Electrogenerated Chemiluminescence

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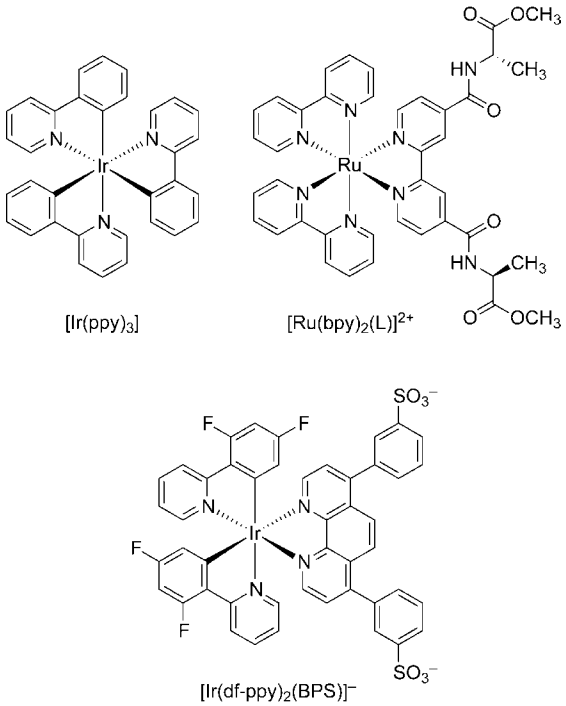
Selective Excitation of Concomitant Electrochemiluminophores: Tuning Emission Color by Electrode Potential**

Egan H. Doeven, Elizabeth M. Zammit, Gregory J. Barbante, Conor F. Hogan, Neil W. Barnett, and Paul S. Francis**

The electrogenerated chemiluminescence (ECL) of tris(2,2′-bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) ions with co-reactants in aqueous solution[1] is a remarkably sensitive and versatile detection system[2] that is now exploited in commercial instrumentation throughout the world for rapid screening and quantification of clinical biomarkers, food borne pathogens and biowarfare agents.[3] New applications of the ECL of $[Ru(bpy)_3]^{2+}$ and related complexes continue to appear in areas as diverse as light-emitting devices,[4] anion sensing,[5] multiplexed (microarray) immunoassays,[6] detection of damaged or sequence-specific DNA,[7] and molecular encoding–decoding.[8]

The pursuit of superior ECL reagents and strategies that underpin such applications remains intensive,[2f,h] and is spurred by advances in the manipulation of reagent properties (for example ECL efficiency, solubility, spectral distribution) derived from extensive investigation of ruthenium, iridium, and other metal complexes,[9] and innovations in nanotechnology.[10]

Considerable attention has been focused on controlling the emission color,[9d,e,11] with the enticing prospect of simultaneously detecting multiple, spectrally distinct electrochemiluminophores for multi-analyte quantification or internal standardization.[2e,9e,11,12] The implementation of these approaches, however, is complicated by the small number of available complexes with high ECL efficiency and solubility in analytically useful solvents,[10c,13] and is fundamentally limited by the width of the emission bands,[11a,14] which can span hundreds of nanometers. ECL spectra obtained by Richter et al. using a mixture of $[Ru(bpy)_3]^{2+}$ and either $[Ir(ppy)_3]$ (ppy = 2-phenylpyridine) or $[Ir(df\text{-}ppy)_2(pic)]$ (df-ppy = 2-(2,4-difluorophenyl)pyridine, pic = 2-carboxypyridine; see Scheme 1) in acetonitrile with tripropylamine (TPA) as a co-reactant show considerable spectral overlap despite differences in emission maxima of 100 and 150 nm, respectively.[11a,12a,15]



Scheme 1. Metal complexes selected for dual-emitter investigations (L = N^4,$N^{4'}$-bis((2*S*)-1-methoxy-1-oxopropan-2-yl)-2,2′-bipyridyl-4,4′-dicarboxamide). The position of the sulfonate groups in the BPS ligand is dependent on the source of the material.[17] The synthesis and characterization of $[Ru(bpy)_2(L)]^{2+}$ and $[Ir(df\text{-}ppy)_2(BPS)]^-$ have been described previously.[9d,18]

If we compare ECL with photoluminescence, where selectivity is routinely derived not only from the wavelengths of emission, but also the energy required to attain the electronically excited state of the fluorophore,[16] the question arises of whether the excitation processes of ECL can also be exploited for selectivity between multiple emitting species. Herein we demonstrate that such systems can indeed be controlled through electrode potential (Figure 1). We then extend this concept through the repeated acquisition of ECL spectra during cyclic voltammetry experiments to derive three-dimensional (intensity versus wavelength and potential) resolution of electrochemiluminophores.

The ECL of metal complexes with an oxidative-reduction co-reactant, such as TPA, involves several interrelated pathways,[19] the most dominant of which is dependent upon reaction conditions. Inspection of several key reaction steps

[*] E. H. Doeven, Dr. C. F. Hogan
Department of Chemistry, La Trobe Institute for Molecular Science
La Trobe University, Victoria 3086 (Australia)
E-mail: c.hogan@latrobe.edu.au
E. M. Zammit, Dr. G. J. Barbante, Prof. N. W. Barnett, Dr. P. S. Francis
Centre for Biotechnology, Chemistry and Systems Biology
School of Life and Environmental Sciences
Deakin University, Geelong, Victoria 3216 (Australia)
E-mail: paul.francis@deakin.edu.au

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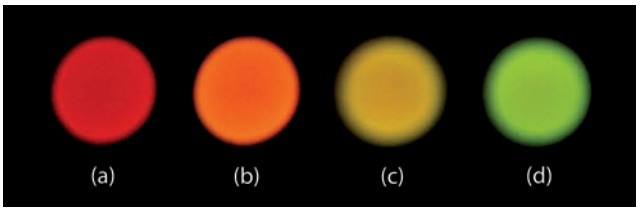

Figure 1. Photographs of electrogenerated chemiluminescence from a single solution of two metal complexes at four electrode potentials: a) 0.8 V, b) 0.95 V, c) 1.23 V, and d) 1.40 V (versus the ferrocene/ferrocinium couple). The solution contained 0.95 mM $[Ir(df\text{-}ppy)_2(BPS)]^-$ and 0.05 mM $[Ru(bpy)_2(L)]^{2+}$ (see Scheme 1 for chemical structures), with 30 mM TPA co-reactant, and 0.1 M phosphate buffer in 1:1 v/v water/acetonitrile. The colors were first observed by the naked eye, and then photographs were taken at values of applied potential that provided the greatest differences. Longer exposure times were used for the redder emissions to achieve uniform image brightness.

[for example Equations (1)–(5)] reveals two theoretical sources of selectivity for different metal complexes: the electrode potential and the co-reactant.

$$[Ru(bpy)_3]^{2+} - e^- \rightarrow [Ru(bpy)_3]^{3+} \quad (1)$$

$$[Ru(bpy)_3]^{3+} + TPA \rightarrow [Ru(bpy)_3]^{2+} + TPA^{\bullet+} \quad (2)$$

$$TPA - e^- \rightarrow TPA^{\bullet+} \quad (3)$$

$$TPA^{\bullet+} - H^+ \rightarrow TPA^{\bullet} \quad (4)$$

$$[Ru(bpy)_3]^{3+} + TPA^{\bullet} \rightarrow [Ru(bpy)_3]^{2+}\,* \quad (5)$$

Changes in the relative emission intensities from various metal complexes (in separate solutions) with different co-reactants have been observed,[13,20] but the changes are difficult to predict because the reactivity of both the co-reactant and its radical oxidation products must be considered[19,21] and they cannot be modified without replacing the reaction media. On the other hand, the electrode potential can be easily tuned and applied at multiple levels to the same system. We initially explored this idea under conditions akin to those utilized by Richter and co-workers in their study of the emission from two metal complexes at a single electrode potential,[11a,12a] which in our case comprised $[Ir(ppy)_3]$ and $[Ru(bpy)_2(L)]^{2+}$ (Scheme 1) in acetonitrile with TPA as a co-reactant. The addition of the electron-withdrawing amide groups on the parent $[Ru(bpy)_3]^{2+}$ complex imparts a significant bathochromic shift in the emission ($\lambda_{max} = 666$ nm) by lowering the π* (LUMO) energy level,[9d] thus increasing the spectral separation from $[Ir(ppy)_3]$ (ca. 150 nm between peak maxima). The two complexes also have distinct oxidation potentials: $[Ir(ppy)_3]$ at 0.32 V and $[Ru(bpy)_2(L)]^{2+}$ at 1.01 V versus ferrocene.

When subjected to an electrode potential sweep capable of oxidizing both complexes (0 to 1.04 V versus ferrocene), the ECL spectrum for this combined system exhibited the two characteristic emission bands of the respective charge transfer (^{3}MLCT and/or ^{3}LC) states (Figure 2a; spectrum A), similar to that demonstrated by Muegge and Richter[11a] for $[Ir(df\text{-}ppy)_2(pic)]$ and $[Ru(bpy)_3]^{2+}$. However, by selecting a voltage between the oxidation potentials of the two complexes, we were able to generate the $[Ir(ppy)_3]$ ECL peak alone (Figure 1a; spectrum B) from the same reaction mixture.

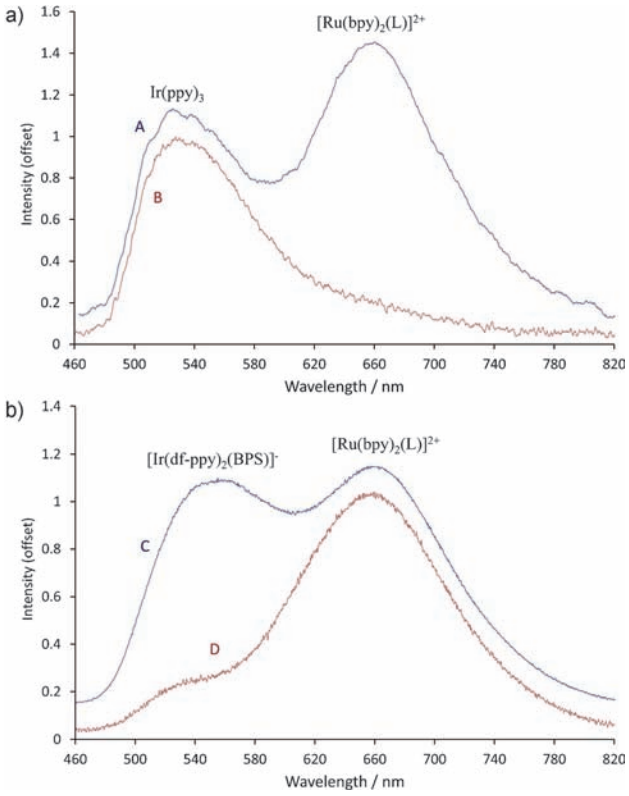

Figure 2. a) ECL spectra generated using an electrode sweep at 0.05 Vs^{-1} from 0 to 1.04 V (spectrum A) or 0 to 0.44 V (spectrum B) vs. ferrocene for 0.5 mM $[Ir(ppy)_3]$ and 0.025 mM $[Ru(bpy)_2(L)]^{2+}$ in acetonitrile/0.1 M $TBAPF_6$ (TBA = tetrabutylammonium) with 10 mM tripropylamine (TPA) as co-reactant. b) ECL spectra generated using chronoamperometry pulses at C) 1.30 V or D) 1.04 V vs. ferrocene for 0.1 mM $[Ir(df\text{-}ppy)_2(BPS)]^-$ and 0.01 mM $[Ru(bpy)_2(L)]^{2+}$ in 0.1 M phosphate buffer, 1:1 v/v water/acetonitrile with 10 mM TPA co-reactant (see the Supporting Information for further details).

Figure 2a shows that the ECL from the iridium complex emerged at lower electrode potentials than its ruthenium counterpart, but this order can be reversed by modifying ligand structure. Substitution of a cyclometalating phenylpyridine of the iridium complex with an ancillary diimine ligand raises the oxidation potential[9a,22] and can improve the solubility in more analytically useful polar solvents.[9e] Herein we selected the dianionic BPS ligand to incorporate additional polar functionality. The deleterious bathochromic shift induced by the diimine ligand was countered by the addition of electron-withdrawing fluorine groups on the phenyl rings of the remaining ppy ligands,[9e,23] which stabilizes the HOMO level from the metal and ligand orbitals and further increases the $Ir^{IV/III}$ potential.[9e,24] The emission maximum of $[Ir(df\text{-}ppy)_2(BPS)]^-$ ($\lambda_{max} = 547$ nm)[18] is still 120 nm lower than that of $[Ru(bpy)_2(L)]^{2+}$, but the iridium complex is less readily oxidized, with an oxidation potential of 1.25 V versus ferrocene (see the Supporting Information). Unlike $[Ir(ppy)_3]$, both $[Ir(df\text{-}ppy)_2(BPS)]^-$ and $[Ru(bpy)_2(L)]^{2+}$ are

highly soluble in 1:1 v/v water/acetonitrile. The characteristic ECL bands of both complexes were observed when this system was subjected to 1.30 V versus ferrocene (Figure 2b; spectrum C) and lower electrode potentials now imparted considerable selectivity towards the ruthenium complex (Figure 2b; spectrum D), albeit with lower overall emission intensities.

The light emitted from a mixture of $[Ir(df\text{-}ppy)_2(BPS)]^-$ and $[Ru(bpy)_2(L)]^{2+}$, with TPA as co-reactant, at the electrode surface was visually inspected through the transparent base of the electrochemical cell, with experiments conducted in a dark room. We observed a clear change in the color of the emitted light, from red to green, with increasing electrode potential (Figure 1).

The emission of concomitant electrochemiluminophores over wide ranges of both electrode potential and emission wavelength can be rapidly explored by automated acquisition of ECL spectra using a CCD camera coupled with cyclic voltammetry experiments. This novel approach enables the rapid generation of three-dimensional ECL spectra for a comprehensive characterization of the luminescent redox system. Figure 3a, for example, shows the data acquired during the forward electrode potential sweep (1.0 V to 1.3 V vs ferrocene) for a mixture of 0.1 mM $[Ir(df\text{-}ppy)_2(BPS)]^-$ and 0.01 mM $[Ru(bpy)_2(L)]^{2+}$. A total of 30 spectra were obtained, each an average of 40×18 ms scans. The entire measurement therefore required about 22 s. The analogous three-dimensional photoluminescence plot is shown in Figure 3b. For this combination of complexes over these excitation ranges, the ECL and photoluminescence plots both show the emergence of the ruthenium complex followed by the iridium complex, as the respective excitation energy is modulated. Although the system is sufficiently robust to allow a large number of measurement cycles, it is clear that further work is needed to improve the ECL efficiency of the green emitting components for real-world applications.

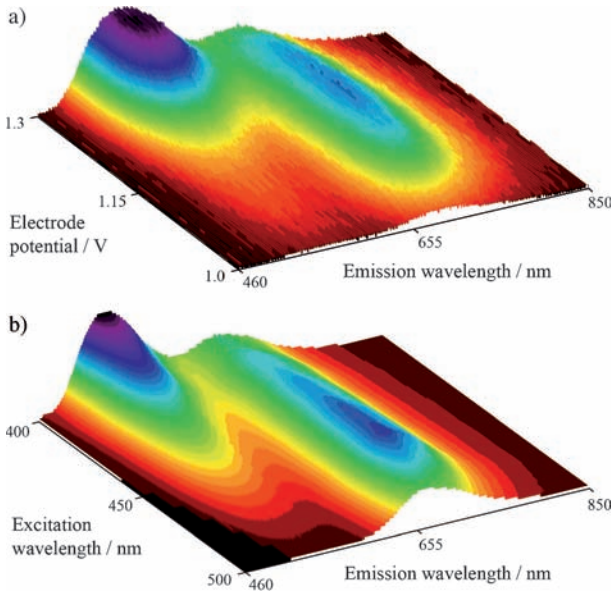

Figure 3. 3D graphs of: a) ECL intensity as a function of applied potential and emission wavelength, created by an automated acquisition of ECL spectra throughout the forward sweep of a cyclic voltammogram; and b) photoluminescence intensity as a function of excitation and emission wavelengths. Conditions: a) 0.1 mM $[Ir(df\text{-}ppy)_2(BPS)]^-$ and 0.01 mM $[Ru(bpy)_2(L)]^{2+}$ in 1:1 v/v water/acetonitrile with 10 mM TPA; b) 0.05 mM $[Ir(df\text{-}ppy)_2(BPS)]^-$ and 0.025 mM $[Ru(bpy)_2(L)]^{2+}$ in 1:1 v/v water/acetonitrile (see the Supporting Information for further details).

We have also examined the analogous chemically initiated luminescence (chemiluminescence) from two metal complexes ($[Ir(df\text{-}ppy)_2(BPS)]^-$ and $[Ru(bpy)_2(L)]^{2+}$) in 1:1 v/v water/acetonitrile with a tertiary amine co-reactant (ofloxacin or codeine) by merging the solution with 1 mM cerium(IV) sulfate (in 0.05 M H_2SO_4) in a spiral glass flow-cell positioned within a Cary Eclipse fluorescence spectrophotometer. Under these conditions we observed the simultaneous emission from the two complexes (data not shown), similar to ECL spectrum C in Figure 2b. Selective chemical excitation of the two luminophores is theoretically possible, but unlike ECL, the oxidation potentials are limited to those of available chemical reagents. The chemiluminescence of ruthenium[2b] and iridium[13,18] complexes is most often initiated with lead dioxide or cerium(IV), although permanganate[25] and bromate[26] have also been used. However, none of these oxidants were suitable to elicit an intense emission from only one complex in the presence of the other.[27]

This approach to electrogenerated chemiluminescence, which exploits differences in redox and luminescence properties, opens new avenues for multianalyte ECL detection and also for the characterization of the photophysical properties and energy transfer mechanisms of luminescent metal complexes. Moreover, the ability to tune the emission ratio of multiple electrochemiluminophores with distinct spectral distributions creates new possibilities for color selection in light-emitting devices.

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[1] I. Rubinstein, A. J. Bard, *J. Am. Chem. Soc.* **1981**, *103*, 512–516.
[2] a) M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003–3036; b) B. A. Gorman, P. S. Francis, N. W. Barnett, *Analyst* **2006**, *131*, 616–639; c) R. Pyati, M. M. Richter, *Annu. Rep. Prog. Chem. Sect. C: Phys. Chem.* **2007**, *103*, 12–78; d) W. Miao, *Chem. Rev.* **2008**, *108*, 2506–2553; e) M. M. Richter, in *Optical Biosensors, 2nd Ed.* (Eds.: F. S. Ligler, C. Rowe Taitt), Elsevier, Amsterdam, **2008**, pp. 317–384; f) R. J. Forster, P. Bertoncello, T. E. Keyes, *Annu. Rev. Anal. Chem.* **2009**, *2*, 359–385; g) A. Kapturkiewicz, in *Electrochemistry of Functional Supramolecular Systems* (Eds.: P. Ceroni, A. Credi, M. Venturi), Wiley-Interscience, Hoboken, N. J., **2010**, pp. 477–522; h) L. Hu, G. Xu, *Chem. Soc. Rev.* **2010**, *39*, 3275–3304.
[3] a) J. D. Debad, E. N. Glezer, J. Wohlstadter, G. B. Sigal, J. K. Leland, in *Electrogenerated Chemiluminescence* (Ed.: A. J. Bard), Marcel Dekker, New York, **2004**, pp. 359–396; b) X.-H. N. Xu, Y. Zu, in *New Frontiers in Ultrasensitive Bioanalysis*

(Ed.: X.-H. N. Xu), John Wiley & Sons, Hoboken, New Jersey, **2007**, pp. 235 – 267.
[4] K. Ide, M. Fujimoto, T. Kado, S. Hayase, *J. Electrochem. Soc.* **2008**, *155*, B645 – B649.
[5] E. Berni, I. Gosse, D. Badocco, P. Pastore, N. Sojic, S. Pinet, *Chem. Eur. J.* **2009**, *15*, 5145 – 5152.
[6] F. Deiss, C. N. La Fratta, M. Symer, T. M. Blicharz, N. Sojic, D. R. Walt, *J. Am. Chem. Soc.* **2009**, *131*, 6088 – 6089.
[7] a) L. Dennany, R. J. Forster, B. White, M. Smyth, J. F. Rusling, *J. Am. Chem. Soc.* **2004**, *126*, 8835 – 8841; b) W. Cao, J. P. Ferrance, J. Demas, J. P. Landers, *J. Am. Chem. Soc.* **2006**, *128*, 7572 – 7578.
[8] P. Ceroni, G. Bergamini, V. Balzani, *Angew. Chem. Int. Ed.* **2009**, *48*, 8516 – 8518.
[9] a) J. I. Kim, I.-S. Shin, H. Kim, J.-K. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 1614 – 1615; b) S. Campagna, F. Puntoriero, F. Nastasi, G. Bergamini, V. Balzani, *Top. Curr. Chem.* **2007**, *280*, 117 – 214; c) L. Flamigni, A. Barbieri, C. Sabatini, B. Ventura, F. Barigelletti, *Top. Curr. Chem.* **2007**, *281*, 143 – 203; d) G. J. Barbante, C. F. Hogan, D. J. D. Wilson, N. A. Lewcenko, F. M. Pfeffer, N. W. Barnett, P. S. Francis, *Analyst* **2011**, *136*, 1329 – 1338; e) S. Zanarini, M. Felici, G. Valenti, M. Marcaccio, L. Prodi, S. Bonacchi, P. Contreras-Carballada, R. M. Williams, M. C. Feiters, R. J. M. Nolte, L. De Cola, F. Paolucci, *Chem. Eur. J.* **2011**, *17*, 4640 – 4647; f) D. J. E. Piper, G. J. Barbante, N. Brack, P. J. Pigram, C. F. Hogan, *Langmuir* **2011**, *27*, 474 – 480.
[10] a) P. Bertoncello, R. J. Forster, *Biosens. Bioelectron.* **2009**, *24*, 3191 – 3200; b) S. Zanarini, E. Rampazzo, L. Della Ciana, M. Marcaccio, E. Marzocchi, M. Montalti, F. Paolucci, L. Prodi, *J. Am. Chem. Soc.* **2009**, *131*, 2260 – 2267; c) S. Zanarini, E. Rampazzo, S. Bonacchi, R. Juris, M. Marcaccio, M. Montalti, F. Paolucci, L. Prodi, *J. Am. Chem. Soc.* **2009**, *131*, 14208 – 14209.
[11] a) B. D. Muegge, M. M. Richter, *Anal. Chem.* **2004**, *76*, 73 – 77; b) A. Kapturkiewicz, T.-M. Chen, I. R. Laskar, J. Nowacki, *Electrochem. Commun.* **2004**, *6*, 827 – 831; c) A. Kapturkiewicz, J. Nowacki, P. Borowicz, *Electrochim. Acta* **2005**, *50*, 3395 – 3400.
[12] a) D. Bruce, M. M. Richter, *Anal. Chem.* **2002**, *74*, 1340 – 1342; b) C. Cole, B. D. Muegge, M. M. Richter, *Anal. Chem.* **2003**, *75*, 601 – 604.
[13] R. V. Kiran, E. M. Zammit, C. F. Hogan, B. D. James, N. W. Barnett, P. S. Francis, *Analyst* **2009**, *134*, 1297 – 1298.
[14] V. V. Grushin, N. Herron, D. D. LeCloux, W. J. Marshall, V. A. Petrov, Y. Wang, *Chem. Commun.* **2001**, 1494 – 1495.
[15] Further refinement of this approach could be expected from new strategies to prepare water-soluble complexes exhibiting intense green and blue ECL: S. Zanarini, M. Felici, G. Valenti, M. Marcaccio, L. Prodi, S. Bonacchi, P. Contreras-Carballada, R. M. Williams, M. C. Feiters, R. J. M. Nolte, L. De Cola, F. Paolucci, *Chem. Eur. J.* **2011**, 17, 4640 – 4647; and the application of spectral deconvolution: W. R. Algar, U. J. Krull, *Anal. Bioanal. Chem.* **2010**, 398, 2439 – 2449.
[16] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Springer, New York, **2006**.
[17] a) G. P. McDermott, E. M. Zammit, E. K. Bowen, M. M. Cooke, J. L. Adcock, X. A. Conlan, F. M. Pfeffer, N. W. Barnett, G. A. Dyson, P. S. Francis, *Anal. Chim. Acta* **2009**, *634*, 222 – 227; b) R. V. Kiran, C. F. Hogan, B. D. James, D. J. D. Wilson, *Eur. J. Inorg. Chem.* **2011**, 4816 – 4825.
[18] E. M. Zammit, N. W. Barnett, L. C. Henderson, G. A. Dyson, M. Zhou, P. S. Francis, *Analyst* **2011**, *136*, 3069 – 3072.
[19] W. Miao, J.-P. Choi, A. J. Bard, *J. Am. Chem. Soc.* **2002**, *124*, 14478 – 14485.
[20] M. M. Cooke, E. H. Doeven, C. F. Hogan, J. L. Adcock, G. P. McDermott, X. A. Conlan, N. W. Barnett, F. M. Pfeffer, P. S. Francis, *Anal. Chim. Acta* **2009**, *635*, 94 – 101.
[21] C. M. Hindson, G. R. Hanson, P. S. Francis, J. L. Adcock, N. W. Barnett, *Chem. Eur. J.* **2011**, *17*, 8018 – 8022.
[22] Y. Ohsawa, S. Sprouse, K. A. King, M. K. DeArmond, K. W. Hanck, R. J. Watts, *J. Phys. Chem.* **1987**, *91*, 1047 – 1054.
[23] A. B. Tamayo, B. D. Alleyne, P. I. Djurovich, S. Lamansky, I. Tsyba, N. N. Ho, R. Bau, M. E. Thompson, *J. Am. Chem. Soc.* **2003**, *125*, 7377 – 7387.
[24] R. Ragni, E. A. Plummer, K. Brunner, J. W. Hofstraat, F. Babudri, G. M. Farinola, F. Naso, L. De Cola, *J. Mater. Chem.* **2006**, *16*, 1161 – 1170.
[25] Z. Lin, X. Chen, Z. Cai, P. Li, X. Chen, X. Wang, *Talanta* **2008**, *75*, 544 – 550.
[26] K. P. Zeyer, F. W. Schneider, *J. Phys. Chem. A* **1998**, *102*, 9702 – 9709.
[27] The preliminary off-line oxidation of $[Ir(df\text{-}ppy)_2(BPS)]^-$ and $[Ru(bpy)_2(L)]^{2+}$ with solid lead dioxide was precluded by the relative instability of the respective oxidized forms of the complexes in comparison with $[Ru(bpy)_3]^{3+}$. On-line oxidation with Ce^{IV} produced an intense emission simultaneously from both chemiluminophores (similar to that of the ECL spectrum (C) in Figure 2b). The emission obtained using either bromate or permanganate was insufficient to establish the spectral distribution.

