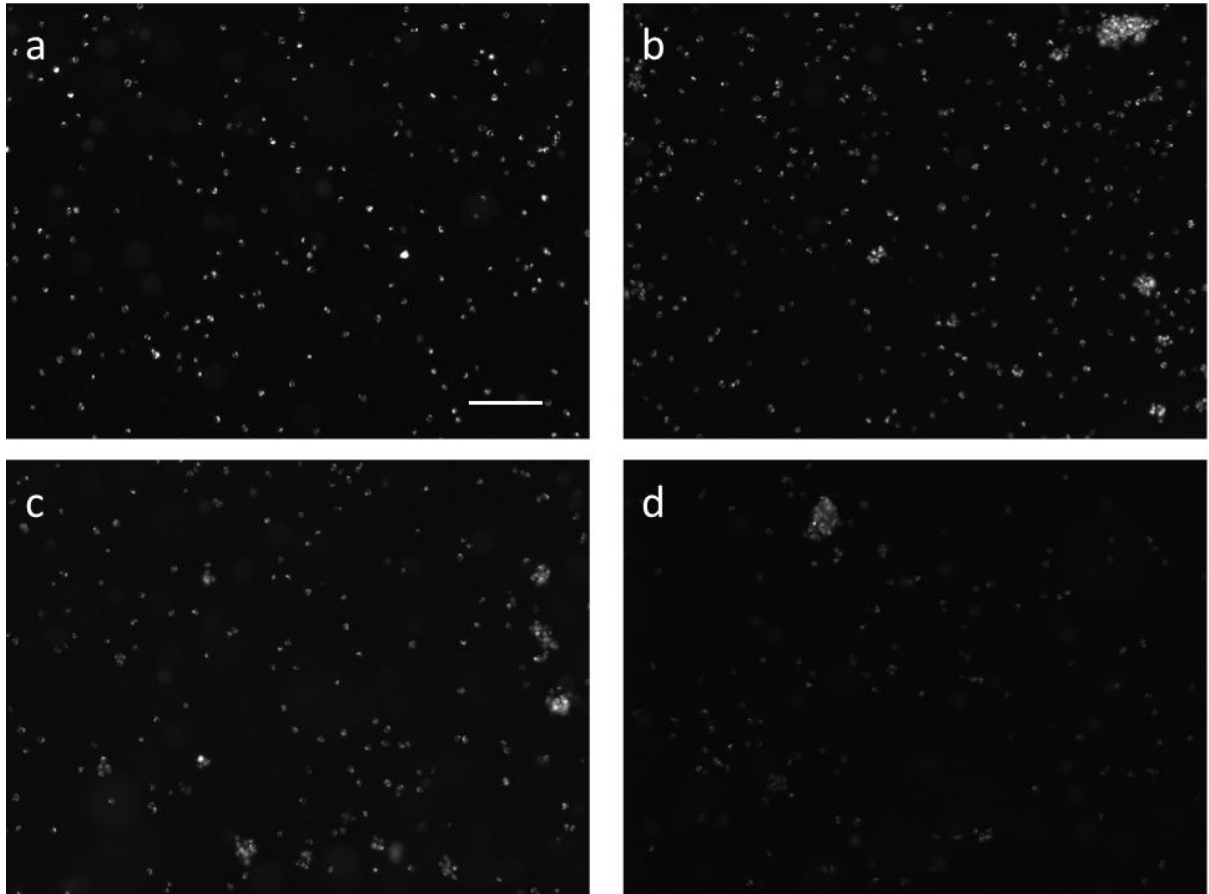


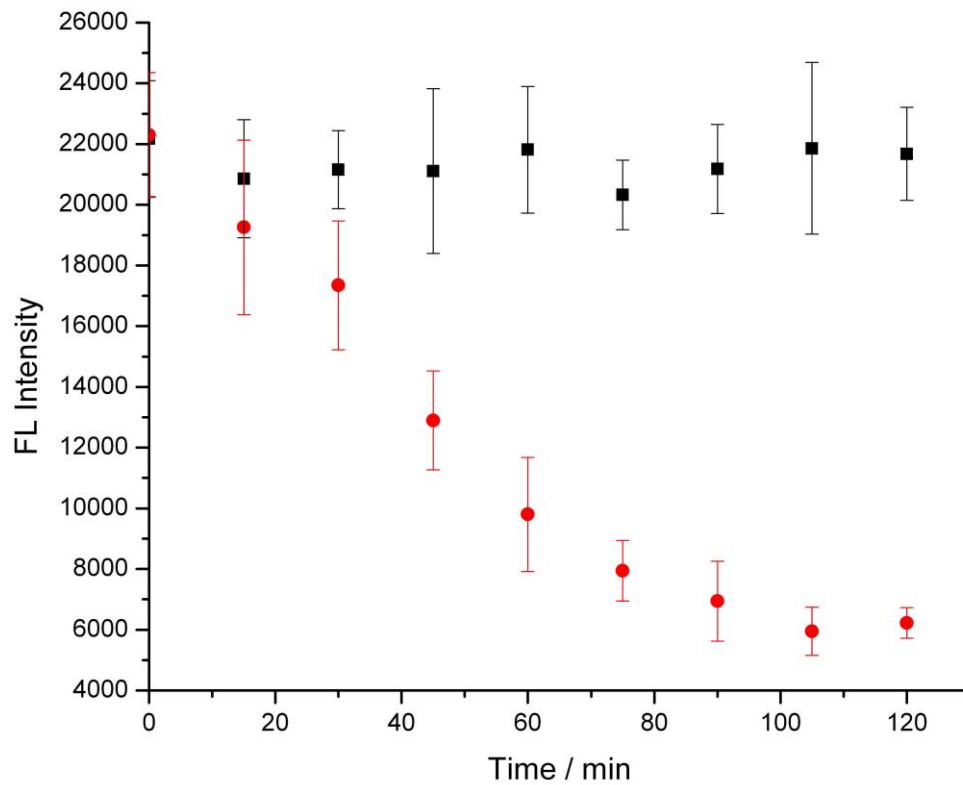
**High temporal resolution confocal fluorescence measurements for ultrasensitive detection of early stage apoptosis**

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

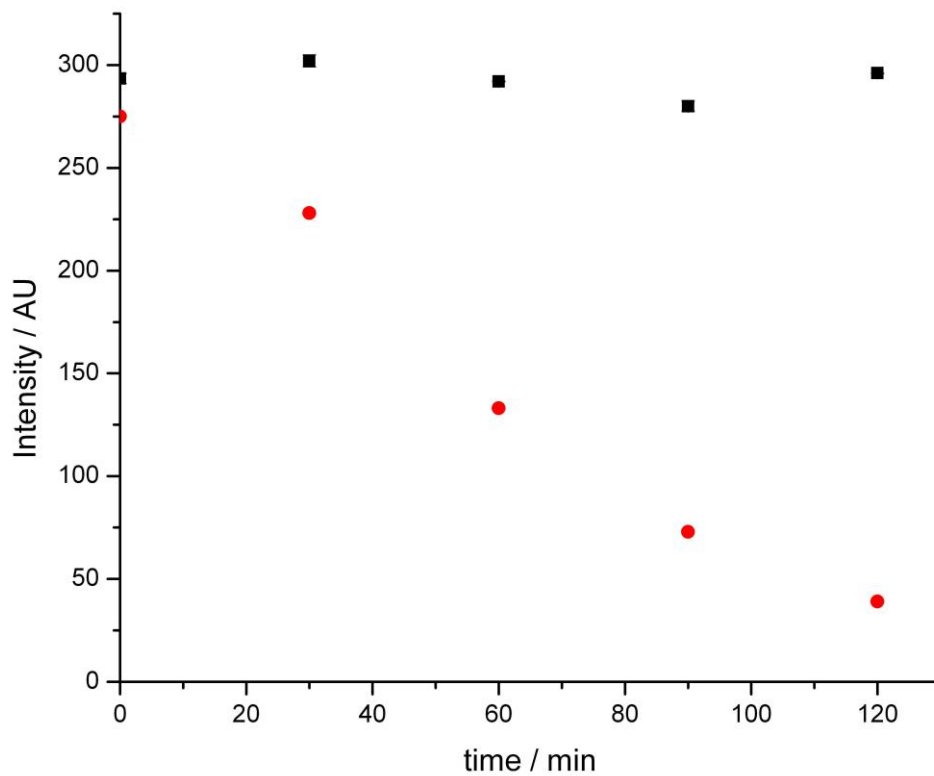
**Supporting Information**



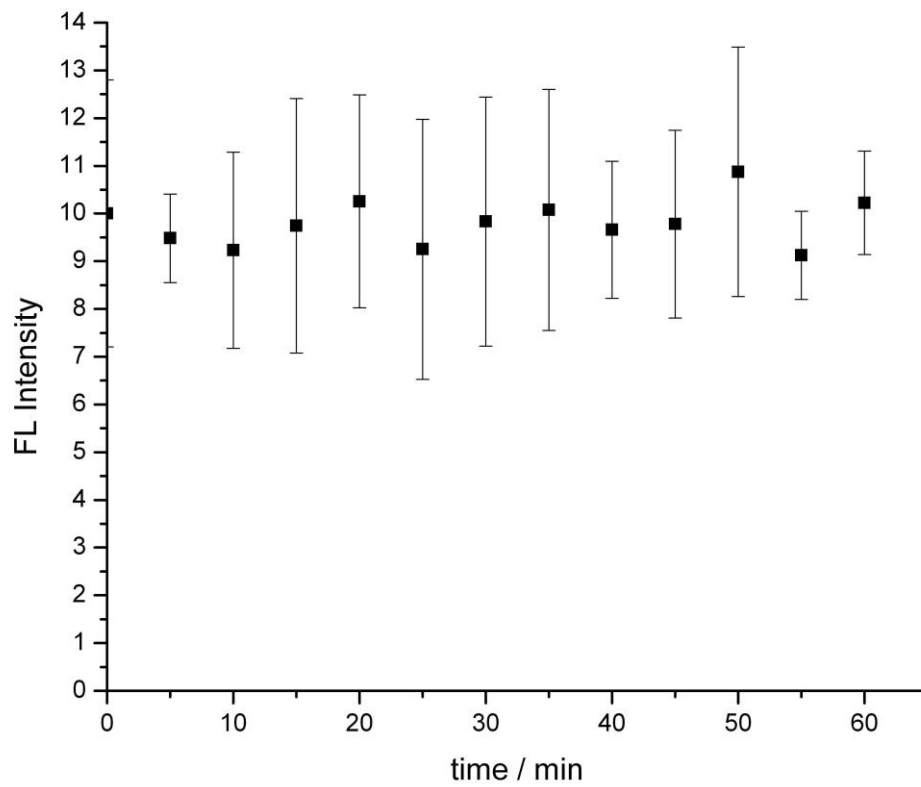
**Figure S1.** Fluorescence images (x10 magnification) of Ramos cells stained with 0.1  $\mu\text{M}$  MitoTracker deep red at (a) Control (Avg intensity = 22301, stdev = 2051), (b) 30 minutes (Avg intensity = 17345, stdev = 2119) (c) 45 minutes (Avg intensity = 12897, stdev = 1629) (d) 60 minutes (Avg 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  $\mu\text{m}$



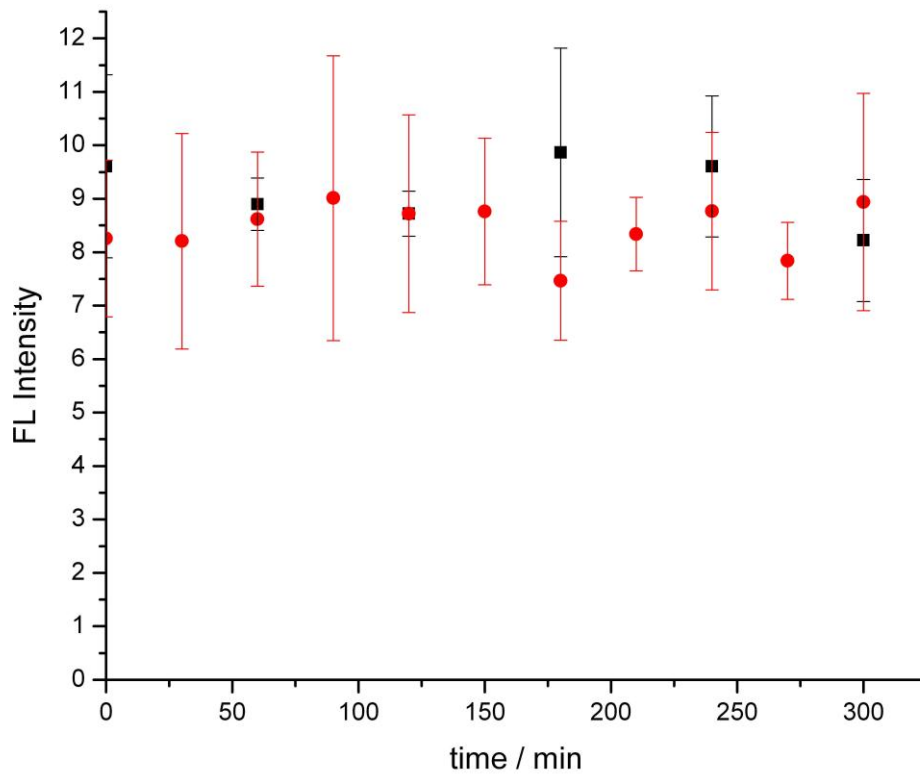
**Figure S2:** The decrease in fluorescence intensity of the sample as a function of time was exponential, whereas the control intensity remained constant for 2 hours. To quantitatively determine the average intensity of apoptotic cells for each image (Fig S1), the mean intensity per pixel of ~20 cells 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  $\mu\text{M}$  MitoTracker deep red and samples were induced with 4  $\mu\text{M}$  staurosporine.



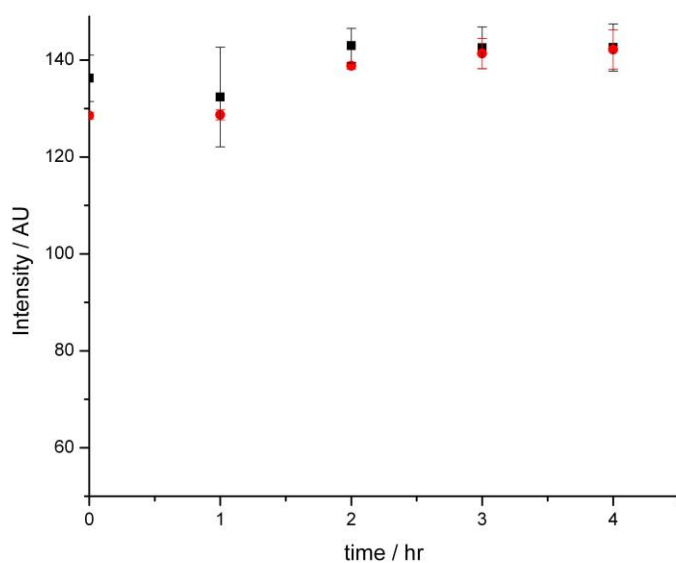
**Figure S3:** MitoTracker deep red (MTDR) intensity measurements extracted from flow cytometer FL 4 histogram after inducing with staurosporine as a function of time to confirm the results obtained by microscopy. Control cells are represented as black squares and drug induced samples as red circles. All cells were stained with 0.1  $\mu$ M MTDR and samples were induced with 4  $\mu$ M 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  $\mu\text{M}$  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  $\mu$ M MTDR and samples were induced with 1.6  $\mu$ g/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  $\mu\text{M}$  MTDR and samples were induced with 1.6  $\mu\text{g/ml}$  anti-CD95. The microscopy results were confirmed by flow cytometry.