Oxidative stress plays an important role in cell physiology. Photodynamic treatment (PDT) is an emerging procedure for the therapy of cancer, based on photosensitizers, compounds that generate highly reactive oxygen species on illumination with visible light. Photodynamic peroxidation of cellular lipids is a consequence of PDT associated with cytolethality. Initiation and propagation of lipid peroxidation in living cells after PDT have not been studied at the microscopic level.

We used a novel fluorescent ratiometric oxidation-sensitive probe, C11-BODIPY<sup>581/591</sup> (C11-BO), which reports on lipid peroxidation, for visualizing oxidative stress in cells subjected to PDT with a phthalocyanine photosensitizer Pc4. With C11-BO loaded into the cells before or immediately after PDT, we observed a prolonged oxidation, which continued up to 30 min after illumination. In contrast, H<sub>2</sub>O<sub>2</sub> caused oxidation of C11-BO only when the cells were in direct contact with H<sub>2</sub>O<sub>2</sub>. Pretreatment with H<sub>2</sub>O<sub>2</sub> and subsequent loading with the probe did not reveal the delayed oxidation.

Intriguingly, the intracellular localization of oxidative stress generated by PDT did not coincide with the intracellular localization of the photosensitizer. The photosensitizer showed a broad localization in the cytoplasm, whereas the oxidative stress was mostly limited to vesicular perinuclear organelles, most likely lysosomes. Acridine Orange staining of lysosomes dissapeared as a result of PDT, indicating that lysosomes were damaged. We hypothesize that the lysosomal localization of the prolonged oxidative stress is a consequence of the presence in the lysosomes of redox-active iron, which can stimulate a prolonged lipid peroxidation.

In conclusion, we have found that oxidative stress induced in cells by PDT differs from one induced by H<sub>2</sub>O<sub>2</sub> in respect of induction of prolonged oxidation of lipids. The oxidation-sensitive probe C11-BO will be helpful for the characterization of particular photosensitizers with respect to intracellular localization and propagation of oxidative stress in living cells.