correction for photobleach and photoconversion using nonlinear fitting, cell motion compensation based on hierarchical block matching, and spatiotemporal filtering. Two *xy* maps are generated to represent the maxima of fluorescent intensity change and the corresponding time, respectively. Flash events are identified via thresholding and segmentation based on these maps. Validation of the algorithm is performed on SNR-controlled synthetic data sets produced by embedding typical flash events in real images. With the aid of Flash Sniper, we examined ~1600 flashes in cardiac myocytes and revealed distinct substructures in their rising kinetics. In sum, the analytical tool developed in this study will facilitate the studies of the mechanisms of superoxide flash and its application in health and diseases.

2735-Pos Board B705

Design Of An ELISA Protocol For A Rapid Quantification Of Activated/oligomerized Bax During Apoptosis

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The release of cytochrome c from mitochondria into the cytosol is considered to be the commitment step of early apoptosis. During this step, the activation/oligomerization of the pro-apoptotic protein Bax leads to the formation of the Mitochondrial Apoptosis-Induced Channel (MAC) through which cytochrome can be released. Remarkably, Bax activation/oligomerization leads to the exposure of the previously inaccessible N-terminal domain of the pro-apoptotic protein Bax. This property was extensively used to detect activated/oligomerized Bax by immunocytochemistry and immunoprecipitation but these assays are long and tedious; and they are only qualitative or semi-quantitative at best.

During this study, we developed an ELISA protocol based on the utilization of monoclonal (6A7) and polyclonal (N-20) antibodies which specifically recognize Bax N-terminus (i.e. the activated/oligomerized form of Bax). Monomeric human recombinant Bax (hBax) was used in all the experiments. As previously described, activation/oligomerization of hBax was artificially triggered by an exposure of the recombinant protein to the detergent Triton X-100. We first confirmed that the N-20 and 6A7 antibodies were able to fully immunoprecipitate the activated/oligomerized form but not the monomeric form of hBax. We then develop a sandwich ELISA using the 6A7 and the N-20 respectively as capture and detection antibodies. Reproducible standard curves were obtained when using increasing amounts of activated/oligomerized hBax. Monomeric hBax was never detected during these assays, even when previously mixed with activated/oligomerized hBax. Taken together, these results show that this ELISA allows a specific and quantitative detection of activated/oligomerized recombinant Bax. Finally, experiments of quantification of activated/oligomerized Bax with this technique in protein extracts from control and apoptotic cells are currently underway.

Supported by NYU College of Dentistry Research Faculty Funds to LD.

2736-Pos Board B706

Cytoprotective Effects Of Mitochondrial Potassium Channel Opener BMS-191095

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Mitochondrial potassium channel openers (KCO's) were shown to be cytoprotective in models of ischemia-reperfusion induced injury in brain, heart and skeletal muscle tissue. The aim of this study was to identify the cellular events responsible for observed protection.

We have investigated the cytoprotective potential of BMS-191095, an opener of the mitochondrial ATP-regulated potassium channel (mitoK$_{ATP}$), in C2C12 myoblasts. BMS-191095 did not protect the cells against tert-butyl hydroperoxide or H_2O_2-induced injury, but prevented calcium ionophore A23187-induced cell death. A23187 caused a transient increase in cytosolic calcium levels, which was not affected by the presence BMS-191095. In contrary, the opener prevented a mild elevation of calcium levels observed after the initial peak.

This study was supported by Polish Mitochondrial Network MitoNet.pl and Polish Ministry of Science and Higher Education grants no. N30105331/1617 and P-N/031/2006.

2737-Pos Board B707

Measuring Intra-Cellular and Intra-Mitochondrial Zinc Concentrations Following Hypoxia/Hypoglycemia with an Expressible Ratiometric Fluorescence Biosensor

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Zinc is a ''trace'' metal necessary for proper cellular function. Studies have shown that the intra- and extra-cellular concentrations of labile zinc increase dramatically in models of cerebral ischemia (1) (2). Substantial evidence indicates that mitochondrial dysfunction plays a significant role in neuronal death following ischemia. Both mitochondrial dysfunction and increased intracellular zinc concentrations have been associated with increased reactive oxygen species (ROS) production and ultimately apoptosis (3, 4). We modified our fluorescent zinc biosensor (5) to be selectively expressed in the mitochondria of PC12 cells, enabling us to ratiometrically image the intra-mitochondrial concentration of labile zinc even at resting (picomolar) levels. We used this expressible biosensor and our previous sensor in cells which have undergone oxygen /glucose deprivation (OGD). Our initial results indicate that the concentration of labile, intra-mitochondrial zinc may not increase to the degree that we observed in the cytoplasm during hypoxic/hypoglycemic conditions, and may be lower than the concentrations observed in cells in more physiological conditions.

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2738-Pos Board B708

Ion Channels From Inner Mitochondrial Membrane From Rat Heart - Single Channel Properties

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Mitochondrial ion channels are objects of electrophysiological and pharmacological studies for over ten years. It is known that they are involved in cytoprotection and apoptosis.

In our study we investigated ion channels from inner mitochondrial membrane of heart mitochondria. We recorded single channel activity using patch - clamp technique. Anion channel in inner mitochondrial membrane from rat heart was observed. In symmetrical 150/150 mM KCl solution we recorded chloride channel with conductance 120 pS. The effect of different channel inhibitors and activators (DIDS, SITS, DCEBIO) on the anion channel activity was studied. We plan to characterize its electrophysiological and pharmacological properties.

This work was supported by Ministry of Science and Higher Education grant N30105331/1676 and by Polish Mitochondrial Network MitoNet.pl

2739-Pos Board B709

Bidirectional Ca^{2+}-dependent Control Of Mitochondrial Dynamics By The Mitochondrial Rhogtpase, Miro

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Mitochondrial motility (mito-motility) is regulated by cytoplasmic [Ca^{2+}] ([Ca^{2+}]$_c$) oscillations in a homeostatic manner to optimize the mitochondrial contribution to intracellullar Ca^{2+} buffering, ATP production and signal transduction. To control mitochondrial movements, Ca^{2+} targets a yet unidentified cytoplasmic factor that does not seem to be a microtubular motor or a kinase/phosphatase.

We here studied the dependence of Ca^{2+}-sensitive mito-motility on Miro (mitochondrial Rho GTPases), an integral mitochondrial membrane protein that has two Ca^{2+}-binding site of EF-hands. Mitochondrial matrix targeted YFP expressing H9c2 cardiac myoblasts were co-transfected with Miro plasmid DNA or siRNA, and then loaded with fura-2 (5 μM) to monitor the mito-motility and [Ca^{2+}]$_c$ simultaneously.

(1) At resting [Ca^{2+}]$_c$ (< 40 nM), mito-motility was enhanced by Miro overexpression irrespective of the presence of the EF-hands and was suppressed when Miro was depleted by siRNA.

(2) When [Ca^{2+}]$_c$ was increased (0.1 - 2 μM) directly or by agonist stimulation, the Ca^{2+}-induced arrest of mito-motility was also promoted by Miro overexpression and was suppressed when either the Miros were depleted or their EF-hand was mutated.

(3) Miro also enhanced the fusion state of the mitochondria at resting [Ca^{2+}]$_c$ but promoted mitochondrial fragmentation at high [Ca^{2+}]$_c$. The Miro effects on mitochondrial morphology seem to involve Dynamin related protein **1 (**Drp1). Thus, Miro serves as a [Ca^{2+}]$_c$-sensitive bidirectional regulator of both the motility and fusion-fission dynamics of mitochondria.