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Combining DNA Nanotechnology and Fluorescence Polarization Microscopy to Determine the Orientation of DNA Bound Fluorophores

Deborah K. Fygenson, Christoph Schneider, Hunter Banks.

We determine the orientation of the transition dipole moment of a fluorophore bound to dsDNA using fluorescence polarization microscopy and compare with published results. We use DNA nanotubes to present the dsDNA in a known orientation and query a variety of intercalating (e.g., YOYO, TOTO), groove-binding (e.g. DAPI) and covalently linked (e.g., Fluorescein, Cy3, Cy5) dyes. We use a de Sénarmont prism in front of the camera to record simultaneous images of fluorescence polarized perpendicular and parallel to the DNA nanotube axis, making the associated polarization ratios insensitive to photobleaching. Our results suggest this technique can be used to detect helical supertwist, and possibly other nanoscale structural features, of DNA nanostructures.

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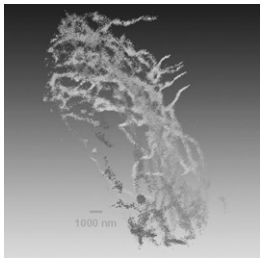
3D Visualization of Mitochondrial Network and Nucleoids of mtDNA in Ins1E and HepG2 Cells at 30 Nm Resolution by Biplane FPALM Microscopy

Andrea Dlaskova, Jitka Santorova, Michael J. Mlodzianoski, Tomas Spacek, Katarina Smolkova, Jan Tauber, Joerg Bewersdorf, **Petr Jezek**.

Three-dimensional (3D) super-resolution microscopy, using a biplane detection scheme, termed biplane photo-activated localization microscopy (Biplane FPALM), enables imaging of volumes as thick as whole cells and could reveal unknown details of cellular organization. Hence, we attempted to visualize mitochondrial reticulum via the matrix space loaded with mitochondria-addressed Eos, while transfecting cells by lentiviral expression. Our 3D images of single Eos molecules in the matrix space have proven the continuous character of mitochondrial reticulum tubules, i.e., an existence of a highly interconnected major mitochondrial reticulum in insulinoma Ins1E and oxidative-phosphorylation-dependent glutaminolytic hepatoma HepG2 cells (Fig.1). Also, using Eos-conjugate of mitochondrial transcription factor-A (TFAM), we have imaged nucleoids of mitochondrial DNA (mtDNA) in which TFAM represents a major assessor protein. Using PA-CFP2-TFAM and mitochondria-addressed Eos, the first 3D two color super-resolution images were obtained for mitochondrial reticulum together with the distribution of mt nucleoids in it. In intact cells we have found mt nucleoids of a narrow size distribution.



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Photoactivatable Hemicyanine Chromophores as Fluorescent Lables

Na Liu, Stacy Wilson, Leslie M. Loew.

A series of photoactivatable hemicyanine chromophores containing azido group instead of the usual amino donor group has been designed and synthesized. Before photolysis, these compounds have negligible fluorescence. Upon photolysis, a molecule of nitrogen is lost from the azido group leaving behind a highly reactive nitrene moiety, which can react with and crosslink neighboring molecules to produce an amine. This fully restores the ''push-pull'' environmentally sensitive hemicyanine chromophore. If the molecule is in a cell membrane it will covalently crosslink lipid or protein molecules and remain relatively immobile. The photoactivity of the dyes was tested on neuroblastoma cells by selectively shining near UV or visible light on a small region of the cell and then observing the whole cell's fluorescence using a confocal microscope. This experiment shows strong fluorescence primarily in the illuminated area, which proves that we can selectively and stably stain a portion of the cell membrane using these photoactivatable hemicyanine dyes. The photoreleased dyes are fluorescent in the membrane because the amino-chromophore is similar to our voltage sensitive ASP and ANEP chromophores. These compounds are promising as targetable sensors of membrane potential. (This work is supported by Human Frontier Science Program grant RGP0027/2009 and NIH grant R01EB001963.)

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Nuclear Receptor (PXR), Ligand and Co-Activator Interactions Measured by Total Internal Reflection Fluorescence Microscopy

Punya Navaratnarajah, Bridgett L. Steele, Matthew R. Redinbo, Nancy L. Thompson.

The pregnane X receptor (PXR) is a member of the nuclear receptor family. PXR acts in a ligand-dependent manner, in concert with its dimerization partner, the retinoid X receptor, and co-regulators, to modulate the expression of proteins that metabolize endogenous and exogenous compounds. Here, we report the use of total internal reflection fluorescence microscopy (TIRFM) for examining interactions between the ligand-binding domain of PXR (PXR-LBD), its ligands, and a peptide derived from a co-activator. TIRFM is a surface-specific technique that enables us to study the behavior of fluorescent species close to or at interfaces. Our experimental system consists of biotinylated PXR-LBD immobilized on a fused silica substrate coated with NeutrAvidin and ovalbumin, a ligand (e.g., the antibiotic rifampicin) and a fluorescently labeled, 25 amino acid fragment of the steroid receptor co-activator-1 (F-SRC-1). Using TIRFM, we measured the surface-associated fluorescence as a function of the F-SRC-1 concentration at fixed ligand concentrations. These curves were fit to a model of single-site binding to obtain apparent equilibrium constants. The apparent equilibrium constants as a function of ligand concentration were fit to an appropriate model to obtain binding constants for the ligand/PXR-LBD interaction, F-SRC-1 binding to apo and ligand-bound PXR-LBD and ligand binding to F-SRC-1-bound PXR-LBD. The approach yielded four, previously unmeasured, binding constants. These values indicate that the increase in PXR's affinity for its co-activator peptide upon binding an activating ligand is modest. This observation implies that for gene expression to be significantly upregulated, the signal arising from the activation of PXR must be amplified downstream - possibly when co-activators like SRC-1 start recruiting the cell's transcription machinery. Kinetic data obtained by combining TIR illumination with fluorescence recovery after photobleaching and/or fluorescence correlation spectroscopy may also be discussed.

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Measuring Surface Binding Thermodynamics and Kinetics by using Total Internal Reflection with Fluorescence Correlation Spectroscopy: Practical Considerations

Xiang Wang, Punya Navaratnarajah, Nancy L. Thompson.

The combination of total internal reflection illumination and fluorescence correlation spectroscopy (TIR-FCS) is an emerging method useful for, among a number of things, measuring the thermodynamic and kinetic parameters describing the reversible association of fluorescently labeled ligands in solution with immobilized, nonfluorescent surface binding sites. However, there are many parameters (both instrumental and intrinsic to the interaction of interest) that determine the nature of the acquired fluorescence fluctuation autocorrelation functions. In this work, we define criteria necessary for successful measurements, and then systematically explore the parameter space to define conditions that meet the criteria. The work is intended to serve as a guide for experimental design; in other words, to provide a methodology to identify experimental conditions that will yield reliable values of the thermodynamic and kinetic parameters for a given interaction. In vitro experiments to verify the theoretical predictions will be also provided.

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Hyperspectral Raman and Fluorescence Microscopy of Individual Algal Cells for Biochemcial Analysis

Aaron M. Collins, Howland D.T. Jones, Thomas E. Beechem III, Ryan W. Davis, Qiang Hu, Anthony E. McDonald, Jerilyn A. Timlin.

Green algae are photosynthetic microorganisms, similar in many ways to plants, that fuel cellular processes with biochemical energy derived from sunlight. Algae are known to produce and accumulate triacyglycerol (TAG) or lipid under certain stress conditions. Subsequently, algae have garnered significant research interest as candidates to produce the next-generation of advanced biofuels because lipds are convertible into biodiesel. Therefore, there is significant interest to develop methodologies and analytical tools to characterize and understand lipid biosynthesis in living algal cells.

Raman spectroscopy offers excellent chemical and spatial resolution of the carotenoid, TAG and TAG precursor compounds, while fluorescence spectral information provides complimentary and confirmatory information about the photosynthetic pigments. The combination of Raman mapping and hyperspectral fluorescence imaging can provide critical insights into the spatial biochemical makeup of algal cells. Additionally, since each algal species has its own unique set of carotenoids, the carotenoid signatures present an opportunity for identifying algae at the taxonomic group and possibly even at the species level. Here, we present a novel use of confocal Raman mapping and hyperspectral confocal fluorescence microscopy paired with multivariate curve resolution to investigate carotenoid and lipid spectral signatures in several algal strains. We will 1) Identify algal species from mixed samples based on carotenoid spectroscopic signatures 2) localize, identify and quantify carotenoid distribution in individual algal cells and 3) determine the lipid content of individual cells from several algal species. This novel approach will demonstrate the feasibility of visualizing biochemical processes in live algal cells using spectroscopic imaging techniques.