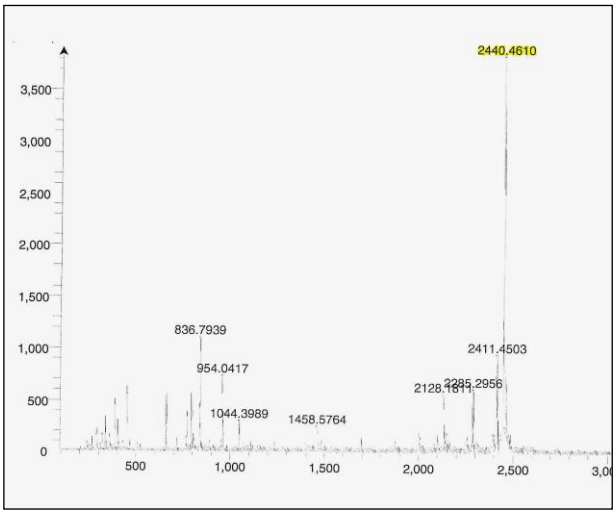

Figure S2 MALDI-TOF of $[Ru(dpp)_2PIC\text{-}\beta Ala\text{-}NF\kappa B]^{6+}$ conjugate using α-cyano-4-hydrxycinnamic acid matrix.

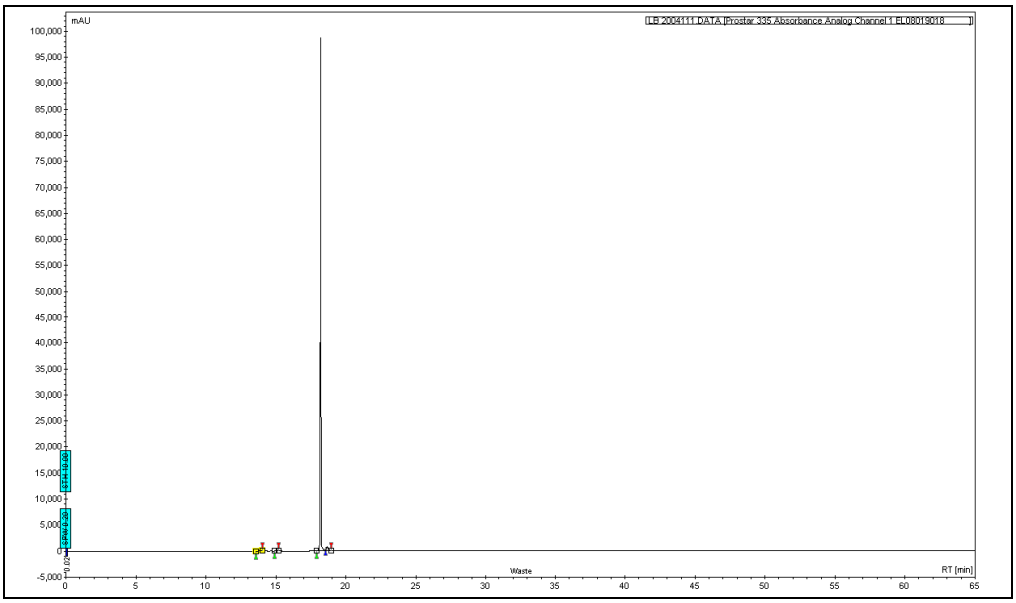

Figure S3 HPLC Trace of $[Ru(bpy)_2PIC\text{-}\beta Ala\text{-}NF\kappa B]^{6+}$ conjugate, Varian chromatography systems with reverse-phase C18 column.

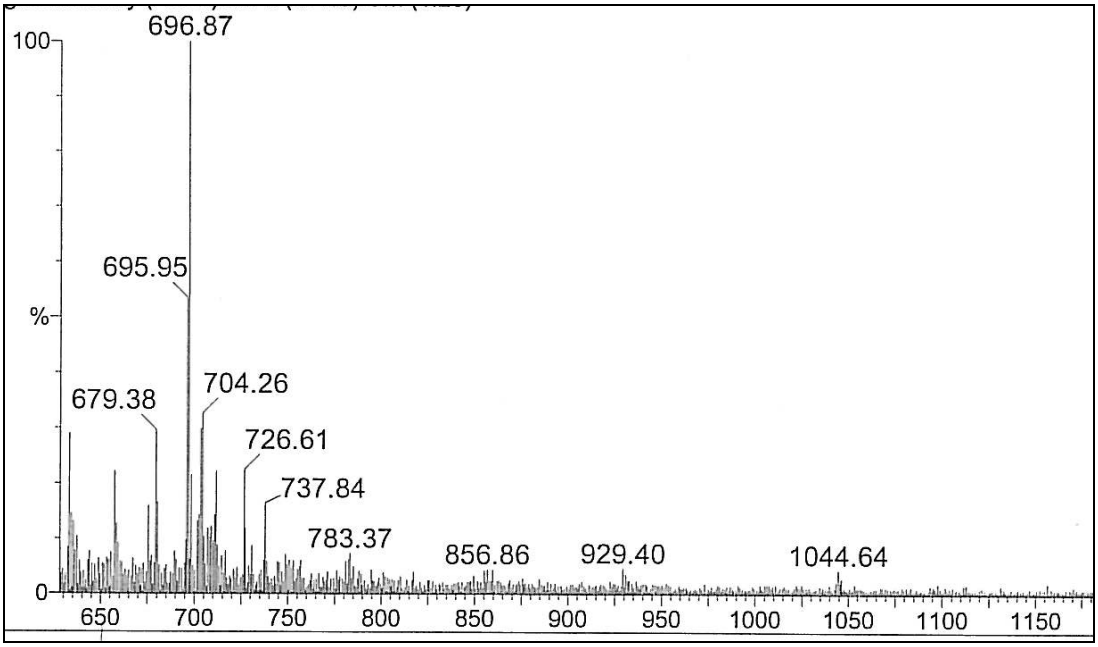

Figure S4 Mass Spectrum of $[Ru(bpy)_2PIC\text{-}\beta Ala\text{-}NF\kappa B]^{6+}$ conjugate.

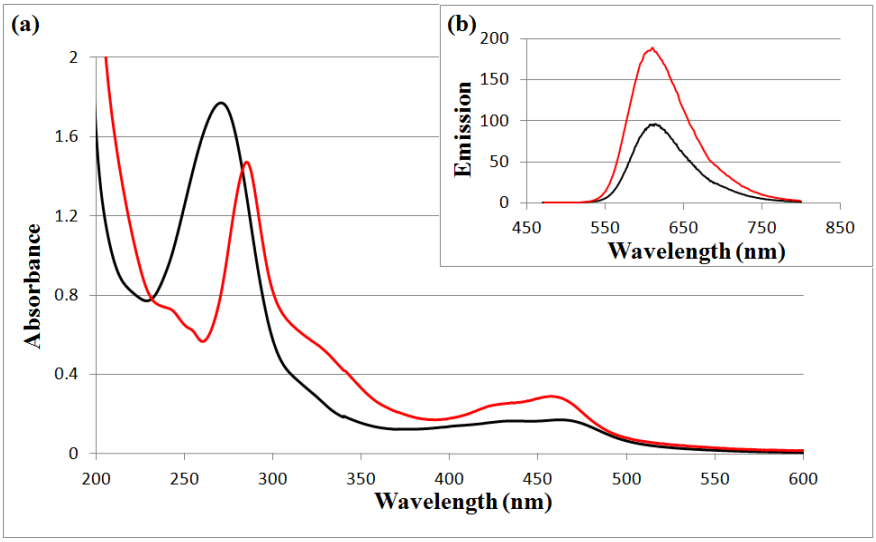

Fig S5 The absorbance (a) and emission, insert (b) spectra in aqueous PBS of $[Ru(dpp)_2PIC\text{-}\beta Ala\text{-}NF\text{-}\kappa B]^{6+}$ black line, and (b) $[Ru(bpy)_2PIC\text{-}\beta Ala\text{-}NF\text{-}\kappa B]^{6+}$ red line.

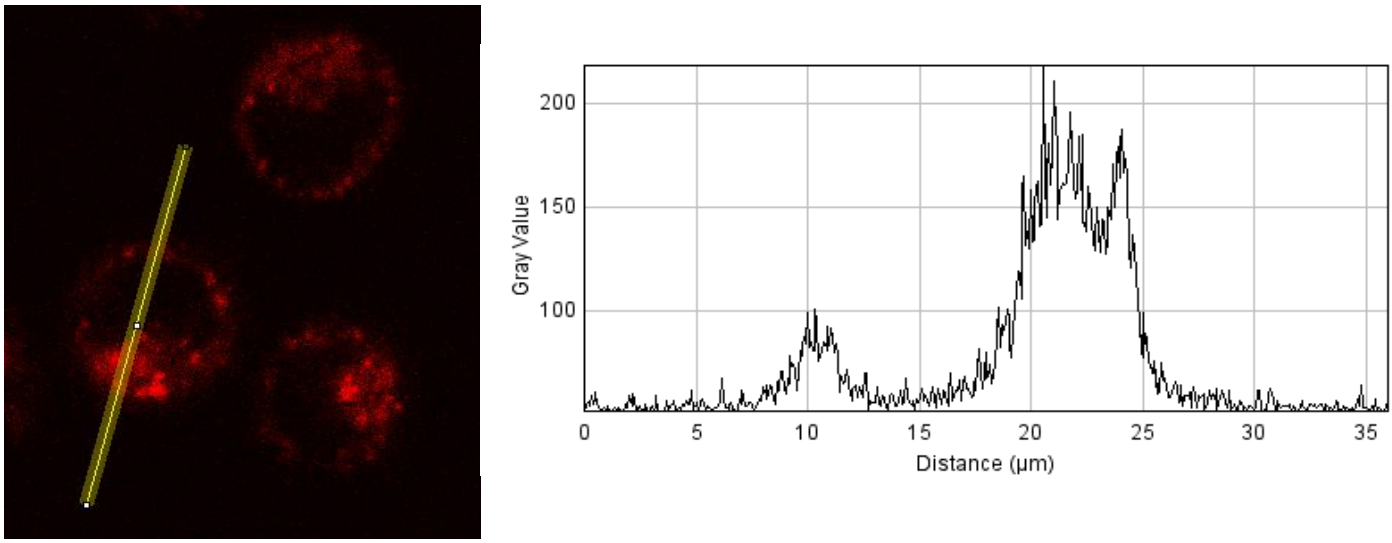

Figure S6 Confocal Luminescence Image of CHO cells incubated overnight $[Ru(dpp)_2PIC]ClO_4$ (0.5 ml of parent complex (40 µM)) in PBS buffer containing 0.5% v/v DMSO (left). The lateral luminescence intensity across the range shown by the yellow line is shown on the right and indicates that the complex does not penetrate the nuclear envelope in the absence of the peptide.

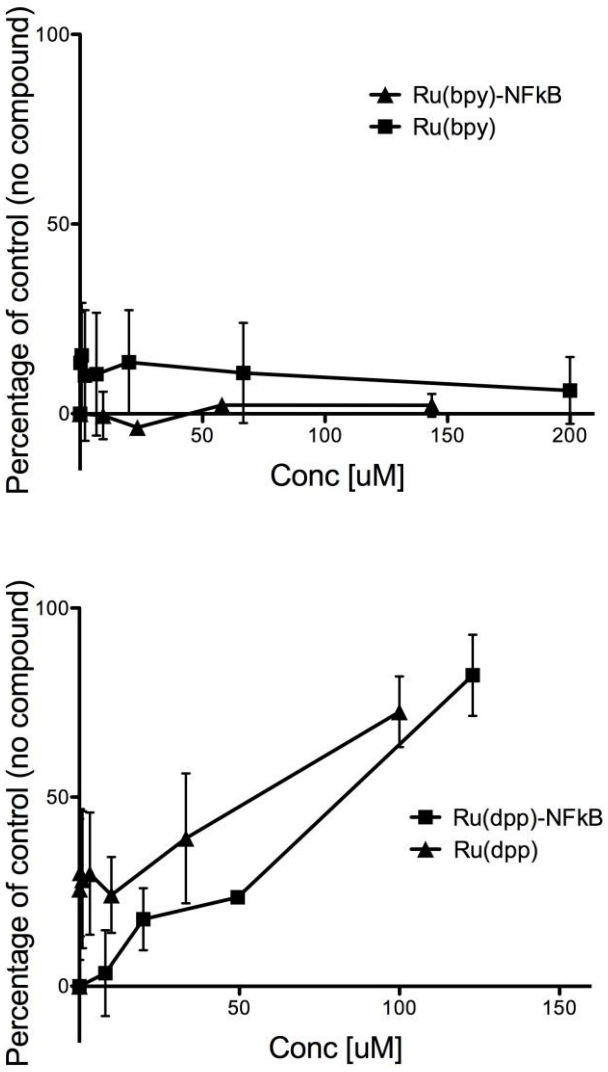

Figure S7: Cytotoxicity assay conducted on CHO cells treated with ruthenium $[Ru(bpy)_2PIC]ClO_4$ βAla-NF-κB conjugate (A) and $[Ru(dpp)_2PIC]ClO_4$ βAla-NF-κB conjugate (B). Cells were seeded in 96-well plates for 24 hours prior to the addition of Ru complexes and incubated in the dark overnight at 37 ^oC. Resazurin reagent was added to cells, incubated at 37^oC for 7 hours and absorbance measured using a Tecan plate reader at 570 nm with background correction at 600 nm, (n=3 ± SD).

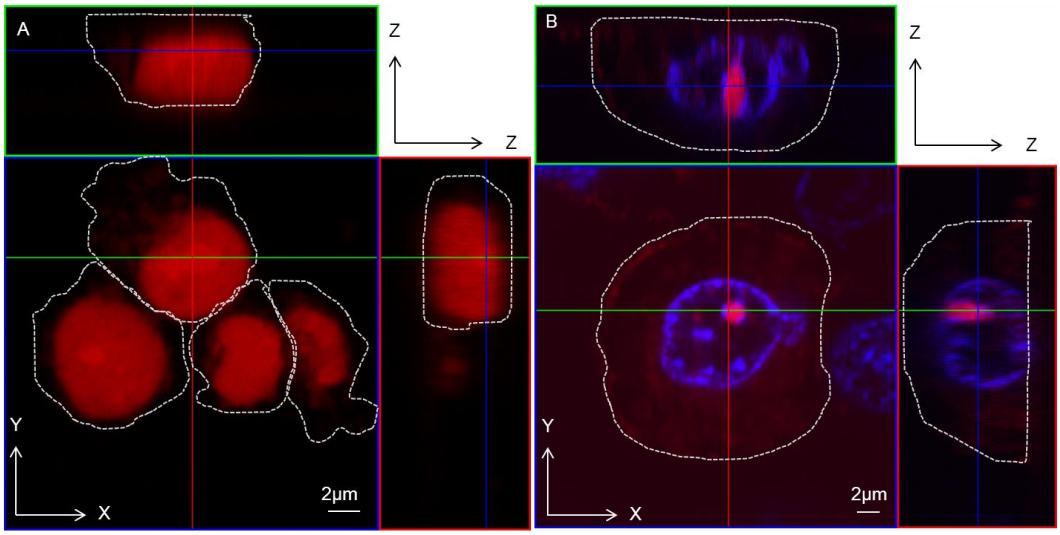

Figure S8 Ruthenium NF-κB conjugates permeate throughtout the cell. Z-stack Confocal Luminescence Images of SP2 murine myeloma cells with $[Ru(bpy)_2PIC]ClO_4$ βAla-NF-κB (A) and CHO cells with $[Ru(dpp)_2PIC]ClO_4$ βAla-NF-κB and DAPI nuclear stain (B). Dyes were incubated overnight with 40μM in culture media, washed in PBS and formaldehyde fixed (3.8% for 10 mins at room temperature). Outline of the cells in dashed lines were obtained by drawing around the backscatter reflection of the cells.

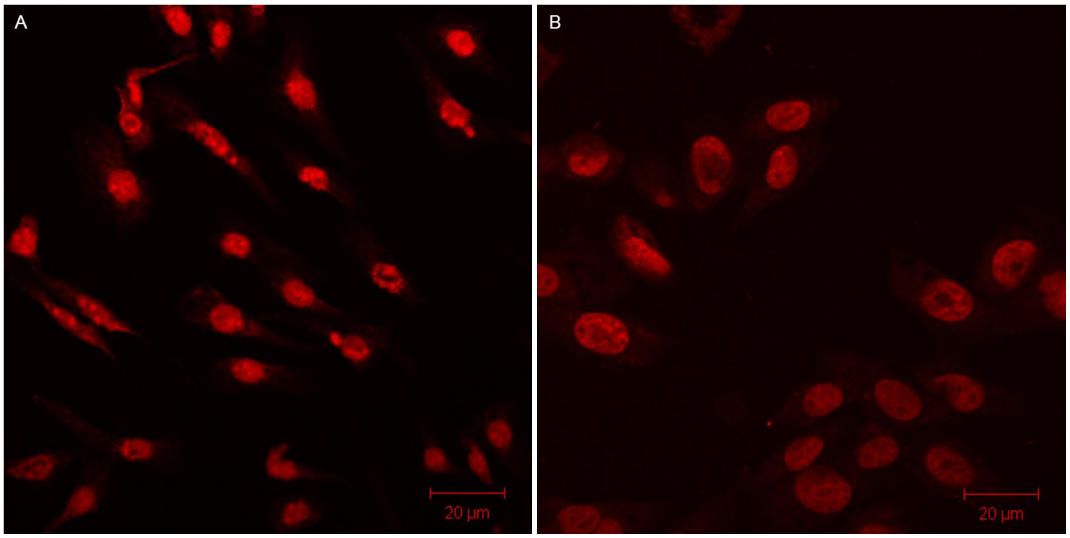

Figure S9 Large field of view confocal images showing nuclear localisation within the overall population of CHO cells with $[Ru(bpy)_2PIC]ClO_4$ βAla-NF-κB (A) and $[Ru(dpp)_2PIC]ClO_4$ βAla-NF-κB (B). Dyes were incubated overnight with 40μM in culture media, washed in PBS and formaldehyde fixed (3.8% for 10 mins at room temperature), n=3.

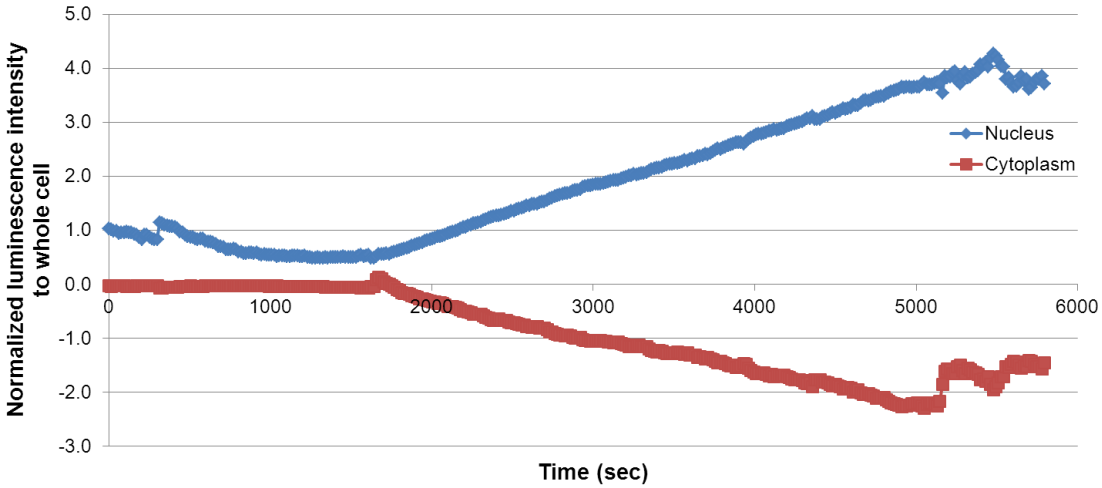

Figure S10. Time trace of luminescence intensity from cytoplasm and nucleus from Live CHO cells following exposure to $[Ru(bpy)_2PIC]ClO_4$ βAla-NF-κB over 95 minutes. . Total luminescence was recorded from five cells, averaged and normalised to total cell loading. Migration of the dye from the cytoplasm to the nucleus is observed after approximately 1800 seconds.

References

[i] Y. Pellegrin, R.J. Forster, T. E Keyes. Inorg. Chim. Acta, 2009, 362, 1715.

[ii] J.E. Dickeson and L.A. Summers, Aust. J. Chem. 1970, 23, , 1023.

[iii] B.P. Sullivan, D.J. Salmon, T. J., and Meyer, Inorg. Chem, 1978, 17, 3334.