PHOTODARKENING AND PHOTOBRIGHTENING OF BIO-CONJUGATED QUANTUM DOTS AFTER ELECTRON-BEAM IRRADIATION
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Understanding the role that different biomolecules play in biological processes requires their identification within the cellular ultrastructure. A major challenge is in the resolution gap between the techniques commonly used for biomolecule identification, i.e., fluorescence microscopy (FM), and ultrastructural determination, i.e., electron microscopy (EM). Superresolution (SR) fluorescence techniques can bridge this gap but correlation between SR-FM and EM is challenging and affected by distortions introduced in sample preparation steps needed between SR and EM imaging. Direct excitation of biomarker fluorescence by an electron beam (so called cathodoluminescence) would allow to visualize biomarkers directly in the EM image. Nanophosphors have recently been explored as cathodoluminescent markers [1-4], but their size is typically in the 100nm range and size polydispersity is an issue. Also, nanophosphor biofunctionalization is only very recently being explored [2]. Biofunctionalized colloidal quantum dots would be an attractive alternative but their observation by cathodoluminescence has thus far remained challenging.

Here, we investigate the cathodoluminescence imaging of bio-functionalized colloidal quantum dots (qdots). Qdots were conjugated to epidermal growth factor and imaged in whole cells fixated after uptake and intracellular transport. We show that the qdots can be imaged with cathodoluminescence using low-energy (<5keV) electron excitation to reduce the background signal[4] from the underlying glass substrate. However, high-dose electron exposure leads to a darkening of the qdots, which makes it impossible to perform high magnification cathodoluminescence imaging and visualize single qdots. We use fluorescence excitation and detection during electron beam irradiation to report on the (on/off) state of the quantum dots in the electron microscope. This reveals a complicated on-off switching upon electron-beam exposure. Interestingly, low-dose exposures, insufficient to generate detectable cathodoluminescence signal, are found to lead to fluorescence enhancement. Higher dose exposures lead to reversible darkening and, eventually, bleaching. These results can be partly understood in terms of charge carrier dynamics and electron trapping in the quantum dot core-shell geometry, which is also linked to quantum dot fluorescence blinking.