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Interaction of β-cyclodextrin with nile red in a single live CHO cell: An initiative towards developing a prospective strategy for excretion of adsorbed drugs from the cell membrane

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Electronic Supplementary Information

Experimental Section

Materials

Laser-grade dye, nile red (Sigma), dimethyl sulphoxide (DMSO, Sigma, biological grade for cell culture) and β-cyclodextrin (Sigma) were used as received.

Methods

Cell Preparation

As discussed in the previous publications,[29,30] Chinese Hamster Ovary (CHO) cells were grown in phenol red free Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 1% Pen Strep Glutamine (From Gibco) in an atmosphere of 5% (v/v) CO_2 enriched air at 37°C. For microscopy, a 35-mm glass bottom petri dish was used for cell culture. For the confocal microscopy and fluorescence lifetime measurement, after rinsing the cells three times with phosphate buffered saline (PBS) buffer solution, 10 μL 2 μM nile red dye solution in ethanol was added to the petri dish containing 2 mL of the phenol red and FBS free DMEM medium. Then it was incubated for about 30 min. This gives ~ 200 nM dye concentration in the culture petri-dish.

The stained cells were rinsed with PBS solution for 5-6 times. After staining, cells were used for microscopic study within 10 min. To elucidate the effect of β-cyclodextrin, requisite amount of solid β-cyclodextrin was added to the petri-dish to make a final concentration of 6 mM. However, the final situation in the petri-dish was not a homogeneous solution since there was not much scope to make the solution homogeneous by shaking (only 2mL solution spreading over a relatively large area of the petri-dish base) since too much of mechanical agitation leads to the destruction of the living cell. All experiments were carried out in ~ 25 °C. All experiments were repeated at least three times.

Experimental Set-up for one Photon Microscopy

Experimental set-up for one photon microscopy has been described in the previous publications.[29,30] Briefly, a combination of confocal microscope (Olympus IX-71) and TCSPC setup (PicoQuant, MicroTime 200) have been used in this study. A water immersion objective (magnification 60X and numerical aperture (NA) ≈ 1.2) and a pulsed picosecond diode laser (PDL 828-S "SEPIA II," PicoQuantat 470 nm) were used. Thus the diffraction limited spot size is $0.6\lambda/1.2 \sim \lambda/2$. For live cell studies, we kept laser power at or below ~ 0.27 μW. Fluorescence from the dye in the cells was separated by a dichroic mirror (Z405RDC, Chroma). To block the exciting laser light, a suitable filter (490LP, Chroma) was used before the detectors. The fluorescence was focused through a pinhole (30 μm). The emitted light with different polarizations were separated using a polarizer cube (Chroma) and were detected by two single-photon counting avalanche photodiodes (SPAD1 and SPAD2). Appropriate narrow band-pass filters were used (e.g. XBPA510, 540, etc. Asahi Spectra) to collect TCSPC decay at specific emission wavelengths. The signal was subsequently processed by the PicoHarp-300 time-correlated single photon counting module (PicoQuant) to generate TCSPC histogram. Lifetime histogram was generated from the photon bursts with a binning time of 10 ms.

The emission spectra of nile red in live CHO cell was recorded using an electron multiplying charge-coupled device (EMCCD, ANDOR Technology) attached to a spectrograph (ANDOR Technology, Shamrock series).