Electronic Supporting Information

Monitoring Excited-State Relaxation in a Molecular Markers in live cells – the Case Study of Astaxanthin

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Experimental details

Cell culture procedure and sample preparation. MCF-7 cells were incubated in 25 cm^2 cell culture flask with RPMI 1640 medium, which was supplemented with 10% fetal bovine serum, and 1% streptomycin-penicilin, at a 37°C with 5% CO_2 incubator. At approximately 90% confluency, the cells were split and seeded in μ-dish glass bottom and incubated with the above stated conditions for 24 h. The cells in μ-dishes were washed with phosphate-buffered saline (PBS) (Sigma-Aldrich) and stained with astaxanthin (AXT) for 24 h (the stock solution was prepared in dimethyl sulfoxide (DMSO) solvent). This was followed by washing the cells thoroughly for three times to remove any unwanted residue of AXT residing on the cells. After the measurements, the cells were treated with Trypan Blue Solution (0.4%) (gibco, USA) for cell viability testing. The cells were rinsed twice with Hanks' balanced salt solution (HBSS) (Sigma-Aldrich) after staining with Trypan Blue for 2 minutes following which the irradiated area of the cell was imaged by a combination of Raspberry Pi camera and Carl Zeiss Axiovert 25 microscope with the objective EC Plan-Neofluar 10X/0.30 M27.

Cell viability test. The cell viability tests of MCF-7 cells without AXT (Fig. S3(a)) and with AXT (staining for 24 h) (Fig. S3(b)) were done by Hemacytometer (Hausser Scientific, USA). The cells were detached from petri dish by using Trypsin-EDTA solution (ATCC, USA) followed by addition of 10 μL PBS and 10 μL Trypan Blue to the cells.

Cells' viability was calculated by using the equation:

$$viable\ cells = \frac{total\ number\ of\ viable\ cells}{total\ number\ of\ cells} \times 100\%$$

The cell viability obtained for the control and AXT stained cell were ~97 %, and ~93 % respectively.

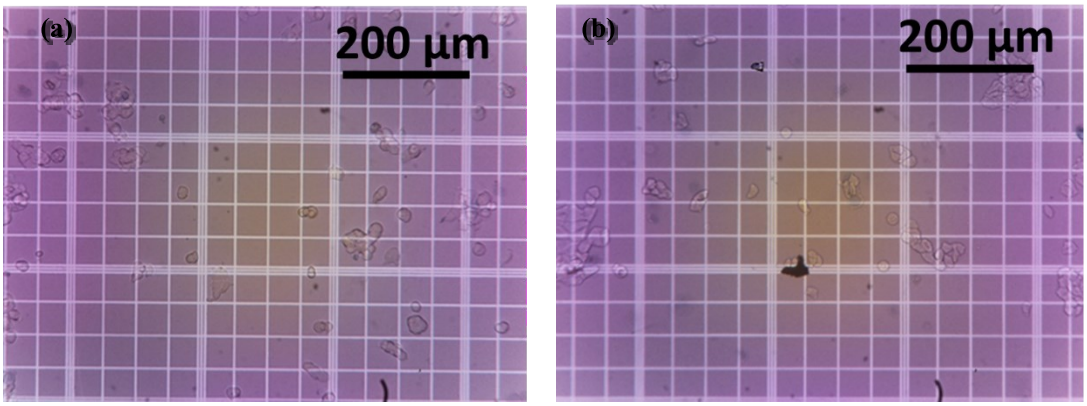

Figure S1 (a) MCF-7 cells stained by trypan blue. (b) MCF-7 cells incubated with AXT for 24 h stained by trypan blue.

Transient absorption spectroscopy measurements. All femtosecond (fs) transient absorption spectra were obtained with a home-built setup[1] where the pump beam with a time duration of ~110 fs was generated using TOPAS-C, Lightconversion Ltd. Throughout the measurement, the pump power was set to 20 μW at the sample position. A white-light supercontinuum light generated by focusing a residual of the fundamental laser on a rotating CaF_2 plate was used to probe the absorbance of the sample between 350 to 750 nm. The probe beam was delayed in time w.r.t. the pump beam by passing through an optical delay line with the polarization between the probe and pump beam set at the magic angle (~ 54.7°). For white light transient absorption experiment, AXT was dissolved in DMSO with an optical density ca. 0.3 at the excitation wavelength recorded in a 1 mm quartz cuvette. Prior to analysis, the data was chirp-corrected and subsequently globally fitted using a sum of exponentials.

Transient absorption microscopy measurements. Transient absorption microscopy measurements were performed using a home-built setup which was described in detail elsewhere.[2] The laser system consisted of two OPAs pumped by an ultrafast

high-power fiber laser running at 1030 nm. Two synchronized pulse trains were provided one of which was used as the pump at 470 nm with a repetition rate of 125 kHz, while the other beam was used as the probe at 625 nm with a repetition rate of 250 kHz. The probe was delayed in time w.r.t. the pump by allowing it to pass through an optical delay line. The temporal resolution of the system is ca. 1 ps.[3] For this transient absorption microscopy measurements, MCF-7 cells were fixed with 4% phosphate-buffered formaldehyde (Carl Roth, Germany), and scanned in a raster pattern by means of a galvanometer-based scanner system (Cambridge Technologies). The laser pulses were focused with an objective of Nikon CEI PLAN APO Lambda 20X/0.75 DIC.

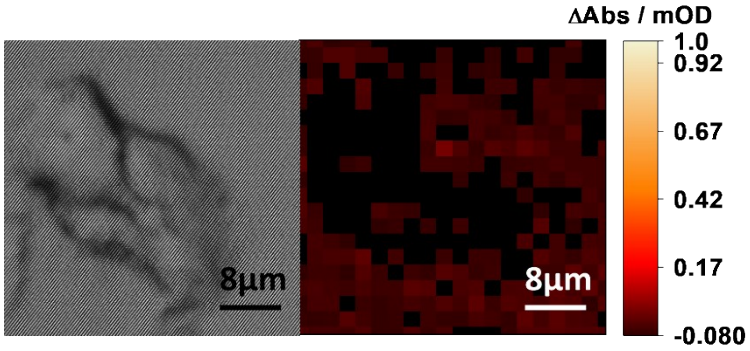

Figure S2 Phase image (left panel) showing the inside of the MCF-7 cell and its corresponding image (right panel) obtained by Transient absorption microscopy (λ_{pump} = 470 nm, λ_{probe} = 625nm).

Time resolved pump-probe setup on bulk of cells measurements. A home-built time-resolved pump-probe setup was used to perform the measurements in bulk of cells.[4] The 510 nm pump beam was generated from non-collinear optical-parametric amplifier (TOPAS-C, Lightconversion Ltd.), while the generation of the probe beam was also obtained via a non-collinear optical-parametric amplifier (TOPASwhite, LightConversion Ltd.). The probe beam was split into two sets after a beam splitter. One of the beams was sent to a reference photodiode while the other one was allowed to pass through the sample then recorded by photodiodes and read out by the detections system purchased from Pascher Instruments AB. After obtaining spatial and temporal overlap of the pump and probe beams, the transient absorption data were recorded in two experimental runs in each measured position in the µ-dish.

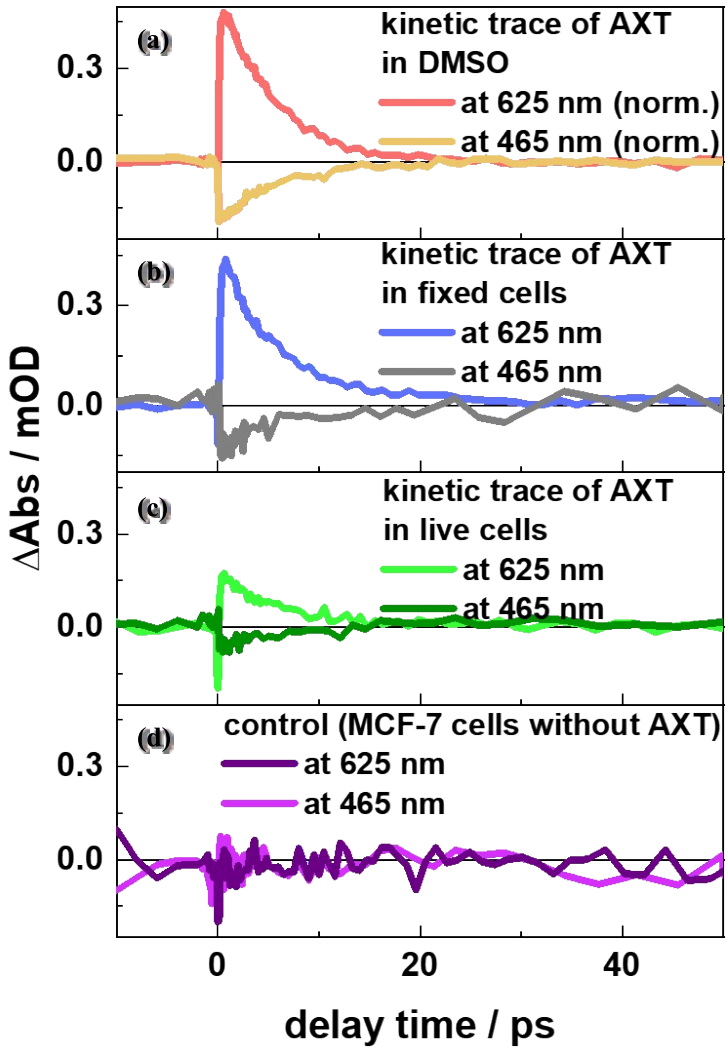

Figure S3: The kinetic traces of AXT probed at 625 nm and 465 nm measured in (a) DMSO solvent, (b) fixed cells, (c) live cells, (d) control-cells without AXT.

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