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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.

## 923-Symp Intracellular calcium ($Ca^{2+}$) signals have distinct spatial and temporal components

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Intracellular calcium ($Ca^{2+}$) signals have distinct spatial and temporal components. Spatially, cytosolic $Ca^{2+}$ changes have been linked to cell events such as secretion whereas nuclear $Ca^{2+}$ changes are necessary for transcription, regenerative processes and cell death. Temporally, the frequency of $Ca^{2+}$ oscillations and the rate of $Ca^{2+}$ wave propagation have each been linked to cell-specific responses. To investigate the basis of these differences we have taken several approaches: structural, immunocytochemical, electrophysiological, and imaging. The structure of purified InsP3R was obtained from single particle reconstruction of cryo-electron microscopic images. This reconstruction shows the InsP3R as an uneven cylinder with lateral arms extending into the cytoplasmic domain. These arms appear to move when ligand and cofactors bind to the receptor. Using immunocytochemical methods, we found that the distribution of InsP3R isoforms was non-uniform both in tissue (hippocampal slices) and individual cells (epithelial and neuronal cells). Single channel measurements were used to study the functional implications of ligand and cofactor binding. Channel activity could be modulated by addition of cofactors to either the lumenal or the cytoplasmic side of the channel. Many of these modulators also are non-uniformly distributed in tissues and cells, adding another layer of complexity. $Ca^{2+}$ imaging revealed a regional specificity in signal initiation and propagation. In many cell types the rate of InsP3 production and surface-to-volume effects play minor roles in determining temporal and spatial $Ca^{2+}$ signaling patterns. Conversely, the combination of a non-uniform distribution of InsP3R isoforms and unique isoform-specific single channel properties do play a role in establishing patterns. Similarly, the reagents found to modulate single channel activity were also important in altering subcellular $Ca^{2+}$ signals. These functional interactions provide the basis for understanding the consequences of channel modulation for intracellular $Ca^{2+}$ signaling.

**Minisymposium 2: Structural Refinement & Modeling Guided by Low-Resolution Experimental Poster**

## 923.01-Minisymp Merging Data from Different Resolutions to Reveal Biomolecular Function

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A key to understanding how biological systems function is to look at their structures captured at work. Experimental techniques reveal different levels of macromolecular structure: high-resolution techniques such as X-ray chrystallography yield atomistic details, but the structures captured are often in undefined functional states; alternatively, techniques such as cryo-electron microscopy (cryo-EM) capture the system in a biologically functional state, albeit at lower resolution. Computational techniques can help bridge the resolution gap by adapting high-resolution crystallographic structures to electron microscope density maps, providing the details of the molecules in different functional states, and thus revealing astonishing views of cellular processes.

We developed a novel method to fit atomic structures into EM maps using molecular dynamics simulations. EM data are incorporated into the simulation as an external grid potential added to the molecular dynamics force field, allowing all internal features present in the EM map to be used in the fitting process, while the model remains fully flexible and stereochemically correct. The novel method has been applied to several macromolecular systems. As an example application, a high-resolution structure of the bacterial ribosome bound to the ternary complex derived from a 6.7Å EM map will be presented.

## 924-Minisymp Improving Structures of Supramolecular Complexes and Membrane Proteins at Moderate Resolutions of X-ray Diffraction

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We report a novel X-ray crystallographic refinement protocol for modeling anisotropic thermal parameters of supramolecular complexes and membrane proteins. Based on that, a very small set of low-frequency normal modes (e.g., 25 $\sim$ 50 modes) was used to reconstruct the thermal motions in X-ray diffraction. The method was applied on a series of supramolecular complexes and membrane proteins, all of which structures were solved at moderate resolutions. The results universally showed that the Rfree values of the normal-mode-refined models were lower than the original isotropically refined models. Most importantly, the refinement resulted in improvement in electron density maps that allowed for building of a substantial amount of missing atoms including those from functionally important residues. The distribution of anisotropic thermal ellipsoids also revealed structure flexibility that is functionally important. We believe that the new protocol will help to significantly improve the structures of many highly-flexible supramolecular complexes and membrane proteins, for which further refinement is beyond any currently available methods.

## 925-Minisymp Modeling Protein Complexes by Combining High-Resolution Structure with Small-Angle X-ray and Neutron Contrast Variation Data

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Small-angle solution scattering yields low-resolution structural information that complements high-resolution techniques such as crystallography and NMR. The ever increasing desire to understand