

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 (fwhm) 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 prob-

ability 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, tetrapyrroles 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 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 Phycocourobilin and Erythrobilin were determined. In this report the quantum yield, half life were determined for hexameric PE, for the alpha and beta subunits and for purified chromophorylated proteolytic fragments from 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 (20 μM 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,