Time dependence of singlet oxygen luminescence provides an indication of oxygen concentration during oxygen consumption

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Abstract. Singlet oxygen plays a major role in photodynamic inactivation of tumor cells or bacteria. Its efficacy depends critically on the oxygen concentration \([O_2]\), which can decrease in case oxygen is consumed caused by oxidative reactions. When detecting singlet oxygen directly by its luminescence at 1270 nm, the course of the luminescence signal is critically affected by \([O_2]\). Thus, it should be feasible to monitor oxygen consumption during photo-oxidative processes. Singlet oxygen was generated by exciting a photosensitizer (TMPyP) in aqueous solution (H\(_2\)O or D\(_2\)O) of albumin. Chromatography shows that most of the TMPyP molecules are unbound, and therefore singlet oxygen molecules can diffuse in the solution. A sensor device for oxygen concentration revealed a rapid decrease of \([O_2]\) (oxygen depletion) in the solution during irradiation. The extent of oxygen depletion in aqueous albumin solution depends on the radiant exposure and the solvent. When detecting the luminescence signal of singlet oxygen, the shape of the luminescence signal significantly changed with irradiation time. Thus, local oxygen consumption could be monitored during photodynamic action by evaluating the course of singlet oxygen luminescence. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2821151]

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1 Introduction

Reactive oxygen species, in particular singlet oxygen, have been the focus of intense investigation due to their important role in biological processes. Singlet oxygen is a highly reactive oxygen species that can effectively oxidize lipids and proteins of cells leading to cell damage, gene regulations and even cell death. The reactions of singlet oxygen are frequently non-diffusion-controlled processes, and the extent of reaction in a complex biological system is determined by the bimolecular rate constant and the local concentration of singlet oxygen.

Singlet oxygen is generated either by chemical reactions or by energy transfer of a light absorbing photosensitizer. The photosensitizers can localize in different cellular compartments and generate singlet oxygen, which damages lipids or proteins in the immediate vicinity. In addition to chemoluminescence techniques, a direct and noninvasive detection of singlet oxygen is the time-resolved measurement of its luminescence at 1270 nm. Although singlet oxygen is short-lived on a microsecond scale or even shorter, it can diffuse into different environments that may consist of various cellular membranes, proteins, and water. Since the respective environment of singlet oxygen determines the rise and decay time of the luminescence, the localization and partition of singlet oxygen in a microheterogeneous system should be feasible. Consequently, the time-resolved measurement has become an important tool to detect singlet oxygen and its localization in complex structures like living cells or tissue.

However, the determination of singlet oxygen by its luminescence depends critically on the oxygen concentration \([O_2]\), in particular the rise and decay rate of luminescence can have a different meaning for different \([O_2]\). Usually, the decay time of luminescence is attributed to singlet oxygen decay. In the case of photosensitized generation of singlet oxygen at low \([O_2]\), the decay time of luminescence at 1270 nm is in all likelihood the decay time of the triplet \(T_1\) state of the photosensitizer. This might be true in an cellular environment of singlet oxygen, where \([O_2]\) is consistently low showing values of 4 Torr \(([O_2]=7.5 \text{ µM})\).

Moreover, \([O_2]\) may change while singlet oxygen is generated due to chemical reactions between singlet oxygen and biomolecules such as lipids and proteins. This oxidative process can lead to oxygen consumption at the site of singlet oxygen generation, which has already been investigated by detecting the phosphorescence quenching of certain
metalloporphyrins. The clinical application of singlet oxygen is the photodynamic therapy of tumors and bacteria. In these therapeutic procedures, oxygen depletion is recognized and clinicians try to avoid it by fractionation of fluence rates.

To elucidate the role of oxygen depletion by photosensitized singlet oxygen generation, we chose a rather well defined system that consists of a water-soluble photosensitizer in aqueous solution (H$_2$O, D$_2$O) and bovine serum albumin. The light of a pulsed laser system (532 nm) excited the photosensitizer and singlet oxygen is generated. Singlet oxygen was detected time resolved by its luminescence at 1270 nm. Simultaneously, the oxygen concentration in the solution was measured.

2 Material and Methods

2.1 Materials
TMPyP [5,10,15,20-Tetrakis(1-methyl-4-pyridinio) porphyrin tetra(p-toluenesulfonate)] (purity >97%) was purchased from Sigma-Aldrich (Steinheim, Germany) and bovine serum albumin (purity >98%) was purchased from Biomol GmbH (Hamburg, Germany).

2.2 Singlet Oxygen Detection
The solutions were transferred into a cuvette (QS-100, Hellma Optik, Jena, Germany) and hermetically sealed. The sensitizer was excited using a frequency-doubled Nd:YAG laser (PhotonEnergy, Ottensoos, Germany) with a repetition rate of 2.0 kHz (wavelength 532 nm, pulse duration of about 100 ns). The laser pulse energy for luminescence experiments was 50 μJ. The singlet oxygen luminescence at 1270 nm was detected in the near-backward direction with respect to the excitation beam using an IR-sensitive photomultiplier (R5509-42, Hamamatsu Photonics Deutschland GmbH, Herrsching, Germany) with a rise time of about 3 ns. At the entrance of the photomultiplier, two interference filters on top of each other were used with a maximum transmission at 1270 nm, and a half width of 25 nm (Schott, Mainz, Germany), or 13 nm (L.O.T. Oriel GmbH & Co. KG, Darmstadt, Germany). The signal of the photomultiplier was amplified by a preamplifier (Model 6954, Philips Scientific, Ramsey, New Jersey). The signal was processed by a 7886S Dual Input Multiscaler (time of flight, Photon Counter, FAST Com Tec GmbH, Oberhaching, Germany) plugged into a personal computer. The channel width of the Dual Input Multiscaler was set to 128 ns. Absorbance was measured with a Beckman DU640 spectrophotometer (Beckman Instruments GmbH, Munich, Germany).

2.3 Measurement of Oxygen Concentration
The [O$_2$] was determined by measuring the partial vapor pressure (% air saturation) of oxygen in solution during irradiation (MICROX TX, PreSens GmbH, Regensburg, Germany). To achieve a uniform oxygen depletion, the cuvette was completely filled, hermetically closed, and permanently agitated by a magnetic stirrer (Color squid, IKA, Staufen, Germany). To best of our knowledge a value for oxygen solubility in D$_2$O is lacking. Therefore, we used an estimation to calculate oxygen concentration in D$_2$O based on the known ratio for CO$_2$ in D$_2$O and H$_2$O (0.88).

2.3.1 Chromatography
The solutions containing TMPyP or TMPyP/albumine were fed into a modular 3-D high performance liquid chromatography (HPLC) system with 1100 Photo-Diode-Array-Detector Mod. Nr. G1315b (1100 Agilent Technologies Waldbronn, Germany). A HPLC-3-D-ChemStation Rev. A.10.01 was used for data analysis. The analytical column used was a Reversed Phase Jupiter C4 (250×2.0 mm i.d., 5-μm particle size, 300-Å pore size) from Phenomenex (Aschaffenburg, Germany). Gradient elution was achieved with water obtained by a Millipore Water-Purification-System (MilliQ-Gradient-A10) with 0.01 M tetrabutylammonium hydrogensulfate (>99% Fluka) as the ion pair chromatography (IPC) reagent (solvent A) and acetonitrile HPLC gradient grade quality (solvent B) at a constant flow rate of 400 μL/min. A gradient profile of solvent B was applied [t (min), %B]: (0, 10), (10, 40), (40, 80), (45, 80). The compounds described were monitored at 220 nm. The injection volume was 15 μL.

3 Results and Discussion
After light absorption in a photosensitizer, the energy can be transferred from the triplet state of the photosensitizer to molecular-oxygen-generating singlet oxygen. The time-resolved technique yields the time course of luminescence and reflects the solution of a coupled differential equation system. This describes the population of the photosensitizer triplet state $T_1$ and the first excited state of oxygen (singlet oxygen, O$_2$).

$$\frac{d[T_1]}{dt} = \left(-k_{T_1} - (k_{T_1}\text{O}_2 + k_{T_1}\text{O}_2)\right)[T_1] = -K_{T_1}[T_1], \quad (1)$$

$$\frac{d[O_2]}{dt} = \left(-k_\Delta + k_\text{Q}[Q]\right)[O_2] + k_{T_1}[O_2][T_1]$$
$$= -K_{\Delta}[O_2] + k_{T_1}[O_2][T_1], \quad (2)$$

where ($k_{T_1}\text{O}_2 + k_{T_1}\text{O}_2$) is the deactivation of the $T_1$ state by oxygen, $k_\Delta$ describes the generation of singlet oxygen, and $k_{T_1}$ is the deactivation of the $T_1$ state to the ground state of the photosensitizer. The deactivation of singlet oxygen by the solvent and quencher at the concentration $[Q]$ is given by $k_\Delta$ and $k_{\text{Q}}$, respectively. The rates $K_{\Delta}$ and $K_{T_1}$ represent the deactivation of singlet oxygen and of the triplet state of the photosensitizer, respectively.

The population of these states is initiated by the laser pulse (532 nm), which is absorbed by the photosensitizer. After excitation, the population of triplet state $T_1$ and singlet oxygen decay, which is described by the temporal solution of the Eqs. (1):
\[
[1^1O_2](t) = \frac{C}{\beta_1 - \beta_2} [\exp(-\beta_2 t) - \exp(-\beta_1 t)],
\]

where \(\beta_1\) and \(\beta_2\) are the rise and the decay rates of the luminescence detected at 1270 nm, which are fitted to the luminescence using the constant \(C\). The luminescence rises with the rate \(\beta_1\) showing two meanings with \(\beta_1 = K_{T_1}\) for \(K_{T_1} > K_\Delta\) and \(\beta_1 = K_\Delta\) for \(K_{T_1} < K_\Delta\). The luminescence decays with \(\beta_2\), showing two meanings with \(\beta_2 = K_\Delta\) for \(K_{T_1} > K_\Delta\) and \(\beta_2 = K_{T_1}\) for \(K_{T_1} < K_\Delta\).

When solving Eqs. (1) and (2), \([O_2]\) is assumed constant. Normally, the decay rate \(\beta_2\) is equal to \(K_\Delta\), and the reciprocal value of \(K_\Delta\) represents the lifetime of singlet oxygen. The rise rate \(\beta_1\) is equal to \(K_{T_1}\), and the reciprocal value represent the lifetime of the triplet state of the photosensitizer. However, at low oxygen or high quencher concentrations, the rise rate \(\beta_1\) describes the deactivation of singlet oxygen and \(\beta_2\) describes the deactivation of the photosensitizer triplet state \(T_1\). The analytical solution of Eqs. (1) and (2) does not consider the time-dependent decrease of \([O_2]\), which is caused by chemical reactions of singlet oxygen with proteins during irradiation. To consider oxygen consumption in our experimental setup, singlet oxygen was generated in an aqueous solution of albumin with photosensitizer, and \([O_2]\) was measured simultaneously.

The photosensitizer TMPyP is a water soluble porphyrin molecule that is chemical very stable on irradiation. The change of absorbance at 532 nm was less than 3% after irradiation (532 nm) of TMPyP in \(H_2O\) (50 \(\mu\)M) for 60 min even at a high power of 250 mW. This correlates well with the findings in HPLC when comparing peak areas before and after irradiation. The absorption cross section of TMPyP is \(3.6 \times 10^{-17}\) cm\(^2\) at 532 nm and the absorbed energy is effectively transferred\(^{27}\) to the triplet state of TMPyP (\(\Phi_T = 0.92\)). TMPyP sufficiently generates singlet oxygen showing\(^{27}\) a quantum yield of \(\Phi_3 = 0.77\). The lifetime of the triplet \(T_1\) state in non-aerated aqueous solution was determined\(^{27}\) yielding a value of 0.165 ms, which is in good agreement with our own findings\(^{14}\) (0.14\(\pm\)0.03 ms).

### 3.1 Oxygen Depletion in Albumin Solutions

Singlet oxygen can readily oxidize serum albumin,\(^{16}\) which may lead to a decrease of \([O_2]\) in the solution.\(^{18,29,30}\) To quantify this oxygen consumption during generation of singlet oxygen, serum albumin (30 \(\mu\)M) was dissolved in either \(H_2O\) or \(D_2O\), TMPyP (100 \(\mu\)M) was added and filled in a glass cuvette. The cuvette was completely filled (3.3\(\pm\)0.1 ml), hermetically closed, and magnetically stirred during irradiation to avoid local gradients of \([O_2]\).

Using the pulsed laser system (repetition rate 2 kHz) at a pulse energy of 50 \(\mu\)J, the respective solution was excited for 500 (\(H_2O\)) or 50 s (\(D_2O\)). The oxygen sensor measured the \([O_2]\) in the cuvette during irradiation. Figure 1(A) shows the results for serum albumin in \(H_2O\). At the energy of 0 J (prior to irradiation), \([O_2]\) is calculated to be 270 \(\mu\)M (air saturated). During the pulsed irradiation, \([O_2]\) continuously decreased until the oxygen sensor showed no oxygen at total energy of about 45 J. In \(D_2O\) [Fig. 1(B)], the air-saturated concentration of oxygen is calculated to be 240 \(\mu\)M. The oxygen depletion in \(D_2O\) during irradiation is much faster than in \(H_2O\). The sensor detected no oxygen after application of about 5 J. The shape of the curves in Fig. 1 is compatible with a second-order equation for chemical reactions.

After generation by TMPyP, singlet oxygen can diffuse in the solution during its lifetime. Singlet oxygen can be deactivated by the solvent or by the interaction with albumin as a quencher. The physical quenching of singlet oxygen leads to ground state oxygen without consuming oxygen. Chemical quenching of singlet oxygen by albumin leads to the binding of oxygen molecules to the protein. Therefore, oxygen is consumed during irradiation and the concentration of oxygen in the solution should decrease. Due to the singlet oxygen lifetime of 3.5\(\pm\)0.5 ms in \(H_2O\) and 67\(\pm\)5 ms in \(D_2O\), the diffusion length of singlet oxygen in \(H_2O\) is shorter than in \(D_2O\). Therefore, the probability of singlet oxygen to hit serum albumin and oxidize serum albumin is more likely in \(D_2O\). As a consequence, the depletion of singlet oxygen in \(D_2O\) is faster than in \(H_2O\) (see Fig. 1).

### 3.2 Chromatography (HPLC) of Albumin and TMPyP Solutions

To elucidate the different results for \(H_2O\) and \(D_2O\), the localization of the photosensitizer was investigated. Singlet oxygen can be generated either by unbound photosensitizer or by pho-
tosensitizer molecules bound to albumin. The latter has been shown for photosensitizers such as tin ethyl etiopurpurin or chlorine p6 (Ref. 16).

When performing HPLC for TMPyP solutions (100 μM) with or without albumin (30 μM), the TMPyP peak at a retention time of 20.070 min showed an area under the peak of 4204.2 with albumin and 4807.8 without albumin. When comparing these peak areas, it is calculated that about 12.5% of TMPyP is bound to albumin. In case the bound TMPyP generates singlet oxygen, it could be immediately quenched by albumin, which shows a high quenching rate constant.

Since the major part of TMPyP molecules is unbound, singlet oxygen can diffuse in H2O or D2O. This is in agreement with findings that after incubation of cells with TMPyP, the photosensitizer molecules show a non negligible amount remaining homogeneously distributed in the cytoplasm. Diffusion of singlet oxygen in the respective aqueous solvent plays an important role. Diffusion is quite different for pure H2O and D2O. Assuming \( \Delta r = 6D\tau (D = 2 \times 10^{-5} \text{ cm}^2/\text{s}; \tau: \text{decay time of singlet oxygen}) \), the diffusion length is about 0.9 in D2O and 0.2 μm in H2O. As already mentioned, the difference may explain the rapid oxygen depletion in D2O as compared to H2O.

When performing HPLC after laser irradiation of the solution containing albumin and TMPyP, the shape of the albumin peak is significantly altered. Obviously, this might be due to the oxidative damage of the protein, which has been already shown by the change of intrinsic protein fluorescence. The peak area of albumin in HPLC was changed by about 30% as compared to untreated control. Due to the concentration ratio of albumin and oxygen, we suggest multiple oxidations of albumin molecules.

### 3.3 Singlet Oxygen Luminescence in H2O

TMPyP in H2O generated singlet oxygen and the luminescence at 1270 nm was detected without and with albumin in a long-run experiment, whereas \([O_2]^-\) was measured simultaneously.

#### 3.3.1 Without albumin

We dissolved 100 μM TMPyP in H2O without albumin. Singlet oxygen was generated and the luminescence signal measured by summing up 2000 pulses of irradiation for each experiment. The rise rate \( \beta_1 \) and decay rate \( \beta_2 \) of the luminescence of singlet oxygen was measured for different \([O_2]^-\) (0 to 280 μM) by flowing the solution prior to irradiation with nitrogen. During the variation of oxygen and the variation of the concentration of TMPyP the sum of rate constants \( (k_{Tt,O2} + k_{Tt,a}) = 1.8 \pm 0.2 \mu s^{-1} \text{mM}^{-1} \) \( k_{Tt,a} = 0.007 \pm 0.001 \mu s^{-1} \), and \( k_{A} = 0.29 \pm 0.02 \mu s^{-1} \) were determined. Our value for TMPyP triplet state quenching by oxygen is in good agreement with literature (1.6 \( \mu s^{-1} \text{mM}^{-1} \) in Ref. 33). However, the authors detected a clear decrease of this rate when TMPyP was irradiated in the presence of DNA.

This could be partially caused by local oxygen depletion due to the reaction of singlet oxygen with DNA (see in the following).

#### 3.3.2 With albumin

The quencher rate constant \( k_{AQ} \) was fixed to 0.45±0.10 \( \mu s^{-1} \text{mM}^{-1} \) using a variation of albumin concentration at a constant oxygen concentration, which is in good agreement with literature (0.5 \( \mu s^{-1} \text{mM}^{-1} \), Ref. 30). Taking these values, \( K_A \) and \( K_{Tt} \) were calculated and added as lines to Fig. 2.

After that, albumin was added to H2O, yielding a concentration of 30 μM. After adding TMPyP at a concentration of 100 μM, the cuvette was completely filled with the solution (3.3±0.1 ml), hermetically closed, and magnetically stirred during irradiation. In contrast to experiments without albumin, no nitrogen gas was applied to change the \([O_2]^-\). Here, the variation of \([O_2]^-\) was intrinsically achieved by the generation of singlet oxygen and its subsequent reaction with albumin (peroxidation). At the same time, the oxygen sensor monitored the respective \([O_2]^-\). To measure the respective \([O_2]^-\) during irradiation procedure, a stepwise decrease of \([O_2]^-\) was performed. After applying 2000 laser pulses, the irradiation was temporarily stopped and \([O_2]^-\) was measured. This was repeated until \([O_2]^-\) reached zero.

After every 2000 laser pulses, the rise rate \( \beta_1 \) (square point) and decay rate \( \beta_2 \) (circle point) of the luminescence of singlet oxygen were determined. The oxygen sensor determined the respective \([O_2]^-\) and therefore the values could be added to Fig. 2. Within experimental accuracy, the measured rise and decay rates from experiments with albumin fit to the lines \( (K_A \text{ and } K_{Tt}) \), which represent the values of experiments without albumin. Consequently, the variation of oxygen either by flowing the solution with nitrogen or by chemical quenching in serum albumin yielded comparable results regarding the luminescence of singlet oxygen.

We used a very short irradiation time of 1 s (2000 laser pulses). Nevertheless, note that \([O_2]^-\) also slightly decreased during this short irradiation time (2000 pulses=0.1 J) of 1 s. Hence, the measured signal of luminescence is a superposition of 2000 single experiments at different \([O_2]^-\). For a closer
examination of this effect, two additional experiments were performed using a substantially longer irradiation time. At a starting oxygen concentration of 110 μM, the luminescence signal was measured by summing up 120,000 (6 J) and 400,000 (20 J) single pulses. The rate $\beta_1$ (closed triangle points in Fig. 2) seems to be constant within experimental accuracy. This is not surprising, because for $[O_2]$ of about less than 110 μM, the rise rate $\beta_1$ reflects the decay rate of singlet oxygen, which should be independent of $[O_2]$. In contrast, the measured rate $\beta_2$ (open triangle points in Fig. 2) began to decrease with decreasing $[O_2]$. In H$_2$O and at $[O_2]=110$ μM, $\beta_2$ describes the deactivation of the triplet $T_1$ state. When applying up to 400,000 laser pulses to the solution, oxygen is increasingly consumed leading to a decreasing $[O_2]$. Consequently, the deactivation of the triplet $T_1$ state by oxygen is hampered, and the $T_1$ deactivation is more pronounced inside TMPyP to its ground state, which changes the lifetime of the triplet state. The measured decay rate $\beta_2$ yielded an intermediate value at an intermediate $[O_2]$, which is not exactly the decay rate that is measured at the start of the measurement. This fact seems to explain the drift of each measurement with 2000 pulses. Each decay rate seems to be smaller than the theoretical value determined without serum albumin.

The change of the lifetime of the TMPyP triplet state in DNA solutions was previously reported. The lifetime increased from 2.5 to 27.8 μs with increasing DNA concentration, which could indicate local oxygen consumption.

### 3.4 Singlet Oxygen Luminescence in D$_2$O

Comparable to experiments with H$_2$O, singlet oxygen was generated by TMPyP in D$_2$O and the luminescence at 1270 nm was detected without and with albumin in a long-run experiment, whereas $[O_2]$ was measured simultaneously.

#### 3.4.1 Without albumin

We dissolved 100 μM TMPyP in D$_2$O. The rise rate $\beta_1$ and decay rate $\beta_2$ of the luminescence of singlet oxygen were measured for different concentrations of oxygen, TMPyP. The sum ($k_T=1+K_T\lambda\Delta$) yielded a value of $2.2\pm0.2 \mu s^{-1} mM^{-1}$, $k_T=0.004\pm0.002 \mu s^{-1}$, and $K_T=0.015\pm0.001 \mu s^{-1}$. Again, our value is in good agreement with literature (1.86 μs$^{-1}$ mM$^{-1}$). As with H$_2$O, the decrease of this rate by about a factor 10 in the presence of DNA could be partially caused by oxygen depletion. Unfortunately, the authors truncated the time course of singlet oxygen luminescence at 15 μs, and therefore the information about the rise rate of the signal is lacking.

#### 3.4.2 With albumin

The use of albumin showed a $k_{\lambda\Delta}=0.6\pm0.2 \mu s^{-1} mM^{-1}$ and taking all these values, $K_T$ and $K_T\lambda\Delta$ were calculated and added as lines to Fig. 3.

After that, serum albumin was added to the solvent of 100 μM TMPyP in D$_2$O at a concentration of 30 μM. As shown in experiments with H$_2$O, the cuvette was completely filled (3.3±0.1 ml), hermetically closed, and magnetically stirred during irradiation. Starting with air saturation (240 μM oxygen), a step such as the decrease of $[O_2]$ was achieved by the generation of singlet oxygen and its subsequent binding to albumin, whereas for each step, 2000 laser pulses were applied (see earlier in the paper). The rise rate $\beta_1$ (square point) and decay rate $\beta_2$ (circle point) of the luminescence of singlet oxygen were determined for the respective $[O_2]$ and added to Fig. 3.

Due to the fast depletion of oxygen in D$_2$O by serum albumin [see also Fig. 1(B)], the change of the measured rise rate $\beta_1$ is even more striking as compared to H$_2$O. For $[O_2]>10$ μM, the rate $\beta_1$ was consistently smaller than the values of experiments without albumin (dashed line in Fig. 3). Since $\beta_1=K_{T_1}^\lambda$ for $T_1$, $K_T^\lambda=K_{T_1}^\lambda=K_T^\lambda T_1(kT_1O_2+kT_1\Delta)\times[O_2]$, the deactivation of the triplet $T_1$ state is correlated to $[O_2]$. Thus, the measurement of $K_T^\lambda$ is affected in case singlet oxygen is produced and chemically quenched by biomolecules such as proteins or lipids. If this effect is not considered, the measured value of $K_T^\lambda$ might be too small and should depend on the light energy or the time of irradiation.

To elucidate this dependency of the rate $\beta_1$ on the irradiation energy, the total irradiation energy was varied from 100 pulses (5 mJ) to 4000 pulses (200 mJ) at $[O_2]=190$ μM and fresh solutions were used for each. With a decreasing number of pulses, the rise rate $\beta_1$ increased due to less oxygen consumption (insert Fig. 3). The values of $\beta_1$ are approaching the rates, which were measured for D$_2$O without serum albumin and consequently for the case of no oxygen consumption. Additionally, when reducing the number of laser pulses for irradiation, the intermediate $[O_2]$ approaches the oxygen concentration at the start of each measurement step. For $[O_2]=190$ μM, the decay rate $\beta_2$, which corresponds here to the decay time of singlet oxygen, was constant within experimental accuracy. However, with decreasing $[O_2]$, the measured values of $\beta_2$ clearly deviate from the line calculated for no oxygen consumption.

![Fig. 3 Rates $\beta_1$ and $\beta_2$ ($K_T^\lambda$ and $K_T^\Delta$) in D$_2$O solutions (100 μM TMPyP) determined by fitting the singlet oxygen luminescence curves at 2000 excitation pulses. The lines represent the calculated values based on experiments without albumin and changing $[O_2]$ by nitrogen gas. The squares represent the rise rates and the circles are the decay rates when adding serum albumin (30 μM) to the solution; the oxygen variation is due to chemical quenching of singlet oxygen. The vertical rectangles show the values for different numbers of pulses.](https://example.com/fig3.png)
3.5 Outlook: Singlet Oxygen Luminescence in More Complex Environments Such as Cells

After being generated, singlet oxygen can diffuse, can decay due to an interaction with solvent or quencher molecules (physical quenching), or can chemically react with other molecules (chemical quenching). The time-resolved detection of luminescence is a powerful tool for direct detection of singlet oxygen. At the same time, the detection can be used to elucidate the localization and reaction mechanisms of singlet oxygen, in particular in cells and tissue.

Our current experiments were carried out in a protein solution that is a more or less simple environment for singlet oxygen. However, the interpretation of time-resolved singlet oxygen luminescence remains rather complex. Among others, two important conditions clearly influence the time course of luminescence. These are the local oxygen concentration \([O_2]\) and its change due to oxidative processes mediated by singlet oxygen (chemical quenching). Even at a very short irradiation time (2000 pulses), the generation and decay is affected by oxygen consumption; that is, chemical quenching reduces the population of singlet oxygen and, at the same time, affects the rise and the decay rate of luminescence.

The detection of singlet oxygen in a more complex environment, such as in cells or tissue, should be substantially more difficult. Snyder et al. made a significant effort to measure the lifetimes in single cells. They found long-lived mon perception of short lifetimes but fits with our own recent findings. However, in regard to singlet oxygen decay in a cellular environment, it is much more difficult to interpret the rise and decay rate of singlet oxygen luminescence. Looking at Fig. 2, the meaning of the rates should change due to the usual very low \([O_2]\) in cells (4 Torr, \([O_2] = 7.5 \mu M, \text{Ref. 14}\)). It is likely that the decay rates of luminescence measured do not reflect the lifetime of singlet oxygen but represent \(K_T\).

Looking at Fig. 3 (\(D_2O\) solution), it is more likely that in cells with \(D_2O\) the decay rate of luminescence reflects the lifetime of singlet oxygen. However, it is usually unclear to what an extent \(D_2O\) can replace \(H_2O\) inside the entire cells, in particular, at the subcellular localization of the photosensitizer. In addition, small amounts of remaining \(H_2O\) can affect the lifetime of singlet oxygen. Ten percent of \(H_2O\) in \(D_2O\) shortens the lifetime by more than 50%.

Despite our recent findings in bacteria or tissue, a challenging gap remains between our present investigations and the complexity of a cell. However, we could show that the luminescence rates of singlet oxygen clearly depend on the respective oxygen concentration. In pure solvents, \([O_2]\) can be easily changed by replacing the oxygen molecules with nitrogen molecules. When adding a protein such as albumin, the oxygen is not replaced by other gas molecules but is consumed due to chemical reactions of singlet oxygen with the protein (chemical quenching). In both cases, oxygen is removed from the solution. Owing to this chemical quenching of singlet oxygen, \([O_2]\) decreases from its initial concentration to a value that is correlated with the absorbed energy in the protein solution. Thus, singlet oxygen luminescence could be used to monitor the respective oxygen concentration in a biological environment, which here was a protein solution.

References


