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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 shuttling 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 evidences obtained in live, unperturbed cells.

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