used fluorescence correlation spectroscopy (FCS) instead, to detect the fluorescence blinking caused by the reversible protonation reaction, and to determine both the fraction of fluorophore in each state and the associated relaxation time. We studied how various environmental conditions, pH, temperature, ionic strength and buffer concentration, influenced the blinking of pyranine. We found that knowledge of both the fraction of protonated pyranine and the blinking relaxation time can be used to measure two of these external variables at the same time, something that cannot be achieved with the ratiometric method. As a first application, we showed that pyranine can be used to measure both pH and temperature during the mixing of two solutions in a microfluidic channel. More relevantly for biological applications, this method can now be applied to measure both pH and ionic strength inside different cellular compartments.

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Components of intrinsic fluorescence revealed by Metabolic Modulation Matrix in isolated rat cardiac myocytes

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Aim: Recent developments in multi-wavelength fluorescence lifetime spectroscopy brought simultaneous measurement of fluorescence spectra and lifetimes in complex samples, namely cell and tissue intrinsic fluorescence. However, emission spectra of endogenous fluorophores are often superimposed in broad bands over the 400-600 nm wavelength region, making their separation particularly difficult. We therefore present a newly developed method of separation. Based on Metabolic Modulation Matrix approach, this method is built on evaluation of spectral modulation of endogenous fluorescence following changes in the cell metabolic state. **Methods:** Spectral fingerprints of time-resolved fluorescence are determined in isolated cardiomyocytes after excitation by 375-nm pulsed picosecond laser diode using SPC-830 TCSPC measurement system (Becker-Hickl on Zeiss Axiovert 200). Metabolic modulation was induced by respiratory chain and/or oxidative stress regulators. The number and profiles of the most significant spectral components were identified by time-resolved area-normalized emission spectroscopy and principal component analysis. **Results:** The Metabolic Modulation Matrix approach applied to time-resolved spectroscopy data in living cardiac cells demonstrated the presence of at least 3 significant spectrally-distinct components of NAD(P)H fluorescence corresponding to: i) NAD(P)H in water-like environment, ii) NAD(P)H in restricted-motion environment and iii) a flavin-type component. Lifetimes, revealed by fluorescence decay analysis showed values of τ_1~0.4 $\pm$ 0.1 ns and τ_2~1.0 $\pm$ 0.2 ns for component i), τ ~3.2 $\pm$ 0.8 ns, for component ii), and τ~2.5-5 ns for component iii). **Conclusions:** Presented Metabolic Modulation Matrix concept, in conjunction with spectrally-resolved fluorescence lifetime detection of the cell intrinsic fluorescence, is a promising, highly versatile tool for quantitative assessment of oxidative metabolism in living cells.

2068-Pos Board B38

Random Fluorescently Labeled Proteins: Label Distribution and Effect on Binding

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Proteins for fluorescence measurements are often labeled randomly by covalent linkage of fluorescent dyes to amine groups on the target protein and subsequently purified. Such labeling results in a heterogeneous population of protein molecules containing a varied number of labels, which may depend on the number and location of available lysine residues. We explore the extent to which protein labeling techniques result in a Poissonian distribution of protein-fluorophore complexes using fluorescence fluctuation spectroscopy (FFS). The fluctuation amplitude in an FFS measurement is related to the number of labeled proteins and is not sensitive to unlabeled protein. We model the expected fluctuation amplitude as a function of average incorporated fluorophores assuming the distribution is governed by Poissonian statistics. We experimentally fit the model by randomly labeling monoclonal antibody with fluorescent dye and show agreement for incorporation ratios up to ~ 1.5. For greater amounts of incorporated dye molecules, we use mass spectrometry to examine labeled $F(ab')_2$ fragments and show that the distribution is better described by a Gaussian profile. Finally, by performing quenching experiments on a steady-state fluorimeter, we show that randomly labeling antibodies and antigens does not affect measured affinity values within experimental uncertainty.

2069-Pos Board B39

Probing Variations In The Structural Environment Of A DNA Sequence Using Fluorescence Properties Of The Pteridine Analog Probes, 3MI and 6MI

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We explored two different microenvironments in the sequence; 5′-actaGagatccctcagacccttttagtcagtGtgga -3′ in single and duplex form using two similar nucleoside analogs. 3MI and 6MI were each investigated in two different environments, one flanked by thymines (PTRT) and the other, by adenines (PTRA)(shown by G's noted above). Each site is equidistant from a terminus. The probes differ only by the position of a methyl group in either the 3- (3MI) position or the 6- (6MI) position. Both time-resolved anisotropies and lifetimes of the probes depend upon local electrostatics which are impacted by duplex formation. 3MI shows less response to structural change as compared to 6MI. Integrals of lifetime curves compared with quantum yields of each sample reveal that each displays a ''dark'' component which we are unable to detect with TCSPC (e.g.,tau<70ps). For 6MI in the A environment this QSSQ ''quasi static quenching'' eliminates approximately half the molecules, whether in SS or DS form. 6MI in the T environment displays an unexpected increase in the quantum yield upon duplex formation (0.107 to 0.189) apparently the result of escape from QSSQ which simultaneously declines from 66% to 33%. Escape from the dark state is accompanied by doubling of steady state anisotropy of 6MI in PTRT in the duplex. Only 6MI in the T duplex displays a rotational correlation time over 7 ns. The DS A environment fails to constrain local motion and QSSQ remains the same as in SS; in contrast, the flanking T duplex environment restricts local motion and halves the QSSQ.

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A Fluorescence Polarization Displacement Assay for Aggrecanase-1 and -2

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Fluorescence polarization is a valuable technique for characterizing small molecule inhibitors. Competition fluorescence polarization assays involve the displacement of a fluorescent ligand from the enzyme with increasing amounts of a competing compound. The technique allows the determination of inhibitor dissociation constants (Ki values) and evaluation of whether the data fit is consistent with competitive and stoichiometric binding, which enables the potency of small molecule inhibitors to be ranked. Fluorescence polarization assays have the advantage of being robust, non-radioactive, and formatted for 384-well plates. Inhibitor dissociation constants can be obtained without the necessity of separating bound and unbound species. Here we describe the development and characterization of a fluorescent ligand for the Aggrecanase-1 and Aggrecanase-2 enzymes, and present measured competition IC_{50} values and Ki values for hit compounds in the Aggrecanase program that aided the team in lead optimization.

2071-Pos Board B41

Optical force measurements utilizing Lanthanide Binding Tags

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The Lanthanide Binding Tag (LBT) is a motif comprised of 17 amino-acids with the sequence YIDTNNDGWYEGDELLA. The LBT has been crystallized in the presence of Tb^{3+} and thus it is known that this motif forms a loop in which the center is occupied by the Tb^{3+} ion, with the ion held in place by negatively charged amino-acids. Comparison with the highly related structure of an EF-hand revealed that only in the case of the LBT, water molecules are completely excluded from the interior of the loop (Nitz et al, 2004). This explains the high quantum yield of Tb^{3+} bound to the LBT.
However, when encoded into a large ''host molecule'' the environment of the LBT is more constrained and it is possible that if inserted in the correct position, it will sense forces originating from conformational changes within the host. The forces exerted by these conformational changes may lead to a deformation or an unfolding of the motif, which would consequently change the emission properties of the bound Tb^{3+} ion. Here we show that Tb^{3+} emission from LBTs inserted on a particular position of the voltage sensor of Shaker K channels can be quenched by voltage dependent conformational changes. In order to understand this phenomenon, and with the attempt to calibrate this system for optical force measurements, we have employed molecular dynamic simulations. In these simulations an artificial force was applied to the ends of