Markéta Procházková, 175241 Poster for presentation
Doctoral conference XD107 on February 10, 2022

Poster title:

Miniaturized bioluminescence technology for single-cell quantification of caspase-3/7

Abstract

Correct determination of the instantaneous level and changes of relevant proteins inside individual cells is essential for correct interpretation and understanding of physiological, diagnostic, and therapeutic events. Thus, single-cell analyses are important for quantification of natural cellular heterogeneity, which cannot be evaluated from averaged data of a cell population measurements. We developed an original highly sensitive and selective instrumentation and methodology based on homogeneous single-step bioluminescence assay to quantify caspases and evaluate their heterogeneity in individual cells. Individual suspended cells are selected under microscope and reliably transferred into the 7 μl detection vials by a micromanipulator. The sensitivity of the method is given by implementation of photomultiplying tube with a cooled photocathode working in the photon counting mode. By optimization of our device and methodology, the limits of detection and quantitation were decreased down to 2.1 and 7.0 fg of recombinant caspase-3, respectively. These masses are lower than average amounts of caspase-3/7 in individual apoptotic and even non-apoptotic cells. As a proof of concept, the content of caspase-3/7 in single treated and untreated HeLa cells was determined to be 154 and 25 fg, respectively. Based on these results, we aim to use the technology for investigations of non-apoptotic functions of caspases. Detailed presentation of our bioluminescence method and all obtained results for treated and untreated HeLa cells will be presented in the poster.

Literature

Markéta Procházková, Michael Killinger, Lubomír Prokeš, Karel Klepárník, Miniaturized bioluminescence technology for single-cell quantification of caspase-3/7, J. Pharm. Biomed., Volume 209, 5 February 2022, 114512, doi:10.1016/j.jpba.2021.114512.