## **Supplementary Information**

**Fig. S1.** Cyclic Voltammetry (scan rate:  $0.1 \vee s^{-1}$ ). (a)  $[Ir(sppy)_3]^{3^-}$ , (b)  $[Ru(bpy)_3]^{2^+}$  and (c)  $[Ir(ppy)_2(pt-TEG)]^+$ , at 1 mM in 0.1 M PBS (pH 7.5). (d) 100 mM TPrA in 0.1 M PBS (pH 7.5). (e) ProCell solution. The  $E^{0^{\prime\prime}}$  (M<sup>+</sup>/M) (vs SCE) for  $[Ir(sppy)_3]^{3^-}$ ,  $[Ru(bpy)_3]^{2^+}$  and  $[Ir(ppy)_2(pt-TEG)]^+$  (Table 1) were in good agreement with previously reported values.<sup>1</sup>



**Fig. S2**. (a) UV-visible absorption spectra, and (b) photoluminescence emission spectra at ambient temperature, of  $[Ru(bpy)_3]^{2+}$  (red plot),  $[Ir(sppy)_3]^{3-}$  (green plot), and  $[Ir(ppy)_2(pt-TEG)]^+$  (blue plot), at 10  $\mu$ M in deionised (Milli-Q) water, and  $Ir(ppy)_3$  (black plot) at 10  $\mu$ M in acetonitrile. (c) photoluminescence emission spectra of the same metal complexes at 5  $\mu$ M in 4:1 ethanol:methanol at low temperature (85 K). All emission spectra were corrected for the variation in instrument sensitivity over the wavelength range and then normalised.



**Fig. S3.** (a) Co-reactant ECL spectra of 10  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> (red plot), 100  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup> (green plot) and 10  $\mu$ M [Ir(ppy)<sub>2</sub>(pt-TEG)]<sup>+</sup> (blue plot) in ProCell solution. (b) Photographs of the co-reactant ECL at the surface of the working electrode at the stated applied potential *vs* SCE (ISO 3200, f/ 2.8, Exposure times of 16 s, 0.2 s and 0.2 s for the [Ir(sppy)<sub>3</sub>]<sup>3-</sup>, [Ir(ppy)<sub>2</sub>(pt-TEG)]<sup>+</sup> and [Ru(bpy)<sub>3</sub>]<sup>2+</sup>; 0.2 mM in 0.1 M PBS (pH 7.5) with 100 mM TPrA). (c) CIE plot of the ECL emission colour of the three luminophores (CIE 1931 2° observer).



**Fig. S4**. ECL spectra at 0.76 V (grey plot) and 0.81 V (blue plot) vs SCE (10 s pulse) for a mixture of  $[Ir(sppy)_3]^{3-}$  (150  $\mu$ M) and  $[Ru(bpy)_3]^{2+}$  (0.75  $\mu$ M) in ProCell, showing the approximate ECL on-set potential for both luminophores.



**Fig. S5.** Contour plot of ECL *vs* wavelength and applied potential for  $[Ir(sppy)_3]^{3-}$  (150  $\mu$ M) in ProCell solution, prepared by applying the potentials (10 s chronoamperometric pulses) in 50 mV intervals. Fig. 3 shows the contour plots for a mixture of  $[Ru(bpy)_3]^{2+}$  (0.75  $\mu$ M) and  $[Ir(sppy)_3]^{3-}$  (150  $\mu$ M), or  $[Ru(bpy)_3]^{2+}$  (0.75  $\mu$ M) alone.







**Fig. S7.** ECL intensity at 620 nm vs applied potential (10 s chronoamperometric pulses at 50 mV intervals) for 0.75  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> (red plot) or a mixture of 0.75  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> and 150  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup> (black plot), in ProCell solution, extracted from the data shown in Fig. 3. The grey plot shows the ECL intensity at 620 nm vs applied potential for the mixture of 0.75  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> and 150  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup>, after subtracting the intensity for 150  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup> (extracted from the data depicted in Fig. S5). The vertical dashed lines show the  $E^{0'}$  for [Ir(sppy)<sub>3</sub>]<sup>3-</sup>, TPrA and [Ru(bpy)<sub>3</sub>]<sup>2+</sup> in aqueous solution.





**Fig. S9.** ECL spectra for 0.75  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> and 100  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup> (blue plots) in ProCell solution, at an applied potential of (a) 1.16 V or (b) 0.86 V vs SCE, compared to the individual electrochemiluminophores under the same conditions (red and green plots, respectively). (c) Deconvolution of the mixture (*i.e.*, the blue plot of Fig. S9b), where the red and green lines show the deconvoluted emissions from the [Ru(bpy)<sub>3</sub>]<sup>2+</sup> and [Ir(sppy)<sub>3</sub>]<sup>3-</sup> luminophores, and the black line is the sum of the two components showing the excellent fit with the raw data. For clarity, spectra shown in this figure were smoothed by adjacent averaging (15 pt).



**Fig. S10**. ECL spectra generated with an applied potential of (a) 1.16 V and (b) 0.86 V vs SCE for varying concentrations of  $[Ir(sppy)_3]^{3-}$  (1, 5, 25, 50 or 100  $\mu$ M) in ProCell solution, without (green plots) and with (blue plots) 0.75  $\mu$ M  $[Ru(bpy)_3]^{2+}$ . The black plots show the ECL spectra for 0.75  $\mu$ M  $[Ru(bpy)_3]^{2+}$  in ProCell solution without  $[Ir(sppy)_3]^{3-}$ . Spectra were smoothed by adjacent averaging (15 pt). (c) Relative ECL intensity of  $[Ru(bpy)_3]^{2+}$  in the experiments depicted in Fig. S10a and S10b, established by deconvolution of the emission spectra from both luminophores (for example, see Fig. S9c), relative to the emission at each applied potential in the absence of  $[Ir(sppy)_3]^{3-}$ .



**Fig. S11**. Photographs and corresponding images of the extracted RGB data of the ECL at the working electrode surface for 0.1-10  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> in ProCell solution with 100  $\mu$ M [Ir(sppy)<sub>3</sub>]<sup>3-</sup> upon application of 0.86 V vs SCE. The rightmost column shows the data for 5  $\mu$ M [Ru(bpy)<sub>3</sub>]<sup>2+</sup> in ProCell solution without the [Ir(sppy)<sub>3</sub>]<sup>3-</sup> enhancer, under the same experimental conditions. Settings: ISO 10,000, f/ 3.5, shutter time 10 s, electrochemical pulse time: 9.5 s with 0.2 s wait between shutter trigger and chronoamperometric pulse.



**Fig. S12**.  $[Ru(bpy)_3]^{2+}$  calibrations prepared using the R values (red plot) or the ratio of the R to G values from the RGB data extracted from the images (Fig. S11), after initial subtraction of the corresponding value (R) or ratio (R/G) for the blank solution containing the enhancer in ProCell, but no  $[Ru(bpy)_3]^{2+}$ . Error bars represent ±1 standard deviation (n = 3).



## References

X. Guo, Y. Okamoto, M. R. Schreier, T. R. Ward and O. S. Wenger, *Chem. Sci.*, 2018, 9, 5052-5056; A. Fiorani, G. Valenti, Irkham, F. Paolucci and Y. Einaga, *Phys. Chem. Chem. Phys.*, 2020, 22, 15413-15417; L. Chen, D. J. Hayne, E. H. Doeven, J. Agugiaro, D. J. D. Wilson, L. C. Henderson, T. U. Connell, Y. H. Nai, R. Alexander, S. Carrara, C. F. Hogan, P. S. Donnelly and P. S. Francis, *Chem. Sci.*, 2019, 10, 8654-8667.