a catalytic site (C), SOS has a catalytically inactive allosteric Ras-binding site, and together these sites form the catalytic core of SOS (SOScat). An N-terminal region containing Pleckstrin Homology (P), Dbl Homology (D) and Histone fold (H) domains in SOS-HDPC is known to auto-inhibit catalytic activity. We have previously shown that the allosteric site allows SOS to concentrate at Ras presenting membranes, dramatically increasing the Ras-GDP turnover rate. Here, we use single molecule fluorescence microscopy and spectroscopy to study conformational and functional dynamics of SOScat and SOS-HDPC enzymes.

Nuclear Pores

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Super-Resolution by Feedback Imaging: Mechanisms of Translocation through the Nuclear Pore Complex
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Nuclear pore complexes (NPCs) are the gateways for nucleocytoplasmic exchange. Because single molecules undergo rapid transport, it is challenging to follow their motion in live cells. Hence fundamental questions remain in regard to the nanomechanical basis of selective gating of molecules through the NPC in live cells.

We set out to address these issues by a combination of fluorescence correlation spectroscopy (FCS) and real-time tracking of the center of mass of single NPCs in live cells. The center of mass tracking allows us to create an “Einstein trap” in which the thermal motion of the entire pore is compensated so that we observe the shuffling of single molecules in the reference frame of the pore. Using this setup we demonstrate that the transport of Karyopherin-β1 (Kapβ1) receptor is regulated to produce a characteristic narrow correlation time within the NPC, which is the signature of directed-motion events. We also show that the back and forth components of Kapβ1 transport are coupled by energy-consuming processes. Analogously, the dynamics of nucleoporin-153 (Nup153) at the nanoscale is characterized by a similar correlation pattern between two separate positions within the NPC. By means of the pair correlation function (pCF) analysis we separate the two components of Nup153 exchange: a fast collapse into compact conformations (cytoplasm-to-nucleus) and a slightly slower release into extended conformations (nucleus-to-cytoplasm). We demonstrate that this signature activity is directly linked to the functional import of classical transport receptors and cargoes.

Thus, we propose that the selective gating through intact NPCs may be powered by spring-like molecular engines. As far as we know, this is the first time that a selective gating model is proposed on the basis of experimental evidence obtained in live, unperturbed cells.

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The Behavior of the Intrinsically Disordered FG Nups Determined by in Cell NMR
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The Nuclear Pore Complex (NPC) mediates all transport between the nucleus and cytoplasm. The channel of the NPC is lined with “FG Nups”, a family of intrinsically disordered proteins characterized by phenylalanine-glycine repeat motifs. FG nups form the exquisitely selective filter of the NPC; non-binding proteins are excluded while the binding of transport factors to the FG Nups facilitates their passage through the NPC. Like other intrinsically disordered proteins, the FG Nups appear to be very sensitive to their environment, showing vastly different behavior in different experimental conditions; in vitro, the observed behavior of the FG Nups varies from rigid gels to flexible random-coil polymers. We used NMR to probe the behavior of model FG Nups of both flavors, low charge (cohesive) and high charge (non-cohesive), within a variety of environments and when bound to transport factors. Our results have important implications for the various current models regarding the molecular mechanisms of nucleocytoplasmic transport and behavior of weak cellular interactions generally.

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The Supramolecular Assembly of Intrinsically Disordered Nucleoporin Domains is Tuned by Inter-Chain Interactions
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Nuclear pore complexes (NPCs) are the gates that mediate the exchange of all macromolecules between the cytoplasm and the nucleus. They are highly selective: inert proteins bigger than 30 kDa cannot diffuse through NPCs unless they are bound to nuclear transport receptors (NTRs). This selectivity arises from a supramolecular assembly of intrinsically unfolded nucleoporin domains which contain phenylalanine-glycine (FG)-rich repeats, so called FG-repeat domains (FGRDs). Different models for the size and species selectivity of the permeability barrier have been suggested that build on different putative supramolecular assemblies of FGRDs. A controversially discussed question today is whether and how inter-FGRD interactions contribute to the function of the permeability barrier. We have studied the inter-FGRD interactions in monolayers of end-grafted FGRDs which constitute a biomimetic nano-scale model system of the NPC’s permeability barrier. A toolbox of biophysical characterization techniques enabled us to relate the strength of inter-FGRD interactions of different FGRD types to monolayer formation and morphology. Our main finding is that inter-FGRD interactions strongly affect the kinetics of formation, the morphology (thickness and lateral homogeneity), and the mechanical properties of FGRD monolayers. Based on these results, we propose that the strength of inter-FGRD interactions in the NPC has evolved to promote the formation of a homogeneous meshwork with a small mesh size. Our results highlight the importance of inter-FGRD interactions for the functionality, and hence contribute important information to refine the model of transport across the nuclear pore permeability barrier.

619-Pos Board B388
Importin-Beta and Ran Regulate the Passive Permeability Barrier in the Nuclear Pore Complex
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The nuclear pore complex (NPC) is a large, multi-protein assembly that mediates the selective transport of molecules between the cytoplasm and nucleus in eukaryotic cells. While the proteins involved in the transport pathway have largely been identified, the physical mechanism by which this complex can support both efficient and selective molecular transport remains unclear. Using fluorescence microscopy and super-resolution imaging techniques, we have examined how nuclear transport receptors influence the permeability properties of the NPC for both active and passive transport processes. We find that importin-β binding to the nucleoporin Nup153 significantly slows passive transport through the NPC; however, Ran in its GTP-bound form reverses this effect. FRAP studies reveal that RanGTP weakens the binding interaction of importin-β to the NPC. Furthermore, STORM imaging of individual importin-β localizations inside the NPC show that RanGTP dramatically changes the importin-β distribution within the channel. These results suggest that importin-β in conjunction with Nup153 is an integral component of the NPC permeability barrier which is regulated by RanGTP.

620-Pos Board B389
Three-Dimensional Mapping Nuclear Export of Single mRNAs with Exceptional Spatiotemporal Accuracy
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In eukaryotic cells, the flow of genetic information is regulated by selective nucleocytoplasmic transport of individual messenger RNA:protein complexes (mRNPs) through the nuclear pore complex (NPC). Each NPC, ~200 nm in length and ~50 nm in inner diameter, is a large assembly of multiple copies of ~30 nucleoporins. However, the nuclear export kinetics, three-dimensional (3D) pathway and selectivity of mRNPs remain poorly understood. Here we employ single-molecule fluorescence microscopy with an unprecedented spatiotemporal super-accuracy of 8 nm and 2 ms to characterize nuclear mRNP export in living cells. We find that, mRNPs exiting the nucleus are decelerated and selected at the narrowest region of the NPC, and adopt a fast-slow-fast diffusion pattern as they translocate through the NPC. 3D mapping translocation pathway for mRNPs further indicates only one third of all mRNAs successfully transits during their