method is employed to produce mutants of fluorescent chromophore for color shifts [4, 5]. However, due to the uncertainty and complexity of the mutation effect on the wavelength of emitted light, real color modulation for target marking remains unavailable. In this work, we report a top-down method for the accurate and continuous color tuning of firefly chromophore (oxyluciferin) by controlling the surrounding polarization electrostatic fields. Systematic investigations of the absorption spectra of oxyluciferin molecules are carried out in the framework of time-dependent density functional theory. Results show that the polarization electrostatic field applied on the long molecular axis significantly changes the optical properties. However, if the field is applied on the out-of-plane axis, its effect is almost negligible. Under long axis electric fields, the wavelength of the two main peaks shifts continuously, covering a wavelength range of about 100 nm. Such a wide range of wavelength shift provides us a realizable modulation technique for very accurate color tuning of fluorescent proteins. The need of any special marking application can be met by careful design of the local polarization electrostatic fields. On the other hand, the peak intensity is also associated with the electrostatic fields, which shows that the efficiency of light emission can be well enhanced as well.

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3009-Pos

Sequence-Dependent Enhancement of Cy3 Fluorescence on DNA

Billie J. Harvey, Perez Claudia, **Marcia Levitus**.
Arizona State University, Tempe, AZ, USA.

Cy3 is a cyanine fluorescent dye extensively used as a fluorescent probes in molecular biology, biochemical and biophysical applications. We investigated the fluorescent properties of Cy3 covalently attached to the 5' terminus of DNA oligonucleotides, and demonstrated that its fluorescence efficiency and lifetime depend strongly on DNA sequence. Fluorescence quantum yields and mean fluorescence lifetimes ranged from 0.18 to 0.39 and from 533 ps to 1.2 ns respectively. DNA sequence determines the extent and nature of the interactions between the dye and the DNA bases, which are responsible for the unusual enhancement in fluorescence observed for a large number of oligonucleotides. Results are discussed in terms of a photoisomerization mechanism that deactivates the excited state and thus competes with fluorescence. The efficiency of isomerization decreases when Cy3-DNA interactions prevent rotation around the double bonds, resulting in an increase in the lifetime of the singlet excited state. We have shown that the ability of Cy3 to interact with DNA depends on the flexibility of the oligonucleotide and the presence of purines in the chain.

3010-Pos

Determination of Fluorophore Orientation and Energy Transfer from MD Simulations

Evelyne Deplazes, Ben Corry, Dylan Jayatilaka.
University of Western Australia, Crawely, Australia.

Fluorescence Resonance Energy Transfer (FRET) spectroscopy is a technique that is widely used to obtain co-localization and structural information of proteins in their native environment. The technique is based on the mechanism of energy transfer by dipole-dipole induced, non-radiative interaction between a fluorescent donor and a suitable acceptor. While the rate of energy transfer depends on the distance between the donor and acceptor, the use of FRET as a spectroscopic ruler is complicated by it also being dependent on the relative orientations of the fluorescent probes. In general these orientations are difficult to determine experimentally making the technique uncertain for measuring absolute distances.

Simulations may offer an alternative means of understanding the behavior of the fluorophores at the molecular level, thus enabling distances between specific sites in the sample to be determined more accurately by calculating the orientation factor for a given system. To examine this possibility, we attempt to simulate FRET in a simple model that allows atomistic simulations in the 10s of ns. The system contains individual donor and acceptor molecules in an aqueous solution.

Preliminary results from standard MD simulations show that the simulation accurately predicts the probability density of the orientation factor κ^2 and reproduces experimental values of the anisotropy decay for donor and acceptor molecules. By simulating FRET in a simple system we hope to gain insight into the process of the energy transfer and the factors affecting the behavior and orientation of the fluorophores in order to better understand and analyze data from FRET experiments. The results of this study might also be useful as indications of when simulation may help to understand and analyze data from more complicated FRET experiments.

3011-Pos

Fretting About FRET: Breakdown of the Ideal Dipole Approximation

Aurora Munoz-Losa[1], Carles Curutchet[2], Lydia R. Hartsell[3], **Brent P. Krueger[3]**, Bendedetta Mennucci[1].
[1]Universita degli Studi di Pisa, Pisa, Italy, [2]University of Toronto, Toronto, ON, Canada, [3]Hope College, Holland, MI, USA.

Fluorescence-detected resonance energy transfer (FRET) experiments have been a useful tool in structural biology for four decades and have enjoyed resurgence in the last several years due to improved fluorescent labeling techniques and the rapid growth of single-molecule methods. As modern experiments examine a variety of complex systems, the validity of the assumptions that underlie analysis of FRET data is unclear. In this talk I will examine one of these, the ideal dipole approximation (IDA). Calculations showing the breakdown of the IDA in several commonly-used FRET probes (e.g. Fluorescein, AlexaFluor 488 and 594, Cy3, Cy5) will be presented and connections will be drawn to the impact on FRET experiments. In particular, breakdown of the IDA exacerbates problems due to limited sampling of dye orientations (i.e. the kappa squared problem). Guidelines will be suggested for planning a FRET experiment to avoid potential issues with the IDA and other assumptions employed in analysis of FRET data.

3012-Pos

The First All-Nucleobase Analog FRET Pair

Søren Preus[1], Karl Börjesson[2], Kristine Kilså[1], Afaf H. El-Sagheer[3], Tom Brown[3], Bo Albinsson[2], L. Marcus Wilhelmsson[2].
[1]Department of Chemistry, University of Copenhagen, Copenhagen, Denmark, [2]Department of Chemical and Biological Engineering/Physical Chemistry, Chalmers University of Technology, Gothenburg, Sweden, [3]School of Chemistry, University of Southampton, Southampton, United Kingdom.

The fluorescent nucleobase analogs of the tricyclic cytosine (tC) family are promising nucleic acid probes capable of being inserted into double-stranded DNA as a replacement for one of the natural bases without perturbing the overall double helical structure. The high fluorescence quantum yields in both single- and double stranded DNA, combined with a rigid and well-defined position inside the DNA double helix, make these molecules particularly well suited as fluorescence resonance energy transfer (FRET) probes in nucleic acid studies. Recently we reported the first all-nucleobase analog FRET-pair, consisting of tC^O as the donor and the newly developed tC_{nitro} as acceptor which will be the focus of this presentation.[1-3] The FRET-pair successfully monitors distances covering up to more than one turn of the DNA duplex and, more importantly, the rigid stacking of the two base analogs, and consequently excellent control of the their exact positions, results in a very high control of the orientation factor in the FRET efficiency. A set of DNA strands containing the FRET-pair at wisely chosen locations will, thus, make it possible to accurately distinguish distance- from orientation-changes using FRET. We believe the development of this new tool opens up a wide range of possibilities in the structural investigation of nucleic acids, *e.g.* in characterizing DNA-protein complexes and in monitoring the inherent dynamics and the structural changes of nucleic acids in response to all kinds of stimuli.

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3013-Pos

A Fluorescent Indicator Monitors in Vivo Acetyl-Transferase Activity

Fernanda Ricci[1,2], Daniele Arosio[2], Fabio Beltram[1,2].
[1]IIT≅NEST, Center for nanotechnology Innovation, Pisa, Italy, [2]NEST, CNR-INFM and Scuola Normale Superiore, Pisa, Italy.

Lysine acetylation was identified in histones as a posttranslational modification that plays an important role in chromatin regulation. Following that discovery many other nuclear and cytoplasmatic proteins have been identified as targets of this modification. Acetylated proteins are often involved in the regulation of DNA transcription, cell growth, differentiation and epigenetic information. Moreover, aberrant levels of acetylation were reported in various human diseases such as neuropathologies and cancer (Watson, J.A. et al. 2009). To date, however, no methods for real-time monitoring of acetyltransferase activity are available for application in living cells.

We shall present the first cell-permeable fluorescent indicator of acetyltransferase activity in live cell cultures. The sensor consists of the basic domain of the HIV-1 trans-activator protein (Tat) labeled with a pair of fluorescent dyes

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

Syed S. Ahsan, Huimin Chen, Mitk'El B. Santiago-Berrios,
Hector D. Abruna, Watt W. Webb.
Cornell University, Ithaca, NY, USA.

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

Geoffrey F. Strouse, Tim Logan, Steven Hira.
Florida State University, Tallahassee, FL, USA.

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

Chia-Pin Pan[1], Pedro L. Muíño[2], Mary D. Barkley[3], **Patrik R. Callis[4]**.
[1]SRI International, Menlo Park, CA, USA, [2]St. Francis University, Loretto, PA, USA, [3]Case Western Reserve University, Cleveland, OH, USA,
[4]Montana State University, Bozeman, MT, USA.

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

Jose R. Sepulveda-Ugarte, Maximiliano Figueroa, José Martínez-Oyanedel, **Marta C. Bunster**.
Universidad de Concepcion, Concepción, Chile.

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

Lauren A. Regueyra, Erik J. Alquist, Joshua Jasensky, M. Junaid Farooqi, Paul Urayama.
Miami University, Oxford, OH, USA.

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

Hsin-Chih T. Yeh, Jaswinder Sharma, Jennifer S. Martinez,
James H. Werner.
Los Alamos National Laboratory, Los Alamos, NM, USA.

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,