



Detection of Singlet Oxygen using Fluorescent Chemical Traps in Sub-Cellular Domains of a Single Cell



Anita Gollmer and Peter R. Ogilby

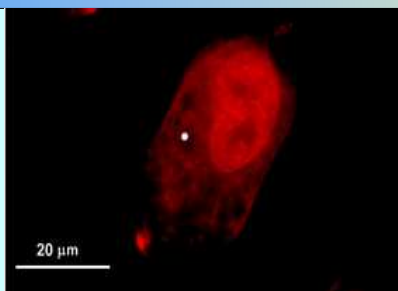
Center for Oxygen Microscopy and Imaging, Department of Chemistry, Aarhus University, Denmark

Singlet oxygen ($^1\text{O}_2$), the lowest excited electronic state of molecular oxygen, plays a major role in many chemical and biological processes, e.g. in photodynamic therapy. $^1\text{O}_2$ can be monitored either directly by its phosphorescence at 1270 nm or indirectly using fluorescent probes specific for $^1\text{O}_2$.¹⁻²

Our present technique

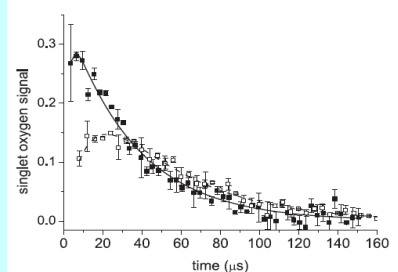
At present, we can selectively create and directly detect $^1\text{O}_2$ at the single cell level with sub-cellular spatial resolution by measuring its weak near-IR phosphorescence at 1270 nm (Figures 1-3).³

Figure 1: Image of a HeLa cell incubated with a $^1\text{O}_2$ photosensitizer which localizes mainly in the nucleus. The white spot in the cytoplasm represents the resolution in our experiments.



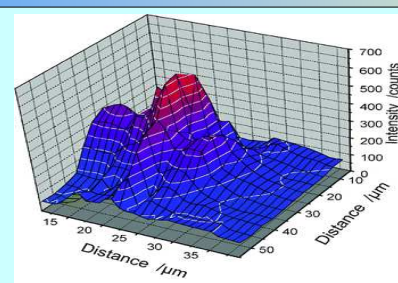
Spatial resolution

Figure 2: Time-resolved $^1\text{O}_2$ phosphorescence signals recorded upon sensitizer irradiation in the nucleus of HeLa cells. Cells were exposed to an atmosphere containing 50% oxygen (\square) and 100% oxygen (\blacksquare).



Intracellular kinetics

Figure 3: Contour plot showing the intensity of photosensitized $^1\text{O}_2$ phosphorescence from a portion of a D_2O -incubated HeLa cell. The data were generated by raster-scanning the sample through the focused laser beam.



Intensity profile

Advantages of this direct technique

Such measurements respond to the inherent heterogeneity of a cell and provide insight into a variety of $^1\text{O}_2$ -dependent phenomena, including the photoinitiated death of cells.

Disadvantages

This method suffers from weak signals because of the low efficiency of $^1\text{O}_2$ phosphorescence.

The complementary strategy

The use of fluorescent probes provides a powerful tool to detect $^1\text{O}_2$ in sub-cellular domains of a single cell with higher sensitivity.

Main goal

The design and characterization of fluorescent probes specific for $^1\text{O}_2$ to better understand the photodynamic behavior of $^1\text{O}_2$ in sub-cellular domains of a cell.

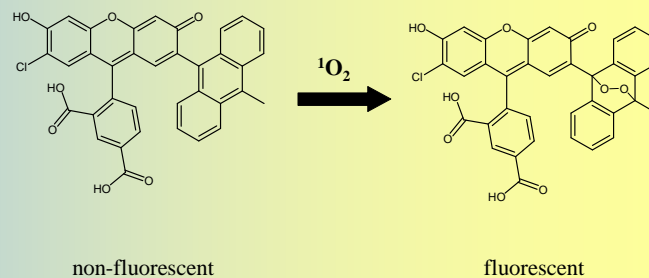


Figure 4: Singlet Oxygen Sensor Green. A commercially available fluorescence probe specific for $^1\text{O}_2$.

Chemical traps which are normally non-fluorescent due to internal electron transfer can react with $^1\text{O}_2$ forming a fluorescent endoperoxide.

Advantages of this indirect technique

- Higher detection sensitivity
- $\Phi_{fl}(\text{Endoperoxide}) \gg \Phi_{ph}(^1\text{O}_2) = 10^{-6} - 10^{-7}$
- Cells are less at risk of being damaged by high $[^1\text{O}_2]$
- Flexible design of fluorescent traps for intracellular location

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¹ Snyder, J.W., et al., *Singlet oxygen microscope: From phase-separated polymers to single biological cells*. Acc. Chem. Res., 2004, **37**(11): p. 894-901.

² Soh, N., *Recent advances in fluorescent probes for the detection of reactive oxygen species*. Analyt. and Bioanalyt. Chem., 2006, **386**(3): p. 532-543.

³ Breitenbach, T., et al., *Photosensitized production of singlet oxygen: spatially resolved optical studies in single cells*. Photochem. & Photobiol. Sci., 2009, **8**: p.442-452.