UVA light and singlet oxygen quantum yield of endogenous photosensitizers determined directly by its luminescence

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Introduction

- UVA light (320 – 400 nm) has been shown to produce deleterious biological effects. Skin is frequently exposed to sunlight and UVA exposure is thought to cause skin aging and skin cancer mainly by singlet oxygen.
- Substances like flavins or urocanic acid were considered to act as endogenous photosensitizers. After UVA light absorption, the excited endogenous photosensitizer transfers energy to generate singlet oxygen.
- However, precise data regarding the singlet oxygen generation are missing.
- **AIM OF THE STUDY:** Comparable to exogenous photosensitizers, the efficacy of singlet oxygen generation (quantum yield) must be determined for endogenous photosensitizers.

Experimental Setup

- Singlet oxygen is directly detected by time resolved measurement of its luminescence at 1270 nm in near-backward direction with respect to the excitation beam using an infrared sensitive photomultiplier.
- Riboflavin, Flavin Mononucleotide (FMN), Flavin Adenine Dinucleotide (FAD), β-Nicotinamide Adenine Dinucleotide (NAD), and β-Nicotinamide Adenine Dinucleotide Phosphate (NADP), urocanic acid, oleic acid or cholesterol are excited by the third harmonic of a Nd:YAG-Laser in the center of UVA at 355 nm.

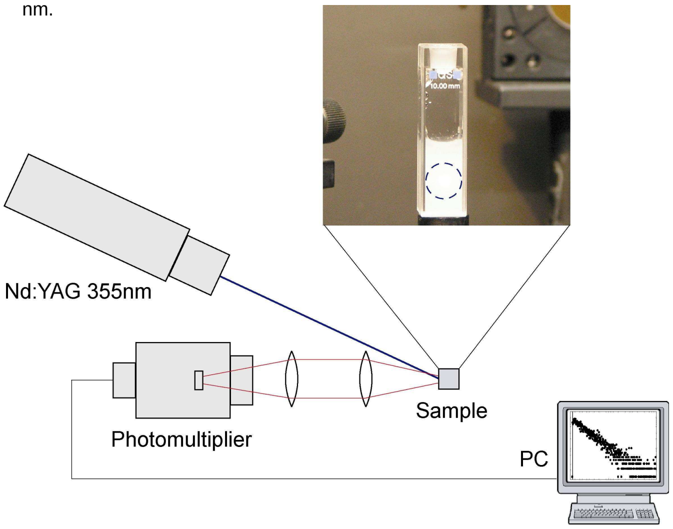

Figure 1. Experimental Setup. The photosensitizers are dissolved, filled in a cuvette and irradiated by the laser (355 nm) at a given spot size (blue ring)

Results

- No luminescence signal at 1270 nm was detected for NAD, NADP or Cholesterol, which might be due to there low absorption coefficient at 355 nm.
- For urocanic acid and oleic acid, singlet oxygen luminescence was clearly detected but the signal was too weak to quantify the respective quantum yield.
- When exciting Riboflavin, FMN or FAD the singlet oxygen luminescence was detected (see more on the right hand side).

References

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J. Baier, M. Maier, R. Engl, M. Landthaler and W. Bäumler, **Time-Resolved Investigations of Singlet Oxygen Luminescence in Water, in Phosphatidylcholine, and Aqueous Suspension of Phosphatidylcholine or HT29 Cells**, J. Phys. Chem. B 109 (2005) 3041

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Results for flavins

- For Riboflavin in air-saturated solution of water the luminescence of singlet oxygen at 1270 nm rises with a time constant of 2.8 ± 0.5µs and decays with a time constant of 3.2 ± 0.5µs, which is the decay time of singlet oxygen in water within the experimental accuracy (Figure 2a).
- The luminescence signal can be doubtless attributed to singlet oxygen by repeating the measurement spectrally resolved between 1160 to 1380 nm (Figure 2b).

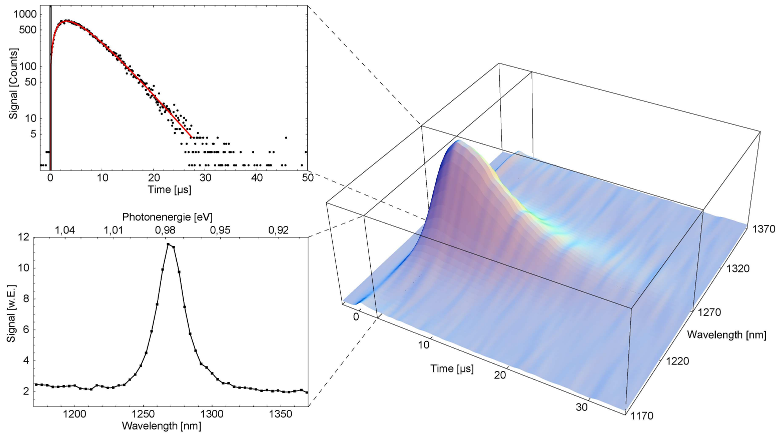

Figure 2. Time- and spectrally resolved luminescence of singlet oxygen for Riboflavin.

- Exciting Riboflavin, FMN and FAD the singlet oxygen luminescence could be detected. For these substances the quantum yields were determined using perinaphtenone as reference: Riboflavin Φ_Δ = 0.54 ± 0.07, FMN Φ_Δ = 0.51 ± 0.07 and FAD Φ_Δ = 0.07 ± 0.02 (air saturation).
- The singlet oxygen quantum yield depends critically on the oxygen concentration, i.e. the oxygen partial pressure (pO_2) in the respective experimental setup. That is important when comparing experiments of *in vitro* (pO_2 ~ 150 mmHg) and conditions *in vivo* such as the skin (pO_2 ~ 20 mmHg). The results show a decrease of Φ_Δ with decreasing oxygen concentration (Figure 3).

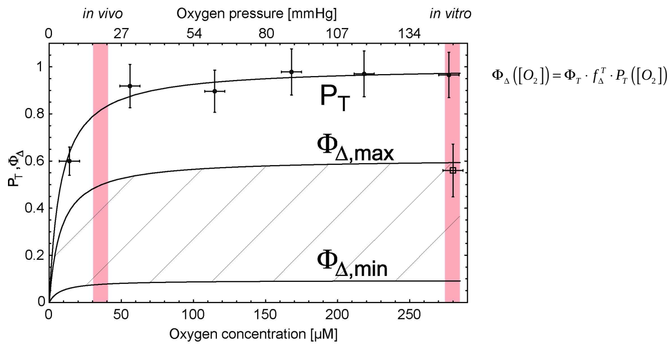

Figure 3. Dependence of the Riboflavin T_1 state deactivation efficacy P_T and the singlet oxygen quantum yield Φ_Δ on oxygen concentration.

Summary

- Our investigations provide evidence that UVA light at 355 nm generates singlet oxygen in endogenous sensitizers such as flavins, urocanic acid or fatty acids.
- The respective quantum yields for Riboflavin and FMN are higher than for exogenous photosensitizers like Photofrin used in Photodynamic Therapy (PDT) to kill cells.
- The efficacy of singlet oxygen generation clearly decreases with decreasing oxygen partial pressure, which is important for conditions *in vivo* (skin pO_2 ~ 20 mmHg).
- **OUTLOOK:** The current setup should be capable to detect UVA-induced singlet oxygen directly by its luminescence *in vivo*.