Supporting information

Bimodal Electrochemiluminescence Microscopy of Single Cells

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Table of contents

Experimental section

Image analysis

ImageJ was used to process all the micrographs. Micrographs of the cells were false-colored: green for PL, red for PECL and cyan for SECL using default ImageJ lookup tables. Rectangular regions of interest along the direction denoted in the PL micrograph (Figure 2) were used to extract PL and ECL intensity profiles of the imaged cells. The profiles were reduced to the same baseline and the SECL intensity values were multiplied by a factor of 10 to improve the visibility and to enable easier interpretation of the profiles. It is important to note that PL and ECL profiles are on distinct scales because the images are taken using different cameras (CCD for PL and EM-CCD for ECL). Furthermore, all intensity values are in arbitrary units, so the range of a specific profile is more informative than the absolute intensity values, which is why reducing the profiles to the same baseline does not influence their interpretation.

Structure Similarity Index Measurement - SSIM

To examine the information revealed using different microscopy modes (PL, PECL and SECL), we constructed structure similarity index measurement (SSIM) maps. SSIM is a method used to measure the similarity between two images. It is calculated by dividing the images into smaller blocks and comparing the quality factors for each block between the images. The SSIM method takes into account three different image quality factors: luminance, contrast, and structure. Luminance represents the total brightness of the object (which is a product of the illumination and the reflectance) and is calculated as the mean value of pixel intensity. Contrast is a measure of the difference between the light and dark pixels, determined as a standard deviation of the intensity. Structure properties are all properties of the image unrelated to the lighting conditions (textures, edges, and shapes). Structure properties are expressed mathematically as signals normalized by their standard deviation. While these factors are relatively independent, the SSIM index is calculated by one equation which is a combined function of luminance, contrast and structure functions.[1]

Considering the differences in lighting and intensity between PL and ECL, as well as the complementarity of the PECL and SECL approach, our goal was to focus on the structural differences between the micrographs. For this reason, to create the maps, it was necessary to threshold the original micrographs (ImageJ - Image - Adjust - Threshold) to condense the total

information about the intensities of pixels to only two - black and white. The SSIM maps with corresponding indexes are automatically generated from the down-sampled (thresholded) images using a plugin in ImageJ (ImageJ - Plugins - SSIM index created by Gabriel Prieto Renieblas from Complutense University Madrid, Spain, https://imagej.nih.gov/ij/plugins/ssim-index.html).

ECL mechanisms in PECL

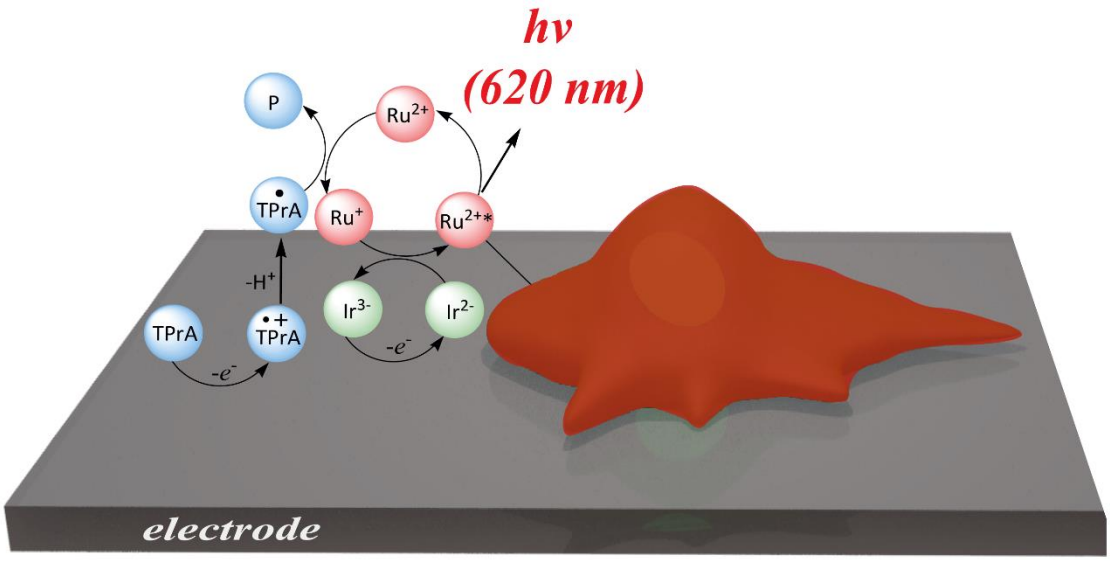

Figure S1. Schematics of a potential heterogeneous ECL route in PECL involving immobilized $[Ru(bpy)_3]^{2+}$ complex (SA@Ru) on the plasma membrane with dissolved $[Ir(sppy)_3]^{3-}$ and TPrA. Ru^{2+} and Ir^{3-} represent the ECL SA@Ru label and $[Ir(sppy)_3]^{3-}$, respectively.

$$[Ir(sppy)_3]^{3-} - e^- \rightarrow [Ir(sppy)_3]^{2-} \quad (1)$$

$$[Ir(sppy)_3]^{2-} + TPrA \rightarrow [Ir(sppy)_3]^{3-} + TPrA^{\bullet+} \quad (2)$$

$$TPrA^{\bullet+} \rightarrow TPrA^{\bullet} + H^+ \quad (3)$$

$$SA@Ru^{2+} + TPrA^{\bullet} \rightarrow SA@Ru^{+} + P1 \quad (4)$$

$$SA@Ru^{+} + TPrA^{\bullet+} \rightarrow SA@Ru^{2+*} + P2 \quad (5)$$

$$SA@Ru^{2+*} \rightarrow SA@Ru^{2+} + h\nu\ (620\ nm) \quad (6)$$

Scheme S1. Sequence of reactions involved in the "electrocatalytic" route potentially involved in the PECL emission of SA@Ru.

Emission spectra and filters

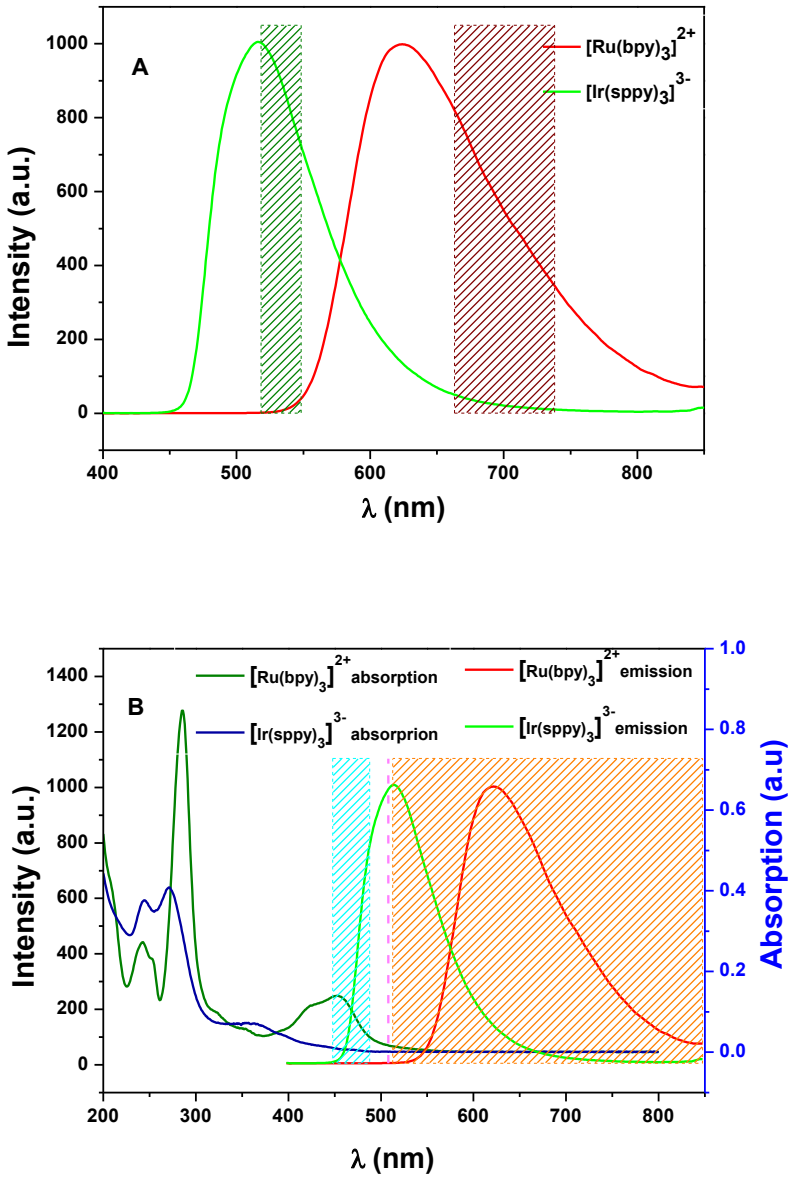

Figure S2. (A) Normalized photoluminescence emission spectrum of $[Ru(bpy)_3]^{2+}$ (red plot) and $[Ir(sppy)_3]^{3-}$ (green plot) with corresponding suppression filters – FITC (olive hatched region) and Y5 (wine hatched region). (B) Absorption spectrum of $[Ru(bpy)_3]^{2+}$ (olive plot) and $[Ir(sppy)_3]^{3-}$ (royal blue plot) and normalized photoluminescence emission spectrum of $[Ru(bpy)_3]^{2+}$ (red plot) and $[Ir(sppy)_3]^{3-}$ (green plot) with FITC/LP excitation (blue hatched region), suppression (orange hatched region) filters and dichroic mirror (magenta dashed line) overlayed. Complex concentration: 10 μM in ultra-pure (Milli-Q) water at ambient temperature. Reproduced from reference 2.

PL, PECL and SECL imaging

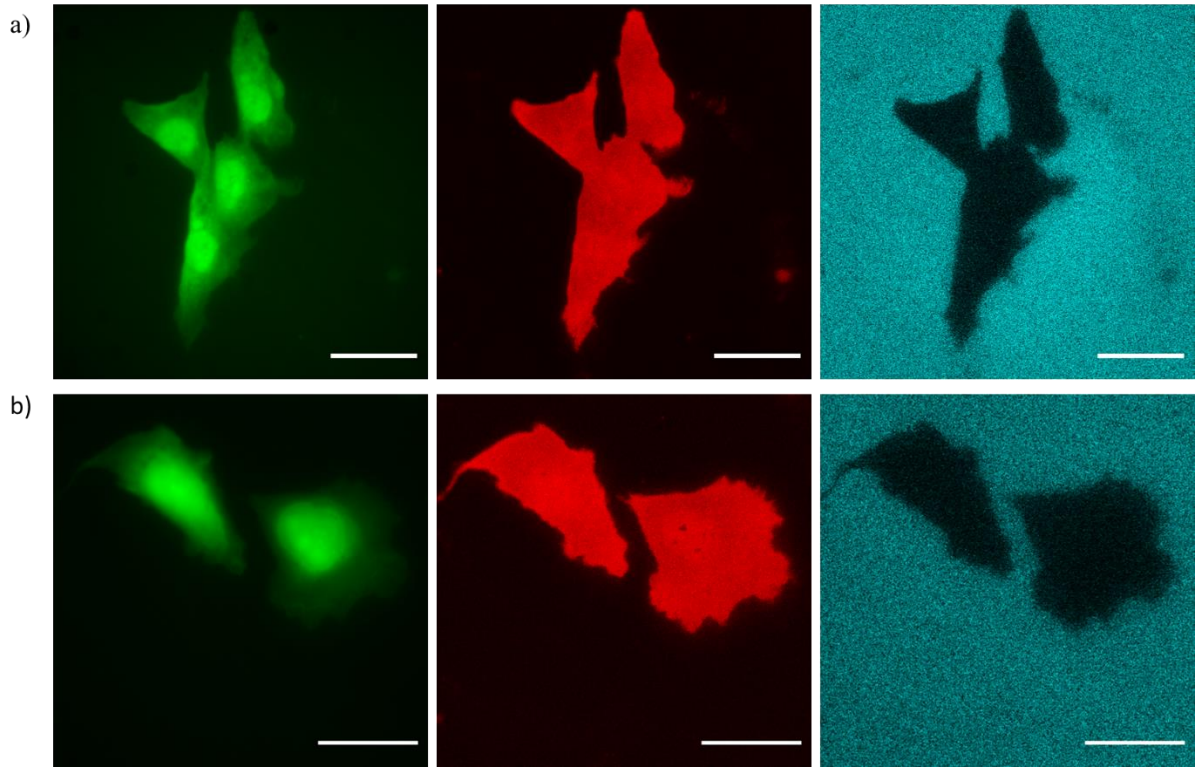

Figure S3. (a-b) Images of different CHO-K1 cells recorded in PL (left), PECL (middle), and SECL (right). Same experimental conditions as in Figure 2. Scale bar: 20 µm.

3D images

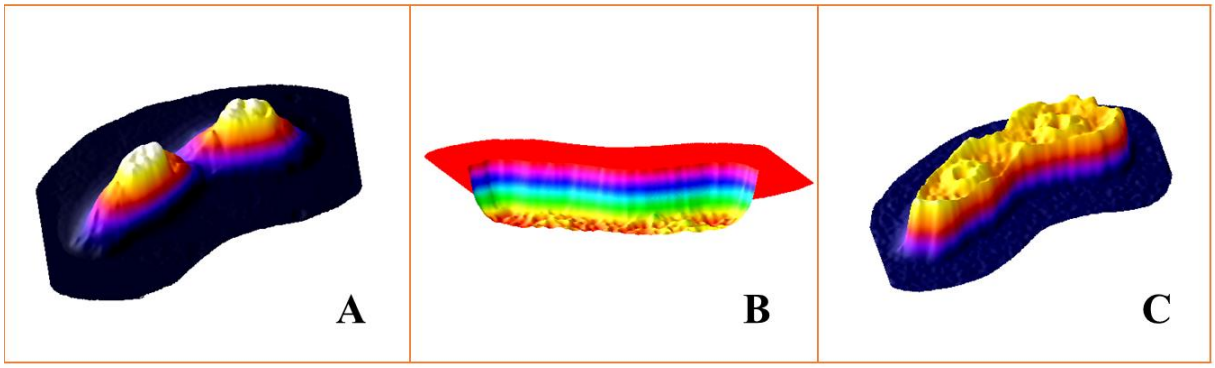

Figure S4. (A) PL, (B) SECL, and (C) PECL 3D images of the same region of interest showing two single cells.

References

1. Wang, Z., Bovik, A. C., Sheikh, H. R. & Simoncelli, E. P. Image quality assessment: from error visibility to structural similarity. *IEEE* **13**, 600–612 (2004).
2. Kerr, E. *et al.* A redox-mediator pathway for enhanced multi-colour electrochemiluminescence in aqueous solution. *Chem. Sci.* **13**, 469–477 (2022).