

Electronic Supplementary Information:

‘Solid-supported chemiluminescence and electrogenerated chemiluminescence based on a tris(2,2’-bipyridyl)ruthenium(II) derivative’

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Instrumentation. Unless otherwise stated, nuclear magnetic resonance (NMR) spectra were obtained at room temperature as solutions in deuteriodimethylsulfoxide (*d*-DMSO) or deuteriomethanol (*d*-MeOH) using a Joel GX400 spectrometer. The chemical shifts are given in parts per million (ppm) with coupling constants in Hertz (Hz). Infra-red spectra were recorded in the solid state (4000 to 600 cm⁻¹) using a Perkin Elmer Paragon 1000 FT-IR spectrometer and peaks (ν_{max}) reported in wavenumbers (cm⁻¹). Inductively coupled plasma-mass spectrometry (ICP-MS) measurements were made at 349.89 nm and 240.27 nm using a Perkin Elmer Optima 5300DV instrument. The pH of buffered solutions was measured using a Hanna Instruments HI 8520 pH meter (Hertfordshire, UK) which was calibrated using the appropriate reference solutions. Chemiluminescence emission was detected using a photomultiplier tube (B2EBA/RFI, Thorn EMI, New York, USA) with a current converter (3000R, Thorn EMI, Ruislip, UK) maintained at an operating voltage of 600 V and recorded using a Chessell chart recorder (Kipp and Zonen, Netherlands). Electrochemically induced chemiluminescence was performed using a three-electrode system coupled to a PalmSens potentiostat (Palm Instruments BV, Netherlands). All CL and ECL measurements were performed within an in-house fabricated light-tight box, containing a zero aperture iris diaphragm shutter (Edmonds Optics, York, UK) to enable easy access to the reaction cells.

Reagents. All reagents were of analytical grade and used as received. Ruthenium trichloride hydrate (35-40 % Ru) was purchased from Acros Organic (New Jersey, USA), trimethylorthosilicate (+ 99 %), triethylamine (99.5 %), 3-aminopropyltriethoxysilane (99 %), 4,4’-dimethyl-2,2’-bipyridine (99.5 %), potassium bromide (+ 99 %, FT-IR grade), sodium hydroxide (97 %), boric acid (99 %), formamide (99 %) and cerium (IV) sulfate hydrate (98 %) were purchased from Aldrich (Gillingham, UK). Sodium dichromate (97 %) and tris(bipyridyl)ruthenium(II)chloride hexahydrate (90 to 95 %) were supplied by Fluka Chemie (Gillingham, UK), hydrochloric acid (38 %) and sulfuric acid (98 %) by Fisher Scientific (Loughborough, UK), aqueous potassium silicate (21 % SiO₂, 9 % K₂O) by VWR International (Poole, UK), codeine sulfate (99.0 %) and controlled pore glass (120-200 mesh, 500 Å pore size and 51.4 m² g⁻¹ surface area) were purchased from Sigma (Gillingham, UK). Anhydrous solvents employed in this study were purchased from Fluka Chemie (Gillingham, UK) as puriss grade and maintained over the appropriate molecular sieves. Deuteriodimethylsulfoxide (99.98 %) and deuteriomethanol (99.9 %) were obtained from Cambridge Isotope Laboratories (Massachusetts, USA); all other solvents were of HPLC grade and obtained from Fisher Scientific (Loughborough, UK). Platinum wire (0.72 mm x 2.00 cm), silver wire (1.00 mm x 2.00 cm) and type 2 glassy carbon electrodes (1.00 mm x 2.00 cm) were purchased from Alfa aesar (Karlsruhe, Germany).

Synthesis of 2,2’-bipyridine-4,4’-dicarboxylic acid (4).¹ 4,4’-Dimethyl-2,2’-bipyridine **5** (0.79 g, 4.30 mmol) was added slowly to a stirred solution of sodium dichromate (2.54 g, 9.70 mmol) in concentrated sulfuric acid (2.5 ml) to afford an orange slurry. After stirring at room temperature for 30 min, the reaction mixture turned dark green and was subsequently poured into cold distilled water (100 ml) to afford a pale yellow precipitate. The precipitate was then filtered under suction and dissolved in 10 % aq. potassium hydroxide (100 ml). The product was crystallised by acidifying the solution with 10 % aq. hydrochloric acid (pH 2) to afford 2,2’-bipyridine-4,4’-dicarboxylic acid **4** as a white crystalline solid (1.04 g, 97.8 %); δ_{H} (400 MHz, *d*-DMSO) 7.91 (2H, dd, *J* 1.7 and 4.9, ArH), 8.84 (2H, d, *J* 1.7, ArH), 8.91 (2H, dd, *J* 1.7 and 4.9, ArH) and 13.9 (2H, s, OH); δ_{C} (100 MHz, *d*-

DMSO) 119.5 (CH), 123.5 (CH), 139.6 (C₀), 150.7 (CHN), 155.5 (C₀) and 166.0 (CO); $\nu_{\max}/\text{cm}^{-1}$ 1715 (CO₂H), and 2443 (OH).

Synthesis of 2,2'-bipyridyl-4,4'-dicarboxylic acid bis-[(3-triethoxysilylpropyl)amide] (10).² 2,2'-Bipyridine-4,4'-dicarboxylic acid **4** (0.50 g, 2.05 mmol) was dissolved in thionyl chloride (5 ml, 68.55 mmol) and heated to reflux for 3 hr prior to concentrating *in vacuo*. The crude acid chloride **7** in toluene (10 ml) was then added dropwise, over a period of 1 hr, to a stirred solution of aminopropyltriethoxysilane **8** (0.48 ml, 2.05 mmol) and triethylamine **9** (0.29 ml, 2.05 mmol) in toluene (10 ml), under N₂. The reaction mixture was stirred for a further 16 hr prior to filtration, to remove triethylamine hydrochloride, and concentrated *in vacuo* to afford 2,2'-bipyridyl-4,4'-dicarboxylic acid bis-[(3-triethoxysilylpropyl)amide] **10** as a pale yellow oil (1.28 g, 96.00 %); δ_{H} (400 MHz, *d*-DMSO) 0.74 (4H, t, *J* 7.0, CH₂Si), 1.22 (18H, t, *J* 7.0, 6 x CH₂CH₃), 1.80 (4H, qu, *J* 7.0, CH₂CH₂CH₂Si), 3.52 (4H, q, *J* 7.0, NHCH₂CH₂), 3.84 (12H, q, *J* 7.0, 6 x CH₂CH₃), 7.03 (2H, m, NHCH₂), 7.81 (2H, dd, *J* 1.7 and 5.0, ArH), 8.69 (2H, d, *J* 1.7, ArH) and 8.78 (2H, dd, *J* 1.7 and 5.0, ArH); δ_{C} (100 MHz, *d*-DMSO) 7.9 (2 x CH₂Si), 18.3 (6 x CH₂CH₃), 22.8 (2 x CH₂CH₂CH₂Si), 42.5 (2 x NHCH₂), 58.6 (6 x CH₂CH₃), 117.7 (2 x CH), 122.3 (2 x CH), 143.3 (2 x C₀CONH), 150.0 (2 x CH), 156.0 (2 x C₀) and 165.4 (CONH); $\nu_{\max}/\text{cm}^{-1}$ 995 (SiO), 1657 (CONH), 3404 (NH).

Synthesis of *cis*-dichlorobis(bipyridine)ruthenium (11).³ Ruthenium trichloride (0.50 g, 2.41 mmol) and 2,2'-bipyridine (0.75 g, 4.82 mmol) were heated to reflux in *N,N*-dimethylformamide (20 ml) for 3 hr. Upon cooling, acetone (32 ml) was added and the reaction mixture stored at 0 °C overnight. The fine black precipitate was then filtered under suction and washed with water. The precipitate was then suspended in a mixture of ethanol and water (50:50, 100 ml) and heated to reflux for 1 hr. The reaction mixture was again filtered to remove any insoluble material and the filtrate carefully treated with lithium chloride (0.55 g, 12.97 mmol). Ethanol was then distilled off and the resulting solution cooled to 0 °C until dark purple crystals precipitated. The reaction mixture was then filtered under suction and the precipitate dried *in vacuo* to afford product *cis*-dichlorobis(bipyridine)ruthenium **11** (0.73 g, 62.50 %) as a dark purple solid; δ_{H} (400 MHz, *d*-DMSO) 7.10 (2H, dt, *J* 1.1 and 7.0, 2 x ArH), 7.52 (2H, d, *J* 5.6, 2 x ArH), 7.68 (2H, dt, *J* 1.1 and 7.0, 2 x ArH), 7.78 (2H, dt, *J* 1.1 and 7.0, 2 x ArH), 8.06 (2H, dt, *J* 1.1 and 7.0, 2 x ArH), 8.48 (2H, d, *J* 8.2, 2 x ArH), 8.63 (2H, d, *J* 8.2, 2 x ArH) and 9.97 (2H, dd, *J* 1.1 and 5.6, 2 x ArH); δ_{C} (100 MHz, *d*-DMSO) 122.4 (2 x CH), 122.8 (2 x CH), 125.3 (2 x CH), 125.2 (2 x CH), 133.2 (2 x CH), 134.5 (2 x CH), 151.9 (2 x CH), 153.1 (2 x CH), 158.1 (2 x C₀) and 160.1 (2 x C₀).

Synthesis of [4,4'-bis[(3-triethoxysilylpropyl)amide]-2,2'-bipyridine] bis-(2,2'-bipyridine) ruthenium(II) dichloride (3).³ *cis*-Dichlorobis(bipyridine)ruthenium **11** (0.30 g, 0.62 mmol) and 2,2'-bipyridyl-4,4'-dicarboxylic acid bis-[(3-trimethoxysilylpropyl)amide] **10** (0.31 g, 0.62 mmol) were dissolved in absolute ethanol (20 ml) and stirred at 50 °C for 48 hrs. Ethanol was distilled off and the crude reaction products recrystallised from hot acetone to afford [4,4'-bis[(3-triethoxysilylpropyl)amide]-2,2'-bipyridine]bis-(2,2'-bipyridine) ruthenium(II) dichloride **3** (0.52 g, 73.80 %) as a brick red solid; δ_{H} (400 MHz, *d*-MeOH) 0.91 (4H, m, 2 x CH₂Si), 1.31 (18 H, t, *J* 7.3, 6 x OCH₂CH₃), 1.45 (4H, m, 2 x CH₂CH₂CH₂Si), 3.61 (2H, q, *J* 7.0, NHCH₂CH₂), 4.03 (2H, q, *J* 7.0, NHCH₂CH₂), 4.49 (12H, dq, *J* 7.0 and 14.1, 2 x OCH₂CH₃), 7.49 (1H, m, NH), 7.53 (1H, m, NH), 7.80 (2H, dd, *J* 1.6 and 5.9, 2 x ArH), 7.96 (2H, dd, *J* 1.6 and 5.9, 2 x ArH), 8.0 (2H, d, *J* 5.9, 2 x ArH), 8.16 (4H, dd, *J* 1.6 and 5.9, 2 x ArH), 8.72 (2H, m, 2 x ArH), 8.86 (2H, brd, *J* 4.5, 2 x ArH), 8.92 (2H, brs, 2 x ArH) and 9.17 (2H, d, *J* 1.4, ArH); δ_{C} (100 MHz, *d*-MeOH) 10.0 (2 x CH₂Si), 15.3 (6 x OCH₂CH₃), 15.3 (2 x NHCH₂CH₂CH₂Si), 63.9 (2 x NHCH₂CH₂), 64.7 (6 x OCH₂CH₃), 125.8 (4 x CH), 126.6 (4 x CH), 128.7 (4 x CH), 129.9 (4 x CH), 140.5 (2 x CH), 141.1 (2 x CH), 153.3 (2 x CH), 153.6 (2 x C₀), 154.5 (2 x C₀), 159.0 (2 x C₀), 160.0 (2 x C₀) and 165.5 (2 x

NHCO) and $\nu_{\max}/\text{cm}^{-1}$ 1180 (SiO), 1194 (SiC), 1546 (CONH), 1658 (CONH), 2927 (NH), 3066 (NH) and 3398 (NH).

Immobilisation of complex 3 onto controlled pore glass. [4,4'-Bis(3-triethoxysilylpropyl)amide]-2,2'-bipyridine] bis-(2,2'-bipyridine) ruthenium(II) dichloride **3** (0.05 g, 0.044 mmol) was hydrolysed by heating in dilute HCl (0.1 M, 10 ml) for 1 hr; prior to cooling controlled pore glass (10.00 g) was added and the mixture stirred at room temperature overnight. The functionalised CPG was filtered under suction and washed with distilled water, acetone and DCM, to afford an orange free flowing solid, with a Ru loading of 2.61×10^{-3} mmol g^{-1} .

Preparation of a ruthenium (III) complex 3 sol-gel monolith. Hydrolysis of compound **3** (0.05 g, 0.044 mmol) was achieved by heating in dilute HCl (0.1 M, 10 ml) for 1 hr. After cooling, an aliquot of the hydrolysed compound (100 μl , 4.40×10^{-4} mmol) was added to tetramethylorthosilicate (100 μl , 0.68 mmol) and the reaction mixture agitated until a single layer was observed, upon which 50 μl of NaOH (0.1 M) was added to afford an orange sol-gel. The sol-gel was covered and placed in a dessicator overnight (in the absence of a vacuum), upon which ~ 25 % shrinkage was observed; $\nu_{\max}/\text{cm}^{-1}$ 970 (SiO), 1105 (Si-O-Si), 1637 (CONH), 2855 (OCH₃), 2926 (NH) and 3448 (NH). Compared to work previously reported by Lin and co-workers,⁴ whereby the 2,2'-bipyridyl-4,4'-dicarboxylic acid bis[(3-triethoxysilylpropyl)amide] **10** containing sol-gels were cured for 3 weeks at 40 °C, the sol-gels described herein were found to gel in under 2 min at room temperature and solidified in > 24 hr.

Preparation of organosilicate coated carbon electrodes. Hydrolysis of compound **3** (0.05 g, 0.044 mmol) was achieved by heating in dilute HCl (0.1 M, 10 ml) for 1 hr. After cooling, an aliquot of the hydrolysed compound (100 μl , 4.40×10^{-4} mmol) was added to tetramethylorthosilicate (100 μl , 0.68 mmol) and the reaction mixture agitated until a single layer was observed, the carbon electrode (1.00 mm x 2.00 cm) was dipped into the mixture and removed slowly, allowing a thin film to form; the electrode was then placed in borate buffer (pH 8.5, 0.1 M) to induce gelation. Prior to analytical evaluation, the electrode was aged for 24 hr in borate buffer.

Preparation of doped sol-gel coatings. Tris(bipyridyl)ruthenium(II)chloride hexahydrate (0.01 g, 1.336×10^{-3} mmol) was added to a solution containing tetramethylorthosilicate (1.60 ml) and deionised water (1.00 ml), the reaction mixture was thoroughly mixed prior to the addition of dilute hydrochloric acid (50 μl , 0.1 M). The carbon electrode (1.00 mm x 2.00 cm) was then dipped into the reaction mixture prior to placing in borate buffer (pH 8.5, 0.1 M), the electrode was then aged overnight prior to analytical evaluation.

CL Evaluation (Fig. A). CL measurements were performed using an in-house fabricated borosilicate glass flow cell (2.50 mm i.d. x 1.50 cm long, 50 mg of compound **12**) (Fig. 3) and the solutions delivered using a Gilson peristaltic pump at a flow rate of 2.00 ml min^{-1} . The functionalised CPG **12** was retained within the cell *via* the fabrication of micro porous silica frits⁵ at both ends of the packed bed and the flow cell attached to the peristaltic pump using 1/8 " Omnifit connectors (Supelco, UK).

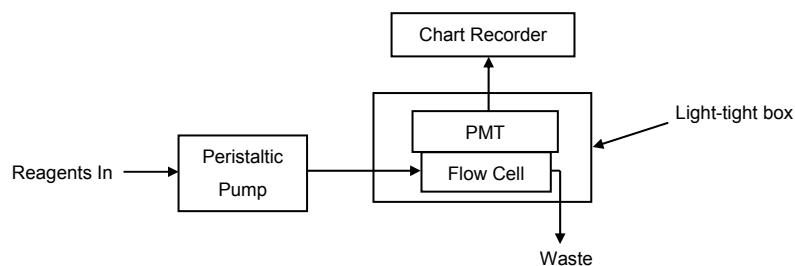


Fig. A Schematic illustrating the set-up used for the chemiluminescent evaluation of ruthenium functionalised CPG **12**.

ECL evaluation (Fig. B). ECL measurements were made using a three electrode set-up illustrated in Fig. 4, in order to maintain an inter electrode distance of 0.80 cm, the electrodes were housed in a 5A terminal block through which connections to the potentiostat were made. The platinum and silver electrodes were polished using several grades of emery cloth followed by diamond paste (Hyprez, Type 1-FS-48) and the carbon electrode polished using a diamond suspension (Buchler, 9 μm). Analytical measurements were performed using the following electrochemical program; 0.7 to 1.3 V, with a step potential of 0.0024 V, a scan rate of 0.01 V s^{-1} and typically 5 cycles. Between analyses, the electrodes were stored in borate buffer (pH 8.5) to prevent drying and cracking of the silicate sol-gel coating.

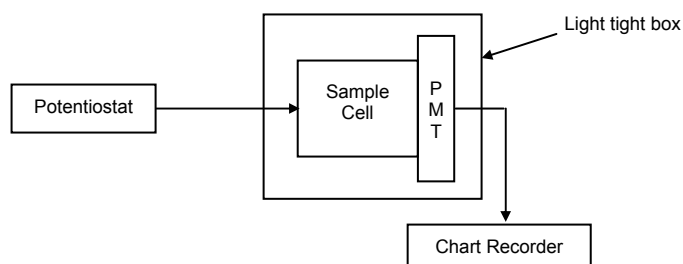


Fig. B Schematic illustrating the set-up used for the electrochemiluminescent evaluation of organosilicate films.

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