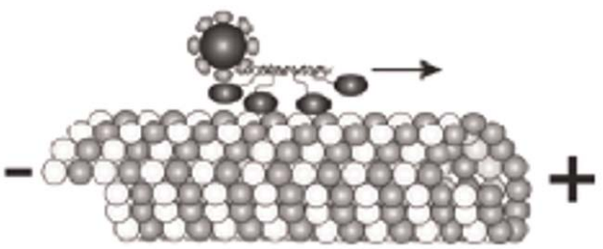
templates containing zinc-finger recognition sequences, teams with a defined number (1–6) of single-headed kinesin motor proteins have been assembled. This allows investigation of the effects of team composition, including the number of heads and the length and flexibility of the linkages between them, on the function of these composite motors.

Microtubule gliding assays show that these shuttles are functional: microtubules move across surface-bound teams, at a velocity similar to that measured with independent, single kinesin for monomeric to tetrameric populations at 24°C. Gliding in the temperature range 15–28°C has been investigated. The motion of single molecular shuttles labelled with quantum dots along static microtubules has also been observed by fluorescence microscopy. This allows measurement of velocity, dwell time and run length of quantum dots attached to single teams.

The use of a double-stranded DNA template has potential not only to specify nanoscale structure, but also to allow dynamic exchange of the protein arrangement, through the use of DNA strand exchange.



918-Plat Programmable Assembly of Vesicle Superstructures using Membrane-Anchored DNA as Biomolecular Combination Locks

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Much interest has been shown over the past decade in the programmable assembly of hard-sphere colloids mediated by the specific interaction between complementary ssDNA sequences. Lipid vesicles potentially offer greater levels of control in structural assembly due to some of their unique properties, including the deformability and fluidity of membranes, and the ease with which the properties of vesicles can be tuned by utilizing the multitude of lipids that are readily available. Due to the role of lipids in nature as biological packaging material, lipid vesicles have been considered for engineering applications as soft, technological containers. The ability to reversibly assemble vesicles into multicompartment containers has many applications in microfluidics and, potentially, drug delivery. We approach this innovative challenge by anchoring single-stranded DNA (ssDNA) to the outer monolayers of lipid vesicles: superstructures are observed to form between different populations of vesicles decorated with complimentary ssDNA strands. The fidelity of vesicle hetero-binding in the superstructures is found to be dependent on the strength of the DNA's hydrophobic anchor to the lipid bilayer. The aggregation is found to be reversible either by heating above the melting temperature of the DNA duplex or by the isothermal decrease of the ionic strength of the solution. Confinement of the DNA to the membrane surface greatly enhances the thermal stability of the double- stranded duplex compared to the stability in free solution. Furthermore, we can engineer DNA clustering by inducing lateral phase separation in multicomponent lipid vesicles. This causes the membrane-bound DNA to partition preferentially into one of the resultant phases, leading to the formation of localized 'sticky' patches on the membrane surface.

Symposium 9: RNA in Action

919-Symp Direct Measurement Of A pKa Near Neutrality For C75 In The Genomic HDV Ribozyme

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The hepatitis delta virus (HDV) ribozyme plays essential roles in the life cycle of the virus. Catalytic activity of the genomic HDV ribozyme is regulated by the binding of two protons and two magnesium ions. The protons bind to C75 and C41, and play catalytic and structural roles respectively. In addition, a magnesium ion binds in the vicinity of each cytosine. I will present solution ribozyme kinetics data that support intrinsic pKas for C75 of 5.9 and 7.2 in the presence and absence of bound metal ion, respectively, and intrinsic pKas for C41 of 7.1 and 5.5 in the presence and absence of bound Mg2+, respectively. In an effort to provide an independent physical measurement of the pKa, we have determined the pKa in crystals of the ribozyme using difference Raman spectroscopy. We grew crystals of the genomic HDV ribozyme bound to a non-cleavable substrate analog. Difference Raman spectra as a function of pH for the wild-type ribozyme provide a pKa of 6.2 in the presence of 20 mM magnesium ions. This value is similar to the pKa of 6.1 obtained in solution kinetics experiments under similar ionic conditions. Moreover, crystals of a C75U variant show no ionization behavior. The mutant data support assignment of this pKa to residue 75. Additional experiments show binding of metal ions in the crystals mirrors binding in solution, which further indicates that ribozyme in the crystal behaves like ribozyme in solution. Together these data show that ribozymes can use their active sites to optimize nucleobase pKa values for proton transfer, providing further support for the importance of general acid-base chemistry by C75 in the HDV ribozyme.

920-Symp Comparing to protein, RNA crystallization is a much more challenging task

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Comparing to protein, RNA crystallization is a much more challenging task. Among the extra features presented in RNA, the most prominent is the negatively charged backbone that adopt intricate scaffold and might make crystal packing more difficult. We are trying to tackle this problem by co-crystallizing the RNA with its Antigen Binding Fragment (FAB). Using a reduced codon antibody FAB library displayed on the M13 phage, we have selected FABs that bind to ΔC209 P4-P6 domain of *Tetrahymena* Group I intron. Two FABs, FAB2 and FAB5, bind to the ΔC209 with affinities of 50 nM and 30 nM, respectively. These FABs are highly specific and do not bind to BP, a ΔC209 mutant, in which tertiary RNA folding has been disrupted. Furthermore, the binding between these FABs and ΔC209 is diminished when [Mg^{2+}] was reduced to zero, showing that these FABs bind to the tertiary structure of ΔC209. FAB2 was cocrystallized with ΔC209 and the structure was solved at 1.95 Å resolution. The Fe-EDTA footprinting assay and the crystal structure reveal that FAB2 does not alter the overall folding of ΔC209 either in solution or in crystal. The crystal structure also shows that, with direct and water-mediated hydrogen bonding network, FAB2 helps ΔC209 achieve its native folding with fewer innersphere coordinated magnesium ions. The protein participated crystal contacts account for 61% of the buried surface area and we expect this method will facilitate the crystallization of RNA by providing crystal contacts and lending structure stability.

Symposium 10: Ca^{++} Signaling: From the Plasma Membrane to the Nucleus

921-Symp Nuclear Calcium Signaling, the Geometry of the Nucleus and Gene Transcription

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The generation of calcium signals following electrical activation is a fundamental property of neurons that controls many processes in the developing and the adult vertebrate nervous system. Activity-induced increases in the intracellular calcium concentration result from calcium entering neurons from the extracellular space through ligand and/or voltage-gated ion channels; these calcium transients can be amplified through calcium release from intracellular calcium stores. The NMDA receptor, a calcium permeable, glutamate-gated ion channel, plays a particularly important role in the mammalian nervous system. Calcium entry through synaptic NMDA receptors activates mechanisms that affect synaptic connectivity and promote neuronal survival; these changes can be long-lasting and depend on nuclear calcium signaling and gene expression mediated by the transcription regulators CREB and/or CBP. The nuclear calcium-regulated genomic pro-survival and plasticity programs triggered by synaptic NMDA receptors are antagonized by a signaling pathway stimulated by calcium entry through NMDA receptors that are localized outside synaptic contacts; extrasynaptic NMDA receptors couple to CREB shut-off and cell death pathways. Synaptic NMDA receptors also control the geometry of the cell nucleus. Three-dimensional reconstruction of cell organelles revealed that many nuclei from hippocampal neurons are complex, highly infolded structures, which often form unequally sized nuclear compartments that can function as signaling microdomains. Compared to near-spherical nuclei, infolded nuclei have a smaller diffusion relevant diameter, a larger surface, and contain more nuclear pore complexes. Mathematical modeling and calcium imaging experiments indicate that infolding-induced compartmentalization optimizes the propagation of calcium signals from the cytosol to the nucleus.

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922-Symp Growth Factors, Cell Growth, and Calcium Signaling In The Nucleus

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Background: Growth factors stimulate cell proliferation by activation of receptor tyrosine kinases, which in turn increases free Ca^{2+} within the cytosol and nucleus, but the relative role of cytosolic and nuclear Ca^{2+} in this process is unclear. Our aim is investigate the role of growth factors in the generation of Ca^{2+} signals within the nucleus and therefore in cell proliferation.

Methods: Liver cells were used to characterize the intranuclear Ca^{2+} network using fluorescent dyes and confocal microscopy. We used selective buffers of nucleoplasmic or cytoplasmic Ca^{2+} and $InsP_3$ to determine that cell proliferation depends upon Ca^{2+} signals within the nucleus rather than in the cytoplasm.

Results: Here we report that c-met rapidly translocates to the nucleus upon stimulation with HGF. Ca^{2+} signals that are induced by HGF result from PIP_2 hydrolysis and $InsP_3$ formation within the nucleus rather than within the cytoplasm. Translocation of c-met to the nucleus depends upon the adaptor protein Gab1 and Importin β1, and formation of Ca^{2+} signals in turn depends upon this translocation. Nuclear Ca^{2+} signals stimulate cell growth rather than inhibit apoptosis and specifically permit cells to advance through early prophase. Selective buffering of nuclear but not cytoplasmic Ca^{2+} signals also impairs growth of tumors *in vivo*.

Conclusions: Translocation of c-suggests a potential route by which such tyrosine kinase receptor selectively activate Ca^{2+} signaling pathways in the nucleus and also may be novel mechanism to explain the duality of response induced by c-met. findings also reveal a major physiological and potential pathophysiological role for nucleoplasmic Ca^{2+} signals and suggest that this information can used to design novel therapeutic strategies to regulate conditions of abnormal cell growth.