Recent Conference Abstracts/Presentations

Phosphorescent probes for the analysis of intracellular oxygen and real-time monitoring of cell respiratory responses

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Molecular oxygen is a useful marker of mitochondrial and cellular function. Analysis of intracellular oxygen (icO2) and its dynamics can provide valuable information on cell metabolic status, responses to various stimuli, signalling and control mechanisms, etc. We have developed a family of phosphorescent probes and techniques, which allow facile, minimally invasive monitoring of icO2 in mammalian cells. One technique allows the analysis of cell populations in standard 96-well plates, using the red-emitting O2-sensing probe MitoXpress™ and detection on a time-resolved fluorescent plate reader, to monitor icO2 gradients and responses to effectors of metabolism in multiple samples. The other technique employs the highly photostable NIR emitting O2-sensing probe and detection on conventional live-cell fluorescent imaging platforms, providing monitoring of individual cells with high spatial and temporal resolution. These complementary techniques have been applied to a range of mechanistic studies with cells (PC12, SH-SY5Y), compound screening and measurements under hypoxia. Demonstrating general simplicity, convenience, high information content and the ability to accurately detect rapid and transient changes in cellular respiration, they are particularly useful for studies of metabolism, cell mitochondrial function and hypoxia research.
(Keystone Symposium on Hypoxia, Vancouver, March 2008)

The use of oxygen and pH-sensitive fluorescent probes for the investigation of perturbed cell metabolism

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In standard in vitro systems, the main sources of cellular ATP are glycolysis and oxidative phosphorylation. The balance between these two related energy generating systems can therefore inform on disease states and toxicities where perturbed metabolism is implicated. A highly informative approach to the investigation of such metabolic perturbations is to measure both cellular oxygen consumption and extracellular acidification rate (ECA). Analysis of oxygen consumption gives specific information on OxPhos, while measurement of ECA, under the appropriate conditions, provides information on non-aerobic metabolism. Here we present a new method of assessment of ECA of both adherent and suspension cells, using a long-decay pH-sensitive fluorescent probe and convenient 96-well plate format. As probe emission lifetime is used as the readout, a simple transformation, allows the generation of ECA rates in units of [H+] per unit time. We also outline how these probes may be combined with the MitoXpress oxygen consumption assay providing a highly informative dual-parameter metabolic analysis. Such parallel measurements allow inferences to be drawn regarding the site of an observed metabolic insult; thereby allowing altered glycolytic activity to be delineated from direct mitochondrial effects. We examine how such an

analytical approach may be deployed for the examination of the perturbed metabolism using compounds of known metabolic impact including antimycin, 2-deoxyglucose, oxamic acid and dichloroacetate as models. This analysis is then extended to more relevant models analyzing the effect of a panel of biguanides on cellular metabolism and relating these observations to their proposed mechanism of action.
(European Bioenergetic Congress, Dublin, July 2008)

Analysis of respiratory responses of neuronal cells to the decrease of extracellular calcium

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A profound decrease in extracellular Ca2+ (eCa2+) occurs during neuronal activity or ischemia, while Ca2+-free conditions are commonly used in biological experiments. In this study we examined the dynamics of respiration of neurosecretory PC12 cells and Cerebellar Granular Neurons upon sequestration of eCa2+. By monitoring intracellular oxygen (iO2) by means of dedicated iO2-sensing probe and time-resolved fluorescent detection, we observed a marked transient activation of respiration in response to chelation of eCa2+. Subsequent depolarization of the plasma membrane with high eK+ (1-12 min after treatment with EGTA) had an additive effect on cellular O2 consumption, reducing iO2 to 20-25% of air saturation. The respiratory response was accompanied by Na+ influx, a decrease in cytosolic and mitochondrial Ca2+ and partial depolarization of plasma membrane, while the mitochondrial membrane potential (MMP) and cellular ATP remained unchanged. The effect of EGTA was down-regulated by the depletion of Ca2+ stores and dissipation of proton gradient across the mitochondrial membrane, up-regulated by mitochondria uncoupling and was independent on MMP. The respiratory effect was largely reduced by the inhibition of mitochondrial Na+/Ca2+ exchanger (mNCX) and Na+/H+ exchangers. We suggest that such respiratory response is driven by a non-selective Na+ influx, activation of mNCX and increased mitochondrial Na+/H+ exchange. This leads to the acidification of matrix, loss of mCa2+ and acceleration of mitochondrial proton pumps to restore proton gradient.
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Sensing and imaging of (intra)cellular oxygen by means of the phosphorescent porphyrin probes

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Molecular oxygen (O_2) is the key substrate of aerobic cells and informative marker of cell metabolism. Analysis of cellular O_2 and consumption rate can provide useful information on the metabolic status of the cell, particularly its mitochondrial function. Phosphorescent O_2-sensing probes based and quenched-fluorescence detection allow monitoring of O_2 in complex biological samples in a minimally invasive manner and on a micro-scale. However, application of this methodology to the analysis of mammalian cells and real-time monitoring of their metabolism and responses to various effectors and treatments has been challenging so far.

We have developed several new methodologies for sensing of both extracellular and intracellular O_2 in complex biological samples, particularly those containing cultured or primary mammalian cells. Specially designed supramolecular O_2-sensing probes based on phosphorescent Pt-porphyrins enable new bioassays performed in relatively simple and robust measurement formats. These new probe chemistries and assay formats provide the basis for a range of high-utility applications, including:

- Screening of various effectors and new chemical entities for mitochondrial toxicity using isolated rat liver mitochondria or cultured/primary cells. The assay is based on the measurement of O_2 consumption in multiple samples using extracellular O_2 probe MitoXpress®, standard microtitter plates and fluorescent plate reader detection;
- High-throughput analysis of *intracellular* O_2 in populations of cells using MitoXpress® oxygen probe passively loaded into the cells and phosphorescence lifetime-based detection on a time-resolved fluorescent reader;
- High-content analysis of intracellular O_2 in individual cells using highly-photostable NIR O2 probe and live cell fluorescent microscopy imaging setup.

These O_2 sensing methodology and particular bioassays have been extensively validated with various cells, effector compounds and biological models, and applied to basic studies of cell metabolism and bioenergetics [4]. Several leading pharmaceutical companies have adopted these assays for their drug discovery programs and drug safety assessment. In this presentation we will describe basic detection principles, probe chemistries and experimental set-ups, and illustrate this with experimental data obtained with different cells.

(International Conference on Porphyrins and Phthallocyanines, Moscow, July 2008)