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The Earths predictable light/dark cycle has led to the ubiquitous evolution of anticipatory mechanisms known as circadian rhythms which provide organisms with elevated survival and reproductive fitness. Investigations into the circadian core clock oscillator have revealed its control of an astounding amount of cellular physiology. However, the mechanistic details of the molecular oscillator are still poorly understood. The oscillator is comprised of a transcription-translation feedback loop and one of its intriguing and conserved features is the extensive level of intrinsic disorder in the negative arm elements. Given this observation, we sought to ask if these disordered regions contribute to clock timing or output, hypothesizing that plasticity could serve as a tunable mechanism for clock homeostasis. In the model organism *Neurospora crassa,* the negative arm of the clockis centered around the IDP FREQUENCY (FRQ) which has two known isoforms produced via temperature-induced alternative splicing. To investigate this role, we created mono-isoform strains to delineate any functionality they may individually impart. We discovered that the isoforms differ in their resistance to protease treatment, their cycloheximide half-life, and their interactome. To provide a mechanistic explanation for these differences, we surveyed the molecular grammar of the disordered N-terminus of L-FRQ, revealing potential degrons. To further characterize the N-terminus we performed all-atom Monte Carlo simulations with a phosphomimetic approach. Our analysis revealed N-terminal phospho-dependent transient helices which could serve as a binding motif phosphoswitch to regulate the timing of clock output or L-FRQ half-life. Embedded within one of the helices are a key hydrophobic pair that upon alanine substitution dampen out the clock *in vivo*. Summarizing these findings, we propose an isoform-specific mechanistic role for protein disorder in regulation of the circadian clock under different temperatures.

1452-Plat

Mechanistic roles of tyrosine phosphorylation in reversible amyloids, autoinhibition, and endosomal membrane association of ALIX

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ALIX is involved in a myriad of essential cellular processes, most notably acting within the ESCRT pathway to mediate events such as cytokinesis and exosome biogenesis. Regulation of ALIX has been suggested to involve intramolecular, autoinhibitory interactions between individual ALIX domains. Interaction with the tyrosine kinase Src results in inactivation and redistribution of ALIX from late endosomal membranes to the cytosol, suggesting phosphorylation plays a role in ALIX autoinhibition. Yet, the molecular details of these interactions remain unclear owing to challenges relating to characterizing the large, disordered proline-rich domain (PRD) of ALIX. Here we investigate the effects of tyrosine phosphorylation on the interplay between structure, assembly, and interactions of ALIX. We report PRD formed amyloid fibrils that dissolved upon Src-mediated hyperphosphorylation, and which restored upon PTP1b mediated dephosphorylation of conserved tyrosine residues. NMR analyses of the ALIX Bro1 domain elucidated conformational changes originating from its phosphorylation by Src and established that Bro1 interacts with both hyperphosphorylated PRD and anionic lipids through its convex, basic surface. These results uncover the autoinhibition mechanism that relocates ALIX to the cytosol, and the diverse roles played by tyrosine phosphorylation in cellular functions of ALIX.

Platform: Optical Microscopy and Superresolution Imaging II

1453-Plat

Multimodal single-molecule microscopy with continuously controlled spectral resolution (CoCoS)

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Color is a fundamental contrast mechanism in fluorescence microscopy, providing the basis for numerous imaging and spectroscopy techniques. Building on spectral imaging schemes that encode color into a fixed spatial intensity distribution, here, we introduce continuously controlled spectral-resolution (CoCoS) microscopy, which allows the spectral resolution of the system to be adjusted in real-time. By optimizing the spectral resolution for each experiment, we achieve maximal sensitivity and throughput, allowing for single-frame acquisition of multiple color channels with single-molecule sensitivity and 140-fold larger fields of view compared with previous superresolution spectral imaging techniques. Here, we demonstrate the utility of CoCoS in three experimental formats, single-molecule spectroscopy, single-molecule Förster resonance energy transfer, and multicolor single-particle tracking in live neurons, using a range of samples and 12 distinct fluorescent markers. A simple add-on allows CoCoS to be integrated into existing fluorescence microscopes, rendering spectral imaging accessible to the wider scientific community.

1454-Plat

Label-retention expansion microscopy toward molecular resolution

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In the past two decades, single molecule localization microscopy has revolutionized bioimaging with the superresolution. Through a different approach, a more recent technology Expansion Microscopy elevated the resolution again using expandable hydrogel. This talk will decipher a novel hybrid method, Label-Retention Expansion Microscopy (LR-ExM), which uses trifunctional chemical labels to combine these two microscopy approaches for molecular resolution imaging. This method achieved molecular resolution and outstanding labeling efficiency, which allows visualization of individual proteins, genes, and RNA molecules *in situ*. The applications of LR-ExM in cilia, clathrin-coated pits, mitochondria, nuclear lamina, and chromatins will be demonstrated in this talk.

1455-Plat

Use of sub-cellular fluctuation imaging (SCFI) as a rapid antimicrobial susceptibility test

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A rapid Antimicrobial Susceptibility Test (AST) facilitates proper diagnostics and prescriptions, decelerating the progression of antimicrobial resistance. We developed a rapid AST technique called Sub-Cellular Fluctuation Imaging (SCFI) in which bacteria absorbed onto a glass surface are observed using Total Internal Reflection microscopy. Under an evanescent illumination, SCFI detects a fluctuating scattering signal from within a bacterium. To locate the origin of this signal, we measured the SCFI-based fluctuation level at different penetration depths of the evanescent field and found that the relationship is consistent with moving scatterers within the cytoplasm of *Escherichia coli* (*E. coli*). We also found that the effect of cellular energy depletion caused by 2,4-dinitrophenol and carbonylcyanide-m-chlorophenylhydrazone on *E. coli* significantly decreased the amplitude of fluctuation, showing a link between fluctuations and the metabolic activity. When exposing *E. coli* to the bacteriostatic antimicrobial trimethoprim, the fluctuation amplitude decreased and returned to initial values once removing trimethoprim from the solution. When using bactericidal antimicrobials, we could observe the different responses between susceptible and resistant bacteria in both *E. coli* (Gram-negative) treated with kanamycin and *Staphylococcus aureus* (Gram-positive) treated with cephalexin. We also demonstrate a notable improvement in SCFI-based AST performance by implementing a Convolutional Neural Network (CNN) algorithm. Using preliminary data sets, CNN could classify the kanamycin susceptible and resistant *E. coli* with an accuracy of 95.1%. We envisage that a combination of SCFI-based AST and machine learning technology can minimise the time required for an AST below 15 minutes, while maintaining an overall accuracy above 90%.

1456-Plat

Whole-cell quantitative imaging of structural changes induced by SARS-CoV-2 using soft X-ray tomography

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Quantitative analysis of three-dimensional (3D) cell structure and composition is crucial for unveiling mechanisms of virus-host interactions. To study the interaction of virus particles with cells at the sub-cellular ultra-structural level, we are using soft x-ray tomography (SXT) to image the whole cell with minimal sample preparation. SXT takes the advantage of the ''water window'' property to acquire 3D tomographic data non-invasively and free of labeling. Here, we employ soft x-ray tomography (SXT) with a full-rotation stage to examine organelle remodeling induced by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) at the whole-cell level with high spatial resolution and throughput. Our data provide a signature X-ray linear absorption coefficient (LAC) to analyze intracellular changes induced by SARS-CoV-2. This rapid, high-throughput imaging technique allows us to visualize the spatiotemporal changes of cellular organelles during viral infection in a quantitative manner. Our results indicate that the aggregates of SARS-CoV-2 virions and virus-induced intracellular alterations, including double-membrane vesicles, convoluted membranes, and large intracellular compartments, can be quantitatively investigated during viral infection.

1457-Plat

Molecular cartography: charting the sea of molecular organization in live synapses with nanoscale precision

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Understanding live-cell behavior in part requires high precision mapping of molecular species in 3-D dynamic environments. Approaches like single-molecule localization microscopy (SMLM) offer high promise for challenges posed by molecular cartography. Effectively, the precision of these approaches is dependent on the how many photons / second a fluorescent marker is capable of emitting. For this reason, many SRLM experiments are typically done using fluorescent organic dyes (such as Alexa Fluors) in reducing chemical environments which cause some organic dyes to stochastically cycle through dark states, allowing single-molecule localization (e.g. (d)STORM). The need to couple these dyes to antibodies and the harsh reducing conditions makes their application to live cell work problematic. To overcome these limitations, we made use of modifications to Janelia Fluor-based dyes which make them spontaneously cycle through dark states (blink) under physiological imaging conditions. The dyes are spectrally compatible with photo-activatable fluorescent proteins such as mEos and allow for simultaneous 2-color superresolution microscopy. When conjugated to a HaloTag, these artificial dyes can bind genetically encodable targets in live samples, allowing subsequent measurement in a live-cell environment. To correct for nanoscale chromatic aberrations we developed a new machine-learning based approach with reconstruction errors below achievable localization precisions. We show that these methods allow the reconstruction of live synapse surfaces and a variety of the associated molecular machineries with up to 50 nm accuracy in 3 dimensions.

1458-Plat

Diffusion and interaction dynamics of the cytosolic peroxisomal import receptor PEX5

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Peroxisomes fulfil many anabolic and catabolic functions, thereby importing all required proteins post-translationally from the cytosol. Dysfunction of the peroxisomal import process leads to severe diseases making this process a study of utmost importance. Peroxisomal proteins are recognized in the cytosol by the import receptor PEX5 and transported towards the peroxisomal membrane. Here we study the diffusion of the cargo loaded receptor in the cytosol by combining advanced live-cell microscopy and spectroscopy techniques such as fluorescence correlation spectroscopy (FCS) and superresolution STED and Minflux microscopy to present a detailed characterization of the diffusion and thus interaction dynamics as well as spatial organization of the peroxisomal import receptor PEX5. Among other features, we disclose an unexpectedly slow diffusion of PEX5, independent of many factors such as aggregation, target binding or cytoskeleton, but associated with larger cytosolic interaction partners. This sheds new light on the functionality of the receptor in the cytosol. Besides these specific insights, our study highlights the potential of using complementary microscopy tools to decipher molecular interactions in the cytosol via studying their diffusion.

1459-Plat

Single particle tracking on cells reveals new insights into membrane compartmentalization and lipid dynamics

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The study of lipid dynamics on cellular membrane is a topic of high interest. In particular, the compartmentalization observed in lipid diffusion on the cell surface has given rise to several competing models for its nano-organization, such as the picket-fence and the lipid raft hypothesis. However, investigations in this topic always prove challenging, owing to the need for simultaneous high localization precision and high temporal resolution to appropriately probe the dynamics of such systems. In this study, we show how Single Particle Tracking through Interferometric Scattering (ISCAT) microscopy can help us gain new insights in plasma membrane structure and the resulting diffusion modes of the tracked lipids. We adopt a statistics-driven diffusion mode classification pipeline, based on the analysis of the Apparent Diffusion Coefficient, which enables us to estimate relevant physical parameters of the lipid motion and membrane environment. From this analysis, we conclude that the inter- and intra-compartmental diffusion coefficients of the tracked lipids do not significantly differ, in contrast to previous findings, suggesting an underlying model of membrane made up of a continuum of nano-compartments. We sought to confirm these results with the use of simple particle diffusion simulations in a corralled two-dimensional plane. These show a remarkable similarity to the experimental trajectory recorded, thus confirming our previous conclusions. Finally, we introduce the confinement strength (S_{conf}) metric, as the ratio between the inter- and intra-compartmental diffusion coefficients. Through this metric, we are able to both connect the present results to a specific set of simulation parameters, but also to fit results from past studies, with different experimental techniques (such as Fluorescence Correlation Spectroscopy) into the same picture, leading to an increased understanding of lipid dynamics in live cells.

1460-Plat

Brightness correlation spectroscopy tracks transcription factor diffusion as a function of oligomeric state within live cell nuclear architecture

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In the crowded environment of the cell nucleus, transcription factors form homo and hetero-oligomers that modulate the exploration volume available during DNA target search, as well as DNA binding affinity. To directly track this process in a living cell here we present brightness correlation spectroscopy as a method to extract oligomeric transcription factor dynamics within live-cell microscopy data. From correlation of brightness fluctuations that originate from a fluorescently tagged transcription factor this approach has the capacity to extract protein mobility as a function of oligomeric state, and spatiotemporally map the anisotropy of this parameter with respect to nuclear architecture within a rapid single-channel frame scan acquisition. Expanding this method, which can be applied to any oligomeric protein, to a dual-channel frame scan acquisition enables measurement of the spatiotemporal dynamics that underly hetero-oligomeric transcription factor complex formation. Application of one and two channel brightness correlation spectroscopy to the signal transducer and activation of transcription (STAT) family of transcription factors reveals homo and hetero oligomer formation to differentially regulate chromatin accessibility and their interaction with the DNA template.