3299-Pos Board B404
Non-Exponential Decay: Understanding the Correlation of Wavelength and Lifetime caused by Heterogeneity
Patrik R. Callis.
We report work that leads naturally to the missing underlying universal physical principle accounting for the strong correlation between tryptophan (Trp) decay associated (DAS) fluorescence wavelength (λ_{max}) and lifetime (τ_f), *in the absence of solvent relaxation*. The familiar broad fluorescence spectrum of a solvent-exposed chromophore is actually an ensemble average of single molecular λ_{max} values, fluctuating on a femtosecond time scale typically over 4000 cm^{-1} or 40 nm. In this dynamic picture, those conformers having shorter wavelength emission spectra, i.e., higher average energy, have an increased probability for transient fast electron transfer (ET) during large fluctuations in environment that bring a high energy, non-fluorescent charge transfer (CT) state and the fluorescing state (S_1) into resonance. For Trp, the CT state lies well above the S_1 state, *and* the wavelength is quite sensitive to local electric field. In these cases, heterogeneity and relaxation *both* can lead to time dependent red shifting fluorescence, making heterogeneity difficult to prove. Studies using non-natural amino acids expose the reality of heterogeneity by contrasting behavior. The fluorescence decays of 5-fluoroTrp incorporated in proteins are much more nearly monoexponential. This probe is not as easily quenched by ET as Trp because of its higher ionization potential, but it retains full wavelength sensitivity. Abbyad et al.(2007), find that time resolved fluorescence spectra of Aladan behave *oppositely*. When incorporated at several sites in the protein GB1, λ_{max} shifts to *shorter* wavelength on the nanosecond time scale– unequivocally revealing ground-state heterogeneity, because relaxation always requires a red shift in time; this is consistent with the observation that τ_f for this probe *decreases* with increasing solvent polarity by an internal mechanism.

3300-Pos Board B405
Ultrafast (“Quasi-Static”) Quenching of Trp in Proteins and Peptides
Jay R. Knutson, Arianna Biesso, Jianhua Xu.
Femtosecond UV Upconversion Spectrophotofluorometers have, in recent years, provided a very new look at the Trp environment within proteins. For any protein in water, one observes the ~2ps energy loss due to bulk water relaxation; moreover, in some proteins, the signatory risetime in emission intensity at redder wavelengths can include some slower (15-100ps) processes involving relaxation modes of either the protein or coupled water interface.
We have taken more interest, however, in the environmental ***heterogeneity*** that is visible even at these short times. Positive definite preexponential (DAS, decay-associated spectra) terms are seen associated with lifetimes of 20-300 ps ; these require very effective quenching partners. Since this ultrafast quenching process is invisible in regular lifetime instruments, we refer to it as ‘‘quasi-static self quenching’’- a loss in yield without apparent lifetime reduction. QSSQ is found even in simple Trp dipeptides, thus these potential quenchers are ubiquitous . In proteins like crystallins, strong QSSQ is apparently employed to limit lens photodamage from the excited Trp singlet.
These rapid quenching events are reconcilable with QM-MM simulation, and the heterogeneity appears to originate not only from local Trp orientation (i.e. rotamers), but also from more distant conformers that move the quencher (eg. creating more proximate and more distant subpopulations).
Unresolved QSSQ might complicate rotamer assignment and this population of ‘‘dark’’ conformers will often exceed the bright population. QSSQ rates and proportions vary as proteins change conformation.
Finally, we find some QSSQ is too fast for our current (~300fs) resolution; this implies Trp must either have intimate contact with a quencher or some alternative prompt deactivation channel; both potential mechanisms require further exploration.
In all, QSSQ provides a new set of handles on protein conformation and motions.

Computational Methods II

3301-Pos Board B406
Automated Umbrella Sampling Simulations for the Calculation of Multidimensional Potentials of Mean Force
Wojciech Wojtas-Niziurski.
The potential of mean force (PMF) describing the conformational changes of biomolecules is a central quantity that determines the function of biomolecular systems. Calculating a multidimensional PMF (free energy landscape) is a time consuming process. Each additional reaction coordinate drastically increases the required simulation time for conformation sampling, making calculations in four or more dimensions practically impossible. However, in most cases, only a small fraction of such multidimensional space is energetically relevant. PMF calculations in high dimensionality could thus be achieved if one could effectively focus the simulation effort on those region of high interest. We have developed a method based on umbrella sampling (US) that determines, using a feedback mechanism, which regions of the multidimensional space is worth exploring. The first aim of such application is to manage the creation and analysis of sampling windows for the calculation of PMFs involving up to four reaction coordinates. While this approach could in some cases be used to find the minimum free energy pathway underlying large conformational changes, it is not as general as other approaches that were specifically developed in that purpose. However, contrary to most other approaches, our method allows for the simultaneous characterization of several pathways, and not only the most probable one. The current implementation consists in a C++ application with a python interface that manages MD simulations performed with the biomolecular simulation program CHARMM. The feedback mechanism and final PMF calculation involves the unbiasing of simulations using the weighted-histogram analysis methods (WHAM).

3302-Pos Board B407
A Dynamic Model of Furrow Ingression during Cytokinesis
Christopher C. Poirier, Win-Pin Ng, Douglas N. Robinson, **Pablo A. Iglesias**.
Cytokinesis is one of the most elegant transformations in nature, whereby a mother cell is separated into two identical daughter cells. This complex process relies not only on biochemical reactions, but also on the mechanics and geometry. We create a computational model to examine the role geometry, cellular mechanics and forces influence cytokinesis. Using the level set method, an effective tool for studying shape changes, coupled with high fidelity, experimentally obtained stress profiles and material models, we model cell division under a variety of conditions. We carry out a systematic, reverse engineering of the cellular subsystems, and recreate experimentally measured furrow-thinning trajectories measured for several genotypes in Dictyostelim cells. In doing so, we identify the contributions of motor proteins, substrate adhesion and surface tension in shaping the furrow-thinning trajectory. This model allows for the modular addition and removal of cellular subsystems and provides an attractive framework for the study of altered genotypes and conceptual models. In particular, it provides an effective way to study the effects of temporally and spatially molecular and mechanical inhomogeneities have on the furrow trajectory.

3303-Pos Board B408
Maxwell Relations for Single-DNA Experiments: Monitoring Protein Binding and Double-Helix Torque with Force-Extension Measurements
Houyin Zhang, John F. Marko.
Single-DNA stretching and twisting experiments provide a sensitive means to detect binding of proteins, via detection of their modification of DNA mechanical properties. However, it is often difficult or impossible to determine the numbers of proteins bound in such experiments, especially when the proteins interact nonspecifically (bind stably at any sequence position) with DNA. Here we discuss how analogs of the Maxwell relations of classical thermodynamics may be defined and used to determine changes in numbers of bound proteins, from measurements of extension as a function of bulk protein concentration. We include DNA twisting in our analysis, which allows us to show how changes in torque along single DNA molecules may be determined from measurements of extension as a function of DNA linking number. We focus on relations relevant to common experimental situations (e.g., magnetic and optical tweezers with or without controlled torque or linking number). The relation of our results to Gibbs adsorption is discussed.

3304-Pos Board B409
Structural Mapping of MHCII-Eluted Peptides to their Source Proteins: A Preliminary Survey of the Effect of Structure on Immunodominance
Karen Katrina Manalastas, Denise Mirano-Bascos, Neil Andrew Bascos, Pablo Manalastas.
The prediction of which peptides would bind to major histocompatibility complex class II molecules (MHCII) has long been of interest as a method of predicting immunodominance. Most approaches for MHCII binding prediction have been sequence-based (i.e. predicting which amino acids are optimal binders to the MHCII binding groove). Alternatively, a structure-based view for MHCII-peptide binding may be taken. Recent studies suggest that the population of peptides available for binding MHCII may be dependent on exposed protease cleavage sites in the protein structure at different stages of antigen processing. Thus, we hypothesized that a survey of MHCII-binding peptides would reveal them to be clustered at a particular depth in their respective undigested source proteins as these areas would be exposed at appropriate times for loading into MHCII. A population of 127 documented MHC-II binding peptide sequences with available source protein structure files in the RCSB Protein Data Bank was used for this study. An algorithm was constructed to determine the relative position of these peptides in their source protein. The surface of each source protein was estimated by computing the convex hull of their