# Photophysical properties of Radachlorin photosensitizer in solutions, cells and tissues

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Photodynamic therapy is an emerging modality used for treatment of various malignancies and for inactivation of viral and bacterial infections. Its performance is based on photosensitized generation of reactive oxygen species, singlet oxygen in particular. Besides the opportunity for generation of oxidative substances, most photosensitizer (PS) molecules are fluorescent that can be used for estimation of the phototoxic impact on the targeted tissues or pathogens and for localization of malignant tissues. Therefore understanding of photophysical properties of a photosensitizer is essential for assessing its performance and optimization of photodynamic therapy protocols. The fluorescent properties of a PS depend on a number of factors and may vary noticeably in different microenvironments.

In this lecture we present a comprehensive analysis of photophysical properties of a clinically approved chlorin-based PS Radachlorin in bulk solutions, in aqueous solutions sprayed onto different biological surfaces, in living cells and in tissues of a murine model. Experiments were performed using time-resolved analysis of PS fluorescence and singlet oxygen phosphorescence, fluorescence lifetime imaging microscopy (FLIM) and digital holography.

Radachlorin fluorescence lifetimes and corresponding singlet oxygen quantum yields were analyzed in bulk solutions as a function of solution pH, viscosity and polarity and of PS concentration. The kinetics of PS photobleaching sprayed onto biological surfaces were studied at normoxic and hyperoxic conditions and were shown to change significantly depending upon the surface, with characteristic decay times varying from seconds to minutes [1].

The response of living cells to photodynamic treatment with Radachlorin was analyzed in three established cell lines of different origin (HeLa, A549 and 3T3) using holographic and fluorescence microscopy and FLIM. The analysis was performed on different aspects of the treatment procedure including PS accumulation, localization and photobleaching in cells and post-treatment dynamics of changes in cellular morphology at different treatment doses [2]. The developed holographic approach allowed for monitoring cell morphology noninvasively over long time and to distinguish between different pathways of cell death occurring at different treatment doses [3]. The approach was used for analysis of the response of cells obtained from tumor material of individual patients with different localizations of solid tumors to photodynamic treatment and a substantial diversity in responsivity of patients has been demonstrated [4].

The investigation of *in-vivo* accumulation of the PS in a murine model in several types of normal and tumor tissues was based on FLIM-assisted analysis of fluorescence intensity images, time-resolved fluorescence signals and phasor plots. The combined analysis of fluorescence intensity distributions and time-resolved fluorescent images provided qualitative and under some limitations quantitative data on relative uptake of the PS in tissues.

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**Index Terms:** photodynamic therapy, photosensitizer, fluorescence lifetime, FLIM, digital holography.

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