Electronic Supplementary Information

Spectral counterstaining in luminescence-enhanced biological Raman microscopy

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Methods

Cells. Exfoliated human buccal epithelial (epidermal) cells were obtained by gently scraping the oral mucosa (inner side of the cheek) of one of the authors (RP) with a stainless-steel spatula, and suspended in physiological solution (0.9% NaCl).

HeLa cells were grown at 37°C on microslides placed at the bottom of plastic Petri dishes (35 mm diameter), in DMEM culture medium (Dulbecco's modified Eagle's medium) supplemented with 10% heat-inactivated foetal bovine serum (Sigma-Aldrich), 1% glutamax (Gibco), 1% sodium pyruvate (Sigma-Aldrich) and 100 units/ml of penicillin-streptomycin (Lonza). As DMEM contains phenol red as pH indicator (which is fluorescent) it was replaced just before Raman microscopy by MEM culture medium (Eagle's minimum essential medium) containing no phenol red. As living cells didn't tolerate spending too much time outside the incubator and/or lengthy laser beam exposure required for high-resolution Raman microscopy some of them were fixed (at room temperature) as follows: Upon removal of the culture medium (DMEM) from Petri dish, the cells were rinsed with PBS buffer to remove any floating particles, buffer removed, and 1 ml of 10% buffered formalin solution (i.e., 4% formaldehyde) added. After 10 minutes, the formalin solution was removed and the cells rinsed three times with PBS buffer.

Pollen grains of juniper (*Juniperus chinensis*) were collected in spring in Prague, left to dry and stored at room temperature in a Petri dish covered with a sheet of paper (to prevent dust contamination) yet allowing sufficient circulation of air (to prevent mould formation). When needed, a small amount of pollen was dispersed in water. Measurements were carried out upon their hydration manifesting itself in shell (exine) rupture due to pectin expansion of the pollen grain's interior (intine).

Yeast cells (*Candida albicans*, strain SC5314) were grown on YPD solid medium (10 g yeast extract, 10 g peptone, 20 g glucose, 24 g agar and 950 ml water). An isolated colony was picked from an YPD agar plate struck from a frozen glycerol stock, sub-cultured in new YPD solid medium, and incubated at 30°C for 48 hrs. After that (day 0), cells were kept at 20°C, and eventually (day 1 or 2) suspended in physiological solution (0.9% NaCl).

Labelling by Eu-DPA complex. Sodium salt of the Eu-DPA complex, $Na_3[Eu(DPA)_3]$ was employed to label the cells, with DPA (dipicolinic acid or 2,6-pyridinedicarboxylate) as a ligand. It was obtained by mixing europium(III) carbonate and pyridine-2,6-dicarboxylic acid in water (1:3 molar ratio), followed by adjustment of pH to 7 by adding 1 M sodium carbonate.

Cells in suspension (epithelial, pollen and yeast) were subjected to careful trituration with a shortened (wide-open) yellow pipette trip was applied to achieve homogeneous distribution of the complex. They were then placed under a coverslip of standard thickness (0.17 mm), excess solution sucked out, and edges of the coverslip sealed to the microslide with nail varnish to prevent evaporation. Cultured cells (HeLa) were labelled directly on glass coverslips in plastic Petri dishes, while still in culture medium.

Labelling of all cells was carried out at laboratory temperature, and was always at least one hour. Eu-DPA concentrations applied were 100 µM (HeLa cells), 1 mM (buccal and yeast cells) or 1.6 mM (pollen grains).

Microscopy. Raman spectra (λ_{exc} = 532 nm) were obtained at laboratory temperature on an upright confocal Raman microscope (WITec alpha300 R+) equipped with proprietary software. Zeiss EC Epiplan Neofluar DIC x50/0.8 (dry) and Nikon NIR Apo DIC N2 x60/1.0 W (water immersion) objectives were used, as specified in figure legends. Spectral integration time was 100 ms per pixel. Intensity in spectra is offset to reduce background. The luminescence and Raman images were generated using 615 nm (~2539 or 2546 cm^{-1}) and 630 nm (2925 cm^{-1}) spectral windows, respectively. Their width (highlighted in the spectra) was either 77 or 300 cm^{-1} (luminescence), or 250 cm^{-1} (Raman). The wavenumber and corresponding approximate wavelength stated in luminescence/Raman images denote the centre of the spectral window employed to generate the pseudocolour images, using WITec's Project FOUR software package, version 4.1 or newer. Background in images was subtracted based on intensities in front of and behind a relevant peak. Thresholding was additionally applied as appropriate using the same software to optimize imaging contrast, and pseudocolour scales shown in Figs. 2-5 apply to thus processed image. The Eu-DPA spectrum (Fig. 1) was recorded in two parts (subsequently 'glued' at 1800 cm^{-1}) from a tiny crystal grown out of water solution in which the spectrum

looked the same, except Raman band of water. Phase-contrast (positive-type) imaging was carried out on inverted microscopes (Nikon in Fig. 2 and Olympus in Fig. 3).