Protein Dynamics I

1213-Pos

Dynamics and Co-Localization of the Electron Transport Chain of Escherichia Coli: Investigations Through Fluorescence Microscopy

Alex Robson, Mark Leake.
Oxford University, Oxford, United Kingdom.

Total Internal Reflection Fluorescence Microscopy (TIRFM) is a key tool in probing the dynamics and distribution of fluorescently tagged fusion proteins. This non-invasive *in vivo* technique is suited to the investigation of membrane-bound proteins due to the low cytoplasmic response, sometimes allowing for ~millisecond trajectory sampling of membrane bound fusion proteins. In addition, multi-wavelength TIRFM set-ups allow for investigations into co-localization of multiple fluorescently labeled protein species.

Presented here are simultaneous dual-color TIRFM investigations of key proteins involved in the branched Electron Transport Chain (ETC) in *Escherichia coli,* using a series of single and dual-labeled mutant strains. Of interest was the comparative degree to which these proteins reflected a 'free-diffusion-collision' model of protein interactions, to that of a 'solid state' model in which reacting proteins are confined and co-localized.This builds upon previous work that identified a key protein in the ETC as having a highly non-random heterogeneous distribution, being located into functional mobile membrane patches. We investigated the spatial distribution and the degree of co-localization of these protein pairs at the cellular level. We quantified the diffusion behavior of the observed trajectories comparing the relative populations which displayed freely diffusing characteristics (through standard Brownian motion), with the populations that exhibited confined or anomalous modes. We discuss the implications of the observed dynamics at the systems level, and how this relates to the overall functioning of the branched ETC.

1214-Pos

Spectroscopic Properties of Truncated GFP and Synthetic Strand Reassembly

Kevin P. Kent.
Stanford University, Stanford, CA, USA.

Semi-synthetic green fluorescent proteins (GFPs) can be prepared by producing truncated GFPs recombinantly and assembling them with synthetic β-strands of GFP. The yield from expressing the truncated GFPs is low, and the chromophore is either partially formed or not formed. An alternative method is presented in which full-length proteins are produced recombinantly with a protease site inserted between the structural element to be removed and the rest of the protein. The native peptide can then be replaced by cutting the protease site with trypsin, denaturing in guanidine hydrochloride to disrupt the complex, separating the native peptide from the rest of the protein by size exclusion, refolding the truncated protein, and mixing the truncated protein with a synthetic strand. We report the unusual spectroscopic properties of refolded GFP with strand 11 missing, which is surprisingly fluorescent considering the free chromophore in aqueous solution is very weakly fluorescent. We also report the slow reassembly kinetics of strand 11 with the truncated GFP.

1215-Pos

Measurement of Protein Binding Rates in Live Cells with FCS

Ariel Michelman-Ribeiro[1], Davide Mazza[2], Tilman Rosales[2], Timothy J. Stasevich[2], Hacene Boukari[2], Vikas Rishi[2], Charles Vinson[2], Jay R. Knutson[2], James G. McNally[2].
[1]NIST, Gaithersburg, MD, USA, [2]NIH, Bethesda, MD, USA.

In-vivo binding rates of transcription factors are of great biological interest, yet are difficult to measure or verify independently. The commonly used technique of FRAP has certain limitations, such as capturing fast dynamics, and measured association and dissociation constants have not been verified by other techniques (Sprague, B.L. et al., Biophys J 86, 3473 (2004)). The sister fluorescence technique, FCS, is sensitive to fast dynamics, and should be able to provide independent verification of binding parameters, if the appropriate model is applied. We have developed a new procedure for analyzing FCS autocorrelation functions in the presence of diffusion and binding. We present analysis of 2-photon FCS data collected from transcription factor fragments in live cells. We compare the diffusion and binding parameters with those obtained from quantitative FRAP and find consistency. (see Michelman-Ribeiro, A. et al., Biophys. J. 97, 337 (2009))

1216-Pos

Probing the Changes in Antibody Flexibility During Affinity-Maturation Using Single Molecule Spectroscopy

Elias Aba-Milki, Patricia B. O'Hara.
Amherst College, Amherst, MA, USA.

Affinity maturation of an immune response is characterized by the expression of antibody proteins with increasing specificity and selectivity over the course of an organism's response to a pathogen. Immature germ line antibodies contain sites that can bind to a host of related small molecules or small regions of larger molecules with low affinity. Over time, new antibodies with increasing affinity and selectivity are expressed. My experiments will test the hypothesis that affinity maturation is a direct result of the loss of flexibility characteristic of the immature germ line antibody binding site. Binding sites from more mature antibodies are pre-conformed to the small molecule epitopes on the pathogen and therefore not flexible, thus reducing the entropic cost of binding, leading to higher affinity.

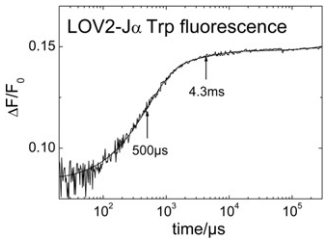
Both intact and Fab fragments of antibodies at various stages of the immune response will be analyzed using fluorescence lifetime imaging microscopy (FLIM) at the single molecule level. This new technique is made possible through the construction of a single molecule fluorescence microscope. Lifetimes and lifetime distributions of donor and acceptor fluorescent labeling dyes tagged to the antibody will be measured for antibodies raised against two classes of small molecule haptens: diketone haptens and PLP-lysine derived haptens. Monoclonal antibodies producing cells have been derived from various points in the affinity maturation process. Lifetime images will be collected with and without hapten.

1217-Pos

Kinetics of Light-Induced Conformational Change in Phot1 LOV2-Jalpha Probed By Transient Tryptophan Fluorescence

Daniel Hoersch[1], Farzin Bolourchian[2], Harald Otto[1], **Maarten P. Heyn**[1], Roberto A. Bogomolni[2].
[1]Freie Universität, Berlin, Germany, [2]University of California, Santa Cruz, CA, USA.

We investigated the kinetics of the light-induced conformational changes of the LOV2-Jalpha domain of oat phototropin1 by transient tryptophan fluorescence spectroscopy from µs to seconds. The figure shows a typical time trace of the transient fluorescence change normalized by the initial fluorescence. Two new transitions were detected in the tryptophan fluorescence signal which are not observed in the UV-VIS absorption photocycle of LOV2. The corresponding time constants at 20 °C are 500 µs for the major component accounting for 88% of the overall amplitude and 4.3 ms for the minor component. The quantum yield of the tryptophan fluorescence is sensitive to the environment and is therefore a good marker for changes in the secondary and tertiary structure of the protein. The fluorescence transitions are presumably coupled to conformational changes of the protein that are not sensed in the chromophore binding pocket. From the temperature dependence of the transient fluorescence signal an activation energy of 18.2 kcal/mol was obtained for the conformational change.



1218-Pos

The Contribution of Fast Protein Dynamics to Cytochrome P450 Molecular Recognition Characterized by Two-Dimensional Infrared Spectroscopy

Megan C. Thielges, Jean K. Chung, Michael D. Fayer.
Stanford University, Stanford, CA, USA.

While there is much interest in the potential contribution of fast protein dynamics to biological function, it remains a hotly debated topic in large part due to the challenges associated with experimentally characterizing fast protein motions. However, recent advances in the technique of two-dimensional infrared (2D-IR) spectroscopy now enable the study of fast protein fluctuations. One biological function where fast dynamics may play a particularly important role is molecular recognition. Molecular recognition of cytochrome (cyt) P450s is recognized as being particularly important to human health. Cyt P450s are a large family of enzymes that catalyze the hydroxylation of a wide variety of substrates and show varying degrees of substrate specificity. While dynamics have been implicated in the substrate specificity of cyt P450s, previous experimental studies have lacked sufficient time resolution. Thus, to overcome this limitation we are applying 2D-IR spectroscopic techniques toward the characterizing the role of dynamics in the substrate specificity of cyt P450 enzymes. Initial studies have focused on how the binding of different substrates affects the active site dynamics of the paradigmatic, relatively substrate-specific cyt P450cam. These and further studies of more substrate-promiscuous members of the cyt P450 family will assess the contribution of dynamics to molecular recognition, and provide a model for the contribution of fast protein motions to other biological systems.