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