

## **450a Size of Nanoparticles Affect Intracellular Trafficking in Live Cells**

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The delivery of therapeutics in nanoparticles capable of quickly traversing the cytoplasm and entering the nucleus via the nuclear pore complex (NPC) has many potential clinical applications. The effectiveness of chemotherapeutics such as alkylating agents, genetic material such as DNA, and transcription inhibitors may increase significantly if delivered directly to the nucleus.

Nanoparticles usually enter cells via endocytosis and proceed to processing within the endo-lysosomal pathway. To avoid degradation in the low pH environment of late endosomes and lysosomes (LE/Lys), endosomolytic agents and proton-sponge polymers have been applied and decreased co-localization with acidic vesicles has been observed. However, transfection efficiencies of these systems remain low compared to viral vectors. Possible limiting factors include the slow release of genetic materials or drugs from nanoparticles, hindered diffusion in the cytoplasm's molecularly crowded environment, or degradation of the therapeutic materials prior to reaching the nucleus.

To overcome these potential bottlenecks, an ideal drug/gene delivery system should deliver its drug cargo directly to the nucleus. Among the many design requirements for such systems, there is a strict size limitation for these nanoparticles due to the fact that the pore size of NPC is 9nm, and expanded to as high as ~25nm during active uptake. It is unclear, however, how such small nanoparticles may traffic within live cells. We sought to uncover the mechanism of intracellular trafficking of nanoparticles smaller than 25nm with a variety of surface modifications.

We used multi-color Confocal Particle Tracking (CPT) to directly correlate the transport of small nanoparticles with biological location (i.e. intracellular vesicles) in live cells and in real time. The high-speed confocal microscope used (Zeiss LSM 510 Meta) possesses the ability to capture fluorescence signals from multiple different fluorophores with the same detector (i.e. Meta detector), thereby allowing for characterization of nanoparticle transport in live cells in four dimensions: x, y, time, and color (biological location). As model polymeric nanoparticles, we incubated 20nm and 40nm carboxylated polystyrene (PS) beads (Molecular Probes) loaded with Cy-5 and yellow-green fluorescence along with LysoTracker-Red (Molecular Probes), which labeled late endosomes and lysosomes, in live HeLa cells. Following cellular uptake, intracellular transport and trafficking of nanoparticles were observed and quantified.

After 4 hrs of incubation, 20nm and 40nm PS beads display distinct localization in live HeLa cells. While Cy-5 and yellow-green fluorescence were both observed in the perinuclear region, yellow-green fluorescence appeared as punctate dots and almost 100% co-localized with the red fluorescence from LysoTracker, indicating that 40nm PS beads co-localized with LE/Lys. On the other hand, Cy-5 fluorescence exhibited <5% overlap with red fluorescence, implying that these nanoparticles entered cells without processing in the endo-lysosomal pathway. The Cy-5 fluorescence appeared as punctate dots in similar fashion to the 40nm PS, suggesting that the 20nm PS may reside in non-acidic vesicles. These results indicate that 20nm and 40nm particles may be sorted and trafficked into distinct vesicles within 4 hrs of incubation with cells.

Multi-color Confocal Particle Tracking is a quantitative tool that allows improved correlation of biology with physical phenomena in live cells. Studies using this technology show the intracellular trafficking of 20nm and 40nm polymeric nanoparticles may be fundamentally different. Current studies are underway to elucidate the effects of different surface modification may have on the intracellular trafficking of 20nm nanoparticles.