Real-time precision opto-control of biomolecular activities

Considering the pivotal role that they play in modulating cell survival, differentiation, and apoptosis, reactive oxygen species (ROS) are rightly termed as double-edged swords. ROS are byproducts of various cellular metabolic processes that maintain cellular homeostasis. Higher concentrations of ROS can oxidize DNA base pairs, thereby fragmenting the DNA and leading to carcinogenic mutations, among many other deleterious consequences. Patients of neurodegenerative diseases such as Alzheimer's and Huntington's have shown greater extents of oxidative damage to both nuclear and mitochondrial DNA in their brains.

It is believed that the major source of ROS is the transmembrane NADPH oxidases and the electron transport chain in mitochondria. However, endoplasmic reticulum (ER)-induced ROS is no less catastrophic. Some studies suggest that under pathophysiological conditions, ER-stress activates the unfolded protein response (UPR) leading to dysregulation of the calcium release and uptake process, with more calcium being released into the cytosol. Most of the released calcium is taken up by mitochondria and this calcium overload initiates a cascade of apoptotic events. However, the exact mechanism is still elusive due to the lack of real-time visualization of such processes.

Using real-time precision opto-control (RPOC) technology recently developed in our lab, organelle-specific control of biological processes in cells can be achieved. We can selectively control light interaction with selected organelle while not affecting unwanted locations. In our work, different dyes are used to label specific organelles, and fluorescence signals from them are used to decide the location of light interaction. A blue laser source (405 nm) is used to selectively interact with different organelles separately and monitor the cellular response in real-time. The effect of light-induced ROS on microtubule dynamics was used as an indicator of the cellular response. We show that shooting a blue laser to ER causes a drastic reduction of the dynamics of tubulin and end-binding protein 3 (EB3), while blue laser interaction with lipid droplets does not give a significant cellular perturbation. We also correlate the energy and dose dependence of laser-induced ROS on ER. In addition, RPOC also shows selective inhibition of tubulin polymerization at subcellular locations with photoswitchable inhibitors.

This research demonstrates a novel approach to studying light interaction with different organelles and site-specific inhibition of molecular processes.