Nanoscale Fluorescence Correlation Spectroscopy of Intact Nuclear Pore Complexes

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Supporting Material

Single NPC tracking setup and data analysis

Tracking of single NPCs was performed using a home-built microscope capable of Single Particle Tracking (SPT), whose details have been already described (1). Briefly, the microscope is built around an Olympus X71 body. A Chameleon Ultra (tunable) Ti:Sapphire laser (Coherent, CA) tuned at 940 nm was used for 2-photon simultaneous excitation of the GFP and mCherry constructs. The scanning of the excitation light was obtained in the x-y plane and in the z-axis through two galvano-motor driven mirrors (Cambridge Technology, MA) and a piezo-objective positioner (Phisik Instrument, Germany) respectively, both driven by a computer card (3-axis card, ISS, Champaign, IL). Fluorescence emission was collected by a 1.2-NA water objective (Olympus UplanSApo 60x), split by a dichroic mirror at 570nm and detected in the 500-550nm (GFP) and 575-645nm (mCherry) spectral ranges by two GaAS detectors H7241P (Hamamatsu, Japan). During the tracking procedure, the two scanning mirrors are moved independently by $\pi/2$-phase shifted sine wave voltages generated in the card so that the laser beam moves in a circular path around the particle. The position of the scanning center is determined by the offset values of the sine waves. The position of the center is updated at each tracking cycle according to the Fast Fourier Transform (FFT)-based algorithm previously described (2). From the FFT of the intensity trace along the orbit, we get the average intensity or DC as the 0th term in a Fourier series and the AC as the coefficient of the 1st harmonic term. The angular coordinate of the particle is given directly by the phase of the AC term, and its distance from the center can be calculated from the modulation of the signal, defined as mod=AC/DC, so that its position can be recovered. The tracking routine changes the coordinates of the center of the scanning orbit in such a way to keep the modulation at a minimum, i.e. to keep the particle always at the center. The tracking procedure started by acquiring a raster scan image of the sample, focusing on the equatorial section of the NE. Then we clicked on a location on the image corresponding to an isolated NPC. The fluorescence intensity is collected at 64 points along a circular orbit around the pore, with a period of 0.5-1 ms and a calibrated scanning start point. The orbit radius (R) is usually set to 180 nm which we found to be optimal for the tracking. The optimal radius for tracking a point-like particle should be on the order of half the size of the PSF (2), but in our case this value is slightly larger due to the finite size of the NPC. The position of the center of the scanning orbit is updated typically every 16-32 orbits (that is defined as the cycle), which is fast enough to follow the NPC movement. The acquisition time for a single NPC tracking measurement typically varied from 15 to 30s. For each NPC we acquired the intensity along the orbit for one or two channels and the trajectory of the center of mass. From the recorded trace of the fluorescence intensity along the orbit the value of the modulation of the signal can be calculated at each cycle. The analysis of the modulation allows to check 'a posteriori' if the particle has been tracked correctly for the entire acquisition or to exclude the portions of the dataset where the particle was temporarily or definitely lost. We selected only the part of the data acquired with the orbit centered and stationary with respect to the center of mass of the NPC. The fluctuation analysis of the data was performed with the SimFCS software (www.lfd.uci.edu, UCI, Irvine) using the scanning-FCS analysis tool, as thoroughly described in previous publications (3-5).

1. Levi, V., Ruan, Q., and E. Gratton. 2005. 3-D particle tracking in a two-photon microscope: application to the study of molecular dynamics in cells. *Biophys J* 88: 2919-2928.
2. Kis-Petikova, K., and E. Gratton. 2004. Distance measurement by circular scanning of the excitation beam in the two-photon microscope. *Microsc Res Tech* 63: 34-49.
3. Digman, M. A., P. Sengupta, …and E. Gratton. 2005. Fluctuation correlation spectroscopy with a laser-scanning microscope: exploiting the hidden time structure. *Biophys J* 88: L33-36.
4. Cardarelli, F., and E. Gratton. 2010. In vivo imaging of single-molecule translocation through nuclear pore complexes by pair correlation functions. *PLoS One* 5: e10475.
5. Hinde, E., F. Cardarelli, …and E. Gratton. 2010. In vivo pair correlation analysis of EGFP intranuclear diffusion reveals DNA-dependent molecular flow. *Proc Natl Acad Sci U S A* 107: 16560-16565.

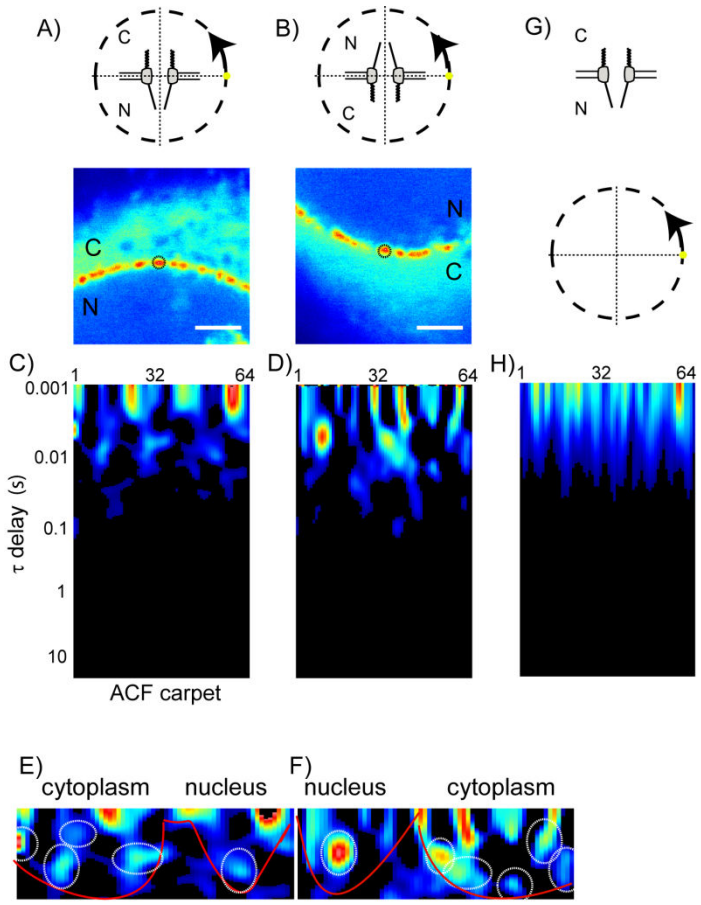

FIGURE S1. Spatial characteristics of the hump. (*A*, *B*) Two different orientations of the NPC with respect to the scanned orbit are represented schematically (upper panels) and by real images (bottom panels, scale bar: 2μm). (*C*, *D*) ACF carpets obtained in the two orientations. Along the orbit we have enough spatial resolution to distinguish different 'hump' regions around the pore. In particular, the cytoplasmic side shows several hump regions while the nucleoplasmic side mainly one. This effect may be linked to the presence of many sparse cytoplasmic filaments for transport initiation on one side and only one nuclear basket for transport termination on the other (*E*, *F*) Magnification of the hump-regions obtained in (C, D) to better visualize the distribution of the hump regions (white circles). (*G*, *H*) If a small-radius orbit is scanned far from the pore (in the nucleoplasm in this case) it does not generate humps in the correlation carpet.