



Ion Selectivity in Acid-Sensing Ion Channels and Epithelial Sodium Channels

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Symposium: Protein and RNA Phase Separation

124-Symp

Organizing Living Matter: The Role of Phase Transitions in Cell Biology and Disease

Simon Alberti.

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Cells that are exposed to environmental fluctuations undergo changes on multiple levels to alter their physiology, metabolism and architecture. Our recent work shows that many of these changes happen in a controlled manner and involve a reorganization of the cytoplasm and the formation of membraneless compartments via a process known as phase separation. This challenges an established paradigm in cell biology, which posits that intracellular compartmentalization requires membranes. Our very recent findings show that the material properties of these membrane-less compartments are widely adjustable and can be modified along a continuum of physical states from liquid to gel to solid. Such changes in the material state endow cells with control over diffusion-limited biochemical processes. Most importantly, we recently discovered that the initially beneficial ability to form membrane-less compartments becomes detrimental with increasing age. This is because many compartment-forming proteins are hypersensitive to changing conditions and have a tendency to form aberrant structures that cause aging-associated diseases. Thus, we propose a new model for many age-related neurodegenerative diseases, where we link the physiological function of compartment-forming proteins with their role in disease.

125-Symp

Dysregulation of Phase Separation in Cancer

Tanja Mittag.

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Liquid-liquid phase separation leads to demixing of proteins from solution and results in a dense, protein-rich phase, which co-exists with a light phase depleted of protein. Recent findings support a model in which phase separation is the biophysical driving force for the formation of membrane-less organelles in the cell, such as stress granules, nucleoli and nuclear speckles. Current open questions are: (i) How is phase separation propensity encoded in the protein sequence, (ii) are dense liquid droplets used as reaction compartments in the cell, and (iii) is physiological phase separation disrupted in disease states? To address them, we study the interaction of the tumor suppressor Speckle-type POZ protein (SPOP), a substrate adaptor of a ubiquitin ligase, with its substrates. SPOP localizes to different liquid membrane-less organelles in the cell nucleus, where it encounters its substrates, but it is never found diffuse in the cell. However, its recruitment mechanism to these organelles is not understood. Here, we show for the first time that SPOP undergoes liquid-liquid phase separation with substrate proteins, and that this mechanism underlies its recruitment to membrane-less organelles. Multivalency of SPOP and substrate for each other drive their ability to phase separate. Moreover, we present strong evidence that the SPOP/substrate assemblies are active ubiquitination compartments *in vitro* and in cells. SPOP cancer mutations reduce the propensity for phase separation and tune the material properties of mesoscale assemblies. In the cell, cancer mutants fail to localize to the proper organelles and to recruit substrate. We propose that SPOP has evolved a propensity for phase separation in order to target substrates localized in membrane-less compartments. Our results provide mechanistic insights into the contributions of structured and disordered domains to phase separate, enzymatic activity inside liquid organelles, and disruption of phase separation by cancer mutations.

126-Symp

Lighting up Intracellular Phase Space

Clifford P. Brangwynne.

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In this talk I will discuss our work showing that phase transitions play an important role in organizing the contents of living cells. We focus on a class of membrane-less RNA and protein rich condensates, known as RNP bodies, which help control the flow of genetic information within cells. The nucleolus is one such nuclear RNP body, which is important for cell growth and size homeostasis. We've shown that a phase transition model explains many features of nucleolar assembly, and that the internal subcompartments of the nucleolus

arise from multi-phase coexistence, which may have important consequences for sequential RNA processing. I will also discuss our new "Optodroplet" approaches, which use light to enable spatiotemporal control of phase transitions within living cells. We are now using Optodroplets to quantitatively map intracellular phase diagrams. This approach has begun to yield rich insights into the link between intracellular liquids, gels, and the onset of pathological protein aggregation.

127-Symp

Physical Mechanisms of Cell Organization on Micron Length Scales

Michael K. Rosen.

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Cells are organized on length scales from Angstroms to microns. But the mechanisms by which Angstrom-scale molecular properties are translated to micron-scale macroscopic properties are not well understood. We have shown that interactions between multivalent proteins and multivalent ligands can cause oligomerization and concomitant liquid-liquid phase transitions, resulting in formation of micron-sized liquid droplets in aqueous solution and micron-sized puncta on membranes. Through this idea of multivalency-driven phase transitions we have explained behaviors of multi-domain proteins, intrinsically disordered proteins and nucleic acids. I will discuss how such transitions may control the spatial organization and biochemical activity of actin regulatory signaling pathways, and contribute to formation and regulation of biomolecular condensates such as PML nuclear bodies and P bodies. Our data suggest a general mechanism by which cells may achieve micron-scale organization based on interactions between multivalent macromolecules.

Platform: Ligand-gated Channels

128-Plat

Ion Selectivity in Acid-Sensing Ion Channels and Epithelial Sodium Channels

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Members of the epithelial Na⁺ channel/degenerin (ENaC/DEG) superfamily are Na⁺-selective channels with a common overall structure. Each of the three subunits within these trimeric channels has two transmembrane domains (M1 and M2), of which M2 forms the pore. Included in this family are the proton-gated acid-sensing ion channels (ASICs), which are formed by identical or homologous subunits. ASICs mediate excitatory Na⁺ currents (relative Na⁺/K⁺ permeability of ~ 10/1) and play key roles in many physiological processes, including nociception and cell death following ischemic stroke. ENaCs are obligate heterotrimers comprised of the principal α subunits, as well as β and γ subunits and display significantly higher Na⁺ selectivity than ASICs (relative Na⁺/K⁺ permeability of ~ 100/1). Previous work had indicated that the selectivity filter in both ENaCs and ASICs is formed by a conserved G-X-S motif in the pore. However, we have recently shown the mASIC1a selectivity filter to be composed of two carboxylate pairs in the lower part of the pore, namely M2 E18' and D21'. As both the G-X-S motif and the carboxylates in 18' and 21' are conserved between ASICs and ENaCs, there are likely other factors contributing to the stark differences in Na⁺ selectivity. We have thus probed the contribution of residues in M1 to ion selectivity in ASICs and ENaCs. By introducing point-mutations, bulky side chains of the ENaC M1 were replaced by less bulky side chains, and the converse was performed in ASIC1a. We show that the bulky residues in M1 are important for Na⁺ selectivity, conceivably by influencing the size of the pore.

129-Plat

Mechanism of NMDA Receptor Channel Block by MK-801 and Memantine

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The *N*-methyl-D-aspartate (NMDA) receptors are neurotransmitter receptors and calcium-permeable ion channels that transduce synaptic signals initiated by binding of glutamate and glycine to the opening of the ion channels. Their activities play important roles in physiological and pathological conditions.