

## QUANTUM DOTS AS SUBSTRATES FOR NUCLEAR-CYTOPLASMIC TRANSPORT

Ulrike Schmitz-Ziffels<sup>1</sup>, Simone Scholl<sup>1</sup>, Thomas Basché<sup>2</sup>, Jan-Peter Siebrasse<sup>1</sup>,  
Ulrich Kubitscheck<sup>1</sup>

<sup>1</sup> Institute of Physical and Theoretical Chemistry, University of Bonn,  
Wegelerstr.12, 53115 Bonn, Germany

<sup>2</sup> Institute of Physical Chemistry, University of Mainz  
Welderweg 11, 55099 Mainz, Germany

**KEY WORDS:** Nuclear-cytoplasmic transport, single molecule fluorescence microscopy, quantum dots.

Nuclear-cytoplasmic transport of macromolecules is accomplished by the nuclear pore complex (NPC) - a transport machine imbedded in the nuclear envelope. The NPC enables high selective translocation across the nuclear envelope, known to be facilitated by the interaction of soluble transport receptors with the NPC's nucleoporins. However, detailed mechanisms and kinetics of the translocation still remain unknown. Single molecule fluorescence microscopy provides a direct observation of processes at the NPC with both excellent spatial and time resolution [1]. We used functionalized biocompatible quantum dots as transport substrates to investigate nuclear import in permeabilized cells at the single particle level. As bright and photostable probes, quantum dots yield an excellent localization precision (< 10nm) due to an extraordinary high signal to noise ratio. This is of great importance when tracking single import complexes, which cross the only ~100 nm long NPC. First experiments with commercially available streptavidin-conjugated CdSe/ZnS-quantum dots and biotinylated proteins showed that specific interaction with the nuclear pores can be achieved, while translocation of these transport complexes was only observed for the smallest, green fluorescing quantum dots. However, upon 488 nm excitation these were not bright enough for a precise localization. In order to yield both smaller and brighter import complexes we now work with taylor-made CdSe/CdS/ZnCdS/ZnS core/shell nanocrystals, which are solubilized by dihydrolipoic acid (DHLLA) [2]. Proteins and peptides can directly be attached to the quantum dots's surface when they exhibit a polyhistidine-tag. The conjugation is due to a complexation of Zn-atoms in the quantum dots by the hystidine-moiety of the protein [3]. This coupling technique results in very small complexes as the quantum dots do not need any additional layer to accomplish a functionalization. Experiments with the nuclear transport receptor Importin $\beta$ 1 conjugated to the multishell QDs demonstrated a specific binding of the complexes to the nuclear envelope.

[1] T. Dange, D. Grünwald, A. Grünwald, R. Peters, and U. Kubitscheck, "Autonomy and robustness of translocation through the nuclear pore complex: a single-molecule study", *J. Cell Biol.*, **183**(1), 77-86 (2008).

[2] R. Xie, U. Kolb, J. Li, T. Basché, and A. Mews, "Synthesis and Characterization of Highly Luminescent CdSe-Core CdS/Zn<sub>0.5</sub>Cd<sub>0.5</sub>S/ZnS Multishell Nanocrystals", *JACS*, **20**, 7480-7488 (2005).

[3] A. R. Clapp, E. R. Goldman, and H. Mattoussi, "Capping of CdSe-ZnS quantum dots with DHLLA and subsequent conjugation with proteins", *Nat. Protoc.*, **1**, 1258 - 1266 (2006).