and deeper penetration. For each region of wavelengths, we have found a mutant, which is 3-4 times two-photon brighter than the benchmark EGFP.

2061-Pos Board B31
Fluorescence, Cyto-, And Photo-toxicity, And Structural Studies Of Substituted Piperidones: Potential Sensitizers For Two-photon Photodynamic Therapy
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Two-photon photodynamic therapy has the advantages of being highly localized in its effects and allows for deeper tissue penetration, when compared to one-photon photodynamic therapy. N-alkylated 3,5-bis(arylidene)-4-piperidones, with a donor-pi-acceptor-pi-donor structure, have the potential to be useful two-photon sensitizers. We have measured two-photon cross sections (using femtosecond excitation), fluorescence quantum yields, fluorescence lifetimes, and x-ray crystal structures for a number of these compounds. Most two-photon cross sections are comparable to or larger than that of Rhodamine B. However, the fluorescence quantum yields are low (all less than 10%) and the fluorescence lifetimes are less than 1 ns, suggesting that there may be a significant energy transfer to the triplet state. This would encourage singlet oxygen formation and increase cellular toxicity. Results of dark cyto-toxicity studies with a number of human cancer cell lines are presented. White light photo-toxicity results are also presented, and suggest that increasing the number of double bonds, from one to two, in the piperidone ''wings'' increases the photo-toxicity with little corresponding change in the dark cyto-toxicity. Two-photon photo-toxicity studies are also underway (exposure in the range of 740 - 860 nm) as well as singlet oxygen detection studies(detection at about 1270 nm).

2062-Pos Board B32
Combretastatin A4 Disodium Phosphate Forms Aggregates In Solution Leading To Exciton Transfer
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Combretastatin A4 disodium phosphate (CA4P) has been effective in the treatment of solid tumors. The side effects of CA4P could be reduced using targeted delivery where CA4P loading/release can be studied using CA4P fluorescence. However, the fluorescence properties of CA4P are poorly characterized. This work reports the effects of drug concentration on the excitation and emission properties of CA4P. The excitation spectrum shows a broad peak with a maximum at 328nm. The spectrum becomes narrow and the emission maximum shifts to 356nm when the CA4P concentration is increased. The emission spectrum also shows a red shift from 398 to 406nm, in the same drug concentration range (0.1-5.0mM). This spectral shift is typical for exciton transfer probably due to the formation of J-aggregates, where an excited monomer in the aggregate transfers its electronic excitation energy through Coulombic interactions to a ground-state monomer in the same aggregate. When excited at 356nm, the emission intensity is proportional to [CA4P] up to ~1.75mM; thereafter, the intensity decreases. When excited at 328nm, the biphasic change persists but occurs at 0.175mM. We propose that the emission upon excitation at 328nm and 356nm comes from CA4P monomers and aggregates, respectively. An increase in drug concentration leads to aggregation, decreasing the number of monomers in solution and therefore the fluorescence intensity due to 328nm excitation drops. The decrease in fluorescence intensity, due to 356nm excitation, observed at [CA4P]>1.75mM is probably due to some subtle changes in the optical properties of the aggregates. These results have been applied to develop an assay capable of following the leakage of the encapsulated CA4P from the liposomes in real time. To the best of our knowledge, this is the first report on optically active aggregates formed by CA4P.

2063-Pos Board B33
Soluble Guanylate Cyclase Conformational Regulation
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Nitric Oxide (NO) is an important signaling molecule that is involved in many physiological processes. In cells, NO is produced by Nitric Oxide Synthases (NOs) then bind to its principal receptor Soluble Guanylate Cyclase (sGC). Upon NO binding, sGC activity increases as it catalyzes the conversion of its substrate GTP to cGMP. As a second messenger, cGMP regulates series of proteins further downstream in the signaling cascade that promotes smooth muscle relaxation, vasodilation, and also inhibits platelet aggregation. Hence, sGC is targeted as a possible therapeutic agent for treatment in pulmonary hypertension and prevention of blood clot formation. Recent interest revolves around the different sGC effectors that may increase the enzyme activity and cGMP production. Intrinsically, CO and NO bind to sGC inducing different activity levels of 5 fold and 400 fold respectively. Synthetic compound, such as YC-1 and BAY 41-2272, activates sGC up to 10 fold independently from NO. However, the presence of both NO and YC-1 molecule pose an additive effect on sGC activity. YC-1 has also been noted to work synergistically with CO increasing activation level that is comparable to NO. Our objective is to distinguish of the sGC activation mechanism between sGC/CO/YC-1 and sGC/NO/YC-1 complex from a structural perspective. Current information has indicated that sGC/CO/YC-1 forms a 6-coordinate complex while sGC/NO/YC-1 forms a 5-coordinate complex. Unfortunately, there are limited information on the binding sites interactions and the overall structure of the enzyme upon activation. We employed fluorescence spectroscopy to observe the global rearrangement of sGC in the presence of effectors by observing the behavior of the reporter residue Trp that is located in each α and β subunit. Fluorescence resonance energy transfer (FRET) will also be utilized to determine the distance between the binding sites in different sGC complexes

2064-Pos Board B34
Sequence-Dependent Cy3-DNA Interactions: Effects On Fluorescence Properties
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Fluorescence has contributed significantly to the understanding of nucleic acid conformation and dynamics. However, the reliable interpretation of fluorescence measurements on probes covalently attached to nucleic acids requires a careful investigation of the spectroscopic and photophysical properties of the fluorescent dyes.
We have carried out an extensive study of the spectroscopic and photophysical properties of Cy3, the most popular fluorescent probe used in single-molecule spectroscopy, fluorescence microscopy and other fluorescence applications. Upon absorption, the molecule isomerizes to a non-fluorescent photoisomer with an efficiency that depends greatly on the environment in which the molecule is located. This process competes with fluorescence, and as a consequence the fluorescence quantum yield and lifetime of Cy3 depend strongly on the location of the probe.
We have shown that Cy3-DNA interactions impact the barrier for isomerization, and as a consequence the brightness of the probe. Surprisingly, Cy3 appears to interact more strongly with ssDNA than when bound to duplex DNA. We have characterized the photophysical properties of Cy3 in a variety of environments on DNA. Here, we'll present results that show that Cy3 interacts with DNA in a sequence-dependent fashion, and as a consequence, its fluorescence efficiency depends strongly on the type of attachment and on the particular sequence in the vicinity of the dye.

2065-Pos Board B35
Peptide-bridged Bis-phenanthridinium Derivatives In Interaction With Double Stranded DNA
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Series of peptide-bridged bis-phenanthridinium derivatives and corresponding monomers were prepared by novel, convergent approach by solid phase peptide synthesis procedures. Spectrophotometric studies revealed that in aqueous, biologically relevant medium bis-phenanthridinium derivatives form significant intramolecular interactions strongly dependent on the rigidity and length of the peptide linker. Furthermore, intramolecular interactions of studied compounds are found to be directly correlated to their affinity towards double stranded (ds) DNA, as well as induced thermal stabilisation effects of DNA double helix. To the best of our knowledge, bis-phenanthridine **3** is the first phenanthridine derivative exhibiting specific fluorescence signal due to the excimer formation, and even more, that fluorescence signal showed to be sensitive to pH as well as on the interactions with ds-DNA. Corresponding properties of pyrene were quite extensively applied and **3** brought new features to it, possessing heterocyclic nitrogen prone to protonation at weakly acidic conditions (pKa 5-6).

2066-Pos Board B36
Combined pH and Temperature Measurements Using Pyranine as a Probe
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Pyranine is a well known fluorescent probe used to measure pH in biological samples with the ratiometric fluorescence method. This method involves the determination of the ratio of the fluorescence emissions recorded with 405 nm and 450 nm excitation. Each of these two excitation maxima corresponds to a different state of pyranine, protonated and deprotonated respectively. In our study, we

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.

2067-Pos Board B37

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.

2070-Pos Board B40

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