Universität Basel

Research Project

Correlative High-Speed Atomic Force Microscopy and Fluorescence Imaging for Resolving the Selective Barrier Mechanism Inside Native Nuclear Pore Complexes

Third-party funded project

Project title Correlative High-Speed Atomic Force Microscopy and Fluorescence Imaging for Resolving the Selective Barrier Mechanism Inside Native Nuclear Pore Complexes
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Nucleocytoplasmic transport (NCT) underpins macromolecular traffic between the cytoplasm and nucleus in eukaryotic cells. This occurs through aqueous channels that perforate the double-membrane nuclear envelope (NE) known as nuclear pore complexes (NPCs). Importantly, each NPC regulates the selective translocation of signal-specific cargoes whilst rejecting non-specific macromolecules. Tethered within each 50 nm-diameter NPC lie numerous intrinsically disordered proteins known as phenylalanine-glycine (FG) nucleoporins (i.e., or FG Nups) that enforce this selective barrier functionality. Cargo-carrying transport receptors (known as karyopherins or Kaps, importins or exportins) exclusively traverse the NPC via multivalent interactions with the FG Nups.

After more than two decades of research, the NPC selective barrier mechanism is still unresolved. As a case in point, how the FG Nups promote selective Kap-cargo traffic while simultaneously blocking smaller non-specific macromolecules remains speculative based on the following reasons: First, the FG Nups still elude direct visualization inside native NPCs. Thus, barrier models that derive from *in vitro* experimentation remain invalidated. Second, nucleocytoplasmic transport *in vivo* proceeds through NPCs in a matter of milliseconds. Hence, to be precise, it is the dynamic spatiotemporal behavior of the FG Nups rather than their static properties (i.e., time-independent) that governs NPC function. Third, it remains puzzling how multivalent Kap-FG Nup binding interactions (i.e., avidity) expedite fast transport. Fourth, it is unknown if NPCs can structurally deform to aid the translocation of large cargoes.

Here, we will interrogate and dissect dynamic FG Nup behavior directly inside native NPCs from *X. laevis* oocytes and *S. cerevisiae* (including FG mutant strains) at transport-relevant timescales (100 ms) and under various trafficking conditions (e.g., RanGTP). Our work centers on high-speed atomic force microscopy (HS-AFM), which resolves FG Nup behavior approaching the spatiotemporal resolution of coarse-grained computer simulations (Sakiyama *et al*, Nature Nanotechnology, in press). We will correlate this data to authentic transport events by integrating the HS-AFM onto a fluorescence microscope. This will be complemented by surface plasmon resonance (SPR) measurements of Kap-FG Nup binding and fluorescence recovery after photobleaching (FRAP) of isolated nuclei. Finally, we will “ground truth” our findings by reconstituting authentic transport functionality using biomimetic NPCs fabricated from glass nanocapillaries (GNCs) and interpret the data via ongoing model building efforts.

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