View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

1094-Plat
Labeling Freedom for the Single Molecule Microscopist
Tilman Plass, Sigrid Milles, Christine Koehler, Carsten Schultz, **Edward A. Lemke**.
EMBL, Heildelberg, Germany.
Observation of single molecule fluorescence has matured into a central tool to study biomolecular structure and dynamics. As hardware and data analysis technology dramatically improved over the last decade, site-specific labeling of proteins with small but highly photostable fluorescent dyes has turned into a major bottleneck for biological applications. In vitro, traditional approaches to label natural amino acids are the most widely applied, but due to the high abundance of even rare cysteines in larger biological machineries, few protein systems are accessible. Still more difficult to achieve is the ultimate goal of visualizing protein structure and dynamics in living cells and organisms. Compared to small organic fluorophores, however, fluorescent proteins, such as GFPs, are bigger and typically have worse photophysical properties, but since they can be genetically attached to any protein, they are the usual choice for in vivo studies. We have now developed a semi-synthetic strategy based on a novel artificial amino acid that is easily and site-specifically introduced into any protein by the natural machinery of the living cell. Expressed proteins only differ from their natural counterparts by very few atoms, constituting a ring-strained cyclooctyne functional group. We show that this completely inert and non-toxic group can be stably incorporated into any protein and readily reacts with commercially available single molecule fluorophores without the need of special reagents, catalyst or non-physiological buffer conditions. Similarly to fluorescent proteins, the dye attachment site is genetically encoded and will thus facilitate precise labeling of proteins in vivo by only changing a single amino acid. In fact, the speed and specificity of this method holds great promise for applications of single molecule and super resolution techniques in living cells, and new experimental results demonstrating this potential will be presented.

1095-Plat
Computational Predictions of Exponential and Non-Exponential Tryptophan Fluorescence Decay in NATA, the Villin Headpiece Subdomain, and other Proteins
Jose R. Tusell, Patrik R. Callis.
Montana State University, Bozeman, MT, USA.
Decay of fluorescence from a single Tryptophan (Trp) in a peptide or protein is sometimes exponential and sometimes non-exponential. Understanding the details of this behavior has proven elusive. We have had qualitative success predicting average fluorescence quantum yields for a variety of proteins based on the stabilization of the charge transfer (CT) state arising from electron transfer from the aromatic ring to a backbone amide by the protein electrostatic environment. Here we report 100ns - 1 microsecond MD simulations, augmented by quantum mechanical (ZINDO) computations of the fluorescing and CT states, on N-acetyl-tryptophan-amide (NATA) and 20 single Trp proteins, including the highly studied fast folding villin headpiece. Although the environment for NATA in water is very similar to that of solvent exposed Trps on the surface of a protein, which almost always exhibit non-exponential decay, NATA shows a surprisingly pure single-exponential decay. In our simulations, all possible rotamer states are well represented, and transitions between rotamers happen at a rate of about 1 per 5 ns. Preliminary results indicate that rare conformations in which an amide carbonyl is H-bonded by 2-3 waters produce spikes of high quenching, more or less independent of rotamer state. Survival curve averaging of the 600 ns trajectory yields a single exponential decay of near 3 ns. For folded villin at 300 K, we find that rotamer transitions on Trp occur only every ~100 ns and that quenching by the nearby His+ happens only during these transient events, although the helix is always intact. Villin is therefore predicted to show extreme heterogeneity in lifetimes. This provides a mechanism for Trp fluorescence to report the global folding rate. Heterogeneity for the other proteins will also be discussed.

1096-Plat
Kinetics of Biotin Derivatives Binding to Avidin and Streptavidin
Roberto F. Delgadillo[1,2], Lawrence J. Parkhurst[2], Timothy C. Mueser[3], Katie A. Carnes[2].
[1]Stanford University, Palo Alto, CA, USA, [2]University of Nebraska, Lincoln, NE, USA, [3]The University of Toledo, Toledo, OH, USA.
The on-rate constants (k_{on}) of biotin (B) binding to avidin (AV) and streptavidin (SAV) are believed to be diffusion limited (10^9 $M^{-1}s^{-1}$). In this study; we asked whether these reactions were actual diffusion controlled, what association model and thermodynamic cycle describe the process, and what are the functional differences between AV and SAV. We have studied the B association by two stopped flow methodologies that used: I) fluorescent probes attached to B; and II) unlabeled B and HABA [2-(4'-hydroxyazobenzene)-benzoic acid]. The reactions were carried out at several temperatures, pH's and under pseudo first-order conditions with: oregon green biocytin (BcO), biotin-4-fluorescein (BFl), biotin-DNA duplex, and unlabeled B. We obtained the spectroscopy properties of the bound dye-biotin complexes to have an insight of the chemical environment surrounding B. The association data showed not cooperativity between the 1st and the 4th binding sites of AV. The k_{on} values of SAV were faster than AV's, but in both cases were slower than those expected for a diffusion limited reaction. Furthermore, the Arrhenius plots revealed strong temperature dependence with large activation energies (6-15 kcal/mol) that did not correspond either to a diffusion limited process (3-4 kcal/mol). The outcomes indicated that *AV binding sites were deeper and less accessibility than SAV*. In addition, we are reporting, *for the first time*, a second order displacement rate constant of a bound SAV complex when challenged with free B; results that are relevant for the purification technology base on these proteins. Finally, we propose a simple reaction model with a single transition state whose forward energetic parameters complete the thermodynamic cycle in excellent agreement with previous studies.

1097-Plat
Nonequilibrium Molecular Dynamics of Trp Zwitterion in Water: Picosecond Fluorescence Measurements Versus Computer Simulations
Dmitri Toptygin, Ludwig Brand.
Johns Hopkins University, Baltimore, MD, USA.
This is an experimental test of MD simulations on the picosecond timescale. Tryptophan zwitterion in TIP3P water at 278°K was simulated using CHARMM22 forcefield with the excited-state Trp atomic charges from [Toptygin et al., *J. Phys. Chem. B* **2010,** *114,* 11323]. Six stable excited-state rotamers of Trp sidechain were found with the population density peaks near (χ_1,χ_2) = (67°,80°), (−170°,57°), (−65°,115°), (65°,-85°), (−165°,-112°), (−65°,-80°). Curved boundaries between the rotamers on the (χ_1,χ_2) map were drawn along the troughs of the population density. Population density distribution within the boundaries of one rotamer reaches equilibrium in less than 20ps; equilibration between different rotamers takes much longer. At t>20ps rotamer populations can be described by a system of six first-order homogeneous linear differential equations. The solution is a sum of six terms $V_{mn}\exp(-t/\tau_n)$. Population decay of each rotamer is not monoexponential and τ_n is not a lifetime. The same set of τ_n applies to all rotamers, but a different set of V_{mn} corresponds to each rotamer. The rotamers have slightly different fluorescence emission spectra, therefore fluorescence intensity is a sum of six terms $\alpha_n(\nu)\exp(-t/\tau_n)$, where α_n vary with the photon energy hν. We have determined τ_n and $\alpha_n(\nu)$ in the global analysis of spectrally- and time-resolved fluorescence data (time resolution 65ps FWHM). Only four exponential terms could be resolved from the experimental data in H_2O at 5°C (τ_1 = 4780ps, τ_2 = 2500ps, τ_3 = 867ps, τ_4 = 411ps); according to MD simulations the fifth term (τ_5 = 241ps) has a very small amplitude, and the sixth (τ_6 = 22ps) is faster than the time resolution. For a precise agreement between the experimental and simulated values of τ_n it is necessary to lower all potential barriers between rotamers by 0.178kcal/mol. This shows that fluorescence spectroscopy can be used to fine-tune torsional parameters.

Platform: Protein Folding & Stability

1098-Plat
Measurement of Average Transition-Path Time for Protein Folding in Single Molecule FRET Experiments
Hoi Sung Chung, Irina V. Gopich, Kevin McHale, John M. Louis, William A. Eaton.
NIDDK/NIH, Bethesda, MD, USA.
The transition-path is the tiny fraction of an equilibrium molecular trajectory when a transition occurs between two states, and appears as an instantaneous jump in the measured signal in single molecule force or fluorescence experiments. Transition-paths are readily observed in atomistic molecular dynamics simulations for systems with fast kinetics, but have never been observed experimentally for any system in the condensed phase. The importance of the transition-path in protein folding is that it contains all the mechanistic information on how a protein folds and unfolds and is predicted from both theory and simulations to be heterogeneous. As a first step toward observing transition-paths in protein folding, we previously estimated an upper bound of ~200 microseconds for the transition-path time of protein G using single molecule FRET spectroscopy, 10,000 times shorter than the average unfolded-state waiting-time of ~2 seconds (Chung et al., PNAS 2009). The biggest obstacle to resolving a transition-path is to detect a sufficient number of photons during a single transition-path. To overcome this problem, we employed