SWCNT preparations including the helix-angle distribution, diameter distribution, length distribution, bundling properties and intensity distribution. To date quantitative analysis of the three dimensional EEMs has relied heavily on manual estimations and 2-dimensional profiling to deal with overlapping peaks and other features in the EEM surface. The global analysis software and method described facilitates a rapid and statistically robust simulation of the 3D EEM surfaces in either wavelength or energy units to yield crucial coordinate and line-width information on all identified PL peaks. The model parameter initialization is facilitated by derivatization of the surface to identify all major peaks coordinates and widths with adjustable amplitude discrimination. The program accepts EEM data in standard x-y-z columnar format in addition to matrix representation. An analytical form of the Voigt function is included to deconvolute the Lorentzian emission line shape from the Gaussian instrument response. The fitting functions can be fully constrained to ascertain physically realistic model parameterization using conserved themes for related data sets. Global linking/sharing of model spectral parameters is used to model excitation-emission peak coordinates relating the main energy levels (S3, S2 and S1) in addition to sidebands in the spectral emission. The model form can be adapted and constrained to yield information concerning anisotropic features, reabsorption phenomenon as well as energy transfer and quenching processes. The modeling routine also facilitates 3D surface simulations of Raman spectra of the radial-breathing modes of SWCNTs.

2057-Pos Board B27

Elucidating the Molecular Basis of Cellulase Synergism Through High Resolution Quantitative Fluorescence Microscopy

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Converting cellulose into fermentable sugars presents significant challenges to producing bioenergy from lignocellulose. Individual cellulases exhibit low rates and extents of hydrolysis. However, mixtures of cellulases and other cell wall degrading enzymes exhibit rates of hydrolysis that are much greater than would be predicted by summing individual rates. Thus, understanding the molecular mechanisms that give rise to synergistic behavior is essential for engineering more effective enzyme cocktails. Previous studies by the Walker Lab revealed mixtures of cellulases Cel9A, a processive endocellulase, and Cel6B, an exocellulase, exhibited higher extent of binding that would be predicted by summing the individual binding extents. A major question is whether this is driven by intrinsic cellulase binding kinetics or are changes in these two cellulases' diffusion rates into the cellulose macrostrucutre yielding this behavior.

In this study, bacterial microcrystalline cellulose fibrils were immobilized on a solid substrate using polymer lift-off. Cel9A and Cel6B were fluorescently labeled with either of two colors and purified into populations with known degree of labeling. These labeled cellulase populations were tested to validate the previous observation that labeling does not inhibit cellulose depolymerization. The binding of labeled cellulases on immobilized cellulose fibrils was observed using fluorescence microscopy for a period of 95 minutes, with images taken every minute for the first 10 minutes, every 2.5 minutes for the next 10 and every 5 minutes for the remainder. Individual binding curves were established for each enzyme in each color using different populations to characterize binding of enzymes with different numbers of labels. The effect of synergism was investigated by combining Cel9A and Cel6B, labeled in different colors, in varying molar ratios and observing effects of synergism and competition on diffusion and substrate binding in the system.

2058-Pos Board B28

Photophysical characterization of Dye-Encapsulated Calcium Phosphate Nanoparticles

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Organic dyes exhibit rapid photobleaching, low quantum yield, and random blinking under physiological conditions, limiting their utility in *in vivo* imaging. To address these photophysical shortcomings, the Adair group at Penn State has recently developed a novel method for synthesizing dye-encapsulated calcium phosphate (CP) nanoparticles based on a double microemulsion method. In this study, time-resolved single photon counting methods were used to characterize cy3-encapsulted CP nanoparticle size, dispersity, molecular brightness, and fluorescence lifetime (FL). Particle sizes measured using fluorescence correlation spectroscopy (FCS) confirmed the presence of highly mono-disperse 20 nm particles. The brightness of an individual nanoparticle measured using moment analysis was found to be 20 times higher than the free dye, due to a five-fold increase in quantum efficiency and encapsulation of 4 dye molecules per particle. FL of the encapsulated dye was independent of the solvent (water, PBS, DMSO, and 50% glycerol), suggesting that the dye was well-protected in the CP matrix. Furthermore, increased FL in CP nanoparticles compared to free dye suggests that the photoisomerization of cy3 was inhibited due to restricted mobility of the dye in CP matrix. Photostability increased 50-fold likely because the dye was protected from the photobleaching effects of dissolved oxygen. Finally, systemic administration of PEGylated CP nanoparticles in nude mice implanted with breast cancer tumors retained fluorescence signal in tumors even after 96 hours post-injection, demonstrating the utility of CP nanoparticles for long term *in vivo* imaging.

2059-Pos Board B29

Multi-exponential Luminescence Decay of Non-blinking CdTe Quantum dots upon one and two photon excitation

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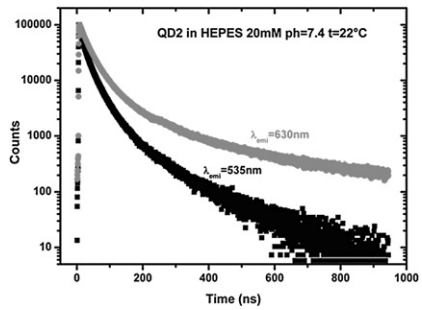
Quantum dots (QD) are semiconductor nanocrystals with quantum confinement of charges carried in limited spaces. They are expected to have high quantum yield, photo and thermal stability, and strongly size dependent emission wavelength. Non-blinking CdTe QDs have been synthetized in water phase by microwave irradiation with mercaptopropionic acid as a stabilizer. They exhibit high QY, good photo and chemical stability in water solution. QD with sizes from 2.1nm to 5nm have been studied. Time-resolved photoluminescence (PL) decays were measured by TCSPC technique upon one and two photon excitation (2PE). The effect of temperature and pH on PL decay was studied at several excitation wavelengths. Lifetime distribution, extracted from PL decay by Maximum Entropy Method of data analysis, display up to five components. PL decay of small QD is slower at longer wavelength (figure). The lifetime distribution of the larger QDs always exhibits three lifetime peaks around 15 ,37 and 100ns respectively , only relative contributions of each peak vary with size.



2060-Pos Board B30

Absolute Two-photon Absorption Spectra Of Orange And Red Fluorescent Proteins

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Two-Photon laser scanning microscopy, which makes use of genetically encoded fluorescent protein (FP) probes, is becoming a method of choice for studying biological systems from sub-cellular to the whole body level. However, reliable information on two-photon absorption (2PA) properties of FPs, specially for the more popular orange and red variants, is still very fragmentary. 2PA spectra, measured in absolute cross section values, will allow us to select the two-photon brightest FP variant with desired fluorescence properties and also to choose the optimum laser system and excitation wavelength. Here we study 2PA spectra of a large set of orange and red FPs, including DsRed2, mRFP, TagRFP, and mFruits series in a wide range of excitation wavelengths, 600 -1200 nm. We have found the the 2PA spectra and maximum cross sections are very sensitive to either changes in chromophore structure (mOrange vs mRFP) or to mutations in chromophore surrounding (DsRed and mFruits series). All red FPs show two pronounced 2PA transitions, the first peaking in the 1000 - 1100 nm region, and second - near 700 - 760 nm. We quantitatively describe the first transition within the framework of two-level model, and the second - within three-level model with strong resonance enhancement. Excitation in the longer wavelength region, accessible for Nd- and Yb-doped short-pulse lasers, has advantages of producing less two-photon autofluorescence

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