DNA nanoprobe for real-time imaging and simultaneous quantification of mitochondrial Ca\(^{2+}\) and pH in neurons induced by superoxide anion and aggregated amyloid beta

Zhichao Liu and Yang Tian
East China Normal University, China

Mitochondria play vital roles in cellular energy production, signal transduction and Ca\(^{2+}\) homeostasis, as well as the cell death. Besides, mitochondrial pH and Ca\(^{2+}\) are closely associated with cellular functions and diseases. Thus, simultaneous imaging and biosensing are essential for understanding inter-relationship between Ca\(^{2+}\) and pH in physiological and pathological processes. Herein, we created a highly selective DNA nanoprobe for real-time imaging and simultaneous quantification of pH and Ca\(^{2+}\) in mitochondria, in which a new Ca\(^{2+}\) fluorescent probe was synthesized and assembled onto a DNA nanostructure together with pH-responsive, inner-reference, and mitochondria-targeted molecules. This new nanoprobe powerfully tracked pH and Ca\(^{2+}\) dynamics at the same localization in response to superoxide anion (O\(^2-\))-induced oxidative stress and aggregated amyloid beta (A\(\beta\)) stimulation with a temporal resolution of milliseconds. Using this new tool, we discovered that acid-sensing ion channel 1a (ASIC1a) channel plays a vital role in O\(^2-\) and A\(\beta\)-induced mitochondrial Ca\(^{2+}\) burst, which may contribute to neuron death. Moreover, psalmotoxin 1 (PcTX1) effectively protects against neuron injury, providing a potential drug for O\(^2-\) and/or A\(\beta\)-induced neuronal death. Using the DNA-assembled nanosensor for determination of pH and Ca\(^{2+}\) at the same localization, we demonstrated that mitochondrial Ca\(^{2+}\) is increased -4-fold in neurons compared with HeLa cells, whereas mitochondrial pH exhibits no obvious difference between the two types of cells. Furthermore, experimental results demonstrated diverse mitochondrial Ca\(^{2+}\) and pH values in different regions of neurons. The close relationship between Ca\(^{2+}\) and pH in mitochondria was discovered. Mitochondrial pH value in neurons obviously increased with increasing Ca\(^{2+}\) concentration, which may be attributed to the function of the Ca\(^{2+}/H^+\) antiporter in mitochondria. On the other hand, the mitochondrial Ca\(^{2+}\) burst can be adjusted by the ASIC1a channel in response to cytoplasmic acidosis. O2•- induces transitory cytoplasmic acidosis, which may activate the ASIC1a channel in the mitochondrial membrane, resulting in alkalization and Ca\(^{2+}\) overload in mitochondria. Mitochondrial Ca\(^{2+}\) overload is possibly one of the important factors in O\(^2-\)-induced neuronal death. These results offer a new view for understanding the signaling pathway of ROS-induced oxidative stress and neuron injury. Aggregated A\(\beta\) is highly toxic to neurons. After stimulation by A\(\beta\)25-35, the pH value in the cytoplasm clearly decreased together with the Ca\(^{2+}\) burst, leading to acidification and Ca\(^{2+}\) overload in mitochondria through ASIC1a. PcTX1 protein protect neurons from death by preventing mitochondrial Ca\(^{2+}\) overload stimulated by O\(^2-\) and aggregated A\(\beta\), suggesting that PcTX1 is a potential drug for O\(^2-\) and/or A\(\beta\)-induced neuronal death.

Speaker Biography
Zhichao Liu, PhD is a student of Analytical Chemistry under the supervision of Prof. Tian in East China Normal University. He received his MS degree in Analytical Chemistry from Nanchang University in 2015. His doctoral research now focuses on the design, synthesis, characterization, and application of fluorescent nanomaterial for sensing and imaging in biological applications.

E: liuzhichao582@sina.com