

Electronic Supplementary Information

for

Neutral N⁺C⁻N terdentate luminescent Pt(II) complexes: synthesis, photophysical properties and bio-imaging application

Alessia Colombo,^{a#} Federica Fiorini,^{b#} Dedy Septiadi,^{b#} Claudia Dragonetti,^{a,c,*} Filippo Nisic,^a Adriana Valore,^a Dominique Roberto,^{a,c} Matteo Mauro,^{b,d,*} Luisa De Cola^{b,*}

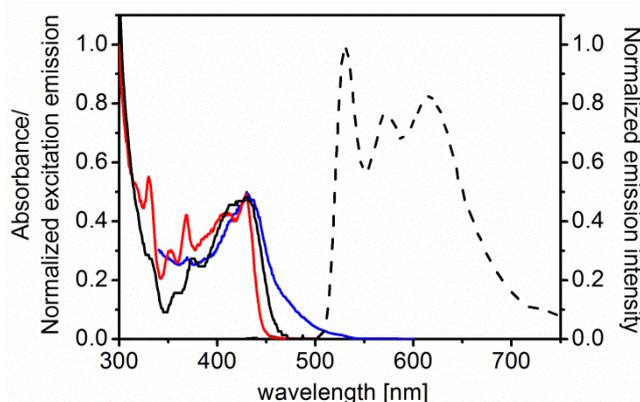


Figure S1. Emission (dashed trace) and excitation spectra for compound **PtL¹NCS** in CH₂Cl₂:MeOH 1:3 glassy matrix at 77 K. The absorption spectra in CH₂Cl₂ at room temperature (black solid trace) is also displayed for comparison. The excitation spectra were recorded at 520 (red trace) and 625 (blue trace) nm. The samples were measured at concentration of 1.0×10⁻⁵ M. For the emission spectra, the sample was excited at λ_{exc} = 400 nm.

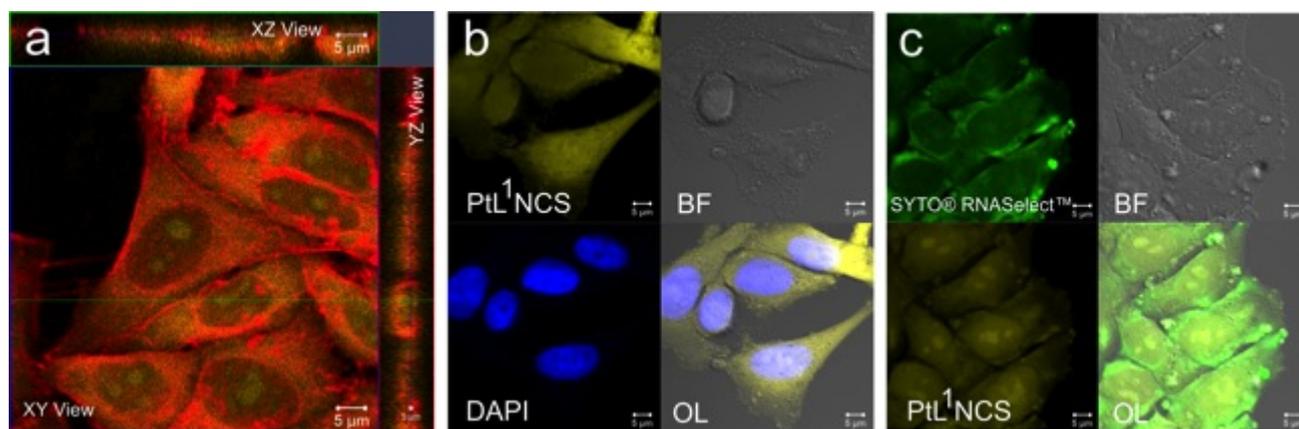


Figure S2. Fluorescence confocal microscopy images of the distribution of **PtL¹NCS** (50 μM <1% DMSO containing PBS) inside HeLa cells and co-localization experiments showing the presence of compound **PtL¹NCS** inside the cell nucleus, nucleoli, and cytoplasmic parts of the cell. (a) Orthogonal views of the image showing **PtL¹NCS** signal (green) coming from inside cytoplasmic and nuclear region of the cells which is stained with Phalloidin Alexa Fluor® 647 (red). (b) **PtL¹NCS**, bright-field (BF) image of HeLa cells, DAPI staining of nucleus, and overlay (OL) of three panels (c) SYTO® RNASelect™ green stains RNA inside cells including nucleoli; BF image, **PtL¹NCS**, and overlay of three

panels. The excitation wavelength for DAPI and **PtL¹NCS** was 405 nm, while SYTO® RNASelect™ and Phalloidin Alexa Fluor® 647 were excited at 488 and 633 nm, respectively.

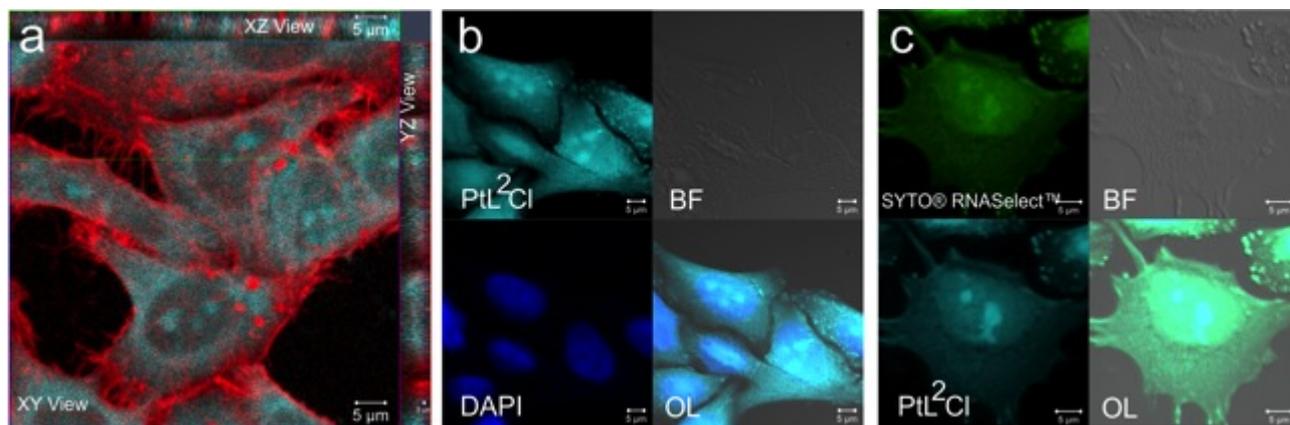


Figure S3. Fluorescence confocal microscopy images of the distribution of **PtL²Cl** (50 μM <1% DMSO containing PBS) inside HeLa cells and co-localization experiments showing the presence of compound **PtL²Cl** inside the cell nucleus, nucleoli, and cytoplasmic parts of the cell. (a) Orthogonal views of the image showing **PtL²Cl** signal (green) coming from inside cytoplasmic and nuclear region of the cells which is stained with Phalloidin Alexa Fluor® 647 (red). (b) **PtL²Cl**, bright-field (BF) image of HeLa cells, DAPI staining of nucleus, and overlay (OL) of three panels (c) SYTO® RNASelect™ green stains RNA inside cells including nucleoli; BF image, **PtL²Cl**, and overlay of three panels. The excitation wavelength for DAPI and **PtL²Cl** was 405 nm, while SYTO® RNASelect™ and Phalloidin Alexa Fluor® 647 were excited at 488 and 633 nm, respectively.

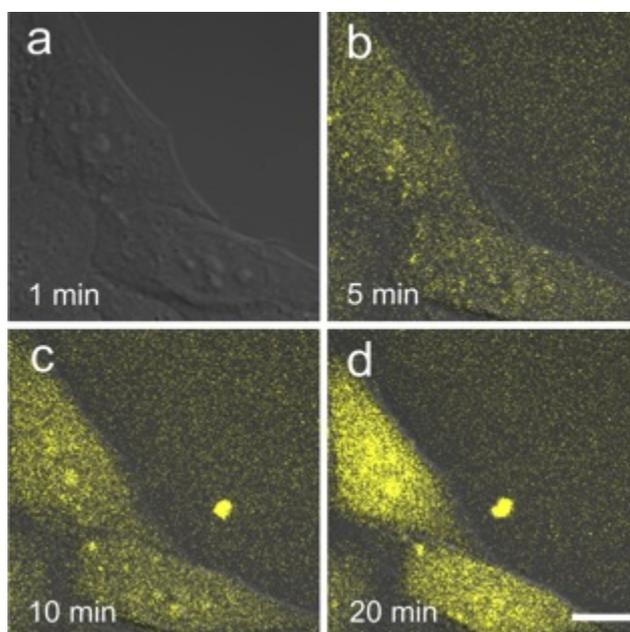


Figure S4. Confocal images of the kinetics experiments of HeLa cells incubated with **PtL¹NCS** at concentration 5 μM in <1% DMSO/PBS at different incubation time: (a) 1 minute, (b) 5 minutes, (c) 10 minutes, and (d) 20 minutes, showing the fast internalization of compound. The samples were excited at $\lambda_{exc} = 405$ nm, respectively. Scale bar is 10 μm.

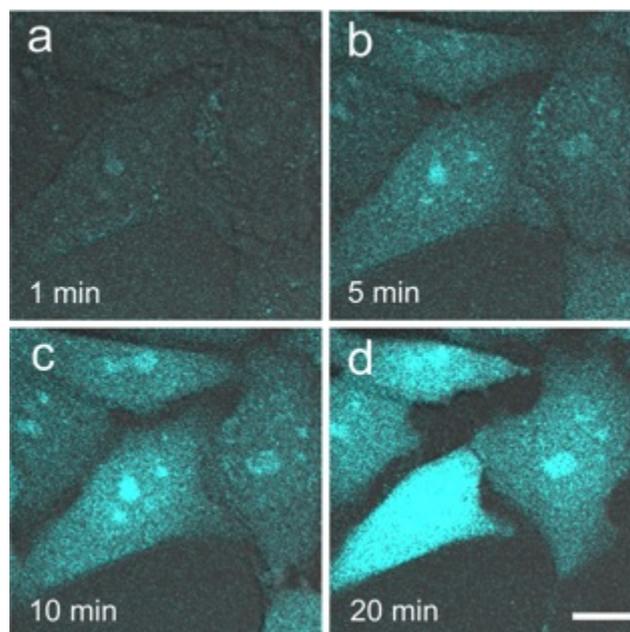


Figure S5. Confocal images of the kinetics experiments of HeLa cells incubated with **PtL²Cl** at concentration 5 μ M in <1% DMSO/PBS at different incubation time: (a) 1 minute, (b) 5 minutes, (c) 10 minutes, and (d) 20 minutes, showing the fast internalization of compound. The samples were excited at $\lambda_{exc} = 405$ nm, respectively. Scale bar is 10 μ m.

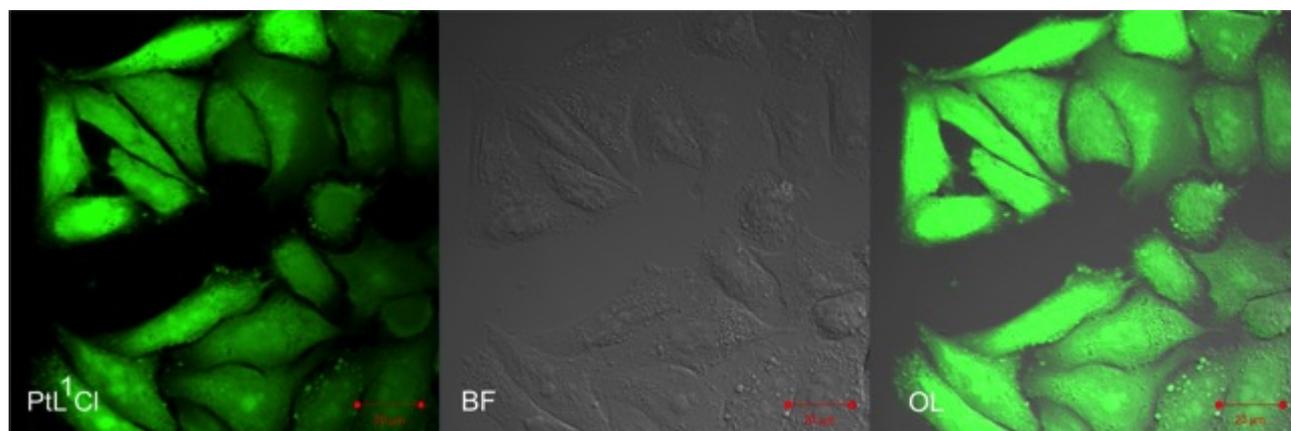


Figure S6. Confocal images of the cellular uptake experiments of living HeLa incubated with **PtL²Cl** 50 μ M in <1%DMSO/normal DMEM culture media.

Table S1. Photophysical data obtained for sample of **PtL²Cl** at concentration of 5×10^{-5} M in air-equilibrated dioxane-water mixture at different ratio.

| # of sample (dioxane:H ₂ O content) | λ_{abs} [nm] ($\epsilon \times 10^{-3} / [\text{M}^{-1} \text{cm}^{-1}]$) | λ_{em} [nm] | PLQY (%) | τ [ns] |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------|-------------|----------------------|
| <i>air-equilibrated</i> | | | | |
| 1 (100:0) | 294 (20), 366 sh (4), 383 (6), 445 (6) | 548 | 1 | 184 (73%) 5 (27%) |
| 2 (80:20) | 293 (19), 362 sh (3), 379 (5), 431 (5) | 546 | 1 | 310 (81%) 5 (19%) |
| 3 (60:40) | 292 (23), 361 sh (4), 376 (6), 423 (6) | 545 | 2 | 474 (76%) 4 (22%) |
| 4 (40:60) | 289 (18), 372 (5), 411 sh (3) | 543 | 1 | 675 (35%) 4 (65%) |
| 5 (20:80) | 288 (17), 369 (5), 413 sh (1) | 539 | 1 | 772 (4%) 4 (96%) |