intrinsic cofactors as natural probes for diagnostic purposes in cancer, diabetes, apoptosis, and neurodegenerative diseases. Here, we used autofluorescence dynamics assay to quantify the concentration and enzyme binding of intrinsic NADH and FAD in living cells. In these studies, cancer (Hs578T) and normal (Hs578Bst) breast cells are used as model systems to examine the sensitivity of the proposed assay to cell pathology. Our non-invasive, quantitative assay includes multiphoton microscopy and spectroscopy of NADH and FAD. Two-photon fluorescence lifetime imaging of cellular autofluorescence, in a calibrated microscope, is used to construct NADH and FAD concentration images in live cells. In addition, time-resolved associated anisotropy of cellular autofluorescence provides direct quantification of the molar fractions of free and enzyme-bound cofactors in both normal and transformed cells. Targeted inhibition of complex IV of the electron transport chain in Hs578Bst, using KCN, confirm the sensitivity of cellular autofluorescence to changes in the respiratory state activities. Comparative studies of the binding kinetics of NADH with mitochondrial malate dehydrogenase and lactate dehydrogenase in solution mimic our findings in living cells. This fluorescence dynamics assay on natural coenzymes (i.e., without the need for exogenous fluorescence dyes) is applicable to other metabolic and signaling pathways in live cells, which contrasts with conventional biochemical techniques that require cell destruction.

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Small but Statistically Reliable Magnetic Field Effect Observed in the Recombination of a Non-Correlated Pair of Biologically Relevant Radicals Nitric Oxide and Superoxide Anion

Tatiana Yu Karogodina[1], Svetlana V. Sergeeva[2], **Dmitri V. Stass[1,3]**.
[1]Inst Chem Kinetics Combustion, Novosibirsk, Russian Federation, [2]Inst Cytology and Genetics, Novosibirsk, Russian Federation, [3]Novosibirsk State University, Novosibirsk, Russian Federation.

Magnetic and spin effects, well studied in photo- and radiation-generated chemical systems involving spin-correlated radical pairs, are often called upon as possible mechanisms underlying magnetic effects (MFE) in highly complex biological systems. Although several biologically relevant systems do exist for which this had been indeed verified, often the radical pair mechanism is invoked solely based on the presence of radicals, such as nitric oxide, in the system. This logic has three serious problems: the complexity of the real biological systems, the lack of correlation in thus reacting radicals, and their difference from ''normal'' partners of the spin-correlated pairs. To address all these issues we created a model chemical system of nitric oxide and superoxide radical recombining to produce peroxynitrite, and studied MFE in it.

The radicals were produced as a pair via decomposition of 3-morpholinosydnonimine (SIN-1) in aqueous phosphate buffer. MFE was monitored by comparing the efficiency of peroxynitrite production in exposed and otherwise identical control samples with additional temperature controls. We used static magnets with induction 0.5T and 4.7T. No statistically significant effects were found in the field 0.5T and in temperature controls. In magnetic field 4.7T magnetic field effect of $(1.8 \pm 0.5)\%$ was obtained.

The effect is small, as expected for a non-correlated pair, but statistically reliable. It is apparently limited by extremely fast relaxation of nitric oxide in liquid due to unquenched orbital momentum in the diatomic molecule with electronically degenerate ground state, and develops in f-pairs via the Δg mechanism. Any MFE due to radical pair involving nitric oxide in biological system would probably require either rather strong magnetic field in the Tesla range, or some internal enhancer of magnetic field.

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Intracellular Diffusion Restrictions In Trout Cardiomyocytes

Nina Sokolova, Rikke Birkedal.
Tallinn University of Technology, Tallinn, Estonia.

Rat cardiomyocytes are compartmentalized by barriers that restrict intracellular diffusion of adenine nucleotides. The exact localization of these diffusion barriers is unknown. Some possible candidates for diffusion restriction are t-tubules, sarcoplasmic reticulum (SR) and outer mitochondrial membrane. Further, rat cardiomyocytes have several parallel rows of mitochondria and myofilaments wrapped in SR, and it is possible that peripheral mitochondria and SR restrict diffusion to more central parts of the cell. Diffusion is facilitated by the creatine kinase system. Trout cardiomyocytes lack t-tubules and have a much more sparse SR. Additionally, single cardiomyocytes have only one layer of myofilaments surrounding a central core of mitochondria. We take advantage of the structural differences between rat and trout cardiomyocytes to study intracellular diffusion restrictions further. We measured the apparent ADP-affinity of trout skinned ventricular fibres at different temperatures to cover the physiological range for rainbow trout. Measurements were performed in the absence and presence of creatine to test whether diffusion is facilitated by the creatine kinase system. Our results show that trout cardiomyocytes are characterized by a low ADP-affinity. The affinity is temperature-dependent and increases with temperature. Creatine increases affinity at all temperatures, but the affinity in the presence of creatine is also temperature-dependent. The low ADP-affinity suggests that diffusion restrictions also exist in trout cardiomyocytes despite their structural difference with much more sparse membrane structures. This makes trout cardiomyocytes a useful model to study intracellular diffusion restrictions further.

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Compartmentation of ATP in Cardiomyocytes and Mitochondria Kinetic Studies and Direct Measurments

Claire Monge[1], Alexei Grichine[1], Tatiana Rostovtseva[2], Dan Sackett[3], Andrey Kuznetsov[4], Valdur Saks[1].
[1]University J.Fourier, Grenoble, France, [2]Laboratory of Physical and Structural Biology, NICHD, NIH, Bethesda, MD, USA, [3]Laboratory of Integrative and Medical Biophysics, NICHD, Bethesda, MD, USA, [4]Department of Transplant Surgery, D. Swarovski Research, Innsbruck, Austria.

High energy demand of heart and brain cells is met by mitochondrial ATP production and energy transfer between ATP compartments mostly by creatine kinase (CK) - phosphocreatine (PCr) system. The aim of this work is to study the diffusion restrictions of ATP at the level of mitochondrial outer membrane as a basis of its compartmentation in cardiomyocytes. In a first part channelling of adenine nucleotides in mitochondria is studied via the direct transfer of the novel synthesized ATP from the adenine nucleotide translocase (ANT) to the mitochondrial creatine kinase (MtCK) by performing the complete kinetic analysis of the MtCK. A partial reconstruction of mitochondrial cytoskeletal environment was performed by incubation of heart isolated mitochondria (apparent Km for exogenous ADP $= 9 \pm 1$ μM) with 1 μM tubulin (apparent Km for exogenous ADP $= 169 \pm 52$ μM) without or with 20 mM creatine (apparent Km for exogenous ADP $= 23 \pm 6$ μM). The results showed a clear restriction in adenine nucleotide diffusion in presence of tubulin by the change of the profile of respiration kinetic linearization. This restriction was found to be overcome by the presence of creatine which can activate the MtCK reaction and increase the rate of ADP/ATP turnover due to functional coupling between MtCK and ANT. In a second part the method of fluorescence correlation spectroscopy was used to study the diffusion kinetics of ATP-Alexa 647 both in solution, isolated mitochondria and cardiomyocytes. However pronounced multicomponent diffusion kinetics was found both in isolated mitochondria and permeabilised cardiomyocytes. This diffusion time seemed to be sensitive to the ordered state of cardiomyocyte and increased when the symmetry was broken in the rounded or apoptotic cells. The fluorescent ATP was use to study ATP compartmentation.

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Kinetic Studies of Intracellular Compartmentalization in Permeabilized Rat Cardiomyocytes

Mervi Sepp[1], Tuuli Kaambre[2], Peeter Sikk[2], Marko Vendelin[1], Rikke Birkedal[1].
[1]Institute of Cybernetics at Tallinn University of Technology, Tallinn, Estonia, [2]National Institute of Chemical Physics and Biophysics, Tallinn, Estonia.

Cardiomyocytes are compartmentalized by intracellular barriers restricting diffusion. The aim of this work is to gain insight into diffusion restrictions in a heart muscle cell. We present a complete set of data, where mitochondrial respiration and its interaction with ATPases has been recorded using oxygraph and spectrophotometer. Although some of these data have been published in the context of different studies, we performed all our measurements in parallel in order to collect a full set of data for each batch of cardiomyocytes. These data were used as input for our new mathematical model. The model describes the dynamics of metabolites during the respiration process assuming that the system can be described as having two compartments - the extracellular and the intracellular. For that a system of ordinary differential equations has been constructed and solved numerically. The model solution was optimized to fit the experimental data. The optimization process consists of applying genetic algorithms and least square method for finding the set of parameters best suited for reproducing experimental results. One of the parameters is the diffusion restriction influencing the rates at which metabolites move between the two compartments of the system. The model gives us estimation of the overall diffusion restriction between intracellular energetic units (ICEU) and extracellular