Correlative Strategies for the Identification and Intracellular Localization of Polymer Nanoparticles

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Amongst others, the use of nanoparticles in biology and especially in medicine is inspired by the idea to transport active agents exclusively to specific cells in the body. This requires a fundamental understanding how nanoparticles interact with complex biological surroundings, with the cell and the combination of both. In order to achieve a deeper insight into the endocytosis processes and the intracellular pathways of polymer nanoparticles its of substantial importance to identify the particles in their cellular context with high-resolution electron microscopy. However, in electron microscopy (EM) an unambiguous identification of polymer nanoparticles in a cellular environment proves to be extremely difficult. In most cases, the preparation for electron microscopy yields a thin section through the plastified cell revealing a confusing variety of many round structures of different sizes. The discrimination of polymer nanoparticles from cell compartments becomes difficult because its based solely on morphological judging of round structures in a confusing surrounding. This becomes even more demanding when trying to identify polymer nanocapsules, because their morphological fingerprint in a thin section is of annular shape, similar to the appearance of many cell compartments.

One strategy to overcome this issue is to add electron dense markers to the nanoparticles so that they can easily be identified in the electron microscope. This involves either loading the nanoparticles with inorganic markers like magnetite or gold nanoparticles or by homogeneously distributiing heteroatoms within the nanoparticles for spectroscopic identification by energy dispersive x-ray or electron energy loss spectroscopy. With these atomic fingerprints the intracellular nanoparticles can be identified unambiguously. However, labeling the polymer nanoparticles with inorganic markers requires additional synthetic effort and is hence not feasible as general approach for electron microscopic visualization. Labeling with a fluorescent dye, on the other hand, is straightforward and can be done easily by copolymerization or dissolving the dye in the polymer during the synthesis process. With this kind of labelling correlative light and electron microscopy (CLEM) would be the method of choice for easy identification of any kind of external polymeric material within a cell.

CLEM has been developed for the localization of fluorescently labeled proteins in a celluar environment. There are no protocols for correlative imaging of nanoparticles yet. We will report on two different model systems how to proceed in terms of preparation protocol and visualization strategies to achieve correlative fluorescence and high-resolution electron microscopical information. Fluorescent nano-diamonds are distinguished by a very stable fluorescence which is not affected by the embedding resin nor the staining agents used for the EM preparation. Accordingly, CLEM of these systems is straightforward and similar to any other protein based system. The other model system we present are polystyrene nanoparticles labelled with a bodipy fluorophore. In this case the fluorescence does not survive the EM preparation and the CLEM protocol has to be modified accordingly. Based on these two different systems we are able to present a versatile strategy for CLEM imaging of polymeric nanoparticles in biological environments.