Supporting Information for

# Ruthenium(II) Complex Enantiomers as Cellular Probes for Diastereomeric Interactions in Confocal and Fluorescence Lifetime Imaging Microscopy

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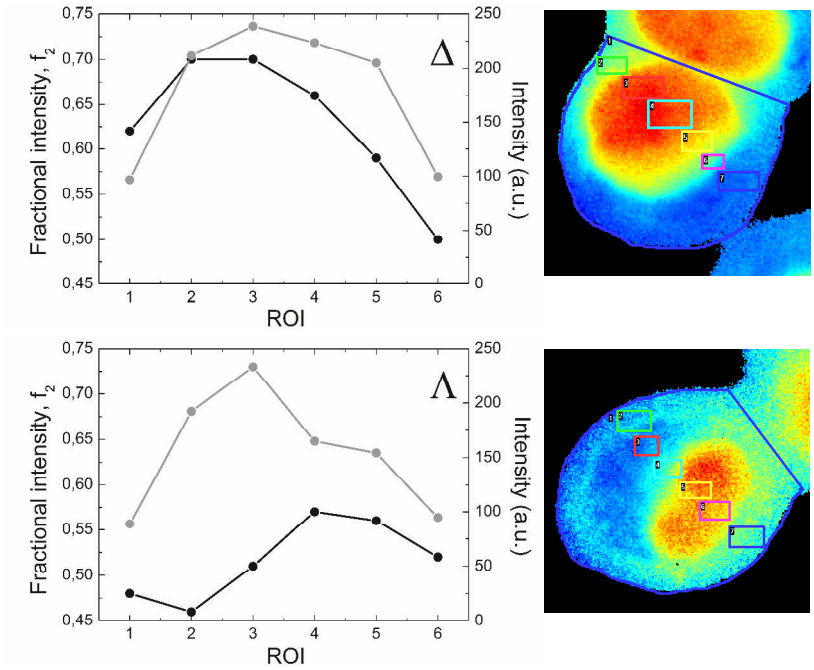


**Figure S1.** Left panels, average fractional intensity (black) and emission intensity (grey) in subsequent regions of interest (ROI) throughout the unfixed cell. Data are fitted with two lifetimes, 64 ns and 470 ns, determined from the best fit of the whole cell, and the fractional intensity for the longer component are shown in the graph. $\chi^2$-values in each ROI are between 9-15 for Δ, and 12-27 for Λ. Right panels, corresponding false color emission lifetime images, fitted with one lifetime in each pixel, with ROI from top to bottom, are presented. For Δ the intracellular variation of the fractional intensity of the long lifetime component correspond well with the emission intensity, whereas for Λ the largest fraction of the long lifetime component is not colocalized with the most intense emission.

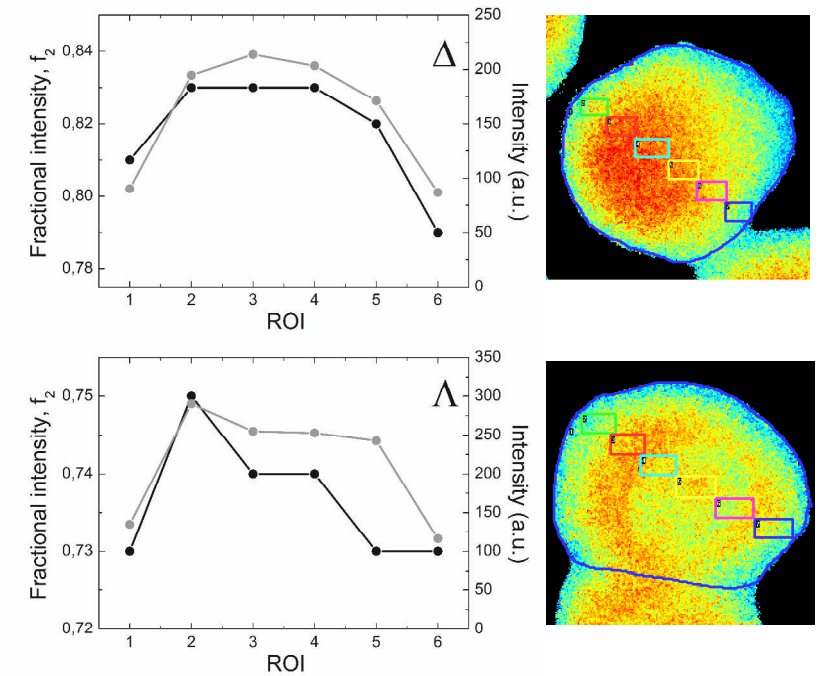


**Figure S2.** Left panels, average fractional intensity (black) and emission intensity (grey) in subsequent regions of interest (ROI) throughout the fixed cell. Data are fitted with two lifetimes, 75 ns and 695 ns for Δ, and 75 ns and 580 ns for Λ, determined from the best fit of the whole cells, and the fractional intensity for the longer component are shown in the graph. $\chi^2$-values in each ROI are between 14-20 for both enantiomers. Right panels, corresponding false color emission lifetime images, fitted with one lifetime in each pixel, with ROI from top to bottom, are presented. For both enantiomers, the

intracellular variation of the fractional intensity of the long lifetime component correlate with the emission intensity.

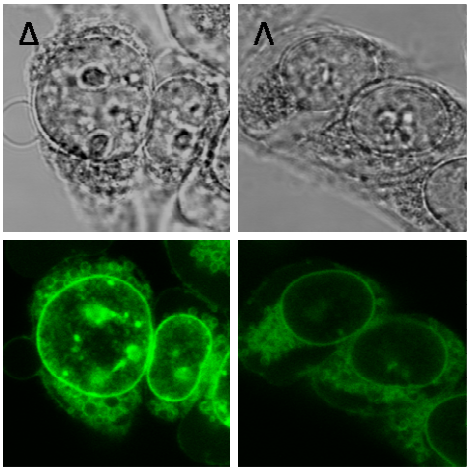


**Figure S3.** Representative transmission (upper) and CLSM (lower) images of the intracellular distribution of Δ- and Λ-D4 in CHO-K1 cells illuminated with the 488 nm Ar laser for 30 min. They have similar intensity in the cytoplasm, whereas Δ show stronger emission intensity in the nucleus.

## EXPERIMENTAL METHODS

### Cell culture

CHO-K1 cells were seeded in glass-bottom dishes (WillcoWells, Netherland) 1-2 days prior to experiment. Cells were fixed by addition of methanol -20°C for 15 min and rinsed once with serum-free medium before addition of complex (5 μM diluted in serum-free medium). Live cells were incubated with complex for 15 min before imaging.

### Multi-frequency fluorescence lifetime imaging microscopy

Multi-frequency FLIM measurements were performed on a LIFA Fluorescence lifetime signal generator system (Lambert Instruments)[1-3] equipped with a LI$^2$CAM on a wide field microscope (Olympus IX70). Samples were excited with an ultra bright LED at 405 nm and a 100× oil immersion objective was used. Measurements were performed using 10 frequencies between 0.2-2 MHz and 12 phase steps with individual collection times of 100-200 ms. For each sample image, a reference image of a fluorescein solution with known lifetime of 4 ns were measured with the same experimental setup. A threshold valued was applied to the FLIM images to remove data from low intensity pixels (with less than 15% intensity). The lifetimes were calculated using the LI-FLIM 1.2.7 software. The multi-frequency fits were produced by the Levenberg-Marquardt algorithm,[4] and $\chi^2$ determined according to:[1]

$$\chi^2 = \frac{40000}{(2N_\omega - N_c + 1)}\left(\sum_\omega \left[\Phi_\omega - \Phi_{c\omega}\right]^2 - \sum_\omega \left[m_\omega - m_{c\omega}\right]^2\right)$$

Lambert Instruments estimate for error in phase and modulation. $N_\omega$ was the number of frequencies and $N_c$ was the number of lifetime components to fit. ν was the number of degrees of freedom, $\Phi_\omega$ was the measured phase, $\Phi_{c\omega}$ was the fitted phase, $m_\omega$ was the measured modulation and $m_{c\omega}$ was the fitted modulation.

The fractional intensity, f, for the long lifetime component obtained from FLIM was converted to pre-exponential factor, α, to be compared with data from spectroscopy measurements, according to:

$$\alpha_i = \frac{\frac{f_i}{\tau_i}}{\sum_i \frac{f_i}{\tau_i}}$$

The apparent lifetime, $\bar{\tau}$, was further calculated according to:

$$\bar{\tau} = \sum_i \alpha_i \tau_i$$

**Confocal microscopy**

Images were acquired using a 63×/1.4 oil immersion objective on a Leica TCS SP Confocal microscope. The 488 ns line of the Ar laser was used for excitation and emission was detected between 600-700 nm. The experiments were repeated three separate days and representative images are presented in this paper.

**Steady State Emission and Excited State Lifetime Spectroscopy**

Steady state emission measurements were performed on a Cary Eclipse Fluorescence Spectrophotometer (Varian, USA) at room temperature. The wavelength of excitation was 440 nm and the emission was measured between 550 and 800 nm. Quantum yields were determined by comparing the absorbance weighted integrated emission intensities with a reference with known quantum yield.

The emission decays were measured with a Nd:YAG laser (Continuum Surelite II-10, pulse width <7 ns) pumping an optical parametric oscillator at an excitation wavelength of 440 nm. Emitted light was sent through a monochromator set at 620 nm and detected by a Hamamatsu R928 photomultiplier tube at an angle of 90° relative the excitation light. For each trace analyzed, four decays were collected and averaged by a 200 MHz digital oscilloscope (Tetronix TDS2200 2Gs/s), stored by a LabView program and analyzed in the Origin software package.

**Preparation of unilamellar lipid vesicles**

Phospholipid vesicles with a diameter of 100 nm of DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine) and DOPG (1,2-dioleoyl-sn-glycero-3-phosphatidylglycerol) at a lipid molar ration of 4/1 were prepared by the extrusion method as described elsewhere.[5] The buffer used was 150 mM NaCl, 10 mM HEPES, 1 mM EDTA, pH 7.4.


(1) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: Singapore, 2006.

(2) Squire, A.; Verveer, P. J.; Bastiaens, P. I. Multiple Frequency Fluorescence Lifetime Imaging Microscopy, *J. Microsc* **2000**, 197, 136-149.

(3) [Anon]. LIFA System for Fluorescence Lifetime Imaging Microscopy (FLIM), *J. Fluoresc.* **2003**, 13, 365-367.

(4) Marquardt, D. W. An Algorithm for Least-Squares Estimation of Nonlinear Parameters, *J. Soc. Ind. Appl. Math.* **1963**, 11, 431-441.

(5) Svensson, F. R.; Matson, M.; Li, M.; Lincoln, P. Lipophilic Ruthenium Complexes with Tuned Cell Membrane Affinity and Photoactivated Uptake, *Biophys. Chem.* **2010**, 149, 102-106.