Supplemental Material:

S1. Experimental

Synthetic Procedures:

2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline (LH_2): 0.21 g of phendione (1 mmol), 0.18 g of 4-carboxybenzaldehyde (1.2 mmol) and 1.54 g of ammonium acetate (20 mmol) were refluxed in glacial acetic acid for 3 hours. The yellow solution was then allowed to cool to room temperature. A yellow solid started to precipitate and addition of water afforded more powder. All solid phases were collected and washed thoroughly with water, methanol and ether. Yield: 0.25g (73%). ^{1}H-NMR (400 MHz, d^6-DMSO): δ 14.0 (s, 1H), 13.15 (s, broad, 1H), 9.05 (m, 2H), 8.95 (m, 2H), 8.40 (d, 2H), 8.18 (d, 2H), 7.85 (m, 2H). IR (KBr): ν(N-H) 3060, ν(C=O) 1707, ν(C=N) 1614 cm^{-1}.

$[Ru(bpy)_2(LH_2)](PF_6)_2$ ($[Ru\text{-}LH_2]^{2+}$): 0.05 g of $[Ru(bpy)_2Cl_2]$ (96 μmol) and 0.033 g of **LH_2** (1 equivalent) were refluxed in ethanol for 16 hours, in the dark. The red-orange solution was then evaporated under reduced pressure. The crude material was then dissolved in the minimum amount of cold methanol and unreacted ligand was removed by filtration. The filtrate was evaporated and the dark red powder obtained was thoroughly washed with dichloromethane; a light orange powder of **$[Ru\text{-}LH_2](PF_6)_2$** was obtained upon addition of aliquots of a concentrated aqueous solution of ammonium hexafluorophosphate. Yield: 0.085 g (85%). ^{1}H-NMR (400 MHz, d^6-DMSO): δ 9.03 (d, 2H), 8.84 (d, 2H), 8.81 (d, 2H), 8.41 (d, 2H), 8.20 (t, 2H), 8.05 (m, 4H), 7.85 (m, 6H), 7.58 (m, 4H), 7.35 (t, 2H). IR (KBr): ν(N-H) 3080, ν(C=O) 1707, ν(C=N) 1605, ν(P-F) 844 cm^{-1}. ESI-MS: 377.0 (M^{2+}).

Synthesis of the oligopeptide on solid support.

The peptides were prepared by standard Solid Phase Peptide Synthesis[1] according to the Fmoc-tBu strategy[2] with HBTU/HOBt/DIEA coupling chemistry, in NMP solvent. Single coupling cycles using a 10-fold excess of Fmoc amino acid derivatives to resin-

bound peptide were employed. The side chain protecting groups were Pbf, for Arg. The syntheses were carried out on a $1.0x10^{-4}$ mol scale.

Assembly of the amino acid sequence, starting from a Rink Amide MBHA resin and attachment of the N-terminal spacer were carried out on an automated peptide synthesizer (Applied Biosystems 433A).

The labelled peptides were then prepared by attachment of the ruthenium polypyridyl complex on an N-terminal spacer (6-aminohexanoic acid) using PyBOP/HOBt/DIEA (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate /1-Hydroxybenzotriazole hydrate/ diisopropylethylamine). This latter reaction was conducted manually in a syringe fitted with a Teflon filter. Peptides were deprotected and cleaved from the synthesis resin using a mixture of 80% trifluoroacetic Acid, 5% water, 5% triisopropylsilane, 10% thioanisole at RT for 4 h. The peptides were precipitated and washed three times with 10 ml portions of diethyl ether. They were then dried, dissolved in distilled water and lyophilized.

This aliphatic linker is introduced after elongation of the peptide sequence, by N-terminal conjugation of 6-aminohexanoic acid which is fluorenylmethoxycarbonyl (Fmoc) protected. Finally, after Fmoc deprotection, the ruthenium complex, $[Ru(bpy)_2(pic)]^{2+}$ was attached to the resin immobilized peptide via amide bond formation through the terminal carboxyl functionality on the ruthenium complex, by PyBOP/HOBt/DIEA (Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate N-Hydroxybenzotriazole/N,N'-Diisopropylethylamine) coupling chemistry. After cleavage and deprotection by treatment with trifluoracetic acid, the ruthenium labelled peptide, was purified by reverse phase HPLC and its structure confirmed by MALDI-TOF mass spectrometry.

Maldi-Tof-MS Ru-Ahx-R_8 and: Mcalc: 2115.4 M_{found}: 2113.155 (M-2H)

Ru-Ahx-R_5, M_{calc}: 1646.9 M_{found} 1644.9 (M-2H)

General Experimental Conditions:

Chromatographic analysis and purification were performed on a BioCAD SPRINT Perfusion Chromatography Workstation (PerSeptive Biosystems) using Gemini columns (5Å, C18, 4.6mmd/250mmL (analytic) 100mmd/250mmL (semi-preparative),

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Phenomenex). A mobile phase was 0.1% TFA in water; B mobile phase was 0.1% TFA in acetonitrile. The gradient employed was 2 to 60% B in 18 column volumes; flow rate: 4 ml/mn; single wavelength detection at 214 nm.

The peptides were characterised by Matrix Assisted Laser Desorption Ionisation – Time Of Flight – Mass Spectrometry (a-cyano-4-hydroxy-cinnamic acid matrix).

UV/Vis spectra were recorded on a Shimadzu UV-Vis/NIR 3100 spectrophotometer.

Steady-state emission spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer, and luminescence lifetimes were obtained using a Picoquant Fluotime 100 TCSPC system exciting at 470 nm and detecting at 600 nm using a narrow band pass dielectric filter.

Confocal luminescence Images (Myeloma) were recorded with a confocal fluorescence microscope (LSM 50, Zeiss) using a 64x oil immersion objective. An argon ion laser provided 458 nm and 488 nm excitation wavelengths. The scanned images contain 512 x 512 pixels.

Lifetime measurements were carried-out with the fluorescence lifetime upgrade kit from PicoQuant, Germany, equipped with a pulsed 405 nm excitation laser and SymPhoTime software.

Confocal luminescence Images (Human Platelets) were recorded on a Zeiss LSM-510 Axioplan 2 using a 63X Apochromat oil immersion objective lens with a numerical aperture of 1.4, to give a final magnification of 400.

S2. Photophysical Properties of complexes

Complex	**Oxygenated**	**deoxygenated**
RuPic, pH 7 in phosphate buffer	Φ_{em} = 0.067 τ_{em} unknown	Φ_{em} unknown τ_{em} = 872 ns
Ru-R8, pH 7 in phosphate buffer	Φ_{em} = 0.06 τ_{em} = 478 ns	Φ_{em} unknown τ_{em} = 775 ns

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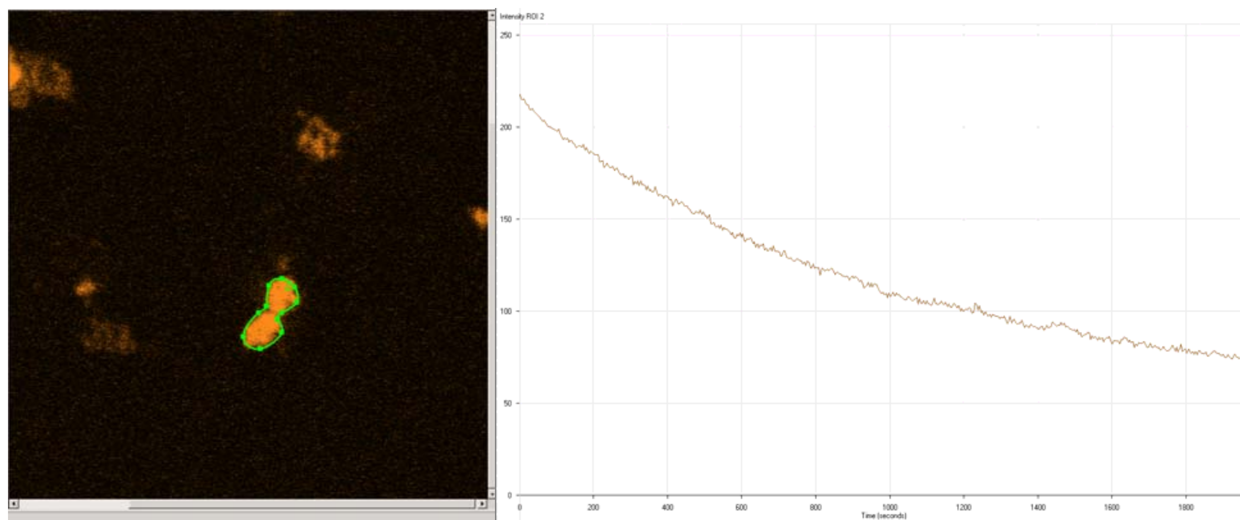
S2. Photobleaching study of Ru-Ahx-R_8 in human blood platelet.



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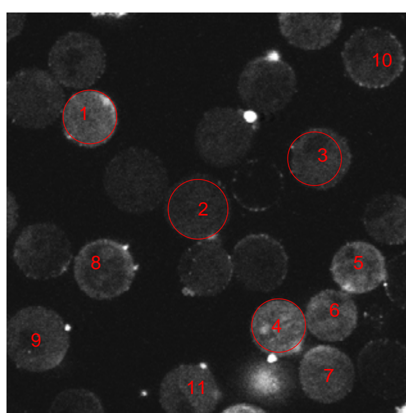
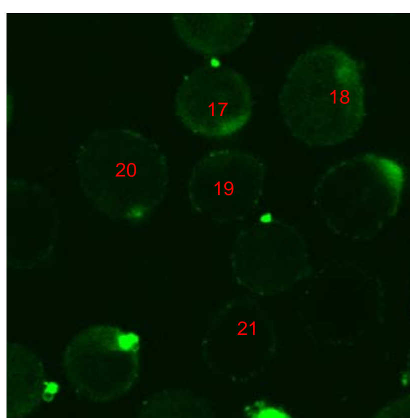
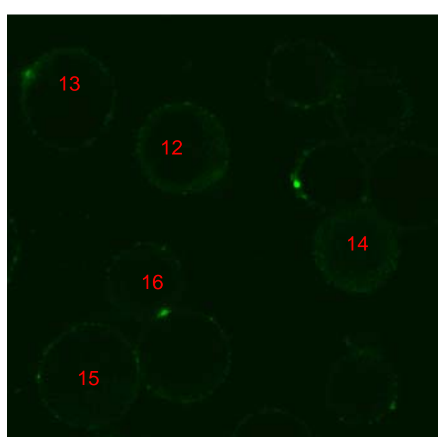
S3. Temperature Dependence of Cellular Uptake of Ru-Ahx-R8 After equilibrating to the right temperature (37°C, 20°C, 4°C) 4 ìl of aqueous Ru-Ahx-R_8 (1.2×10^{-3} M) was added to to 200 ìl aliquots of fresh harvested cells in media. After 20 minutes the cells were investigated under the fluorescence microscope. The same settings (458 nm excitation, reduced laser power 0.05%, pixel time 2.56 ìs, detection with 560 nm longpass filter, gain) were used for each temperature set and the amount of dye inside the cells could be related to the fluorescence counts inside the cells. The average counts inside the cells was determined using imageJ software.

(a) 37°C, Average intensity inside the cells: ca. 950 cts, (b) 20°C, Average intensity inside the cells: ca. 500 cts, (c) 37°C, Average intensity inside the cells: ca. 380 cts







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References

[1] Merrifield, R.B. Solid phase peptide synthesis I. Synthesis of a tetrapeptide. J. Am. Chem. Soc., 1963, 85, 2149-2154. b) Merrifield, R.B., Solid phase peptide synthesis. In Peptides: Synthesis, Structures and Applications (B. Gutte, ed.) Academic Press, New York, pp. 94-169.
[2] a) Carpino, L.A., Han, G.Y., J.Org. Chem., 1972, 37, 3404-3409. b) Atherton, E., Cameron, L.R., Sheppard, R.C., Tetrahedron, 1988, 44, 843-857.