decade of the NPC-related research and discovery, the mechanism of selectivity barrier across the pore remains elusive. This is mainly due to the fact that the exact function and mechanics of the main players of the transport process, i.e. the FG-repeats, has proved difficult to capture during transport. FG-repeat domains are natively unfolded and constitute about one third of the NPC mass. They are believed to coat the most inner layer of the pore and directly interact with cargos, thus founding the selectivity barrier. The FG-repeats are confined to the central channel, so the compact architecture of the channel makes the investigation even more challenging. Here we have established a 3D coarse-grained model of the yeast channel with all 11 known FG Nucleoporins (Nups). We extract the exact sequence of the FG Nups disordered domains and implement their length, hydrophobicity, charge, and the native grafting density in the model. Our results show that the FG-motifs are mainly concentrated toward the central part of the channel, while charged residues are predominantly near the wall. Depending on the pore diameter, FG-repeats can either make a channel-filling hydrogel or a thick lubricating layer, consistent with two different models proposed in the field. We also investigated the effect of the channel shape ranging from a perfect cylinder to an hourglass geometry and observed that the bottleneck of the hourglass shape can affect the conformational behavior of the FG-repeats, depending on its aspect ratio.

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Simple Physical Considerations Explain the Conformational Transitions of the Fg- Nucleoporins Induced by the Transport Factors

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Nuclear Pore Complex (NPC) is a biological ‘‘nano-machine’’ that controls the macromolecular transport between the cell nucleus and the cytoplasm. It is a remarkable device that combines selectivity with robustness and speed. Unlike many other biological nano-channels, it functions without direct input of metabolic energy and without transitions of the gate from a ’closed’ to an ’open’ state during transport. The key aspect of transport is the interaction of the cargo-carrying transport factors with the unfolded, natively unstructured proteins that partially occlude the channel of the NPC and its nuclear and cytoplasmic exits.

Mechanistic understanding of the transport through the Nuclear Pore Complex, and in particular its selectivity, is still lacking. Conformational transitions of the unfolded proteins of the NPC, induced by the transport factors, have been hypothesized to underlie the transport mechanism and its selectivity. These conformational changes are hard to access in vivo; they have been investigated in vitro, generating apparently contradictory results.

We have investigated the biophysical underpinning of these conformational changes, using computational modeling based on the ideas the ideas of the polymer physics. We show that the differences in the experimentally observed behaviors can be explained by rather general physical factors, such as the attraction strength between the transport factors and the unfolded chains, protein density and the transport factor size. We also show how these general behaviors can be modulated by molecule-specific details, such as the aminoacid sequence and the relative arrangement in space of the charged and hydrophobic residues. Finally, we extend the model into a realistic NPC geometry. These results provide new insights into the fundamental principles of transport through the NPC and the control of the behavior of natively unfolded proteins in general.

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Characterization of the Selective Barrier in Nuclear Pore Complex

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The nuclear pore complexes (NPCs) selectively mediate the bidirectional trafficking of macromolecules between the cytoplasm and nucleus in eukaryotic cells. The selective barrier formed by natively unfolded phenylalanine-glycine (FG) nucleoporins (Nups) inside the NPC allows for passive and facilitated transport through the NPC. However, the mechanism of formation and spatial distribution of FG-barrier in the NPC remains unresolved. By a newly developed single-molecule microscopy, single-point edge-excitation sub-diffraction (SPEED) microscopy, we have used various fluorescent transport receptors and FG segments, as probes to determine the structure of FG-Nup barrier in the native NPC with a spatiotemporal resolution of 9 nm and 400 μs. The interactions among FG-filaments enhance a donut-like structure formed at the central of NPC, acting as the primary selective gate for nuclear transport. On either side of the NPC, additionally FG Nups may function as the secondary selectivity for incoming molecules. Finally, the conformations of both selective barriers can be further regulated by major transport receptors.

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Computational Study of Binding Between DDX3 and Hiv-1 MRNA Nucleocytoplasmic Export Complex

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For patients suffering from HIV, there exist several therapeutic drugs that can target various aspects of the HIV life cycle; however, treatment aggressiveness is limited by the very potent and potentially dangerous side effects of these drugs. Molecular traffic between the nucleus and the cytoplasm is exclusively regulated by the nuclear pore complex (NPC), which acts as a highly selective channel perforating the nuclear envelope in eukaryotic cells. HIV exploits the nucleocytoplasmic pathway to export its RNA transcripts across the NPC to the cytoplasm. DDX3 is a RNA helicase necessary for HIV replication which is found to interact with a protein, CRM1, which shuttles HIV mRNA out of the nucleus. Recent work has exposed DDX3 as a promising new target of the viral replication cycle that has not yet been thoroughly investigated due to limitations in experimental methodologies. In the present research we have developed a general computational protocol for analyzing protein-protein binding. This method is based on molecular docking followed by molecular dynamics simulation and accompanied by approximate free energy calculation (MM/GBSA), computational alanine scanning, clustering and evolutionary analysis. This research seeks to explore the details of the structural interaction between DDX3 and CRM1 using the proposed hybrid computational approach. Utilizing this approach to study the HIV mRNA export complex, we have highlighted some of the most likely binding modes and interfacial residues between DDX3 and CRM1 both in the absence and presence of RanGTP. This work shows that although DDX3 can bind to free CRM1, addition of RanGTP leads to more concentrated distribution of binding modes and stronger binding between CRM1 and RanGTP.

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Cellular Metabolism Fluidizes the Glassy Bacterial Cytoplasm

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In eukaryotes, active transport involves motor proteins and cytoskeletal filaments. In contrast, bacteria (which lack cytoskeletal motor proteins) are thought to primarily rely on diffusion for molecular transport and cytoplasmic mixing. However, the physical nature of the bacterial cytoplasm remains poorly understood. Through single particle tracking of protein filaments, plasmids, storage granules and foreign particles of different sizes, we have found that the bacterial cytoplasm exhibits striking similarities to glass-forming liquids. Glass-forming liquids are noted for their metastability near the glass transition where their behavior changes from liquid-like to amorphous solid with even small perturbations. We find that particles of different sizes exhibit distinct dynamics and their mobility changes from fluid-like to glassy with increasing particle size. This size dependency provides an explanation for previous reports of both normal and anomalous diffusion in the bacterial cytoplasm. Moreover, we find that cellular metabolism attenuates the glassy properties of the bacterial cytoplasm. As a result, components that would otherwise be caged in narrow regions of confinement are able to explore the cytoplasmic space under metabolically active conditions. Cytoplasmic dynamics are directly impacted by environmental conditions that impact cellular metabolism. These findings have broad implications for our understanding of bacterial physiology as the glassy behavior of the cytoplasm impacts all intracellular processes involving large cellular components.