1611-Pos Efficient Site-specific Labeling Of Proteins Via Cysteines

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Board B587

Methods for chemical modifications of proteins have been crucial for the advancement of proteomics. In particular, site-specific covalent labeling of proteins with fluorophores and other moieties has permitted the development of a multitude of assays for proteome analysis. A common approach for such a modification is solvent-accessible cysteine labeling using thiol-reactive dyes. Cysteine is very attractive for site-specific conjugation due to its relative rarity throughout the proteome and the ease of its introduction into a specific site along the protein's amino acid chain. This is achieved by site-directed mutagenesis, most often without perturbing the protein's function. Bottlenecks in this reaction, however, include the maintenance of reactive thiol groups without oxidation before the reaction, and the effective removal of unreacted molecules prior to fluorescence studies. Here, we describe an efficient, specific, and rapid procedure for cysteine labeling starting from well-reduced proteins in the solid state. The efficacy and specificity of the improved procedure are estimated using a variety of single-cysteine proteins and thiol-reactive dyes. Based on UV/VIS absorbance spectra, coupling efficiencies are typically in the range of 70~90%, and specificities are better than ~95%. The labeled proteins are evaluated using fluorescence assays, proving that the covalent modification does not alter their function. In addition to maleimide-based conjugation, this improved procedure may be used for other thiol-reactive conjugations such as haloacetyl, alkyl halide, and disulfide interchange derivatives. This facile and rapid procedure is well suited for high throughput proteome analysis.

Fluorescence Spectroscopy - II

1612-Pos The Influence of Computational Method and Basis Set on Electron Transfer Integrals that Determine Tryptophan Fluorescence Quenching in Proteins

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Board B588

The ab initio computed values of electron transfer coupling integrals for photoinduced electron transfer from the ^1L_a excited indole ring state to a local backbone amide play an important role in QM-MM predictions of fluorescence quantum yields for tryptophan residues in numerous proteins. Recently, we have presented a hybrid molecular dynamics (MD)/quantum mechanical method with several advances, including ab initio calculations of the electronic coupling matrix element as a function of conformation for each protein studied (Callis et al., J. Phys. Chem. B **2007**, *111*, 10335). In particular, using the CIS method, we establish for the first time how three basis sets, STO-3G, 3-21G, and D95 influence the computed average values of electron transfer integrals taken along a 150 ps MD trajectory. We now turn our focus to the adequacy of these relatively simple basis sets and of the CIS Hamiltonian matrix to produce realistic coupling elements. We report how the computed values of electron transfer integrals depend on computational method and a further extension of the basis sets. The Generalized Mulliken-Hush (GMH) and direct CIS methods are compared for 20 tryptophans in 17 proteins. We find that the GMH and CIS values of electron transfer integrals don't correlate very well along the MD trajectory, but their average values span the same range of magnitude. Including polarized and diffuse functions in the basis sets barely affected the average value of electron transfer integrals, although for individual conformations variations by a factor of 10–100 were found, especially with small integrals. We conclude that the simplest basis, STO-3G, may be effectively used for computing electron transfer integrals at the CIS level, providing short term fluctuations are unimportant.

1613-Pos Prediction of Fluorescence Quantum Yields for Tryptophan: Improved Classical Sampling by Free Energy Perturbation

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Board B589

Recently we presented an ab initio method for predicting fluorescence quenching of tryptophan (Trp) in proteins [Callis et al J.Phys. Chem. B **2007** *111*, 10335]. The quenching is due to electron transfer from the indole ring in the ^1L_a excited state to a nearby amide, creating a dark CT state. The rate is given by the Fermi golden rule, $4\phi^2/h(V^2\rho_{FC})$. Surprisingly, the electronic coupling elements (V) turned out to be 1-2 orders larger than used in a previous empirical procedure (Callis and Liu, 2004). For agreement with experiment, the much larger coupling requires a much smaller Franck-Condon weighted density of states (ρ_{FC}) that is much more consistent with the expected large CT-La energy gap. The new method is still effective in predicting low and high quantum yields, but agreement is scattered for intermediate cases. The average ρ_{FC} is now much more sensitive to the shape of the extreme tail of the classical probability distribution corresponding to rare conformational fluctuations that have the smallest CT-La gaps. Previously, we assumed the classical probability distributions were Gaussian. As an improvement to the method, we report here calculations based on a more realistic classical distribution far from the equilibrium point by using the free energy perturbation (FEP) method (Warshel and King 1990). The FEP-augmented distributions are not Gaussian, and they show generally improved agreement with experiment.

1614-Pos Experimental and Computed Fluorescence in Single-Trp Mutants of Normal and Modeled Disease-like Prion Protein

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Board B590

The prion protein is responsible for a variety of neurodegenerative diseases. These diseases propagate through an "all-protein" mechanism in which the infectious form of the protein catalyzes conversion of the normal cellular state, leading to the production of aggregate fibrils. Due to the insolubility of the "diseased" form, its structure has not been experimentally determined to high resolution, but is known to be highly enriched in beta-sheet structure whereas the normal form is primarily alpha-helix (NMR structure). Model structures of the disease conformation, the Prusiner/Govaerts beta helix model and a modified version published by Cox, have been developed to incorporate structural changes and predict modes of aggregation. We have measured the steady state fluorescence intensity and wavelength of tryptophan in ten single-tryptophan mutants (D144W, G123W, L125W, N159W, S135W, W99F, W145Y, Y150W, Y163W, Y218W) of the prion protein in both the cellular and disease-like isoforms. Several mutants show dramatic changes in experimental quantum yield (300%) and/or λ_{max} ($>$ 10nm shift) upon conversion, whereas others show only subtle differences, identifying regions of the protein that undergo significant changes in conformation. We have compared the experimental intensities to predictions of hybrid quantum mechanical-molecular mechanical computations (Callis and Liu, J. Phys. Chem B **2004**, *108*, 4248). At this time correlation between experiment and predictions is fair, but compromised by uncertainty in the rotamer conformations of the Trp in the mutated proteins, because only the wild type NMR structure has been determined. If refinement leads to accurate predictions for the normal form, predictions of the changes upon conversion will be made for the disease-like models. Reasonable correlation of the predictions with the observed changes in fluorescence characteristics upon conversion will provide definitive discrimination between the existing models of the infectious form.

1615-Pos Ab Initio Computation of Cofactor Fluorescence Quenching by Electron Transfer to Tryptophan and Tyrosine in Proteins

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Board B591

The quenching of fluorescence from protein cofactors such as oxidized flavins and various dyes ***by*** tryptophan (Trp) and tyrosine (Tyr)–in their ground states– is of considerable interest to those working on proteins at a number of levels. The highest occupied molecular orbitals (HOMOs) of Trp and Tyr lie particularly high in energy (Trp more so than Tyr), so that Trp and Tyr can easily donate an electron from the HOMO to the relatively low energy half-occupied MO of electronically excited oxidized flavin and many dyes. Recently we have developed a method for computing ab initio electron transfer coupling elements, and we have successfully used them with our QM-MM hybrid method for quantitative computation of Trp fluorescence quenching [Callis et al., J. Phys. Chem. B **2007**, *111*, 10335]. Here we report the extension of this method to two cases of interest, FAD in flavin reductase (Fre) and fluorescein in the 4-4-20 fluorescein antibody. In Fre, the fluorescence of FAD is quenched by a single Tyr located about 5 A distant and oriented approximately perpendicular to the isoalloxazine ring. In the antibody, fluorescein fluorescence is quenched by one or more of 2 Trps and 4 Tyrs that line the binding pocket.

1616-Pos Ab Initio Prediction and Mechanism of the Tryptophan Fluorescence Quantum Yield for the Villin Headpiece Mutant N27H

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Board B592

Fast folding of the villin headpiece protein mutant N27H (HP35) has been observed experimentally by Kubelka et al. (PNAS **2005** *102*, 7517) using tryptophan (Trp) fluorescence intensity changes on the μs time scale at pH 5.5. This is presumably because of increased quenching associated with His27 cation stacking with Trp23 in the formation of the helix. Using our ad hoc QM-MM method from 2004, we find a predicted quantum yield for Trp at 300 K of ~0.01, in qualitative agreement with the experimental value of 0.04. Surprisingly, we find that quenching is primarily by electron transfer to the Trp backbone amide instead of the expected His^+ cation, which is a potent collisional quencher of Trp in solution. This conclusion was entirely based on the energy gap between the fluorescing and charge-transfer states, which is governed by electrostatic stabilization. The His^+ is located so as to stabilize electron transfer to the amide, but electron transfer to His is inhibited by its salt bridge with Glu72. We have recently developed a promising way to compute ab initio coupling elements (Callis et al J. Phys. Chem. B **2007** *111*, 10335) that allows good estimates for the His-Trp coupling at any distance and relative orientation. Using this method the average electronic coupling between Trp and the amide is calculated to be ~465 cm^{-1} while the average electronic coupling between Trp and His^+ is only ~0.50 cm^{-1}. This result further brings into question the viability of His^+ as the electron acceptor. With improved estimates of the energy gaps and Franck-Condon factors, we anticipate that our method will be useful for interpreting fluorescence-based folding experiments.

1617-Pos Development Of An Instrument For Measuring Extremely Low Concentrations Of Particles

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Board B593

Improving the quality of screening for single structures, such as bacteria, viruses, among others, has become an important priority in Biotechnology and Medicine. There is a need for a very simple, reliable, inexpensive and valid tool. This current work addresses this need. The goal of this study was to develop and test an instrument capable to detect and analyze extremely low concentrations of different types of structures in solution for use in clinical and biotechnological applications. The detection of the particles is performed using a fluorescence signal generally obtained with antibody labeling.

Our prototype consists of a sample holder for a cylindrical cuvette that can be rotated and vertically translated while having a large volume of solution excited by a laser at the wavelength of 532nm. The excitation focus is centered about 200 μm from the wall of the cuvette inside the sample. A microscope with confocal detection scans the sample for the particles' optical responses that are collected by a photomultiplier. The data is analyzed through fluorescence correlation spectroscopy, that is based on the fluctuations of the particles when they enter the volume of excitation. To achieve high S/N particles are identified with a correlation filter program based on particle passage pattern recognition. Carboxylate-modified latex particles with average size of 284nm were used for calibration and tests. Results on the identification and analysis of somatic cells in milk for the diary industry will be presented.

1618-Pos Decreasing Photobleaching by Silver Nanoparticles on Metal Surfaces: Application to Muscle Myofibrils

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Board B594

Recently it has become possible to study single protein molecules in a cell. However, such experiments are plagued by rapid photobleaching. We have recently shown that the interaction of fluorophores with Localized Surface Plasmon polaritons (LSP) induced in the metallic nanoparticles led to a substantial reduction of photobleaching. In the current paper we investigated whether the photobleaching could be further reduced when the excited fluorophore interacts with the LSP excited in the metallic nanoparticles resident on mirrored surface. As an example we used myofibrils, subcellular structures within skeletal muscle. We compared nanoparticle-enhanced fluorescence of myofibrils in the presence and in the absence of mirrored surface. The proximity of mirrored surface led to enhancement of fluorescence and to a decrease in fluorescent lifetime, much greater than that observed in the presence of nanoparticles alone. We think that the effect was caused by the near field interactions between fluorophores and LSP, and between fluorophores and propagating surface plasmons (PSP) produced in the metallic surface by the nanoparticles. Photobleaching was decreased because fluorescence enhancement allowed illumination with a weaker laser beam and because decrease in fluorescence lifetime minimized the probability of oxygen attack during the time a molecule is in the exited state.

1619-Pos Plasmon-Controlled Fluorescence

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Board B595

Fluorescence methodologies and technologies are widespread throughout nearly all aspects of biological research. The principles and applications of fluorescence have undergone extensive development and commercialization since its introduction to biochemistry in the early 1950's. Future advances in biology and related disciplines depend on the advances and capabilities of fluorescence measurements. However, these fluorescence measurements are governed by the principles of classical fluorescence, with few significant advances being made in the last couple of decades. For example, as fluorescence spectroscopists we develop new probes for imaging and microscopy, as engineers we develop new detectors, software and optical approaches to improve fluorescence detectability. However, these solutions do not alter the fundamental properties of fluorescent molecules, such as the radiative decay rate, its emissive lifetime and / or extent of photobleaching. Moreover, most fluorescence measurements use a classic orthogonal geometry to collect emission, greater than 95 % of which is lost.

However, in the last 10 years, we have seen the emergence of a new technology, *Plasmonics*. While the classic text by Raether appeared in 1988, the potential of surface plasmons laid mostly dormant with regard to fluorescence, until early into the 21st century. Since that time, the authors, their research groups and others, have explored and explained a variety of new concepts which combine surface plasmons and close-proximity fluorophores. With plasmon-controlled fluorescence, also called *Metal-Enhanced Fluorescence,* Surface Plasmon Coupled Fluorescence / emission and Anomalous Transmission, one has the opportunity to modify the intrinsic properties of fluorophores in both the near and far-field. These include enhanced system (metal-fluorophore) quantum yields, photostability and directional and focused emission, to name but just a few. In this paper, we report our recent findings for Plasmon Controlled Fluorescence and the numerous biological applications that are emerging from this collection of novel technologies.

1620-Pos Metal-Fluorophore Interactions: Progress Towards A Unified Description of Metal-enhanced Fluorescence (MEF)

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Board B596

In recent years *The Institute of Fluorescence* has described the numerous favorable interactions and biological outcomes of both plasmon resonant particles and substrates with electronically excited states. These favorable effects have included enhanced fluorescence emission from singlet states, S_1 and S_2, phosphorescence emission from triplet states, T_1 as well as enhanced absorption phenomenon with a variety of metals including, gold, silver, copper, aluminum and platinum, as well as their alloys. In addition, we have observed and described plasmon enhanced chemiluminescence intensities, as well as highly directional emission (surface plasmon coupled fluorescence / emission) as well as angular dependent metal-enhanced fluorescence from non-continuous surfaces. As a result of enhanced triplet yields, we have also observed both enhanced singlet oxygen and superoxide yields, with downstream potential applications in photodynamic therapy and sterilization, respectively.

These favorable influences on the photophysical properties of close proximity excited states to plasmon supporting particles / substrates has led to wealth of biochemical and medical applications, such as the high sensitivity and ultra fast detection of proteins (cardiac markers, bilirubin), DNA (anthrax, Hep C), RNA (ebola), peptides (BNP), as well as the development of a whole range of metal-containing particles with exceptional brightness and photostability for downstream cellular imaging applications. Our current thinking of Metal-Enhanced Fluorescence (MEF) is one whereby a near-field coupling of electronic excited states (irrespective of how excited) to surface plasmons occurs (a surface mirror dipole), the particle in-turn efficiently and rapidly radiating the quanta of the coupled excited state in both the near and far-field. In this paper, we subsequently discuss our latest findings on the development of a general unified description of metal-fluorophore interactions and their biological, medical and analytical applications.

1621-Pos Release and Detection of Anthrax DNA within a Minute using Focused Microwaves and Metal-Enhanced Fluorescence

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Board B597

We recently demonstrated the use of planar aluminum structures to focus microwave fields to a region of interest, with localized fields spatially enhanced up to 40,000-fold, as also predicted by Finite-Difference Time-Domain simulations.[1] We have also achieved spatial and temporal control of enzyme and chemically catalyzed chemiluminescence reactions, which resulted in up to a 500-fold increase in chemiluminescence photon flux using planar aluminum triangle "bow-tie" structures.

Here, we present the use of focused-microwave fields with planar aluminum surfaces, combined with the Microwave-Accelerated Metal-Enhanced Fluorescence (MAMEF) platform technology developed in the Geddes laboratories[2], for both the release and detection of Anthrax DNA within 1 minute. Focused microwaves, for 20 seconds, resulted in the release of exosporium and spore DNA which is subsequently then detected within another 30 seconds using the MAMEF technology. We have calibrated the temperature rise in between the triangles using a thermal imaging camera, and confirmed the release of DNA by gel electrophoresis and the morphological change in the exosporium of the spores with Transmission Electron Microscopy. The MAMEF technology also affords for us to distinguish between *B. anthracis* (causative strain) and *B. cereus* (non-causative closely related strain) as well as the detection of the vegetative form of *B. anthracis* itself. Subsequently, our new technology can both lyse Anthrax spores and detect specific genomic DNA within 1 minute, with an analytical sensitivity of about 100 spores (copies).

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1622-Pos A FRAP Diffusion Analysis Program for Patterned and Anisotropic Samples

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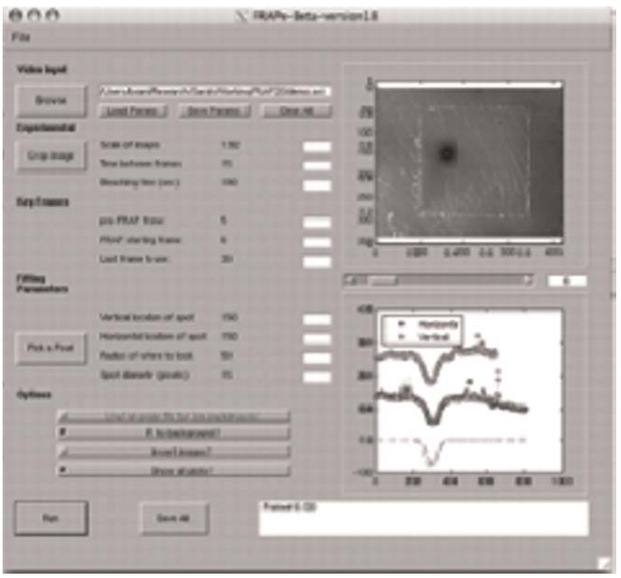
Board B598

Whether in synthetic membranes or living cells, lateral diffusion is often a defining characteristic of a system's dynamic properties. Fluorescent recovery after photobleaching (FRAP) is the prevalent method for measuring a system's intrinsic diffusion coefficient (D). Experimentally it is relatively straightforward and has remained largely unchanged since it was pioneered in 1976, with the notable exception that detection is now conventionally a multi-pixel imaging device.

Here we present a FRAP analysis technique that takes advantage of imaging detectors to enable three extensions of the conventional analysis. First, we extend FRAP to an emerging class of experiments where surfaces that are patterned (by surface-energy, protein content, roughness, etc). The method applies equally well to other inhomogeneous fluorescence distributions such as labeled cells, gradients and even dirty samples. Second, by applying the detect

anisotropic recovery, which we show can be used to infer substrate topology. Third, the approach is flexible with regards to the initial bleaching time is long and/or the bleaching-during-observation. We also present a user-interface to the community for performing FRAP on both homogeneous and inhomogeneous samples, available soon at http://parikh.ucdavis.edu/babak/FRAP.html.



1623-Pos Fluorescence recovery in photobleaching (FRIP)

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Board B599

Confocal microscopes have become widely used as tools to perform photobleaching-based measurements of the mobility of GFP tagged proteins in cells. A limitation of this approach is that the time required to bleach can be significant compared to the characteristic recovery time, especially for rapidly diffusing molecules. As a result, molecules continuously exchange between the bleach region and surrounding area during the bleach. This causes depletion of fluorescence from the area surrounding the bleach region, leading to a systematic underestimation of recovery times and corresponding diffusion coefficients. Here, we described a method that can be used to extract diffusion coefficients from the loss of fluorescence that occurs during the bleach, a technique that we term fluorescence recovery in photobleaching (FRIP).

1624-Pos TIRF-EC Microarrays for Accurate and Rapid Detection of DNA and Protein Molecular Markers

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Board B600

Total internal reflection fluorescence (TIRF) combined with electrochemistry and electric field control (TIRF-EC) is a platform technology, which is capable of detecting thousands of DNA/RNA and protein molecular markers in a matter of several seconds or a few minutes. The detection limit of TIRF-EC is at the level of single molecules. TIRF-EC biosensors are well suited for point-of-care and field applications, since they require no or minimum sample preparation stages. Cartridges of the TIRF-EC biosensors carry microarrays of assays for simultaneous detection of multiple DNA/RNA and proteins. In contrast to traditional DNA and protein arrays, TIRF-EC sensors monitor dynamics of association and dissociation, which allows for discriminating SNP in DNA/RNA targets and distinguishing between close homologs of proteins. In this presentation we report data on sensitivity, selectivity, and rate of responses for autonomous portable TIRF-EC biosensor.

1625-Pos Spectroscopic And Toxicity Studies Of Substituted Piperdones: Potential Two-photon Photodynamic Therapy Chromophores

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Board B601

One treatment for cancer that has shown much promise is photodynamic therapy (PDT). In this treatment selected chromophores are used, that when activated with light cause the production of singlet oxygen, which subsequently triggers apoptosis leading to cell death. Here, we present results from spectroscopic and toxicity studies of a new class of potential two-photon PDT agents, N-alkylated 3,5-bis (arylidene)-4-piperidones. These compounds have a donor-π-acceptor-π-donor structure. The advantages of using two-photon compounds are that PDT treatments can be more highly localized resulting in less non-tumor tissue damage, and the use of near infrared light allows for deeper tissue penetration. Spectroscopic data from fluorescence and absorption studies will be presented, including fluorescence lifetimes and two-photon cross sections. Results from cytotoxicity and phototoxicity tests of these compounds on human tumor cell lines will also be presented. Where possible correlations will be made of spectroscopic properties and molecular structure with biological activity of the PDT compounds.

1626-Pos Encapsulation of Fluorescent Polyelectrolytes in Ribonucleoprotein Vaults

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Board B602

We investigate the incorporation of semiconducting polyelectrolytes, poly(2,5-methoxy-propyloxy sulfonate phenylene vinylene) [MPS-PPV], into ribonucleoprotein vaults—hollow barrel-like protein cages. They are the largest ribonucleoprotein found in higher eukaryotic cells. Although cellular function of these protein cages is unknown, its hollow cavity and subcellular localization suggest that they may be involved in nucleo-cytoplasmic transport. Their hollow capped-barrel shape is measured to be 40 by 70 nm. However, a mechanism for access to the vaults' interior has not been identified. By filling the cage interior with semiconducting polyelectrolytes, whose photophysics is strongly dependent on its environment, we can compare polymer conformation in these confined systems to conformation in solution using fluorescence spectroscopy and Small-angle X-ray Scattering (SAXS). The combined results from fluorescence measurement, fluorescence quenching studies, and SAXS measuments indicates that luminescent semiconducting polymers can be localized in of the vaults interior. And the results indicate that vaults can potentially be used as biologically synthesized nanocapsules for delivery and encapsulation applications.

1627-Pos Using Multiple Luminescent Probes To Report Molecular Mobility In Amorphous Solids

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Board B603

The rates of physical change and chemical reaction in amorphous solid biomaterials are controlled in mysterious ways by the molecular mobility of the closely packed, hydrogen bonded matrix. In an effort to understand how mobility modulates these rates, we have developed a small library of polar luminescent probes that are sensitive to local dynamic properties of the matrix. This study has explored the possibility of using multiple probes simultaneously dispersed within an amorphous solid to investigate the mobility of different components (protein and carbohydrate, for example) with the matrix.

Phosphorescence emission spectra and time-resolved intensity decays were collected for each probe in binary mixtures of erythrosin B, vanillin, and tryptophan dispersed within thin films of amorphous sucrose. Data collected from these mixtures at a variety of temperatures were compared to data collected from sucrose films containing a single probe. Maximum emission energy and bandwidth were determined by fitting spectra to a log normal distribution function while lifetimes were determined using either a stretched exponential decay function (erythrosin B and tryptophan) or a sum of exponentials (vanillin).

Emission energies and lifetimes from the binary mixture of erythrosin B and vanillin probes were indistinguishable from data from films with individual probes, except for an anomalous increase in erythrosin delayed fluorescence in the mixture. Lifetimes from erythrosin B and tryptophan in the binary mixture were also similar to those in films with individual probes. Data from the tryptophan/vanillin mixture, however, differed from the single probe data due to the difficulty of selectively exciting tryptophan in the presence of vanillin. This research thus demonstrates the possibility of collecting mobility data on several components of a complex amorphous solid by simultaneous labeling with multiple probes.

Micro- and Nanotechnology Nanopores

1628-Pos Recent Developments Towards A Centralized Repository For Structural Nanobiology Data

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Board B604

We developed a prototype repository and clearinghouse for the fast exchange of information among nanobiology practitioners, interested in the structural aspects of this emerging field. This repository acts as a prototype engine for analysis tools design and structure dictionary development. New forms of annotation and data integration must be developed to fully integrate structural and biological information. Our observations on data integration and pattern mining will be discussed in this presentation.

1629-Pos Nanopore Cheminformatics Analysis Of Aptamer-target Binding Strength

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Board B605

Aptamers are nucleic acids selected for their ability to bind to molecules of interest and may provide the basis for a whole new class of medicines. They are replacing antibodies as detection reagents due to several advantages: versatility, the creation of a lab-on-a-chip approaches, low detection limits, simpler reactions to perform, and due to the diversity and specificity of aptamer-target binding.

Two varieties of bifunctional 'pseudo-aptamer' (obtained by design, not by SELEX) are examined using a nanopore detector: the three-way dsDNA junction, and the linear dsDNA with bulge. According to our design, the blunt-ended extremity of the dsDNA molecule inserts itself within the alpha-hemolysin channel, producing sensitive, highly modulated, blockade signals, while the free extremity of the DNA molecule is designed to terminate in an