Supporting Information for

Spatially Resolved Electrochemiluminescence Through Chemical Lens

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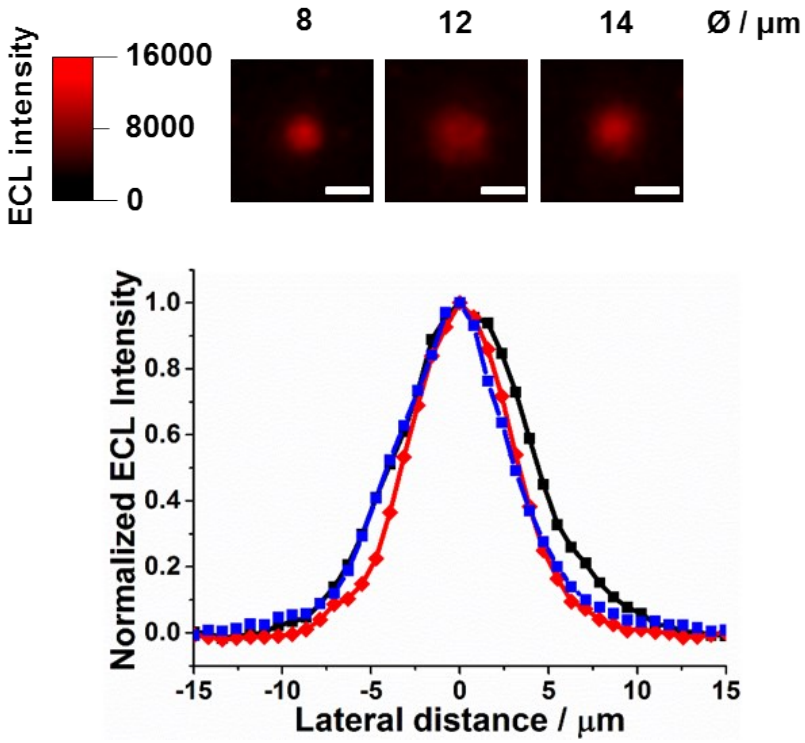

Fig. S1 Top: ECL images of 8 µm, 12 µm and 14 µm microbead labeled with the $[Ru(bpy)_3]^{2+}$ complex recorded in 0.1 M PB by top-view configuration. Bottom: normalized ECL profile of beads for PB 0.1 M, red trace are 8 µm beads, blue trace are 12µm, and black trace are 14 µm beads. Applied potential: 1.4 V vs. Ag/AgCl. Scale bar: 10 µm.

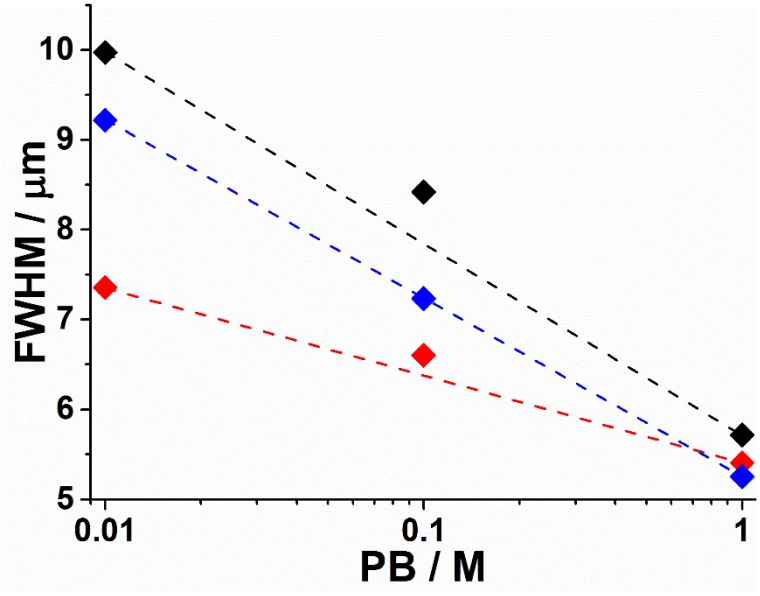

Fig. S2 Full width at half maximum of microbead profiles as function of PB concentration (from Figure 1 and S2) for 8 µm (red), 12 µm (blue), and 14 µm (black).

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from Sigma-Aldrich, unless otherwise stated, and were used as received. Beads were from Spherothech (USA) 8 and 14 μm, and Kisker Biotech GmbH & Co. (Germany) 12 μm.

Preparation of $[Ru(bpy)_3]^{2+}$ labelled beads (8, 12 and 14 μm) used in top-view ECL imaging. This procedure has been described in detail elsewhere.[1]

Briefly, a solution of bis(2,2'-bipyridine)-[4-(4'-methyl-2,2'-bipyridin-4-yl)butanoic acid] ruthenium bis(hexafluorophosphate) ($Ru(bpy)_3^{2+}$–COOH, Cyanagen, Italy) in DMF (70 μL, 0.47mg) was added to 1.5 equivalents of N,N'-dicyclohexylcarbodiimide (DDC) and mixed for 4 h at room temperature. A streptavidin solution (630 μL, 0.665 mg) in 0.1 M borate buffer (pH 9.4) was added to the activated $Ru(bpy)_3^{2+}$–COOH and incubated overnight. The labelled streptavidin was dialyzed against 5 L of PBS 1X. COOH-functionalized beads (200 μL) were washed three times in 0.1 M borate buffer (pH 9.6) and two times in 0.1 M in 2-(N-morpholino)ethanesulfonic acid buffer (MES pH 5.5), and finally re-suspended in 250 μL of MES buffer. The beads solution was added with N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (sulfo-NHS) to a final concentration of 50 mM and 2 mM, respectively, and mixed for 1 h at room temperature. After one washing with MES, biotin cadaverine (500 μL, 9 mM) in 0.1 M borate buffer (pH 8.6) were added and incubated overnight at 48°C. The beads solution was finally washed three times in PBS. Buffer was removed from 50 μL of beads and 50 μL of labelled $Ru(bpy)_3^{2+}$–streptavidin were added and mixed for 2 h at room temperature, followed by three washing steps and re-suspension with PBS.

Preparation of $[Ru(bpy)_3]^{2+}$ labelled beads (12 µm) used in side-view ECL imaging. This procedure has been described in detail elsewhere.[2]

10 µL of NH_2-functionlized beads were washed with PBS (1X, pH=7.4) and re-suspended in 1 mL of PBS. 1 mg of (bis(2,2'-bipyridine)-4'-methyl-4-carboxybipyridine-ruthenium N-succinimidyl ester-bis(hexafluorophosphate) ($Ru(bpy)_3^{2+}$-NHS ester) was dissolved in 100 µL of dimethyl sulfoxide and added to the beads suspension. This mixture was incubated at 4°C for 3 hours with continuous stirring. After the incubation the beads were washed with PBS and re-suspended in 1 mL PBS.

Top-view ECL imaging: The ECL imaging was performed in a PTFE homemade electrochemical cell in a three electrode configuration, glassy carbon working electrode, Ag/AgCl (KCl, 3M) reference electrode and Pt wire counter electrode. ECL images were acquired by an epifluorescence microscope from Nikon equipped with an Hamamatsu Electron Multiplying Charge Coupled Device (EM-CCD) with a resolution of 512 × 512 pixels and long distance objectives from Nikon (20 × /0.40 DL13 mm). The microscope was enclosed in a homemade dark box to avoid interferences from external light, and electrochemical cell positioning was assisted by a motorized microscope stage Corvus Märzhauser. The system included a potentiostat Metrohm PGSTAT 30 for electrochemical measurements.
ECL was triggered by applying 1.4 V for 8 s, and the emission collected by EM-CCD. The pH of all PB solutions has been adjusted to 7.4 by addition of concentrated phosphoric acid and the TPrA concentration was 180 mM.
The ECL emission from top-view ECL images was integrated by ImageJ software. Briefly, a .TIFF image, from the EM-CCD camera, was loaded with ImageJ. The ECL integral (i.e., pixels intensity) was measured for the bead emission with a square of 30×30 px. The same procedure was used to measure the background emission where beads were not present (average of 4 different points). The ECL integral from beads subtracted by the background is the value used in Fig. 2.

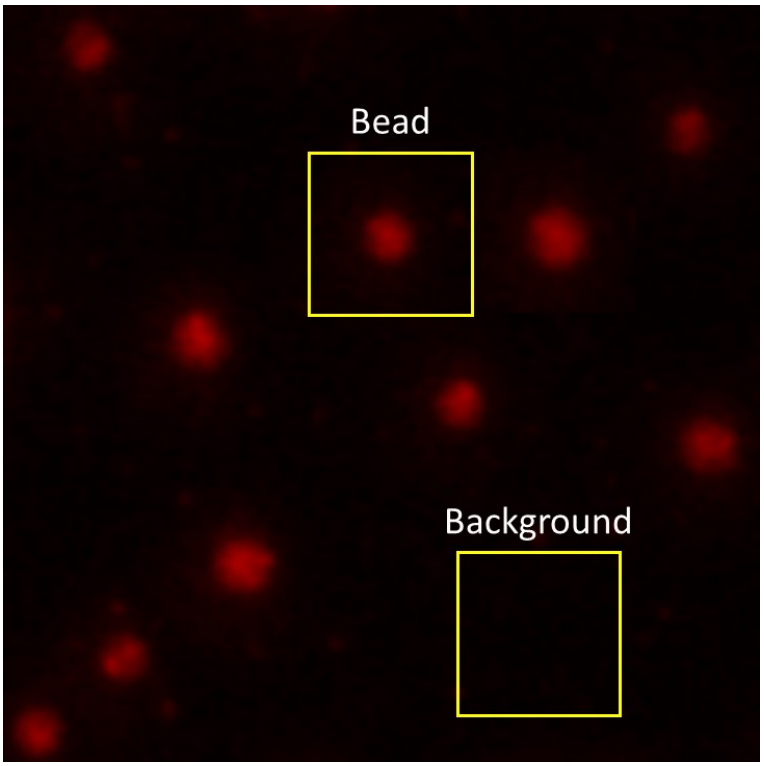

Fig. S3 Example of integration procedure of ECL emission.

Side-view ECL imaging: The electrochemical set-up includes a glassy carbon working electrode, Ag/AgCl (KCl, 3M) reference electrode and Pt wire counter electrode connected to a Metrohm µAutolab type III potentiostat.

A modified epifluorescence microscope (Olympus), as described previously,[3] was used to acquire the PL and ECL images by a 50x microscope objective and an EM-CCD (Hamamatsu).

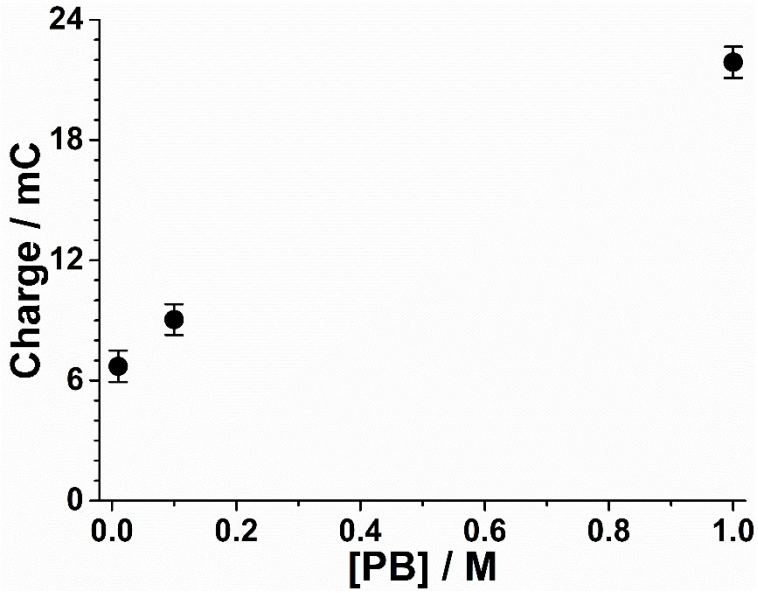

Fig. S4 Charge as function of PB concentration (0.01, 0.1, and 1 M) integrated from chronoamperometry of top-view imaging of beads. Applied potential: 1.4 V vs. Ag/AgCl, time 6 seconds.

References

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