Investigation of photodynamic inactivation of bacteria using the detection of singlet oxygen luminescence

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Introduction

- Worldwide increasing resistance of MRSA against antibiotics
- Photodynamic inactivation of bacteria is a promising new technique
- Photosensitizers like Photofrin generate predominantly singlet oxygen, which oxidizes very effectivly lipids and proteins leading to death of bacteria
- Singlet oxygen is acting at the localisation of its generation, that is localisation of Photofrin
- **AIM OF STUDY:** To determine the localisation of singlet oxygen by time resolved measurement of its luminescence signal at 1270 nm

Methodology

- Bacteria strains: Gram(+) MRSA *S. aureus*, Gram(-) *E. coli*
- Uptake of Photofrin was estimated by spectrophotofluorimetric analysis. Supernatants of Photofrin-incubated bacteria suspensions were collected as well as the corresponding bacteria pellets. Pellets were lysed with 2% SDS o/n prior to analysis (**Fig. 1**)
- Phototoxicity was determined by Colony-Forming Units assay (CFU) after irradiation. Suspensions of 10^8/ml *S. aureus* or *E. coli* were incubated with different concentrations of Photofrin for 10 min and irradiated with 40 mW/cm^2 for 10 min (24 J/cm^2) (**Fig. 2**)
- Protein concentration of bacteria suspensions was measured using the BCA™ Protein assay
- Luminescence experiments: Bacteria suspensions (protein concentration 0 – 1.5 mg/ml) were incubated with 0.3 mg/ml Photofrin. Photofrin was excited using a frequency-doubled Nd:YAG laser (532 nm, pulse duration 135 ns). Singlet oxygen luminescence was detected using an infrared high sensitive photomultiplier at 1270 nm (**Fig. 3 and Fig. 4**)
- In the experiments an exponential rise and decay was observed. The luminescence intensity is given by $I(t) = \frac{C}{\tau_R^{-1} - \tau_D^{-1}}\left[\exp(-\frac{t}{\tau_D}) - \exp(-\frac{t}{\tau_R})\right]$. The constant C was used to fit the luminescence signal. τD and τR are the decay and rise times, respectively. To determine the rise and decay times of singlet oxygen, the least square fit routine of Mathematica 4.2 (Wolfram Research) was used.

Results

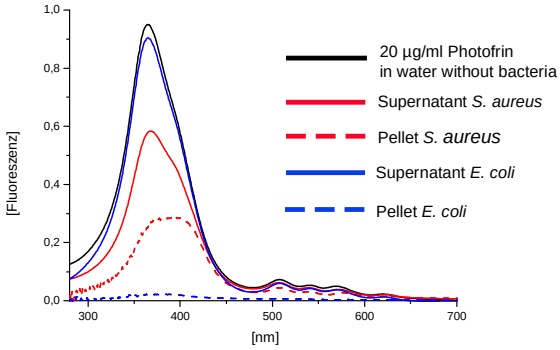

Figure 1. Uptake of Photofrin by *S. aureus* and *E. coli*.
S. aureus or *E. coli* were incubated with 20 µg/ml Photofrin for 10 min prior to spectrophotofluorimetric analysis. Uptake of Photofrin was much higher by *S. aureus* as compared to *E. coli*

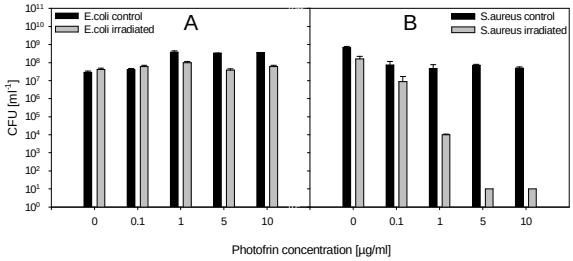

Figure 2. Phototoxicity of Photofrin against *S. aureus* and *E. coli* upon irradiation
Photofrin demonstrate a concentration-dependent phototoxcity against *S. aureus* and not by *E. coli*. **A**) Black line: *E. coli* w/o irradiation, grey line: *E. coli* + irradiation. **B**) Black line: *S. aureus* w/o irradiation, grey line: *S. aureus* + irradiation. n=3, mean ± SD

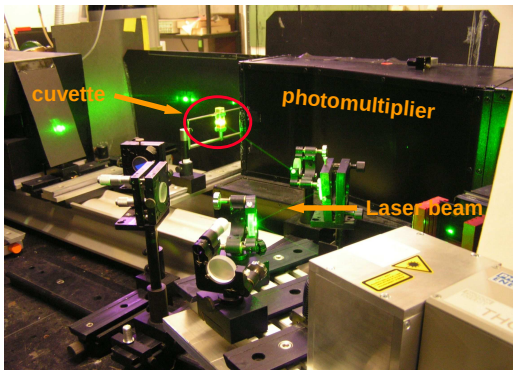

Figure 3. Laser superstruction for detection of singlet oxygen luminescence
Bacteria suspensions were transferred into a cuvette. Excitation was done using a frequency-doubled Nd:YAG laser (532 nm, pulse duration 135 ns)

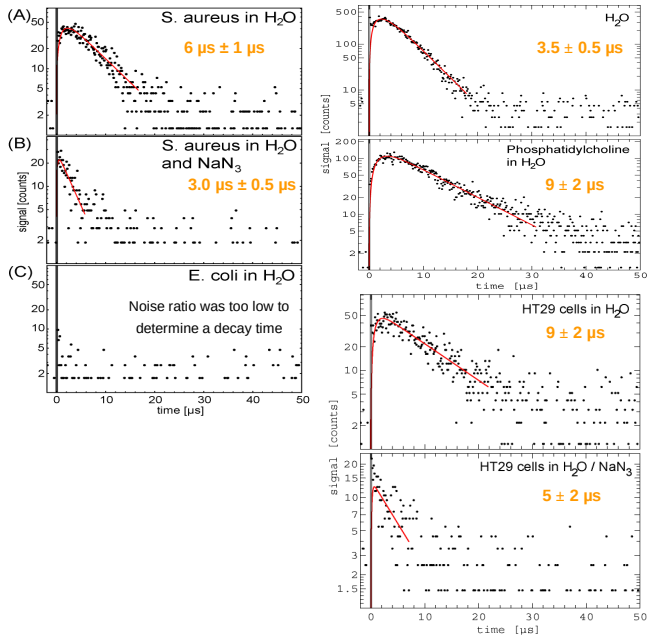

Figure 4. Luminescence of singlet oxygen at 1270nm
Singlet oxygen was generated by 0.3 mg/ml Photofrin vs. time in (**A**) suspension of *S. aureus* (0.125 mg/ml) in H_2O, (**B**) suspension of *S. aureus* in H_2O with 50 nM NaN_3 (Quencher of singlet oxygen) and (**C**) suspension of *E. coli* (0.125 mg/ml) in H_20 and **right panel** in pure H_20, in lipid suspension (Phosphatidylcholine), in HT29 cell suspension, and in HT29 cell suspension containing 50 nM NaN_3

Summary

- Singlet oxygen luminescence decay time of about 6 µs was measured by *S. aureus*.
- That decay time is an intermediate time of singlet oxygen decaying in phospholipids (phosphatidylcholine: 14 µs) of membranes and surrounding water (3.5 µs).
- Thus, singlet oxygen seems to decay in the outer cell wall areas of *S. aureus*, which is then the subcellular localization of Photofrin
- Incubation of *E. coli* with Photofrin revealed no singlet oxygen signal at all being equivalent to nearly no Photofrin within bacteria
- **OUTLOCK:** The results encourage further work on more photosensitizer with different side-chain chemistry to improve photodynamic inactivation of different types of bacteria

Acknowledgement

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