

Mitochondria in Cell Life and Death

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New Fluorescence Probes for Visualizing Cell Structures and Function

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School of Chemistry, University of Melbourne, Parkville, Australia. Organic fluorogens with aggregation-induced emission (AIE) characteristics have demonstrated their potential to be ideal candidates for live cell imaging. Opposite to conventional organic dyes, the AIE luminogens are non-luminescent when molecularly dissolved but highly emissive upon aggregation. As small molecules, the AIE luminogens normally enter cells through diffusion, accumulate in the target location, and generate light emission. Inherently, they possess large Stokes shift (> 100 nm) with appreciable brightness and they are resistant to photo-bleaching and blinking, owing to the formation of aggregates inside the cells. In addition, they are structurally simple and synthetically accessible: the excitation/emission wavelengths as well as the functionalities can be fine-tuned via structural modification. In this study, a series of AIE dyes have been constructed and their applications for specific imaging of different organelles, tracking dynamics of mitochondria as well as sensing intracellular environment in physiological and pathological conditions will be introduced.

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Monitoring Mitochondrial Membrane Potential with MitoView 633: A New Molecular Probe

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¹Physics, Utah State University, Logan, UT, USA, ²Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL, USA, ³Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL, USA. **Background:** Noninvasive mitochondrial inner membrane potential ($\Delta\Psi_m$) measurement has been largely constrained to the use of fluorescent probes. These probes accumulate in the mitochondrial inner membrane according to the Nernst equation. In this manner, $\Delta\Psi_m$ can be measured indirectly by the intensity of the fluorescent dye.

Objective: MitoView 633 is a new fluorescent probe in the far-red spectrum, which has great potentials in bioenergetics studies. However, its application in live cell imaging has not been well characterized. We aimed to determine the spectrum of MitoView using a spectrophotometer and characterized its dynamics in cardiomyocyte during live cell imaging.

Method: H9C2 cells or adult rat cardiomyocytes were stained with MitoView or TMRM. The mitochondrial uncoupler FCCP was used to induce mitochondrial depolarization and the change of $\Delta\Psi_m$ was recorded using a confocal microscope. Images were processed using ImageJ and the dynamics of MitoView and TMRM were compared. In some experiments, cells were co-stained with MitoSOX for simultaneous ROS and $\Delta\Psi_m$ recording.

Results: Spectrum analysis showed that MitoView emission could be detected at 660 ± 50 nm. In addition, MitoView perfectly located in mitochondrial matrix, as demonstrated by its colocalization with Mito-eYFP or TMRM in H9C2 cells and rat cardiomyocytes. Furthermore, the photobleaching of MitoView was insignificant and comparable to that of TMRM and the FCCP-induced decay of MitoView fluorescence was faster than TMRM, indicating its superior sensitivity to $\Delta\Psi_m$. Finally, MitoView could be co-stained with MitoSOX, allowing for simultaneous measurement of ROS and $\Delta\Psi_m$ in live cell imaging.

Conclusion: We showed that MitoView is very sensitive to $\Delta\Psi_m$. Due to its minor photobleaching effect, high sensitivity to $\Delta\Psi_m$, and far-red emission spectrum, MitoView could be an idea molecular probe to monitor $\Delta\Psi_m$ alone or together with other indicators in live cell imaging.

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Biophysical and Biochemical Properties of the Large Conductance Potassium Channel in Fibroblast Mitochondria

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¹Department of Biophysics, Warsaw University of Life Sciences – SGGW, Warsaw, Poland, ²Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland, ³Laboratory of Bioenergetics, Adam Mickiewicz University, Poznan, Poland. Potassium channels have been found in the inner mitochondrial membranes of various cells. The activation of these channels is cytoprotective. Hence, the identification of ion channels present in the inner mitochondrial membrane of fibroblast cells is important in distinguishing possible protective mechanisms in these cells.

In our study single channel activity of a large conductance Ca^{2+} -regulated potassium channel was measured by patch-clamp of mitoplasts isolated from fibroblast cell line. Mitoplast were prepared by addition to a hypotonic solution causing unfolding of the cristae of the inner membrane and consequently

breaking of the outer membrane. Isotonicity was restored by adding a hypertonic solution. A potassium selective current was recorded with a mean conductance of 280 ± 2 pS in symmetrical 150 mM KCl solution. The channel was activated by Ca^{2+} at micromolar concentrations and inhibited irreversibly by paxilline, an selective inhibitor of the BKCa channel. The substances known to modulate BKCa channel activity were found to influence the bioenergetics of mitochondria, isolated from human dermal fibroblast cells. In isolated mitochondria, 10 μ M NS1619 depolarized the mitochondrial membrane potential and stimulated nonphosphorylating respiration. This effect was blocked by 20 μ M paxilline. Our findings indicate presence of the large conductance Ca^{2+} -regulated potassium channels in the inner mitochondrial membranes of human fibroblast. This study was supported by a grant MERIS PBS1/B8/1/2012 from the National Centre of Research and Development.

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Identification of the ATP Regulated Potassium Channel in Mitochondria of Fibroblast Cells

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The transport of potassium ions into the mitochondrial matrix can trigger the protection of the injured cardiac and neuronal tissues. A growing body of evidence suggests that ischemic preconditioning, short episodes of ischemia that increase tissue tolerance to lethal insults, could be mimicked by the administration of openers of mitochondrial potassium channels. Hence, the identification of ion channels present in the inner mitochondrial membrane of fibroblasts is important in distinguishing possible protective mechanisms in these cells.

In our research, inner membrane mitochondrial ion channels of the human fibroblast cell line were investigated using a patch-clamp and biochemical techniques. The single channel activity of mitochondrial potassium channels were investigated using a patch-clamp technique. In the inner mitochondrial membrane of fibroblast we detected ATP-regulated potassium channel (mitoKATP channel) with mean conductance equal to 100 ± 3 pS. The activity of this channel was inhibited by complex of ATP/Mg²⁺ and activated by the potassium channel opener BMS191095 and diazoxide. The influence of substances modulating ATP-regulated potassium channel activity on the bioenergetics, oxygen consumption and membrane potential, of isolated human dermal fibroblast mitochondria was also studied.

Our findings indicate presence of the ATP-regulated potassium channels in the inner mitochondrial membranes of human fibroblast.

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Suppression of Dynamin-Related Protein 1 by Eicosapentaenoic Acid Ameliorates Palmitate-Induced Lipotoxicity in Differentiated H9C2 Myocytes

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Objective: Although previous clinical investigations have reported many preferable effects of eicosapentaenoic acid (EPA; n-3 polyunsaturated fatty acid) against cardiovascular disease, the precise mechanisms are not fully clarified. Since aberrant mitochondrial dynamics plays key roles for the pathogenesis of cardiovascular disease, we investigated the relationship between EPA-mediated cardiac protection and mitochondrial dynamics.

Methods and Results: When differentiated H9c2 myocytes were exposed to palmitate (PAL; 400 μ M, saturated fatty acid) for 24 hours, the PAL-treated myocytes exhibited depolarized mitochondrial membrane potential ($\Delta\Psi_m$: measured with JC10 ratio; 2.1 ± 0.4 vs. 4.5 ± 0.3 of control, $P < 0.01$) and activated caspase3/7 (measured with luminescent assay; 4.1 ± 0.7 fold increase from control, $P < 0.01$), suggesting the PAL-induced lipotoxicity. The PAL-treated myocytes also showed dynamin-related protein 1 (Drp1) up-regulation and fragmented mitochondria (75.3 ± 5.0 % of cells vs. 9.2 ± 3.2 % of control, $P < 0.01$), indicating aberrant mitochondrial dynamics by PAL. When the PAL-treated myocytes were co-incubated with EPA (50 μ M), the caspase3/7 activation by PAL (1.5 ± 0.3 fold increase vs. PAL, $P < 0.01$) was attenuated and the $\Delta\Psi_m$ depolarization by PAL was restored (2.9 ± 0.2 vs. PAL, $P < 0.01$). EPA also suppressed the PAL-induced Drp1 expression and mitochondrial fragmentation (37.6 ± 6.4 % vs. PAL, $P < 0.01$). In addition, EPA alone reduced the Drp1 expression and exhibited elongated mitochondria. **Conclusion:** We conclude that EPA ameliorates the PAL-induced lipotoxicity by the regulation of mitochondrial dynamics through Drp1.