Bel-2 - the major regulating protein - and its interplay with the mitochondrial membrane system are being transferred as physiological consequences at the organelle (mitochondrion) and cellular level is still mysterious. Therefore, we use a biophysical, solid state NMR based approach to develop a mitochondrion based test system, where we can study the physiological response of intact mitochondria of various origin (cancer, healthy, ALS motor neurons) ex vivo upon apoptotic stress ranging from novel cancer drugs to amyloidogenic proteins such as A-beta protein or SOD1 protein, the main culprits in AD and ALS, respectively.

2221-Plat
Near Infrared Light-Dependent Nitric Oxide Effect on Mitochondrial Respiration
Garth Brandal, Phillip F. Pratt, Martin Bienengraeber.
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Reperfusion of a previously ischemic myocardium is a critical life-saving intervention against tissue necrosis, but reperfusion itself also results in significant damage to myocardium. Many therapeutic strategies such as ischemic and volatile anesthetic pre- and postconditioning have been suggested but few have translated effectively to humans. Near infrared and far red light (NIR) has been demonstrated to protect the myocardium against ischemia and reperfusion (IR) injury. The beneficial effect of NIR is mediated in part by an increase in nitric oxide (NO) bioavailability. Under hypoxic conditions, heme-containing proteins such as myoglobin (Mb) and hemoglobin (Hb) exhibit nitrite reductase activity which results in an increase in NO bound to Mb and Hb. NIR treatment is thought to cause a photolysis of MbNO and HbNO which results in an increase in active NO when applied at the time of reperfusion. We hypothesize that NIR-enhanced NO during reperfusion modulates mitochondrial bioenergetics by interaction with the electron transport chain and thereby may induce cardioprotection. Isolated mitochondria were incubated with substrate and ADP in an air tight chamber. Oxygen consumption of mitochondria created a hypoxic environment, simulating conditions of ischemic myocardium. Deoxomyoglobin and nitrite were added to the reaction chamber during the hypoxic period and then reoxygenation was performed in the presence and absence of NIR (670 nm, 50 mW/cm²). A faster reoxygenation of the chamber, corresponding to an inhibition of mitochondrial respiration was observed when the reaction was exposed to NIR during hypoxia and throughout reoxygenation. This mild inhibition of mitochondrial respiration by NIR may decrease reactive oxygen species during reoxygenation and thereby lead to increased cell viability. Interestingly, in a diabetic mouse model (db/db) were ischemic or anesthetic postconditioning fail to protect the myocardium against IR injury, NIR treatment decreased infarct size.

2222-Plat
Spatial Fourier Transform (SFT) for Robust Detection of Mitochondrial Depolarization in Ischemic Myocardium
Paul W. Venable, Tyson G. Taylor, Junko Shibayama, Mark Warren, Kenneth W. Spitzer, Alexey V. Zaitsev.
Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA.
Mitochondrial potential (ΔΨ) loss is a critical event during myocardial ischaemia. Potentiometric dye TMRM accumulates inside well-polarized mitochondria and is used as a ΔΨ indicator. However, during no-flow ischemia (NF-ISCH) total TMRM fluorescence may remain unchanged despite ΔΨ loss. We sought to improve detection of ΔΨ loss during NF-ISCH using SFT. Langendorff-perfused rabbit hearts (n=8) were stained with TMRM (400 nM). Motion was abolished by Blebbistatin (5 μM). TMRM fluorescence was recorded confocally from left ventricular subepicardium (0.5μm/pixel, 20x objective lens) in control, while perfusing mitochondrial uncoupler FCCP (5 μM), and after 65 ± 15 min of NF-ISCH. In control SFT revealed distinct peaks 2.37 ± 0.06 μm⁻¹ along the longitudinal axis of myocytes (Figure), possibly related to the sarcomere length. MITO-PEAKs were greatly depressed after FCCP (not shown) and NF-ISCH (Figure). Normalized spectral power under MITO-PEAKs was significantly different between control and FCCP (p=0.019), and control and NF-ISCH (p=0.016). In contrast, total TMRM brightness failed to reveal significant difference (control vs. FCCP, p = 0.077; control vs. NF-ISCH, p=0.678). Thus, analysis of MITO-PEAKs in SFT spectrum provides a robust method for detecting ΔΨ loss in ischemic myocardium.

Workshop: Measuring, Modeling, and Designing Protein Recognition Specificity

2223-Wkshp
Computational Design of Proteins which Broadly Neutralize Influenza
Sarel Fleishman.
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Design of protein interactions is a key challenge in molecular biology and with the open new routes for biomedical research and biotechnology. We developed a general computational method for designing proteins that bind a surface patch of interest on a target macromolecule. Favorable interactions between disemboiled amino acid residues and the target surface are identified and used to anchor de novo designed interfaces. The method was used to design proteins that bind a conserved surface patch on the stem of the influenza hemagglutinin (HA) from the 1918 H1N1 pandemic virus. After affinity maturation, two of the designed proteins, HB36 and HB80, bind H1 (Spanish influenza) and H5 (avian influenza) HA's with picomolar affinity. Further, HB80 inhibits the HA fusogenic conformational changes induced at low pH. The crystal structure of HB36 in complex with 1918/H1 HA revealed that the actual binding interface is nearly identical to that in the computational design model. Affinity-increasing mutations isolated through experimental affinity maturation helped shed light on crucial elements which are missing in computational design calculations, such as long-range electrostatics. These have been incorporated in design calculations to improve the computational method’s ability to generate de novo designed binders and inhibitors. Such designed binding proteins may be useful as diagnostics, therapeutics, and molecular probes.

2224-Wkshp
Synthetic Proteins Engineered for the Modulation of Cell Signaling
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Over the last decade, therapeutic monoclonal antibodies represent one of the major breakthroughs for the treatment of cancer and other diseases. To date, most therapeutic antibodies have been obtained by the humanization of rodent-derived antibodies, but in recent years, research in antibody engineering has given rise to a new wave of technologies that promise to transform the field. Phage-displayed libraries of “synthetic antibodies” use entirely man-made antigen-binding sites and thus circumvent the need for natural immune repertoires. Using in vitro selections, highly functional antibodies with fully human frameworks can be generated against virtually any antigen in a matter of weeks. Access to the encoding DNA allows for rapid affinity maturation, fine tuning of specificity and recasting into different molecular formats. We have developed particularly simple synthetic antibodies that use a single human framework and limited chemical diversity in restricted regions of the antigen-binding site. These structural simplifications enhance the performance of the libraries, which have yielded highly functional antibodies with fully human frameworks can be generated against virtually any antigen in a matter of weeks. Access to the encoding DNA allows for rapid affinity maturation, fine tuning of specificity and recasting into different molecular formats. We have developed particularly simple synthetic antibodies that use a single human framework and limited chemical diversity in restricted regions of the antigen-binding site. These structural simplifications enhance the performance of the libraries, which have yielded highly functional antibodies with fully human frameworks can be generated against virtually any antigen in futures and recasting into different molecular formats. 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