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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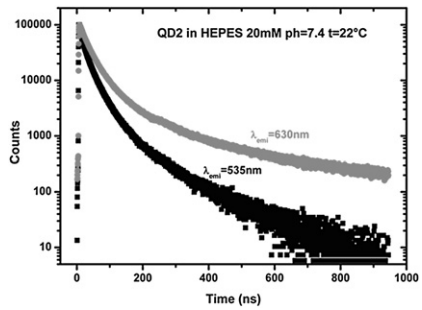
Quantum dots (QD) are semiconductor nanocrystals with quantum confinement of charges carried in limited spaces. They are expected to have high quantum yield, photo and thermal stability, and strongly size dependent emission wavelength. Non-blinking CdTe QDs have been synthetized in water phase by microwave 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