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(importins/exportins), depending if the cargo carries a nuclear localization signal or nuclear export signal (NLS/NES). While the molecular players are known, the spatio-temporal dynamics of the nucleo-cytoplasmic transport remains elusive. In this work, we study the size-dependent dynamics of the nucleo-cytoplasmic transport in space and time. To this end, we designed a series of fluorescently labeled molecular cargo using tandem-mCherry with localization signals, ranging in size 29–135 kDa. Using spinning disk confocal microscopy, we monitor size-dependent nucleo-cytoplasmic transport of the fluorescent cargos in live human cells. In particular, we focus on decoupling the effects of passive and active transport by inhibiting active import/export using biochemical perturbations. In addition, we implement a photo-activatable cargo system, which allows us to control the directionality of its active transport and thus isolate the contributions of active and passive transport. This work holds promise to provide fundamental insights into biophysical mechanism(s) underlying the nucleo-cytoplasmic transport. Such knowledge will aid our understanding of the nucleus in both health and disease. This work was supported by the National Institutes of Health grants R00-GM104152 and R01-GM145924 and by the National Science Foundation grants CAREER PHY-1554880, CMMI-1762506, and PHY-2210541.

1414-Plat

BPS2026 – ESPRESSO: Spatiotemporal omics based on organelle phenotyping reveals dynamic cell state transitions

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Omics technologies, while revolutionizing our molecular understanding of life, often fail to capture the crucial interplay of time and space within the living cell due to the impossibility to follow the state transition of individual cells as a function of time. We present environmental sensor phenotyping relayed by subcellular structures and organelles (ESPRESSO), a novel technique that achieves high-dimensional phenotyping resolved in both space and time at the single-cell level by leveraging morphological and functional information relative to the organelle network. ESPRESSO combines live-cell staining with organelle-specific, environment-sensitive probes, employs confocal hyperspectral imaging and spectral phasor unmixing to resolve multiple, overlapping signals simultaneously, enabling high-speed temporal acquisition. This allows us to quantitatively extract a large feature set of a dimensionality comparable to single-cell RNA sequencing, including the morphological and functional properties (such as mitochondrial membrane potential or lysosomal pH), generating an organelle-based omics signature that reflects the cell's integrated biophysical state. This powerful framework allows us to unravel cellular heterogeneity, unveil distinct stress and drug response pathways based on dynamic organelle reorganization, and trace differentiation trajectories in complex cultures, thus providing a crucial bridge between molecular omics and the active physical state of the cell. Here, we will present ESPRESSO applied to the phenotypic transition in the contexts of stress response, keratinocyte differentiation, macrophage polarization and drug response in breast cancer tumor spheroids, highlighting the power and broad applicability of this methodology.

1415-Plat

BPS2026 – Direct visualization of ER-associated degradation by multicolor single-molecule tracking

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Terminally misfolded or otherwise damaged proteins in the endoplasmic reticulum (ER) are degraded by the proteasome in a process termed endoplasmic reticulum associated degradation (ERAD). To reach the proteasome located in the cytosol, cargo proteins need to be dislocated across the ER lipid bilayer. Retrograde transport is realized by a set of

membrane-spanning multi-protein complexes that are notoriously difficult to study: Despite decades of genetic and biochemical analysis of this dislocation event, there is little consensus regarding its molecular mechanism. Here, we characterize the dynamic assembly of the mammalian Hrd1-complex, one of the most thoroughly studied mammalian dislocons, using in situ multicolor single-particle tracking. Surprisingly, quantitative dual-color tracking reveals that the majority of Hrd1 is assembled into stable homo-tetramers in situ. A herein developed single-molecule assay based on binding competition we show that this tetramerization is driven by a short cytosolic stretch (Hrd1₄₇₉₋₅₃₀, HAF-H) within the cytosolic domain of Hrd1. Combining classical purification assays, quantitative imaging, and structural predictions demonstrates that Hrd1₄₇₉₋₅₃₀ forms a highly stable tetrameric helix bundle via a conserved hydrophobic coiled-coil motif. While higher order assemblies of Hrd1 have been previously implicated, this work offers direct evidence of their role in dislocation, demonstrating that quantitative single-molecule imaging can yield information on species previously hidden for classical biochemistry.

1416-Plat

BPS2026 – VDAC isoform-specific modulation of mitochondria structure and function

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The voltage-dependent anion channel (VDAC) is the major metabolite channel of the mitochondrial outer membrane and the primary regulator of the exchange of ions and metabolites between the cytosol and mitochondria. The three mammalian VDAC isoforms share a 70% sequence similarity, and mouse knockout (KO) studies have revealed isoform-specific differences in their phenotypes, with the VDAC2KO being particularly lethal. To better elucidate the physiological role of each isoform, we studied structural changes in mitochondria of HeLa cells with CRISPR-Cas9 KOs of each VDAC isoform using electron microscopy and fluorescent confocal microscopy. Mitochondria are exceptionally dynamic organelles that can regulate oxidative phosphorylation and reductive biosynthesis in response to nutrient availability and cellular needs. They alter their shape and size through the processes of fusion and fission, and dysregulation of these mitochondrial dynamics is linked to a variety of pathologies, most notably cancer. Our mitochondrial image analysis revealed a variety of mitochondrial morphologies for wild-type (WT) HeLa cells, which may be important for cancer cell survival and adaptivity. The mitochondrial network complexity of the VDAC2KO cells was comparable to that of the WT cells; however, the cristae density was considerably increased. Paired with Seahorse analyzer respiration studies, this indicates increased dependence of HeLa cells on oxidative phosphorylation upon VDAC2KO. VDAC1KO and VDAC3KO decreased mitochondrial size and network complexities, where VDAC1KO decreased cristae density while VDAC3KO resulted in enlarged mitochondria. Despite the drastic effect of VDAC1KO on mitochondrial structure, no effect on mitochondrial respiration and cell growth was observed. Surprisingly, the KO of VDAC3, the minor isoform, displayed the most impact on mitochondrial structure and respiration. Our results suggest that VDAC plays an isoform-specific role in the dynamic regulation of mitochondrial structure and function in cancer cells.

1417-Plat

BPS2026 – Estrogen receptor modulators affect mitochondrial physiology through the voltage-dependent anion channel

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There are three mammalian voltage-dependent anion channel (VDAC) isoforms (VDAC1, VDAC2, and VDAC3) residing in the mitochondria outer membrane (MOM) where they conduct and regulate the fluxes of ions and metabolites between the cytosol and the mitochondria. VDACS interact with various cellular partners and pharmacologic compounds, thus dynamically controlling the MOM permeability and mitochondrial metabolism. The selective estrogen receptor modulators (SERMs) were designed to elicit tissue-dependent effects of endogenous estrogen (17- β -Estradiol, E2). A novel SERM, STX (analog of 4-OH-Tamoxifen) targets proopiomelanocortin (POMC) neurons and in a 5XFAD mice model of Alzheimer's disease (AD) mitigates amyloid- β toxicity and improves mitochondrial function. A