capable of fluorescence resonance energy transfer (FRET). This peptide is known to be a substrate of p300 acetyl-transferase activity (Ott, M. et al. 1999). We shall show that the efficiency of FRET is significantly decreased upon Tat acetylation by p300. Moreover, the choice of this cell-permeable construct allows us to visualize the acetylation states in living cells bypassing cell-invasive procedures. Our results indicate that the sensor can discriminate between basal or altered acetylation states. We shall present results for the case of cells over-expressing p300 or under TSA drug treatment. We shall argue that this method can provide a general approach for screening acetyltransferase activity in live cells.

3014-Pos

Quenching of Alexa Dyes by Amino Acids

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Alexa dyes, rhodamine-derived fluorophores, are popular choices for labeling proteins due to their superior photo-physical properties. They are often used in quantitative fluorescence measurements like Forster Resonance Energy Transfer (FRET) or fluorescence lifetime imaging (FLIM). Consequently, it is important to consider the effects of nearby amino acid residues on the brightness of fluorophores that may influence quantitative measurements of fluorescence intensities or lifetimes. We report on the quenching of Alexa dyes (488, 555 and 594) by various natural amino acids. We observed quenching of Alexa488 by Tryptophan, Histidine, Methionine and Tyrosine. Lifetime measurements indicate that with the exception of Tyrosine, the quenching by the amino acids occur through both static and dynamic processes. Additionally, cyclic voltammetry experiments suggest that photo-induced electron transfer (PET) is a possible mechanism for the quenching of Alexa488.

3015-Pos

Nanometals and Quantum Dots as Optical Markers in Biophysics

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Nanometals and Quantum Dots are finding wide ranging applications in molecular beacon based optical assays. Whether the application is for measuring distances by resonant energy transfer processes or tracking the fate of gene delivery by cellular transfection, nanomaterials are ideal markers for the optical probe. The presentation will probe the use of nanometal Hammerhead RNA, as well as investigate in-vitro release of a gene coding for fluorescent proteins and/or siRNA from a nanometal surface or fluorescent Quantum Dot by live optical microscopy imaging. The applicability of the results to biological, the perturbations arising from non-specific interactions between the nucleic acid and nanomaterial, and cellular cytotoxicity are investigated.

3016-Pos

Understanding Wavelength Dependence of Tryptophan Fluorescence Decays

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Understanding the ubiquitous non-exponential decay exhibited by tryptophan (Trp) fluorescence in proteins is becoming crucial for interpretation of ultrafast decay experiments, especially when the cause of time dependent Stokes shifts may be interpreted equally well by water/protein relaxation or by excited state decay of short-lived blue shifted subpopulations as a result of a heterogeneous microenvironment. Here we report hybrid quantum mechanical-molecular mechanical (QM-MM) simulations of fluorescence wavelengths and lifetimes for a set of 7 cyclic hexapeptides with rigid peptide backbone, a single Trp residue, and 5 other amino acids that do not quench Trp fluorescence in aqueous solution. These were designed specifically to examine possible fluorescence lifetime heterogeneity arising only from different rates of quenching by electron transfer (ET) from the excited indole ring to a nearby backbone amide by different Trp sidechain rotamers– in the absence of nanosecond solvent relaxation. The results capture most of the unexpected diversity in observed spectroscopic properties of the 7 peptides, including the almost universal pronounced correlation of blue shifted decay associated spectra to have the shortest lifetimes. Heretofore no attractive physical mechanism has been advanced to explain why rotamers with blue shifted spectra should almost always have the shortest lifetime component. The familiar broad fluorescence spectrum of a solvent-exposed Trp with emission peak near 350 nm is found to be an ensemble average of single molecular fluorescence peaks fluctuating on a femtosecond time scale over 3000-4000 wavenumbers (fwhw) or 40 nm. This leads naturally to a picture in which those rotamers having shorter wavelength emission spectra (higher average energy) tend to have short lifetimes due to their increased probability for transient fast quenching during the large fluctuations in environment that bring the CT and 1La states into resonance. This mechanism should be operative in proteins.

3017-Pos

The Light Conduction in an Antenna of a Phycobilisome

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Phycobilisomes (PBS) are highly efficient accessory light harvesting protein complexes, responsible for the conduction of light towards the photosynthetic reaction centers. They are exclusive for red algae, cyanobacteria and cryptophyta. Phycobiliproteins (PBP) are the main components of phycobilisomes and they contain bilin chromophores, tetrapyrrols in extended conformation, bound by a thioester bond to cysteines. PBPs have been classified by their spectroscopic properties as Allophycocyanin in the core of the complex (APC, Lambda max 651 nm), from where radiate rods formed by Phycoerythrin, (PE, Lambda max 565 nm) and Phycocyanin (PC,Lambda max 621 nm) (5, 12, 13). Phycobiliproteins in general are formed by heterodimers of alpha and beta subunits and are organized as trimers or hexamers
Phycoerythrin (Chromophores: 1 Urobilin, 4 Erythrobilin per heterodimer) and Phycocyanin (Chromophores: 3 cyanobilins per heterodimer) have been identified as a the phycobiliproteins present in the rods of the phycobilisome of Gracilaria chilensis. To study the energy transfer through a rod formed by two hexamers of phycoerythrin (ID=1eyx) and two hexamers of phycocyanin (ID=2bv8), the spectroscopic parameters of Phycourobilin and Erythrobilin were determined. In this report the quantum yield, half life were determined for hexameric PE, for the alfa and beta subunits and for purified chromophorylated proteolytic fragments form each subunit.Using this information and the values for Cyanobilin, the dipolar moments of each chromophore was calculated, as well as the orientation factor between pairs donor acceptor.The donor acceptor transfer constants using the extended Foster equation for the energy transfer in resonance, were calculated. Using a docking model for a rod, a main pathway for the light transfer in a rod is proposed.

3018-Pos

Pressure Effects on the Solvent Denaturation of NADH Probed using Fluorescence Spectroscopy

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Reduced nicotinamide adenine dinucleotide (NADH) plays a central role in cellular metabolism via a NAD^+/NADH redox reaction. NADH conformational state - whether it is folded or unfolded - has physiological significance because it is in a mostly folded conformation when free and typically takes on an unfolded conformation when protein bound. This study examines the effects of pressure on the solvent denaturation of NADH (20μM NADH in MOPS buffer, pH7.4). Using a quartz capillary-based high-pressure chamber, the methanol-induced folding-unfolding reaction of NADH was examined through fluorescence emission spectroscopy (337 nm excitation) at physiological pressures up to 50 MPa. (The oxidized form NAD^+ is not fluorescent.) Using a two-state solvent-denaturation model to determine thermodynamic parameters relevant to solvent denaturation, the free energy of unfolding with no denaturant was inferred. The change in volume for the folding-unfolding reaction was determined using an Arrhenius relationship. The validity of the two-state assumption in this context is assessed. Results will have significance in understanding cellular piezo-physiological effects on cellular respiratory metabolism.

3019-Pos

Photoinduced-Electron Transfer between Guanine Bases and Silver Nanoclusters Enables Increased Shelf Life

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We studied the interactions between DNA-templated fluorescent silver nanoclusters and nearby guanine bases. Fluorescence quenching by guanine due to photoinduced charge transfer has been reported for many widely used organic dyes. In contrast, we found that for red- and infrared-emitting fluorescent Ag nanoclusters (NCs), formed on DNA templates, interactions with nearby guanine bases tended to protect these NCs against oxidation, making them brighter and more stable in aqueous solution. Nanoclusters formed in the absence of guanine-rich DNA changed from a red-emitting reduced NC into a green-emitting oxidized species in a few hours in air-saturated solutions. In contrast, when guanine bases were brought close to the NCs, through DNA hybridization, guanine served as an electron donor and reducing agent, which prevented the Ag NCs from being quickly oxidized in air-saturated solutions,

increasing the stability from hours to days. In addition, hybridization with guanine-rich DNA could be used to reduce the already oxidized NCs back to the red-emitting reduced ones. Single-stranded DNA templates were also designed with a nanocluster formation sequence and a guanine-rich sequence at each end. Similarly, we found that the guanine-rich tail helped stabilize the fluorescence of the red-emitting NC fluorophores, in comparison to single-stranded templates with only cluster formation sequences but no guanine-rich tails. Using this strategy, we have designed a DNA sequence that produces a highly emissive Ag NC fluorophore with an extended shelf life, which should prove useful in a variety of biological applications, including fluorescence imaging and biosensing.

3020-Pos

Spectroscopic Characterization of Depolarization Via FRET in a BFP-GFP BoNT/A Assay

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The assay is based on depolarization due to Förster Resonance Energy Transfer (FRET) between Blue Fluorescent Protein (BFP) and Green Fluorescent Protein (GFP) moieties linked by a peptide containing residues 134-206 of SNAP-25, the protein substrate for BoNT/A's proteolytic activity. Before cleavage of this recombinant substrate, the polarization observed for the GFP emission, excited near the absorption maximum of the BFP, is −0.04 due to depolarization following FRET from BFP to GFP. After substrate cleavage and diffusion of the fluorescent proteins beyond the FRET distance, the polarization increases significantly to ~0.4, due to observation of the emission only from directly excited GFP. This change in fluorescence polarization allows for an assay (termed DARET for Depolarization After Resonance Energy Transfer) that is robust and sensitive. In this report we characterize the spectroscopic parameters of the system before and after substrate cleavage, including excitation and emission spectra, polarization and time-resolved methods. Our results suggest that the donor and acceptor dipoles are at a large angle (72°) with respect to one another but that the BFP and GFP are in direct contact in the intact substrate. Evidence is also provided to demonstrate the direct interaction of BFP and GFP after cleavage. We have used this assay to determine the enzyme kinetic parameters (K_m, K_{cat} and V_{max}) for BoNT/A proteolysis of the assay substrate. Our conclusions bear on the issue of the common choice of 2/3 for κ^2 for FRET studies in general and on fluorescent proteins in particular. This work was supported by Allergan Inc.

3021-Pos

A Microfluidic Cytometer for High-Throughput and Quantitative Single Cell Red Fluorescent Protein Photobleaching

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Fluorescent proteins (FPs), given their tendency to convert to a non-radiative triplet state and/or undergo rapid photobleaching, remain sub-optimal for today's advanced microscopy techniques which require low-copy cellular expression, high laser intensities and prolonged imaging durations. To overcome these experimental limitations and address FP photobleaching, which is particularly problematic in orange/red emitting fluorescent proteins, we have developed an innovative microfluidic platform capable of screening 30 mammalian cells per second based upon FP photobleaching. More specifically, the fluorescence intensity of a single hydrodynamically focused cell (5 mm/s) expressing the FP of interest is probed before and after exposure to an intense 1 ms. photobleaching laser ($\approx$ 300 kW/cm2). The resulting emission intensities are measured with excellent sensitivity and signal to noise, permitting the change in fluorescence intensity between the first and second probe beams to be measured with high accuracy. As a result, this platform has enabled us to make quantitative and high-throughput photobleaching measurements as well as differentiate a diverse mixture of red FPs (mOr2, DsRed, mCherry, TagRFP & TagRFP-T). These results provide promising potential for library-based sorting and, given the improved selection criteria, may permit vastly improved photostability in future generations of FP mutants.

3022-Pos

Applications of Pulsed Interleaved Excitation in Live Cell Experiments

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To make Fluorescence Correlation Spectroscopy (FCS) measurements more viable in living cells, various new methods have been developed. Among these are Raster Image Correlation Spectroscopy (RICS), and Scanning Fluorescence Correlation Spectroscopy (SFCS).

In RICS, a confocal raster scanning image of a sample is evaluated to extract concentration, diffusion, or colocalization information of fluorescently labeled molecules using both the temporal and spatial information.

Another advantageous possibility, especially in small organisms, is to use SFCS. There, the confocal laser spot is rotated through a small area, effectively increasing the focal volume and reducing fluorophore bleaching.

Especially in live cell measurements, where fluorescent proteins are typically used, signal levels are often weak, and spectral crosstalk can be a significant problem. Therefore we combined both RICS and SFCS with Pulsed Interleaved Laser Excitation (PIE), a technique we developed to avoid the introduction of artifacts by spectral crosstalk. The sensitivity of both RICS and SFCS to detect dually labeled molecules could be significantly improved, rendering them much more useful for biologically relevant applications both in live cells and in vitro.

The principles of PIE-RICS and PIE-SFCS will be presented along with applications on calcium channels and protein interactions in yeast cells.

3023-Pos

Interpreting FRET in Complex Geometries

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Fluorescence resonance energy transfer (FRET) can be utilized to gain low resolution structural information, making use of the fact that the probability of energy transfer is related to the distances between fluorescent molecules. Although the relationship between the efficiency of energy transfer and the distance between sites is well described for a single pair of fluorophores, the situation is more difficult when more than two fluorophores are present. Using a Monte Carlo calculation scheme, we demonstrate how resonance energy transfer experiments can be interpreted when multiple fluorophores are present in complex geometries. We demonstrate the versatility of the approach by calculating the efficiency of energy transfer for individual fluorophores randomly distributed in two and three dimensions, as well as when attached to multimeric proteins. In addition the approach can yield information about the clustering of proteins and their oligomerization state, molecular concentrations and donor:-acceptor ratios.

3024-Pos

Studying Fluorescent Proteins in Living Cells: An Application for Segmented Fluorescence Correlation Spectroscopy

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Fluorescence intensity fluctuations can provide insight into a multitude of molecular properties from localized concentration and transport mechanisms to characteristic rate constants of reactions. These measurements are possible because the movement of particles in solution gives rise to fluctuations in intensity as the fluorescent species traverse the focal volume. Fluorescence correlation spectroscopy (FCS) is a specialized technique for studying these fluctuations and provides high spatial resolution analysis of molecules at low concentrations.

In homogeneous or even quasi-homogeneous samples, the molecular motions that give rise to these fluctuations are directly related to the molecule of interest. However in more complex environments such as the cell cytosol, there can be an abundance of autofluorescent material, or environment-induced aggregation of the labeled proteins of interest. These sparse, but larger fluorescent species distort the correlation curve by emitting large bursts of photons as they move through the observation region. To overcome this limitation we have developed segmented-FCS (sFCS). In sFCS, the raw data stream is screened for bursts of photons arising from large species allowing the uncontaminated data segments to be isolated. These small segments of the photon stream are then correlated, averaged and analyzed to provide insight into the fluorescent species of interest.

We conducted measurements in RBL-2H3 cells stably transfected with GFP using lab built hardware and software. The customized setup is an economical and efficient solution for a variety of fluorescence measurements and is well suited for the post-acquisition software-based correlation of data. In addition, simulated data confirms the robustness of the sFCS protocol. Our data demonstrates that sFCS can accurately measure concentration and characteristic diffusion rate in contaminated signals and provides a solution for studying fluorescence fluctuations in cells.

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3025-Pos

Irregular Excess Energy Transfer Observed with a Cerulean Donor and Multiple Venus FRET Acceptors

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