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Multiple Mine-Associated States and Nucleoid Stabilize Self-Organized Minde Pattern Formation in E. Coli

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E. coli cell division relies on harmonious cooperation between MinCDE oscillations and the protective machinery via nucleoid occlusion to create forbidden zones of Z-ring formation, by which accurate/symmetric septation is secured. Recent reports suggested MinE plays a pivotal role in spatiotemporal pattern formation of MinDE cohorts; yet it remains elusive how collective MinE organization/dynamics is emerged and orchestrated from midcell.

Here, we used fluorescence imaging to detail the mode transitions in distinct MinD/E pattern formations and other fluorescence techniques (FRAP, FCS and Single-Molecule Tracking) to probe the organization/interactions of MinD/E assembly in living cells. Despite E-ring capping has been viewed as a drive of the pole-to-pole MinD oscillations, we found peculiar spatiotemporal expression signatures in MinD/E, corresponding to MinDE patterns other than the E-ring type, correlate to their relative expression levels. Further study by FRAP reveal the organization of MinD/E patterns is actualized through constant remodeling with rapid turnover of their dimers or short filaments. The nature of collective MinD/MinE interactions in living cells are quantitatively determined by FCS and the apparent diffusivity of MinE in single cells were measured by SMT. The results of these biophysical interrogations substantiate a state of MinE associated species with slow mobility and imply the possibility that MinE could commit an unknown interaction other than forming MinD/E assembly. We infer such an interaction related to nucleoid, because, as nucleoid was perturbed (removed), oscillation pattern/frequency of MinDE becomes stuttered (unstable).

Taken together, MinE is proposed to interact with nucleoid for keeping oscillation rhythms on check through buffering MinE molecules around marginal MinD pattern, which is considered as the origin of E-ring capping. Our results suggest a new scenario of how MinDE self-organize dynamic patterns and collaborate with nucleoid to maintain quasi-periodic oscillations.

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Synergistic Interactions of Alzheimer's Aβ40 and Aβ42 on the Surface of Primary Neurons by Single Molecule Microscopy

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Experiments with amyloid-β (Aβ) peptides 40 and 42 have supported the hypothesis that they feature in the synaptic dysfunction and neuronal loss associated with Alzheimer's disease. However, the underlying mechanisms and molecular interactions between these peptides and the neuronal membrane are still not fully understood. Our single molecule study on model membranes has shown that at physiological concentrations both Aβ40 and Aβ42 form dimers quickly on the membrane while they remain mainly monomers in solution. The membrane bound dimers exist in two states: mobile and immobile. Work using live neuron membrane has shown that either Aβ40 or Aβ42 binds tightly to the membrane in the form of small oligomers that do not grow appreciably during up to 48 hours incubation. In contrast, mixed Aβ40/Aβ42 samples (1:1 ratio) form larger oligomers on the primary neurons after 48m hours incubation. To confirm that these two peptides indeed form mixed oligomers on the neuronal membrane, Hilyte-488 (or 555) labeled Aβ40 as the donor and Hilyte-647 labeled Aβ42 as the acceptor were incubated with the primary neurons and the changes in the fluorescence resonance energy transfer (FRET) overtime were measured by fluorescence lifetime imaging microscopy (FLIM). These in vivo studies will serve to provide molecular mechanisms of Aβ40 or Aβ42 on the neuronal membrane at physiological conditions.

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Protein-Protein Interactions In Vivo Studied by Single Plane Illumination Fluorescence Correlation Spectroscopy (SPIM-FCS)

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Characterizing protein mobility and interaction in vivo is a challenging problem, highly relevant for understanding biological function. Here we present first results from a 2D-parallel fluorescence (cross) correlation (F(C)CS) technique using single plane illumination microscope (SPIM). The goal is to understand the function of transcription factors (TFs) through their localization, binding dynamics and transport to their sites of action in the cell nucleus. Our model system is the well-known TF pair c-Fos and c-Jun, which form a heterodimer and participate in proliferation, differentiation, apoptosis, and oncogenesis. using autofluorescent proteins such as GFP and mRFP, the interaction between these TFs in living cells can be quantified by F(C)CS.

Our SPIM uses as detector either an electron multiplying CCD (EM-CCD) camera or a newly developed array of avalanche photodiodes with a time resolution of 10 µs per frame on 32x32 pixel images. By expansion of the single color to two color detection with the use of a second laser and dual-view optics, we can now observe the dynamics and quantify the interaction of c-Fos-GFP and c-Jun-mRFP proteins across an entire living cell. The results are presented in the form of diffusion and concentration maps, characterizing for the first time directly in vivo the action of c-Fos and c-Jun throughout the entire nucleus. This yields important new information about the spatiotemporal organization of c-Fos and c-Jun.

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Single Molecule Measurements of TCR:MHC Binding Kinetics in Living Primary T Cells

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The central question in immunology is how T cells distinguish self from foreign antigens. At the center of this question is the T cell receptor (TCR), which is constantly challenged with the daunting task of recognizing foreign antigens among countless self-peptides presented by MHC molecules. We use live primary T cells and supported lipid bilayers (SLB) to investigate the mechanism of TCR triggering and subsequent T cell activation. using TIRF microscopy and multi-timescale imaging strategy, individual molecules were tracked with temporal resolution spanning from milliseconds to minutes. These observations allow direct readout of pMHC:TCR binding kinetics in situ and are being used to map the physical signature of antigen.

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Investigating Rate of TraI and DNA Transfer during Bacterial Conjugation

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F plasmid TraI (192 kD) is essential for DNA transfer during bacterial conjugation. TraI is composed of a relaxase domain, ssDNA binding domain, helicase domain, and C-terminal domain. Some TraI mutants with a 31 AA insertion (i31) in different domains exhibit higher mating efficiencies than wild-type (WT) TraI. To investigate the higher mating efficiency, we employed a live cell SeqA-YFP fusion protein system that resides in dam- recipient cells and tracks DNA transfer from a dam+ donor. After methylated ssDNA is transferred to the recipient and second strand synthesis begins, a fluorescent focus forms on the hemi-methylated dsDNA, allowing for near-real time analysis of bacterial conjugation. Mean foci formation time for the mutant TraI i31-681 was much lower than WT TraI. To determine if the change in foci formation time was due to second strand synthesis, the assay was repeated, but mating was disrupted at set times. Foci formation was followed over time and the results were similar, indicating the differences between mutants and WT was not due to a difference in second strand synthesis rate. To further investigate where the transfer process is altered, we employed a DNA nicking assay and determined that the nicking activity of the mutants is not altered. Addition of TraDΔN130, a pore coupling protein that increases nicking activity, to the nicking assay does not significantly alter nicking activity of the mutants over WT TraI. Nicking activity being unaffected, the 309-858 region of the TraI mutants will be tested to determine the folding profile of the i31 insertion. Currently we are exploring a possible correlation between increased transfer rates and increased unfolding rates of the domains.

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Tryptophan Uptake via Tat2 Transporters under Hydrostatic Pressure

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The yeast Saccharomyces cerevisiae is useful model organism for gaining insight into effect of stresses on eukaryotic cells because it is easily manipulated and its completely decoded genome. Hydrostatic pressure is a stress related to