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.

1236-Pos Board B80

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

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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.

Oxidative Phosphorylation & Mitochondrial Metabolism

1237-Pos Board B81

Intracellular Diffusion Restrictions In Trout Cardiomyocytes

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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.

1238-Pos Board B82

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

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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.

1239-Pos Board B83

Kinetic Studies of Intracellular Compartmentalization in Permeabilized Rat Cardiomyocytes

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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

solution as well as different restrictions inside ICEUs between ATPases and mitochondria. The model is flexible and allows us to test various hypotheses regarding different compartmentalization of ATPases in the cell. This feature also makes it possible to develop the model into being able to assess a set of diffusion restrictions of more complicated systems.

1240-Pos Board B84

Control and Regulation of Mitochondrial Energetics in an Integrated Model of Cardiomyocyte Function

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In heart there is currently no consensus about the mechanism(s) and relative importance of the processes involved in matching energy supply with demand. This is due to mitochondrial energetics modulating and being modulated by the network of mechano-electrical processes existing in cardiomyocytes. A computational model integrating mitochondrial energetics and EC coupling provides an important analytical tool to understand the regulation and control of the global organ function. Here, we apply a generalized matrix method of control analysis to calculate flux and concentration control coefficients, as well as response coefficients, in an integrated model of Excitation-Contraction coupling and Mitochondrial Energetics in the cardiac ventricular myocyte. Control and regulation of oxygen consumption (VO2) was first assessed in a mitochondrion model, and then in the integrated cardiac myocyte model under resting and working conditions. The results demonstrate that in the model, control of respiration is distributed among cytoplasmic ATPases and mitochondrial processes. The magnitude of control by cytoplasmic ATPases increases under working conditions. The model prediction that the respiratory chain exerts strong positive control on VO2 (control coefficient=0.89) was corroborated experimentally in cardiac trabeculae utilizing the inhibitor titration method. In the model, mitochondrial respiration displayed the highest response coefficients with respect to the concentration of cytoplasmic ATP (ATPi). This was due to the high elasticity of ANT flux towards ATPi. The analysis reveals the complex interdependence of sarcolemmal, cytoplasmic, and mitochondrial processes that contribute to the control of energy supply and demand in the heart. Moreover, by visualizing the structure of control of the metabolic network of the myocyte, we provide support for the emerging concept of control by diffuse loops, in which action on the network may bring about changes in processes without direct mechanistic links between them.

1241-Pos Board B85

Computational Model Of Citric Acid Cycle And Oxidative Phosphorylation In Mitochondria

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Mitochondria are responsible for providing red muscle cells with ATP, the chemical energy of which is converted to mechanical work by sarcomeres. Just as the organism needs to cope with different levels of activity, energy production rate in mitochondria has to be able to adjust to changes in demand.

We study the regulation of mitochondrial energy metabolism by ADP and inorganic phosphate with a computational model. The model consists of a thermodynamically balanced set of equations describing the reactions of the citric acid cycle, electron transfer chain and cross-membrane transport. Reactions for which enzymatic mechanisms are known are modelled accordingly. Furthermore, we account for buffering of protons by mitochondrial metabolites, yielding a system with detailed proton balance - crucial for modelling chemiosmotic energy transduction.

Suitable model parameters with which the system is able to reproduce experimental results are found from the parameter space with a combination of optimization techniques. These computationally intensive operations of solving differential equations and optimizing for parameters are automated and performed on a computational cluster.

1242-Pos Board B86

Application of Proportional Activation Approach to oxidative phosphorylation

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Proportional Activation Approach (PAA) [1] is a simple quantitative method allowing to determine the proportional activation of the producer (P) and consumer (C) of some intermediate metabolite M by some external factor X. M can be e.g. ATP, $\Delta\Psi$ or NADH, while X can be e.g. a hormon or neural/electrical stimulation of muscle. The proportional activation of C and P ($(\Delta C/C)/(\Delta P/P)$) is quantified by the proportional activation coefficient. Application of PAA to the oxidative phosphorylation demonstrates clearly that: 1. $\Delta\Psi$ production and consumption during stimulation of isolated hepatocytes by vasopressin [1]; 2. NADH production and consumption during stimulation of isolated hepatocytes by vasopressin [2]; 3. $\Delta\Psi$ production and consumption during electrical stimulation of rat skeletal muscle [3]; 4. ATP production and consumption during stimulation of perfused heart by adrenaline [4] - are directly activated to a similar extent. These findings confirm the so-called parallel activation idea, saying that different elements of the oxidative phosphorylation system are activated in parallel during low-to-high work transition in different cell types, that was proposed on the basis of computer simulations using a dynamic model of oxidative phosphorylation [5,6].

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1243-Pos Board B87

Cardiolipin's Structure, ATP Synthesis & Barth'S Syndrome

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Resonance stabilizes two phosphate high energy H-bonds with the free hydroxyl of cardiolipin (CL) rendering a bicyclic conformation, but only in bilayers. Thus it is symmetrical, displaying 2 pK's. The pK_2 varies with the length of its fatty acid chains. With 4 $C_{18:0}$ chains the pK_2 is >8.0. Thus the *headgroup surface* is a buffer at neutral pH. CL is on both sides of the IMM. The high pK_2 implies that ATP synthesis is driven by membrane potential rather than by delta pH, lowering the energy demand for ATP synthesis. Nearly all membranes that contain CL also contain F_oF_1, Mammalian mitochondrial CL is generally tetralinoleic, $C_{18:2}$. CL's pK_2 can be altered by chainlength and saturation. It is found on both sides of the IMM so it buffers both headgroup domains. CL binds to all 6 of the ox-phos proteins but no others in the membrane. Its high pK_2, varies with chainlength. FAs apply a symmetrical force on the two sides of the headgroup. The bicyclic structure requires 4 chains. In lyso-CL's pK_2 is reduced to that of PG destroying the bicyclic headgroup. (our control lacks the glycerol OH). Barth's Syndrome's defective gene is a CL acyl transferase. Patients are identified by the presence of lyso-CL (3 chains).

1244-Pos Board B88

Quinine Causes Mitochondrial Uncoupling Independent Of K^+/H^+ Exchange Inhibition

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Introduction: K^+ influx into the respiring mitochondrial matrix is balanced by K^+ efflux via K^+/H^+ exchange (KHE). Quinine (QN) is a reversible inhibitor of KHE. We have shown that QN blocks matrix K^+ efflux when the K^+ ionophore valinomycin is given to increase matrix $[K^+]$. However QN may have other effects on mitochondria. Here we tested the effects of QN on mitochondrial respiration. **Methods:** Guinea pig heart mitochondria were isolated by differential centrifugation and then suspended in either KCl or choline Cl media inside a respirometer. Either the complex 1 substrate pyruvate (10 mM) or the complex 2 substrate succinate (10 mM) with rotenone (10 μM) was added to initiate state 2 respiration. QN (500 μM) was added to inhibit KHE. State 3 was initiated by adding ADP (250 μM) and state 4 occurred when ADP was converted to ATP. **Results:** In KCl buffer with pyruvate, QN increased states 2 and 4 respiration by $56 \pm 8\%$ and by $48 \pm 10\%$, respectively, and decreased state 3 by $15 \pm 2\%$. With succinate and rotenone, QN increased states 2 and 4 respiration by $37 \pm 3\%$ and by $15 \pm 2\%$, respectively, and decreased state 3 by $26 \pm 1\%$. QN had similar effects on respiration in choline Cl buffer. **Conclusion:** The similar effects of QN on respiration in both media suggest a K^+-independent mechanism of QN, which also may be acting as an uncoupler to bring H^+ inside the matrix. Additional experiments show that QN lowers matrix pH without changing membrane potential. More studies with QN and other putative blockers are required to reveal the mechanism by which QN affects mitochondrial transport and bioenergetics.

1245-Pos Board B89

Mitochondrial Redox Responses To Increased Work Intensity In Rabbit Ventricular Myocytes

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Simultaneous measurement of the intrinsically fluorescent metabolic coenzymes NAD(P)H (reduced) and FAD (oxidised) enabled assessment of the