Electronic Supporting Information

for

Iridium(III)-based lab-on-a-molecule for cysteine/homocysteine and tryptophan using triple-channel interrogation

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Abbreviations:

ACN: Acetonitrile

DCM: Dichloromethane

General Information

NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Advance 400 (400 MHz for ^{1}H). Chemical shifts are reported in ppm.

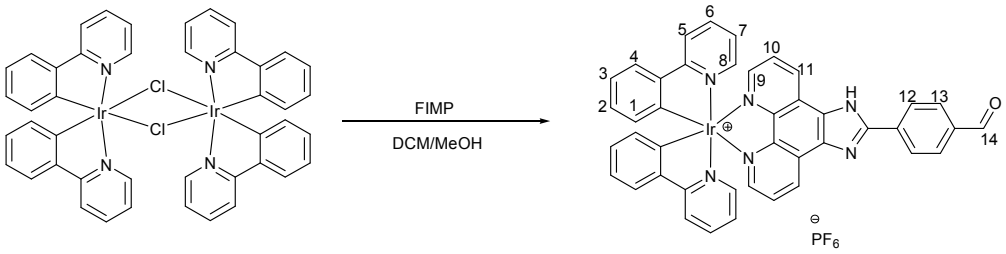
Luminescence and UV-Vis measurements: Using a solution of complex **1** (10 μM) in ACN/aq. 0.1 M HEPES = 50/50, (v/v, pH 7.40), UV-Vis measurements were carried out on a Varian Cary 100 Bio UV-Vis Spectrophotometer and luminescence measurements on a Varian Vary Eclipse Fluorescence Spectrophotometer with exaction slit width set at 10 nm and emission slit width at 5 nm.

Electrochemiluminescence (ECL) measurements: ECL measurements were done on the iridium complex **1** (10 μM) in ACN/aq. 0.1 M HEPES = 50/50, (v/v, pH 7.40) containing tri-n-propylamine (TPrA; 30 mM) as a co-reactant and tetra-*n*-butylammonium hexafluorophosphate (0.05 M) as electrolyte. A standard three-electrode set-up (3.0 mm diameter glass-carbon working electrode), Pt wire auxiliary electrode, and a silver wire as reference electrode) connected to a Princeton Applied Research Model 362 potentialstat was used. To generate the ECL, the potential of the working electrode was swept between 0.5 to 1.8 V (*vs* a silver wire as quasi-reference electrode) at a scan rate v = 100 mV s^{-1}. ECL competition experiments were carried with **1** (10 μM) in presence of 200 equiv. of tryptophan and of 200 equiv. of other amino acids. The resulting emission spectra were obtained with a CCD camera cooled to -50 ^{o}C (0.500 m Imagining Triple Grating Monochromator/Spectrograph), which was connected to spectrometer Spectrapro 2500i (Acton Research Corporation).

Electroanalytical measurements: Cyclic voltammetry (CV) of compounds were measured at a scan rate v = 100 mV s^{-1} using a standard three-electrode set-up (1 mm glass-carbon working electrode, a Pt auxiliary electrode, a silver wire as a quasi-reference electrode) connected to a PARSTAT 2273 Advanced Electrochemical System. The experiments were carried out on compounds (1 mM) dissolved in acetonitrile with 0.1 M tetra-*n*-butylammonium hexafluorophosphate as supporting electrolyte, unless stated otherwise. All potentials are referenced to $FeCp_2$ (Cp = cyclopentadienyl) using $FeCp_2$ as internal standard.

Synthesis and characterisation

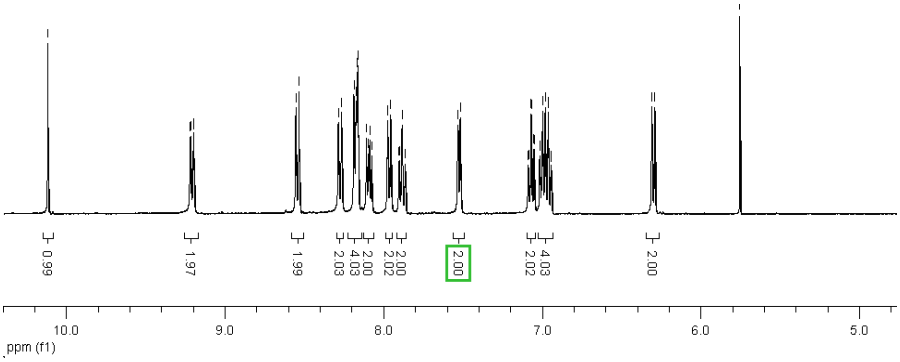
Iridium complex $[(ppy)_2IrCl]_2$ [1] and 2-(4-formylphenyl)imidazo[4,5-*f*][1,10]phenanthroline (= FIMP)[2] were prepared as described in the literature.



Synthesis and characterisation of complex **1**

$[(ppy)_2Ir(\mu\text{-}Cl)]_2$ (53.5 mg, 50.0 mmol) and FIMP (32.4 mg, 100.0 mmol) were dissolved in the mixture of DCM (20 ml) and methanol (10 ml). The solution was heated to reflux for 6 h followed by addition of excess of NH_4PF_6 (100 mg). The solution was stirred for another 1 h at room temperature. The solvent was removed under reduced pressure and the residue was re-dissolved in DCM. The organic layer was washed with water and dried over Na_2SO_4. After removing the solvent, the crude product was purified by column chromatography (silica gel, DCM/Methanol, 20/1, $R_f = 0.3$) to yield an orange-yellow product (74.3 mg, 77%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 10.12 (s, 14-H, 1H), 9.21 (dd, J = 8.2 Hz, J = 1.1 Hz, 11-H, 2H), 8.54 (d, J = 8.2 Hz, 13-H, 2H), 8.28 (d, J = 8.2 Hz, 5-H, 2H), 8.17 (m, 9-H, 12-H, 4H), 8.09 (dd, J = 8.2 Hz, J = 5.1 Hz, 10-H, 2H), 7.96 (dd, J = 7.7 Hz, J = 0.7 Hz, 4-H, 2H), 7.88 (td, J = 8.2 Hz, J = 1.0 Hz, 6-H, 2H), 7.52 (dd, J = 5.1 Hz, J = 1.0 Hz, 8-H, 2H), 7.07 (td, J = 7.7 Hz, J = 0.9 Hz, 3-H, 2H), 6.98 (m, 7-H, 2-H, 4H), 6.30 (dd, J = 7.7 Hz, J = 0.9 Hz, 1-H, 2H) ppm.



^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.6, 166.9, 150.3, 149.2, 148.5, 144.4, 144.0, 140.8, 138.7, 136.8, 134.9, 132.3, 131.2, 130.4, 130.3, 127.2, 127.0, 125.1, 123.8, 122.4, 120.0 ppm.

ESI-MS: $[C_{42}H_{28}IrN_6O]^+$: Calcd.: *m/z* = 825.2. Found: *m/z* = 824.9.

Elemental Analysis $[C_{42}H_{28}F_6IrN_6OP]\cdot 0.25CH_2Cl_2$

Calcd.: C%, 51.20; H %, 2.90; N%, 8.48; Found: C%, 51.16; H%, 2.95; N%, 8.36.

Photophysical and electrochemical measurements

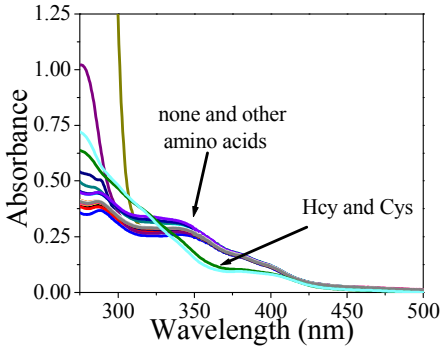


Fig. S1. Absorbance of **1** (10 μM) in ACN/ aq. buffer (0.1 M HEPES, pH = 7.4) = 50: 50 (v/v) in the presence of 200 equiv. of amino acids after a reaction time of 180 ± 1 min.

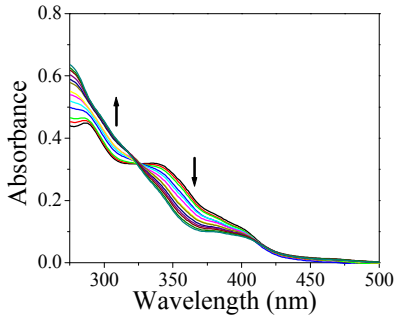


Fig. S2. UV-Vis absorption titration of **1** (10 μM) upon addition of various equiv. of Cys in 0.1 M HEPES buffer solution [ACN/buffer (50/50, v/v), pH = 7.40] after a reaction time of 180 ± 1 min.

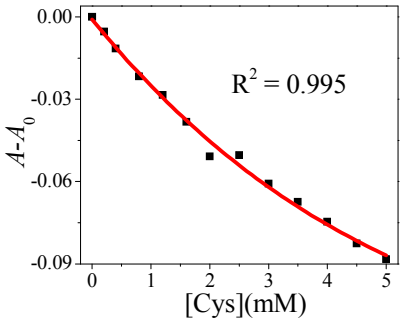


Fig. S3. Δ*A* of **1** (10 μM) at 340 nm upon addition of various equiv. of Cys and non-linear fitting curve.

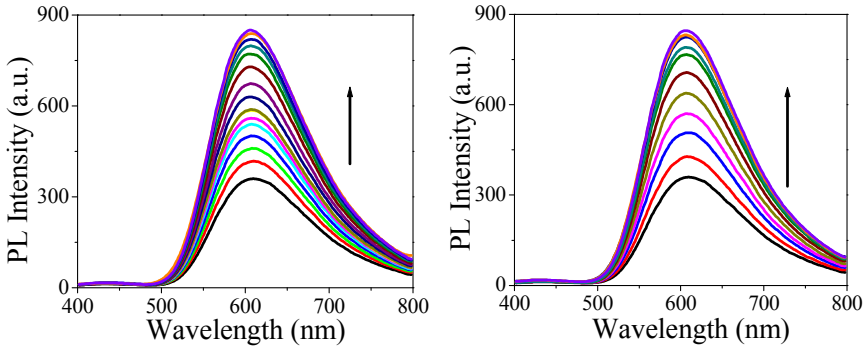


Fig. S4. PL spectra of **1** (10 μM) upon addition of various equiv. of Cys (left) and Hcy (right) in 0.1 M HEPES buffer [ACN/buffer (50/50, v/v), pH = 7.40] after a reaction time of 180 ± 1 min; λ_{ex} = 323 nm.

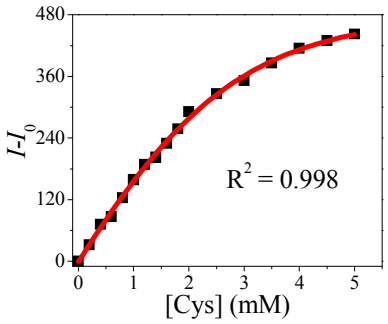


Fig. S5. PL ΔI of **1** (10 μM) at λ_{em} = 606 nm upon addition of various equiv. of Cys using non-linear fitting (R^2 = 0.998).

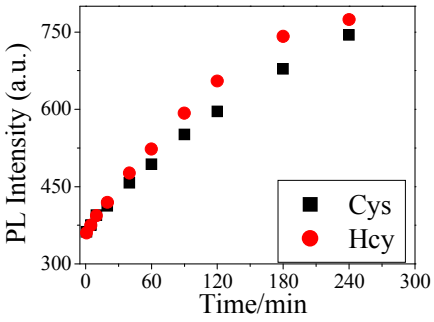


Fig. S6. PL intensity of complex **1** (10 μM) with time in presence of 200-fold Cys (black) and 200-fold Hcy (red) in 0.1 M HEPES buffer [ACN/buffer (50/50, v/v), pH = 7.40]; λ_{ex} = 323 nm, λ_{em} = 606 nm.

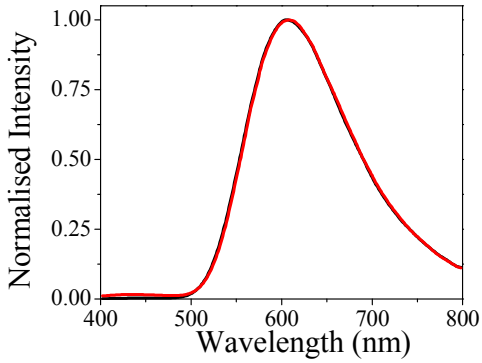


Fig. S7. PL spectra of thiazolidine **2a** (black) and of complex **1** in presence of 200-fold Cys (red) after a reaction time of 180 ± 1 min in 0.1 M HEPES buffer [ACN/buffer (50/50, v/v), pH = 7.40]; λ_{ex} = 323 nm.

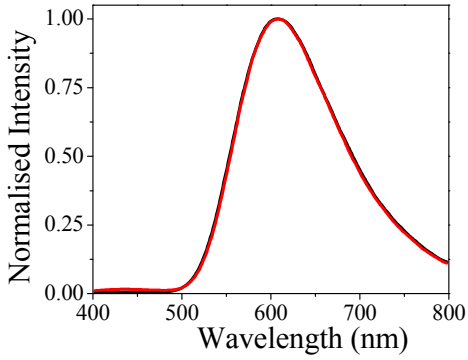


Fig. S8. PL spectra of thiazinane **2b** (black) and of complex **1** in presence of 200-fold Hcy (red) after a reaction time of 180 ± 1 min in 0.1 M HEPES buffer [ACN/buffer (50/50, v/v), pH = 7.40]; λ_{ex} = 323 nm.

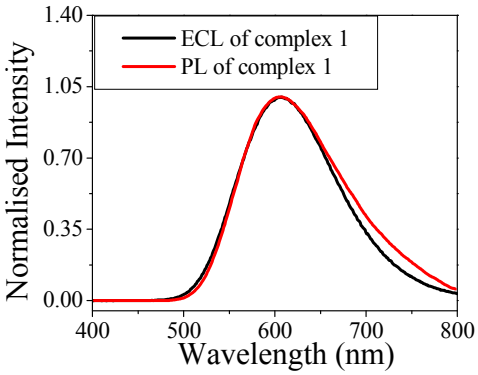


Fig. S9. ECL (black) and PL (red) of complex **1** (10 μM).

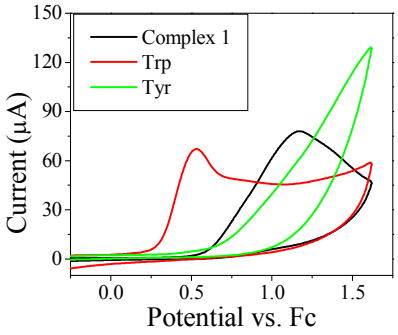


Fig. S10. Cyclic voltammograms of complex **1** (black), tryptophan (red) and tyrosine (green) in ACN (0.1 M nBu$_4$NPF$_6$, v = 100 mV/s).

1 M. Schmittel and H. Lin, *Inorg. Chem.*, 2007, **46**, 9139.
2 P. Das, A. K. Mandal, N. B. Chandar, M. Baidya, H. B. Bhatt, B. Ganguly, S. K. Ghosh and A. Das, *Chem. Eur. J.*, 2012, **18**, 15382.