Photooxidation for ultrastructural localization of fluorescent probes

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Correlative techniques are aimed to combine the advantages of light microscopy, like imaging of fluorescence signals, with the high resolution obtained in the electron microscope, necessary for the exact subcellular localization of molecules. In the spectrum of technologies enabling correlative microscopy, photooxidation is one option. The method relies on the use of fluorescent dyes to oxidize the substrate diaminobenzidine (DAB) thus converting it into an electron-dense reaction product, making fluorescently labelled specimens suitable for electron microscopic examination [1].

Probes that allow easy switching between both imaging modes have given new input into the implementation of a variety of correlative methods [2]. The dyes currently used in immunofluorescence studies primarily were chosen because of their high fluorescence quantum yields (Φ_f); with the implementation of photooxidation it turned out however, that many of them unfortunately have comparatively low yields of singlet oxygen ($^1O_2$), thus having poor photooxidation capacity; dyes used for photooxidation and applications are summarized in Sosinsky et al. 2007 [3].

Our efforts towards a broader use of the method emanate from limited resolutions of light microscopical fluorescence signals in the analysis of cellular biosynthetic and endocytic routes. In our hands several fluorochromes, under optimized conditions, permit differentiated DAB-precipitation combined with excellent fine structural preservation [4]. Here, we refer to prospects and limitations in the use of various fluorochromes in correlative microscopy, recently summarized in 5. Such, one molecule in the centre of interest, the green fluorescent protein (GFP,) despite of its intensity and bleaching stability, turns out to be a conflicting probe. The 3D-structure of GFP, the ß-can, might provide the physicochemical basis of many of the observed features of the protein; the relative resistance to photobleaching on the other hand might be fundamental for the low levels of singlet oxygen production, in turn necessary for DAB-oxidation; multiple copies of GFP and a strong oxygenized DAB solution might favour the generation of sufficient amounts of free radicals upon illumination. Fluorochromes, like Alexa^{488} and Alexa ^{568}, fluorescein isothiocyanate (FITC) or the lipid dye BODIPY, either tracing endocytic uptake of molecules or used in in-situ labelling of endogenous molecules, differently reflected the photooxidation capacity (**Fig 1 and 2**). The DAB-deposits at the plasma membrane and membranous compartments, such as endoplasmic reticulum and Golgi apparatus in combination with the fine structural preservation and high membrane contrast enabled differential topographical analyses, and allowed tomographic reconstructions of complex cellular architectures, such as *trans*-Golgi-ER junctions.

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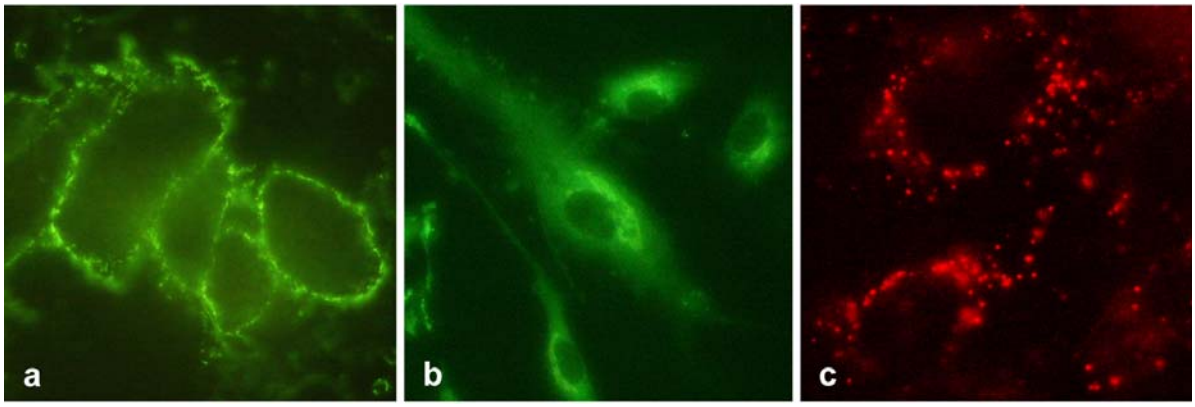

Figure 1. a: Immunofluorescence of human pancreatic epithelial cells (MIA PACa-2) showing CD99 distribution (FITC). b: Bodipy-ceramide accumulation after 30 min. incubation at 37°C in human umbilical venous cells (HUVEC). c: HDL-Alexa^{568} distribution and accumulation in human hepatoma cells (HepG2) after 3 hours internalization.

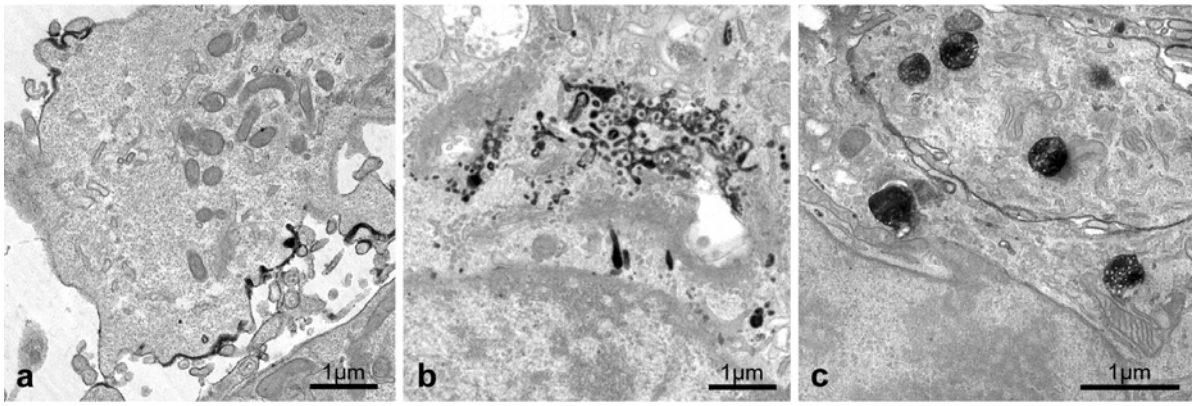

Figure 2. a: The spotty signals of CD99-immunofluorescence are mirrored by the local precipitation of DAB. b: After photooxidation of Bodipy-ceramide, the reaction product is localized at the *trans*-Golgi side and the *trans*-Golgi network. c: Ultrastructural visualization of HDL-Alexa^{568} in multivesicular bodies.

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