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.

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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).

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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.

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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