The linker histone H1 family is the most abundant group of eukaryotic chromatin-binding proteins, in which H1 is known for its role as a nucleosome compactor and an essential component of higher order chromatin structure. However, the mechanism underlying the diverse physiological functions of H1 remains unclear. Here we used single-molecule fluorescence and force microscopy to observe the behavior of H1 on DNA under different tensions in real time. Unexpectedly, we observed that H1 preferentially coalesces around nascent single-stranded DNA, forming condensate-like complexes. Moreover, molecular dynamics simulations revealed that multivalent and transient interactions between H1 and ssDNA mediate their phase separation. Controlled condensate fusion using optical tweezers showed that single-stranded nucleic acids mediate the formation of more viscous, gel-like H1 droplets. Finally, we found the number of H1 puncta in cells increased under stressed conditions, suggesting H1 may function to aid in DNA repair. Overall, our results provide evidence for a new role of H1 to sense different forms of nucleic acids and may orient the field towards viewing H1 as a more diverse player in genome organization in maintenance.

1737-Pos

Role of prion-like domains in the transcriptional reprogramming by FET-family oncofusions

Richoo Davis, Priya R. Banerjee.
Physics, University at Buffalo, Buffalo, NY, USA.

Prion-like domains (PLDs) are disordered polypeptides enriched in uncharged polar and aromatic residues.They are synonymous with RNA-binding proteins (RBPs) that aggregate and are associated with neurodegeneration such as FUS, TDP43, and hnRNPA1. At a molecular level, PLDs can drive the liquid-liquid phase separation of the RBPs and their recruitment to specific cellular membrane-less compartments. Interestingly, chromosomal translocations within cells can lead to the formation of fusion proteins where the PLD of an RBP is fused to a DNA-binding domain (DBD). FET (FUS, EWSR1, and TAF15) family fusions that occur between the prion-like domains of the FET proteins and the DNA-binding domains of the ETS family transcription factors display emergent interactions and aberrant transcriptional reprogramming leading to cancers such as sarcomas and leukemias. We use a FET-oncofusion, FUS-DDIT3, as a model to understand the distinct behavior of the fusion from its parent proteins and the mechanism underlying its function as an oncogenic transcription factor. Utilizing both *in vitro* and *in cellulo* experiments, we show that FUS-DDIT3 undergoes phase separation in the cell nucleus that is mediated via its PLD. Moreover, FUS-DDIT3 condensates enrich essential factors for transcriptional activation such as the chromatin remodeler, mSWI/SNF. The recruitment of mSWI/SNF is driven by heterotypic PLD-PLD interactions between FUS-DDIT3 and several subunits of the mSWI/SNF complex. Our results suggest that both partners in the fusion pair (*i.e.,* FUS and DDIT3) contribute to the neomorphic activity- the DBD^{DDIT3} recruits the fusion transcription factor to specific genomic loci, whereas the PLD^{FUS} drives oncoprotein condensation and engagement with the transcriptional machinery. We discovered other oncogenic fusion proteins with a PLD-DBD domain architecture that aberrantly interact with chromatin remodelers and we propose our model as a general mechanism for such transcriptional regulators to alter gene expression.

1738-Pos

How do antifreeze proteins inhibit Ostwald ripening of ice crystals?: analyzing local surface curvature changes at high resolution

Agata M. Malinowska[1], Tim Huijsmans[1], Siem de Jong[1], Sharon de Jong[1], Roderick Tas[2], Roma Suris Valls[2], Ilja K. Voets[2], Iddo Heller[1].
[1]Physics and Astronomy, Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [2]Chemical Engineering and Chemistry, Eindhoven University of Technology, Eindhoven, Netherlands.

Many lifeforms produce ice-binding proteins, sometimes referred to as antifreeze proteins, that can protect them against frost-damage by controlling the nucleation and growth of ice crystals. Our goal is to elucidate how these proteins can modulate the water-ice phase transition and function as such remarkable cryo-protectants. Specifically, we seek to directly resolve how antifreeze proteins dynamically organize at the solid-liquid interface and interact with advancing ice-crystal planes. To this end, we have developed a new experimental approach that is suited for high-resolution imaging of ice crystals and for fluorescence microscopy for localization of single proteins. We perform quantitative analyses of the Ostwald ripening process in absence and presence of antifreeze proteins where we examine the local and global evolution of the system. Novel image-analysis algorithms have been developed that are capable of extracting the local curvature of ice crystals for quantitative analysis of the boundaries evolution and their pinning due to binding of the proteins. These analyses aim to elucidate crystal-plane specific ice recrystallization and its inhibition by ice-binding proteins.

1739-Pos

Noise regulation by heterotypic biomolecular condensates

Aniruddha Chattaraj, Sujay Champati, Leslie M. Loew.
Center for Cell Analysis and Modeling, UConn School of Medicine, Farmington, CT, USA.

Biomolecular condensates serve as membrane-less compartments inside living cells. These structures emerge via a biophysical mechanism called liquid-liquid phase separation, beyond a concentration threshold of component molecules. For a self-interacting (homotypic) system, dilute phase concentration is buffered at the threshold value and, consequently, molecular fluctuations (noise) are suppressed. We recently extended the buffering framework to multicomponent (heterotypic) systems where a solubility product of concentrations in the dilute phase maintains an approximately constant level (Ksp) above the phase separation threshold. In this study, we used agent based stochastic simulations to explore the possibility of dilute phase noise buffering for a pair of heterotypic binders. We can decompose the total noise into two parts: Intrinsic (stochastic variability even if the initial conditions are identical) and extrinsic (variability arises due to variable initial conditions). Our simulations suggest that heterotypic phase separation does not influence the intrinsic noise much, but it can suppress a wide range of extrinsic noise as long as a stoichiometric ratio of binding partners are present in the system. This buffering capacity is reduced when binders are present in non-stoichiometric amount. However, Ksp serves as an upper bound to regulate the dilute phase compositions. The simulations imply that a heterotypic system can suppress dilute phase noise within a concentration regime that conforms to the Ksp. Thus, our statistical analysis complements the recent theory by Deviri and Safran (PNAS 2021) and provides further computational evidence to extend the Ksp as a useful metric to predict the noise buffering capacity of heterotypic condensates. (Funding: NIH grants R01 GM132859 and R24 GM137787)

1740-Pos

Poly(ADP-ribose) drives condensation of FUS via a transient interaction

Kevin Rhine[1], Morgan Dasovich[2], Joey Yoniles[3], Mohsen Badiee[4], Sophie Skanchy[3], Laura Ganser[3], Yingda Ge[3], Charlotte Fare[5], James Shorter[5], Anthony K. Leung[4], Sua Myong[3].
[1]Biology, Johns Hopkins University, Baltimore, MD, USA, [2]Chemistry, Johns Hopkins University, Baltimore, MD, USA, [3]Biophysics, Johns Hopkins University, Baltimore, MD, USA, [4]Biochemistry & Molecular Biology, Johns Hopkins University School of Public Health, Baltimore, MD, USA, [5]Biochemistry, University of Pennsylvania, Philadelphia, PA, USA.

Poly(ADP-ribose) (PAR) is an RNA-like polymer that regulates an increasing number of biological processes. Dysregulation of PAR is implicated in neurodegenerative diseases characterized by abnormal protein aggregation, including Amyotrophic Lateral Sclerosis (ALS). PAR forms condensates with FUS, an RNA-binding protein linked with ALS, through an unknown mechanism. Here, we demonstrate that a strikingly low concentration of PAR (1 nM) is sufficient to trigger condensation of FUS near its physiological concentration (1 μM), which is three orders of magnitude lower than the concentration at which RNA induces condensation (1 μM). Unlike RNA, which associates with FUS stably, PAR interacts with FUS transiently, triggering FUS to oligomerize into condensates. Moreover, inhibition of a major PAR-synthesizing enzyme, PARP5a, diminishes FUS condensation in cells. Despite their structural similarity, PAR and RNA co-condense with FUS, driven by disparate modes of interaction with FUS. Thus, we uncover a mechanism by which PAR potently seeds FUS condensation.

1741-Pos

Competing interactions regulate the granularity of the nucleolus

Michele Tolbert, Mylene C. Ferrolino, Qi Miao, Pricilla J. Walters, Richard Kriwacki.
Structural Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

Ribosome biogenesis is a critical cellular process and its dysregulation is associated with morbidity and death. Biogenesis and assembly of these complex molecular machines occurs in the nucleolus, a large, multi-compartment membrane-less organelle formed through liquid-liquid phase separation (LLPS). The protein Nucleophosmin (NPM1) is a major constituent of the outer, granular component (GC) region of the nucleolus where it phase separates with ribosomal proteins (r-proteins) and nucleolar proteins containing disordered Arginine-rich regions using its central intrinsically disordered region, and with ribosomal RNAs (rRNA) using its folded C-terminal nucleic acid binding domain. The GC is named because early transmission electron microscopy images revealed heterogeneous biomolecular electron density, giving rise to the term ''granular component.'' How LLPS between these players contribute to ribosome assembly and influence the granularity of the GC is