

VISUALIZATION OF THE INTRA-NPC DYNAMICS OF THE NUCLEOPORINS NUP153 AND NUP214 USING SNAP-TAG TECHNOLOGY

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The exchange of ions, small and large molecules between cytoplasm and nucleus is mediated by the nuclear pore complex (NPC), a highly symmetrical supramolecular complex traversing the nuclear envelope. The NPC is composed of ~50 different proteins, termed nucleoporins (Nups). Nup153 is a well characterized nucleoporin located at the nuclear side of the NPC and believed to play an important role in both export and import [1]. While its N-terminus is tightly incorporated into the NPC-scaffold the C-terminal domain, rich in FG (Phenylalanin-Glycin)-repeats, is highly flexible. A similar structural organization was also shown for another nucleoporin, Nup214, which is located at the cytoplasmic side. From ultrastructural EM studies it was concluded that these long, flexible domains might be directly involved in the translocation of transport receptors and substrates [2,3]. In order to address this question in living cells using light microscopy we functionalized these two nucleoporins with a SNAP-Tag moiety, which allows specific fluorescence labelling *in vivo* [4]. Using conventional confocal laser scanning microscopy we found the SNAP tag fused Nups functionally incorporated in the NPC *in vivo*. Using a single-molecule fluorescence approach [5] we analyze the intra-NPC dynamics of the flexible C-domains of single nucleoporins relative to the nuclear membrane under several transport conditions.

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