Dwell Times of Transport Receptors at the Nuclear Pore Complex

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All molecular traffic between cytoplasm and nucleus occurs via the nuclear pore complex (NPC), which are very large proteinaceous transporters spanning the nuclear envelope. Using single molecule observation based on classical epi-illumination fluorescence microscopy we were able to determine the dwell times of several different transport receptors in digitonin-permeabilized cells [1] and recently also in live cells [2]. The great majority of all receptors resided less then 20 ms at the NPCs. Surprisingly, we found that for most examined transport receptors a small contingent has conspicuously long residence times at the NPC. Here, we focus on the characterization of these long binding events using a technique closely related to HILO microscopy [3]. To this end we illuminate the bottom of the cell nucleus with a refracted beam. This refracted beam is formed by hitting the coverslip/sample interface under an angle just slightly smaller than the critical angle for total reflection. Thereby only the NPCs at the bottom of the nucleus close to the objective lens of the nucleus are illuminated thus reducing strongly the background resulting from out of focus fluorescence. This greatly simplifies the identification of single NPCs and strongly reduces the irradiance of single fluorescently labeled transport receptor molecules prolonging their overall observation time window.

References:

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