

# TRANSLOCATION THROUGH THE NUCLEAR PORE COMPLEX IN LIVING CELLS: ANALYZED ONE MOLECULE AT A TIME

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All protein and RNP traffic between cell nucleus and cytoplasm occurs via the nuclear pore complex (NPC), which is located within the nuclear envelope of eukaryotic cells. NPCs are extremely large (~60 MDa) supramolecular protein complexes with a barrel-shaped geometry of 125 nm in diameter and a length of approximately 120 nm [1]. Proteins and RNP complexes with molecular weights greater than 40 kDa are translocated by the NPC in a signal-dependent manner by transport factors.

We use fluorescence microscopy to study nucleocytoplasmic transport at the single-molecule level. With the MS2 system [2] for labeling of nascent mRNA transcripts in living cells we follow their movement inside the nucleus and their export into the cytoplasm. Therefore the endogenous  $\beta$ -actin gene is modified to code for 24 MS2 stem loop repeats in the 5' untranslated region (UTR) of the  $\beta$ -actin mRNA. Parallel expression of MS2 coat protein fused to eYFP containing a nuclear localization signal (NLS) is used to label the mRNA co-transcriptional. A fusion of Pom121 with a Cyan Fluorescent Protein will be used to localize the NPC. With a fusion of Pom121 with GFP it was possible to localize the NE with a precision of 10 nm [3].

A home build laser based wide field microscope, equipped with a state of the art high speed EMCCD, heating device, a piezo scanner for fast z stacking and customized optics is used to image fluorescently tagged mRNPs at the NPC with a time resolution of approximately 30 ms. The diffraction limited signal of individual mRNPs will be located in each frame and the position of each mRNP will be determined with a localization precision of approximately 20 nm. Individual signals in subsequent frames can be connected to individual traces and should reveal fundamental properties of the mobility of mRNPs inside living cells and how they are transported through the NPC. To measure the interaction time of mRNPs with the pore, the number of frames, each mRNP observed at the nuclear pore, can be counted and translated into a dwell time.

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[3] U. Kubitscheck, D. Grünwald, A. Hoekstra, D. Rohleder, T. Kues, J.-P. Siebrasse and R. Peters, “Nuclear transport of single molecules: dwell times at the nuclear pore complex,“ *J Cell Biol* **168**, 233-243 (2005)