Electronic Supplementary Material (ESI) for Analyst

High temporal resolution confocal fluorescence measurements for ultrasensitive detection of early stage apoptosis

Divya Iyer, Rachel D. Ray, Dimitri Pappas*
Department of Chemistry and Biochemistry
Texas Tech University, Lubbock, TX 79409 USA.

Supporting Information

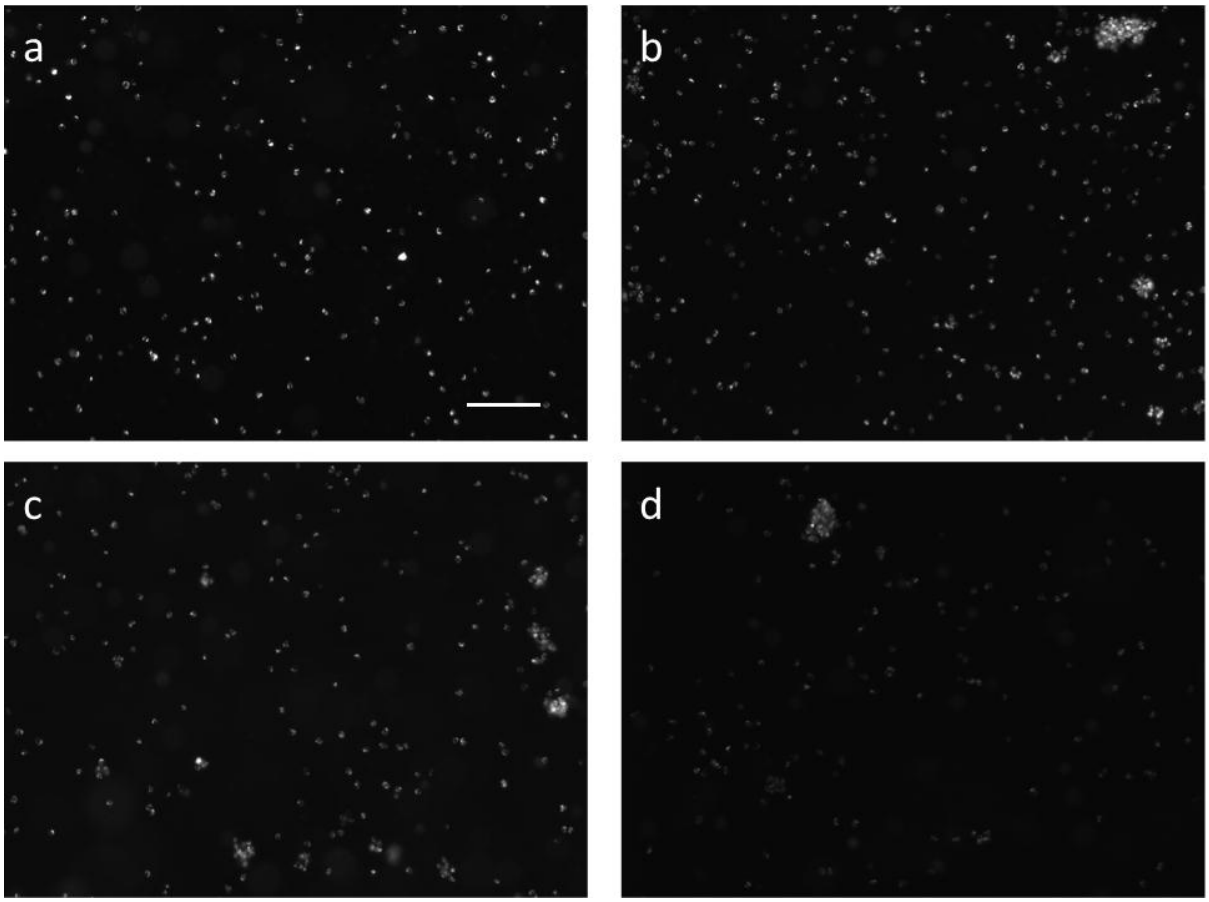

Figure S1. Fluorescence images (x10 magnification) of Ramos cells stained with 0.1 μM MitoTracker deep red at (a) Control (Avg intensity = 22301, stdev = 2051), (b) 30 minutes (Avg intensity = 17345, stdev = 2119) (c) 45 minutes (Avg intensity = 12897, stdev = 1629) (d) 60 minutes (Avg intensity = 9800, stdev = 1882) after staurosporine induction. The fluorescence intensity was observed to decrease after induction, but the earliest changes were not resolved. The image shows illumination of mitochondria in cells. Scale bar = 100 μm

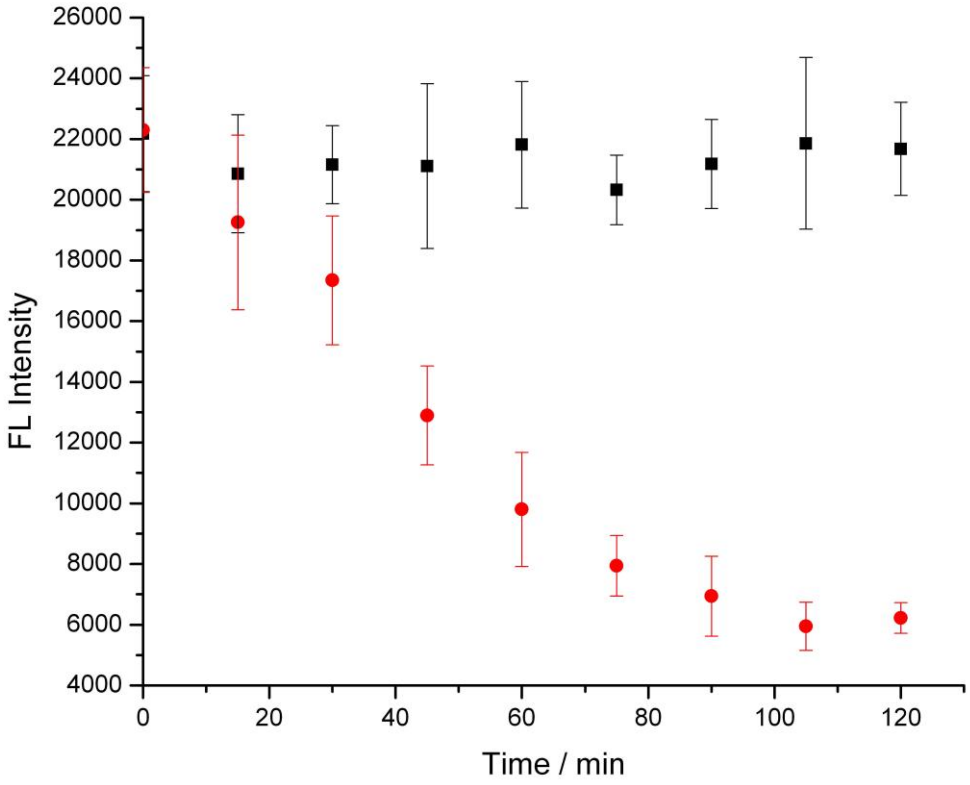

Figure S2: The decrease in fluorescence intensity of the sample as a function of time was exponential, whereas the control intensity remained constant for 2 hours. To quantitatively determine the average intensity of apoptotic cells for each image (Fig S1), the mean intensity per pixel of ~20 cells was measured using ImageJ. Control cells are represented as black squares and drug induced samples as red circles. All cells were stained with 0.1 μM MitoTracker deep red and samples were induced with 4 μM staurosporine.

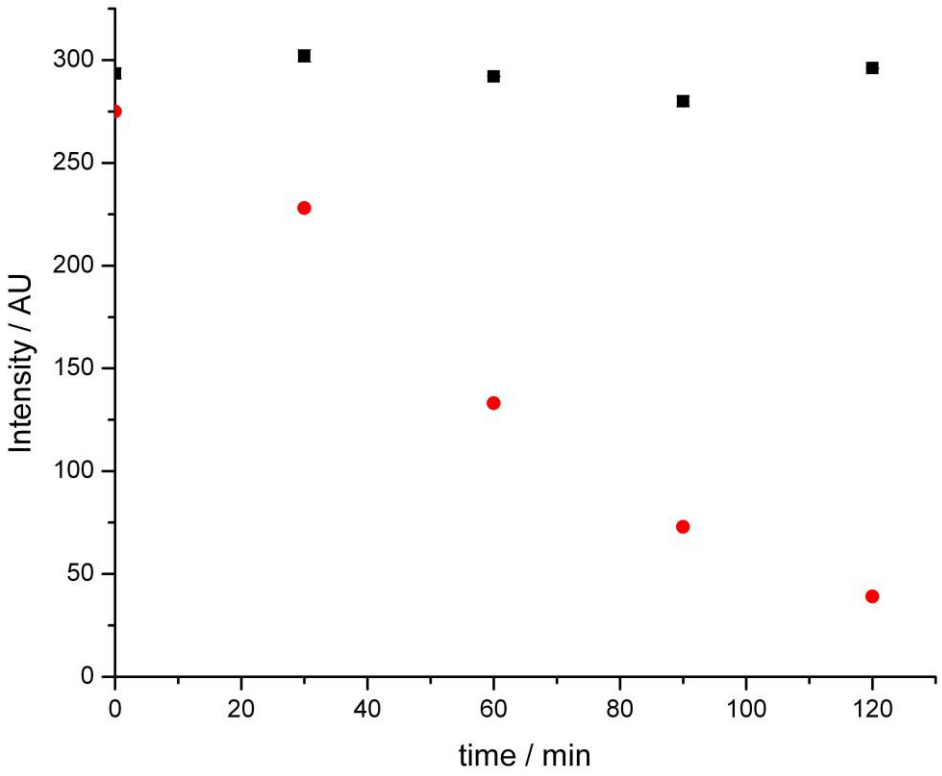

Figure S3: MitoTracker deep red (MTDR) intensity measurements extracted from flow cytometer FL 4 histogram after inducing with staurosporine as a function of time to confirm the results obtained by microscopy. Control cells are represented as black squares and drug induced samples as red circles. All cells were stained with 0.1 μM MTDR and samples were induced with 4 μM staurosporine. Each data point represents mean of triplicate measurements. Standard error of the mean is too small to be visible.

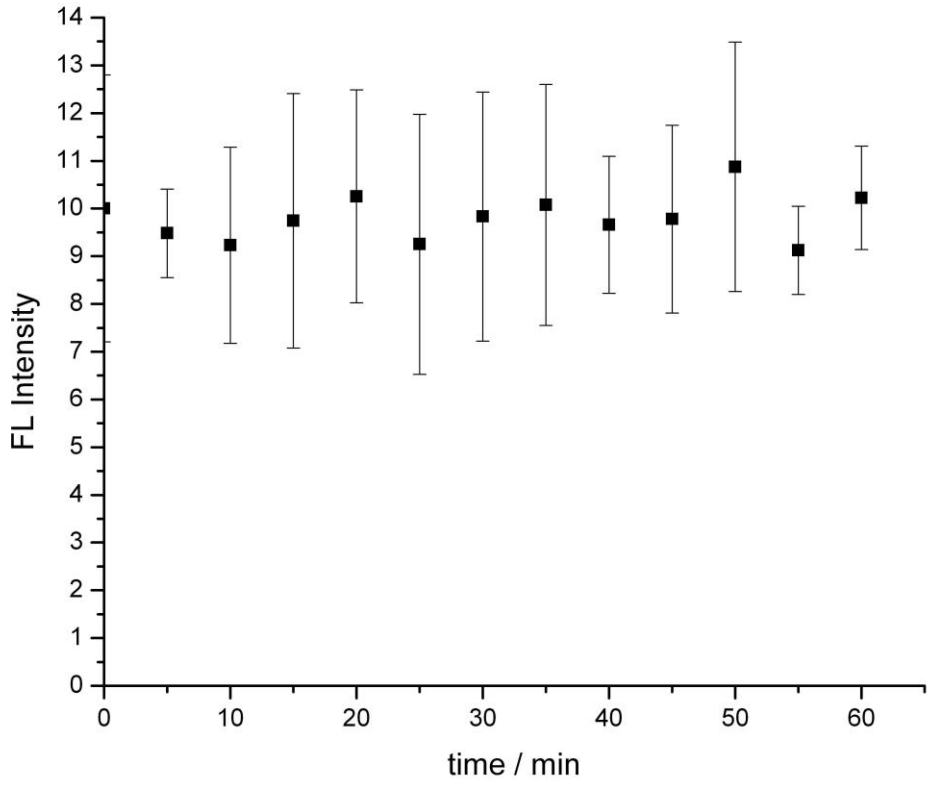

Figure S4: Mean of the median MitoTracker deep red (MTDR) fluorescence intensity as a function of time for five Ramos cells at each time interval by ultrasensitive confocal microscopy. Control cells are represented as black squares and stained with 0.1 μM MTDR.

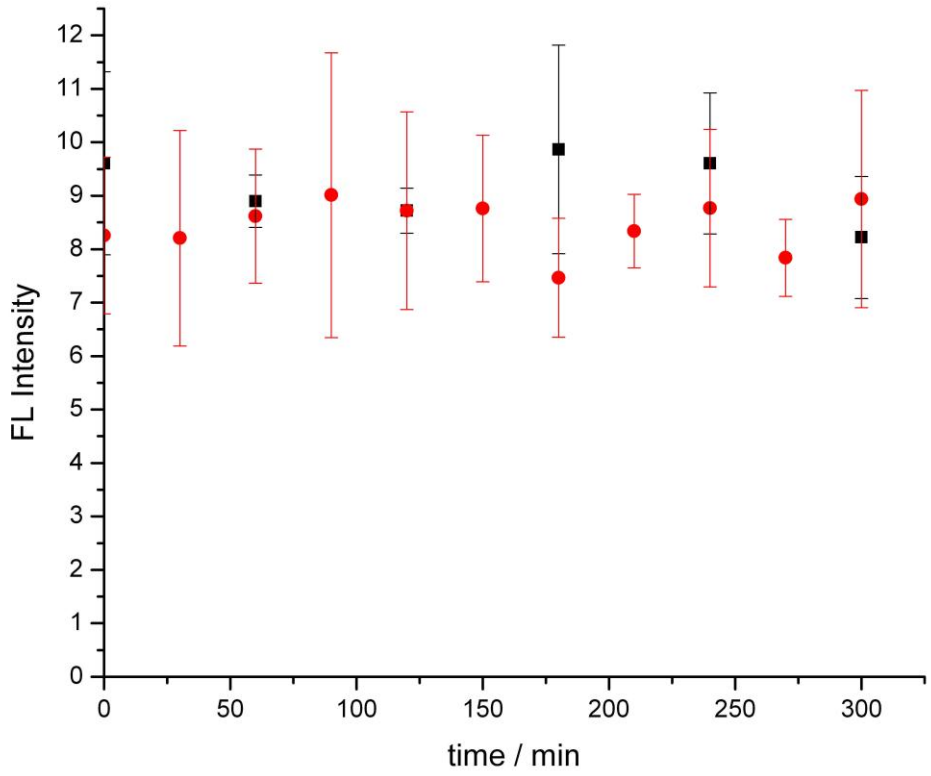

Figure S5: Mean of the median MitoTracker deep red (MTDR) fluorescence intensity as a function of time for five Ramos cells at each time interval by ultrasensitive confocal microscopy. Control cells are represented as black squares and anti-CD95 induced samples as red circles. All cells were stained with 0.1 μM MTDR and samples were induced with 1.6 μg/ml anti-CD95. No significant change in fluorescence intensity was observed between the sample and control cells.

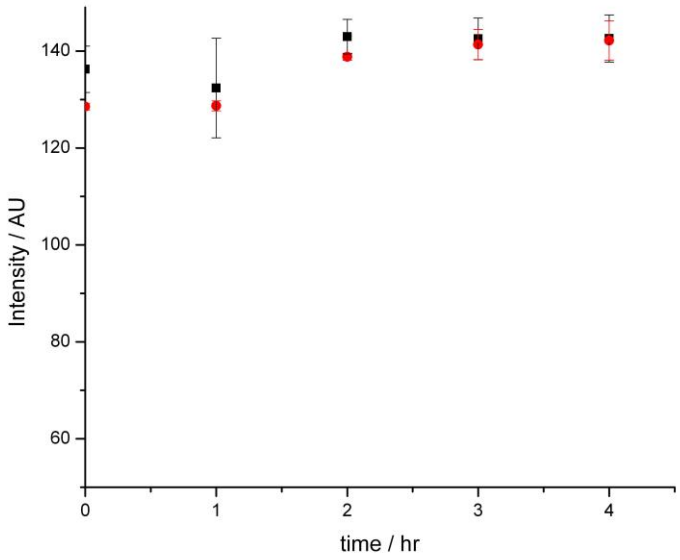

Figure S6: MitoTracker deep red intensity measurements extracted from flow cytometer FL 4 histogram after inducing with anti-CD95 as a function of time. Control cells are represented as black squares and drug induced samples as red circles. All cells were stained with 0.1 μM MTDR and samples were induced with 1.6 μg/ml anti-CD95. The microscopy results were confirmed by flow cytometry.