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Chapter

Photophysical Detection of Singlet Oxygen

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IntechOpen

"Old Man's Advice to Youth: 'Never Lose a Holy Curiosity.'" LIFE Magazine (May 2nd, 1955) p. 64.

Albert Einstein

Abstract

The chemical reactivity of singlet oxygen ($^1O_2$) (SO) derives from its electronically excited state. Being a unique reactive oxygen species SO takes part in many important atmospheric, biological physical, chemical, and therapeutic process and attracted current research interest. To understand the mechanistic pathways in various process the detection and quantification of SO is very important. The direct method of detection is very challenging due to its highly reactive nature. Only direct method of determination of phosphorescence of SO at 1270 nm has been utilised but that also puts some limitation due to very low luminescence quantum yield. Indirect method using UV–Vis spectrophotometric, fluorescent and chemiluminescent probes has been extensively studied for this purpose. Elucidation of various mechanistic processes improvised the use of sophisticated spectroscopic detection probe for SO have been discussed in a simple and lucid manner in this article through citation of literature examples. Four major spectroscopic methods i.e. spectrophotometry, fluorescence, emission and chemiluminescence are elaborately discussed with special emphasis to chemical probes having high selectivity and sensitivity for SO.

Keywords: Recative oxygen species (ROS), Singlet Oxygen (SO), 9,10-Anthracenedipropionic acid (ADPA), 9,10-dimethyl anthracene (DMA), DPA, DPBF, DMAX, ATTA-Eu^{3+}, PATA-Tb^{3+}, MTTA-Eu^{3+}, CLA, MCLA, FCLA

1. Introduction

Reactive Oxygen Species (ROS) is broadly used for those which contain oxygen radical such as superoxide anions ($O_2^{\cdot -}$), hydroxyl radicals ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) (SO). These are usually metabolites of molecular oxygen formed due to normal or abnormal redox function in human body [1]. Numerous studies suggested that ROS can acts as toxins which can reduce the life span by causing ageing [2], synovial fluid degradation [3], neurodegenerative disease [4] etc. Singlet oxygen is known to be a highly reactive oxygen species that can oxidise a broad range of

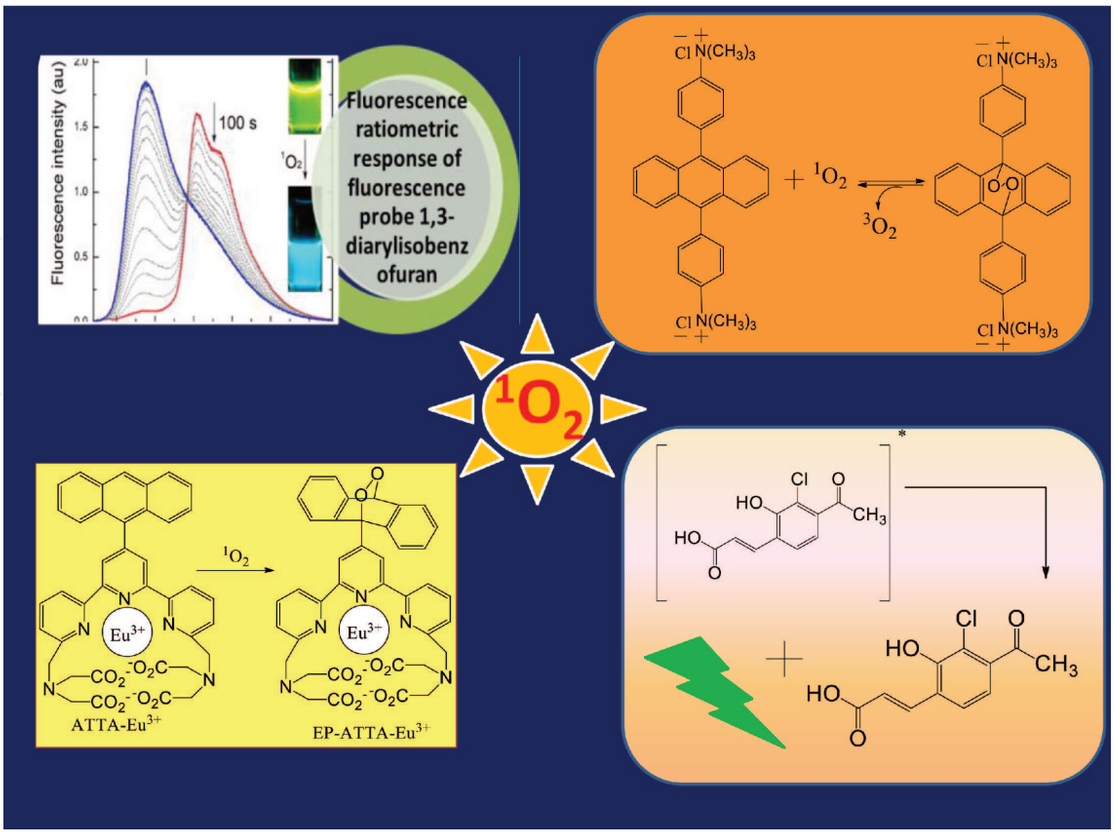


Figure 1.
The various photophysical singlet oxygen detection techniques based on absorbance or luminescence probes have been described pictorially. From top left clockwise (a) fluorescence detection probe (1,3-diarylisobenzofuran, adapted from [13]), (b) UV–Vis absorption based spectrophotometric probe (bis-9,10-anthracene-(4-trimethylphenylammonium) dichloride, adapted from [14]), (c) chemiluminescence probe (SOCL-phenol-dioxetane species, adapted from [15]) and (d) rare earth photoluminescence probe, ([4′-(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+}) (adapted from [16]).

biomolecules including lipids [5], nucleic acids [6, 7] and proteins [8]. The oxidation chemistry of SO provide key mechanism in the regulation of intracellular signalling pathways [9, 10] and eventually connected to human pathophysiology. As SO is a very short lived species ($\tau_{1/2}$ = 10^{-6} to 10^{-5} s) in the aqueous milieu) [11] studies towards the molecular mechanism of $^1O_2$ in vivo has been significantly retarded. Direct monitoring of SO phosphorescence also lacks practical utility due to very low photoluminescence quantum yields (PLQY≈10^{-6}) [12]. Therefore an enormous demand for SO probe with high sensitivity and large dynamic range is required. In this article various photophysical techniques and related probes usually employed in detecting SO has been delineated in separate paragraph (**Figure 1**).

2. UV–Vis spectrophotometric detection probes

2.1 9,10-Anthracenedipropionic acid (ADPA)

Ground state of molecular oxygen exists as triplet state with a lowest lying excited state being a singlet state. The singlet state of oxygen can be generated in solution via energy transfer from excited photosensitizers (S, e.g., humic substance or Rose Bengal; Eq. (1)) [17].

$$S \rightarrow S^{*} \overset{O_2}{\rightarrow} S + {}^{1}O_2 \quad (1)$$

The idea to detect environmentally relevant concentration prompted development of molecular probe that can trap SO and can also be used in studying kinetics. Lindig et.al was the first to introduced 9,10-anthracenedipropionic acid (ADPA) as a quantitative efficient $^1O_2$ detection probe [18]. The benefit of using ADPA is that it is water soluble and reacts rapidly and irreversible with $^1O_2$ to form an endoperoxide with the result of photobleaching of absorbance peak approximately at 378 nm (**Figure 2**) [20]. Craig et.al was the pioneer to demonstrate the use of ADPA for the quantitative measure of $^1O_2$ from the surface of a series of porphyrin-incorporated hydrogels in comparison to the pervious study where ADPA used to ingress into the materials under investigation [19].

2.2 9,10-dimethyl anthracene (DMA)

Another derivative of anthracene i.e. 9,10-dimethyl anthracene (DMA) reacts almost irreversibly with $^1O_2$ in various organic and aqueous medium with a significantly high rate constant (6.8×10^7–5.7×10^{10} $M^{-1}S^{-1}$) with the result of producing non fluorescent 9,10-endoperoxide [21–24]. Elim Albiter et.al reported a facile photosensitized oxidation of 9,10 demethylanthracene with $^1O_2$ in presence of safranin O/silica composite as a heterogeneous photosensitizer [21] in which they reported that oxidation rate does not depend on surface of the composite rather depend only the initial concentration of DMA, light intensity and the amount of composite formed. Their result correlates with the result if the same reaction performed in homogeneous medium. In a similar type experiment Eitan Gross et al. explored DMA inside liposome to study the kinetics of DMA with $^1O_2$ in presence of photosensitizer [22].

2.3 9,10-diphenyl anthracene (DPA)

Addition of two phenyl group in 9 and 10 position of anthacene generates a stable and specific $^1O_2$ trap, 9,10-diphenyl anthracene (DPA) with higher stability of endoperoxide by reaction with $^1O_2$. However, DPA is not a very suitable candidate as the detection method is based on decrease in absorbance at 355 nm band [25]. V. Nardello et al. enhanced the water solubility of 9,10-diphenyl anthracene (DPA) derivative

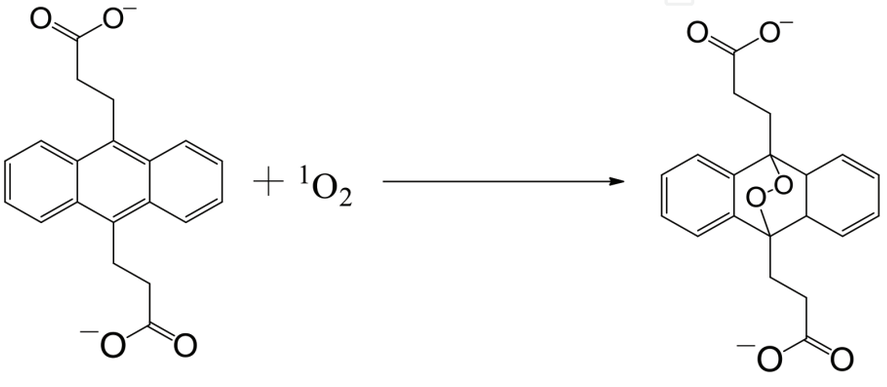


Figure 2.
The formation of endoperoxide upon reaction of ADPA with $^1O_2$ (adapted from [19]).

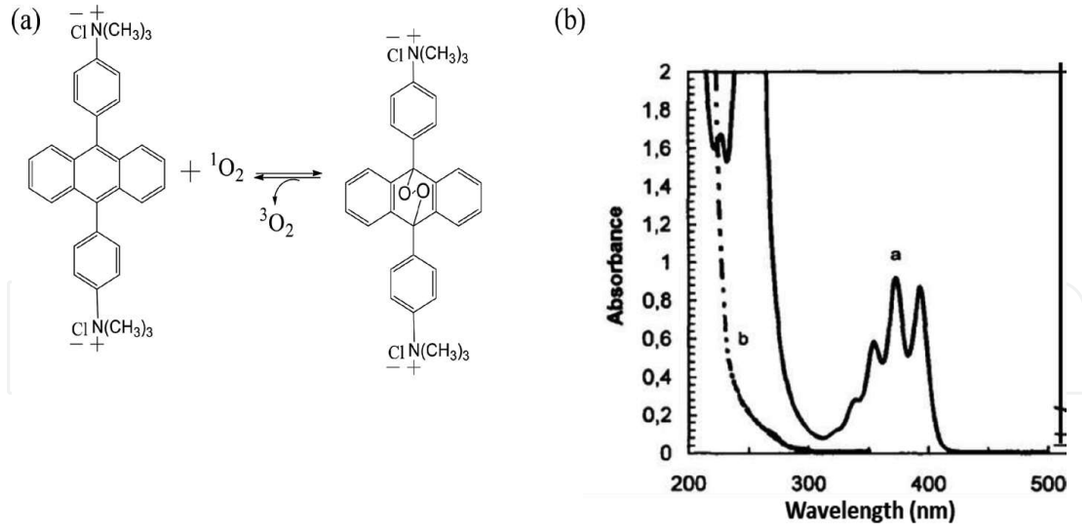


Figure 3.
(a) Interaction of bis-9,10-anthracene-(4-trimethylphenylammonium) dichloride (BPAA) with singlet oxygen resulting into the formation of water soluble endoperoxide (BPAAO$_2$) and (b) UV–Vis absorption spectra of a: BPAA 10^{-4} M in water and b: the corresponding endoperoxide of BPAA, 10^{-4} M in water [adapted from [14]].

by adding two quaternary ammonium functionality with the phenyl ring that do not interfere with $^1O_2$ and also resulting compound [bis-9,10-anthracene-(4-trimethyl-phenylamonium) dichloride] BPAA is very much stable with common oxidising agent (**Figure 3**) [14].

UV–Vis absorption band of BPAA is ranging from 320 to 420 nm which is originated from anthracene core structure and once it binds with $^1O_2$ (**Figure 3**) the absorbance band is quenched totally confirming the formation of endoperoxide.

3. Fluorescent probe for the detection of singlet oxygen species

Among the various available techniques to detect ROS the fluorescence based methodology is an excellent one because of its high sensitivity, high spatial resolution in imaging techniques and also simplicity during data collection [26, 27]. Fluorescent probes are generally non fluorescent before being oxidised by some oxygen species and they are very much specific to some oxidant. Combination of $^1O_2$ trap and a fluorophores like fluorescein [27, 28] reactive dienes [29] including 9,10-disubstituted anthracene [30–33] etc. are the usual method to develop a fluorescent $^1O_2$ probe. In fact $^1O_2$ has huge affinity for biomolecules having cisoid-diene structure and easily undergo [2 + 4] cycloaddition reaction [1, 6, 27]. Thus for most of the probes the fluorescent signals were obtained after [2 + 4] cycloaddition reaction between the probe and $^1O_2$ (**Figure 4**).

3.1 1,3 Diphenylisobenzofuran (DPBF)

D. Song et.al [13] synthesized a series of compounds of 1,3 Diphenylisobenzofuran (DPBF) which can acts as ratiometric fluorescence detection probe having singlet oxygen binding rate constant of 9.6×10^8 $M^{-1}S^{-1}$ in water [13]. Once DPBF reacts with $^1O_2$ forms nonfluorescent endoperoxide or 1,2-dibenzoylbenzene thus fluorescence signal becomes off. To overcome this practical difficulties they synthesised three more derivatives of DPBF namely phenanthrene substituted phenylisobenzofuran

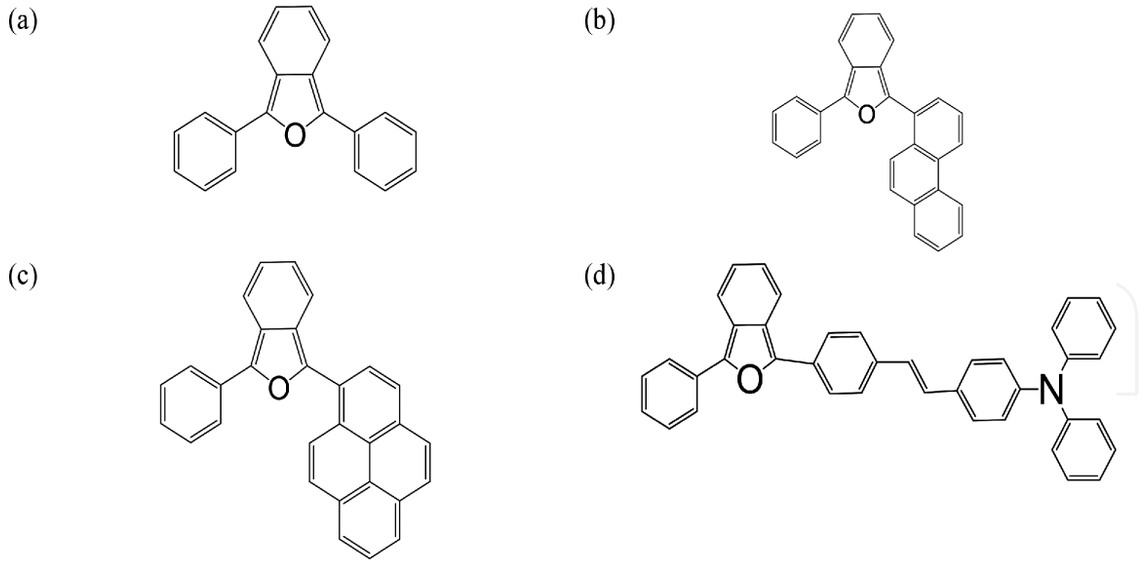


Figure 4.
Chemical structure of fluorescence detection probes for $^1O_2$, (a) DPBF, (b) PPBF, (c) PyPBF and (d) StPBF (adapted from [13]).

(PPBF), pyrene substituted phenylisobenzofuran (PyPBF) and 4-(diphenylamino) stilbene substituted derivative (StPBF) by substituting one phenyl group of DPBF (**Figure 5**) [13].

These $^1O_2$ probes exhibit significant red shift in their emission spectrum as the conjugation increases from DBPF to StPBF.

Upon interaction with $^1O_2$ species the fluorescence signal of DPBF is getting turn off while PPBF, PyPBF and StPBF demonstrate a blue shift of the emission signal with significant ratiometric enhancement of fluorescence as shown in **Table 1**.

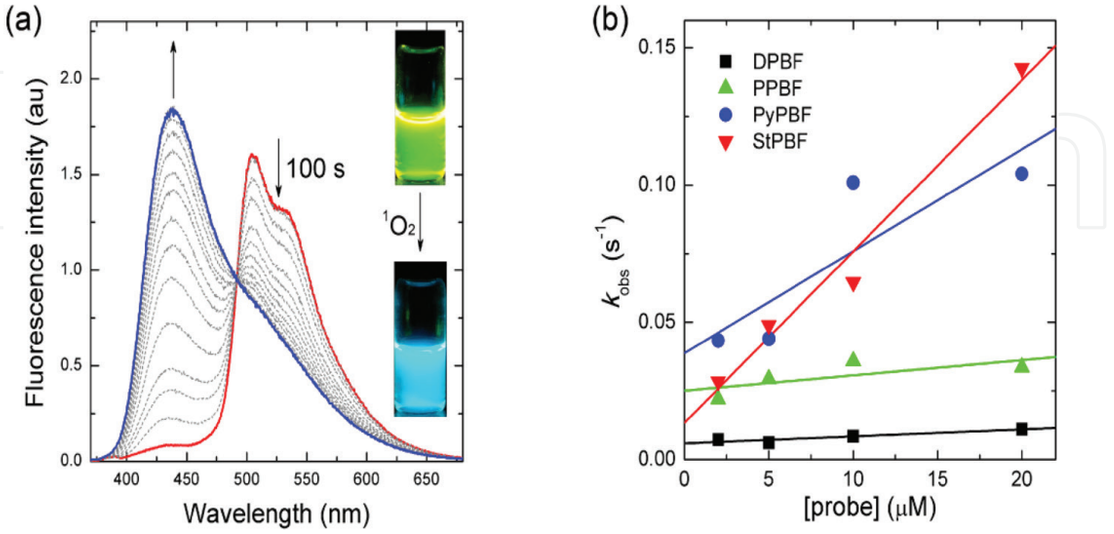


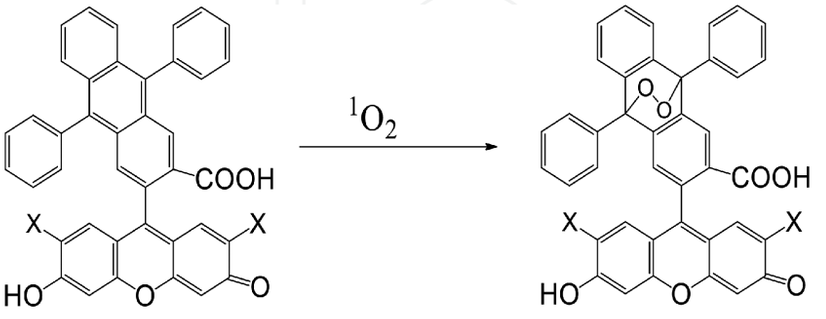
Figure 5.
(a) Fluorescence spectra of StPBF in absence (red in colour) and with gradually increasing concentration of $^1O_2$. The spectra gets blue shifted from 505 nm to 435 nm and fluorescence intensity increases with concentration of $^1O_2$ as directed by arrow in spectra and inset shows the observable colour change from green (in absence) and in presence of $^1O_2$ (blue in colour). (b) Graph of observed rate constant (K_{obs}) vs. concentration of various $^1O_2$ probe (adapted from [13]).

System	λ_{ex} (nm)	λ_{em} nm (probe alone)	λ_{em} (probe +$^1O_2$) nm	Fl ratio
DPBF +$^1O_2$	415	455	Turn off	Not determined
PPBF + $^1O_2$	331	476	370	FI_{476}/FI_{370} = 80
PPBF + $^1O_2$	358	512	398	FI_{512}/FI_{398} = 352
StPBF + $^1O_2$	350	505	435	FI_{505}/FI_{435} = 14

Table 1.
Photophysical data of $^1O_2$ probes [13]

3.2 9-[2-(3-Carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DPAX)

In terms of sensitivity issue a fluorescence probe is always better than probes which work on the basis of absorbance. Umezawa et.al developed a new sensitive and efficient fluorescence probe DPAX namely 9-[2-(3-carboxy-9,10-diphenyl) anthryl]-6-hydroxy-3H-xanthen-3-one, for the detection of $^1O_2$ by fusing a fluorescein moiety, with DPA which serve the characteristics of fluorescence due to fluorescein as well as a good $^1O_2$ trap for the presence of DPA [34]. DPAX and its derivatives show very low fluorescence intensity in aqueous solution but once binding with $^1O_2$ the corresponding endoperoxide (DPAX-ED) shows excellent fluorescence intensity with quantum yield in the range of 0.5–0.7 (**Figure 6**) [35]. The DPAX and its derivatives demonstrate excellent selectivity towards $^1O_2$ as the fluorescence intensity remains unchanged upon reaction with hydrogen peroxide, superoxide and nitric oxide [35]. DPAXs are suitable for application in neutral and basic aqueous solution but the fluorescence intensity is known to be decreased under acidic condition due to the protonation of phenoxide oxygen atom; thus are not suitable for application in acidic conditions [32]. The stability of the fluorescence intensity can be enhanced by incorporating electron withdrawing group like Cl, F at 2 and 7 position of the xanthenes moiety leading to generation of two



X=H, DPAX-1 λ_{ex} =493 nm, λ_{em} =516 nm

X=Cl, DPAX-2 λ_{ex} =507 nm, λ_{em} =524 nm

X=F, DPAX-3 λ_{ex} =493 nm, λ_{em} =514 nm

DPAX-1-EP λ_{ex} =494 nm, λ_{em} =515 nm

DPAX-2-EP λ_{ex} =506 nm, λ_{em} =527 nm

DPAX-3-EP λ_{ex} =494 nm, λ_{em} =515 nm

Figure 6.
Reaction of 9-[2-(3-carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-ones (DPAXs) with $^1O_2$ to produce corresponding DPAX endoperoxides (DPAX-EPs) (adapted from [34]).

derivatives of DPAX namely DPAX-2(Cl derivative) and DPAX-3(F-derivative). This structural change lowered the Pka value of the phenolic oxygen atom [34].

Absorption maxima (A), molar extinction coefficient (ε) and emission maxima (λ_{em}) are more or less same for DPAXs and DPAX-EPs but the quantum efficiencies of fluorescence are altered when DPAXs bind with $^1O_2$. The emission maximum of DPAX-2-EP is shifted to longer wavelength than the corresponding DPAX-2 compound in comparison to other DPAXs compounds (**Table 2**) [34].

DPAX-2 can be utilised as an efficient $^1O_2$ sensor in both basic and neutral medium. Umezawa et al. used two different $^1O_2$ generation system namely, MnO_4^-/H_2O_2 and 3-(4-methyl-1-naphthyl)propionic acid endoperoxide (EP-1) which works in different pH values (10.5 and 7.4 respectively). In both the cases an increase in fluorescence intensity is established when this probe reacts with $^1O_2$ generation system (**Figure 7**).

To confirm the specificity of DPAX-2 towards $^1O_2$, fluorescence experiment was performed in presence of hydrogen peroxide, superoxide and nitric oxide but no appreciable change was observed for those species. These observations suggest the specificity of this probe for $^1O_2$ [34].

Compounds	Absorption maxima(nm)	ε ($\times 10^4$ M^{-1} cm^{-1})	λ_{max} (emission)	Quantum yield (Φf)
DPAX-1	493	6.1	516	0.007
DPAX-1-EP	494	7.9	515	0.53
DPAX-2	507	5.7	524	0.006
DPAX-2-EP	506	8.9	527	0.66
DPAX-3	493	7.6	514	0.006
DPAX-3-EP	494	6.7	515	0.70

Table 2.
Absorbance and fluorescence properties of DPAXs and DPAX-EPs (adopted from [36]).

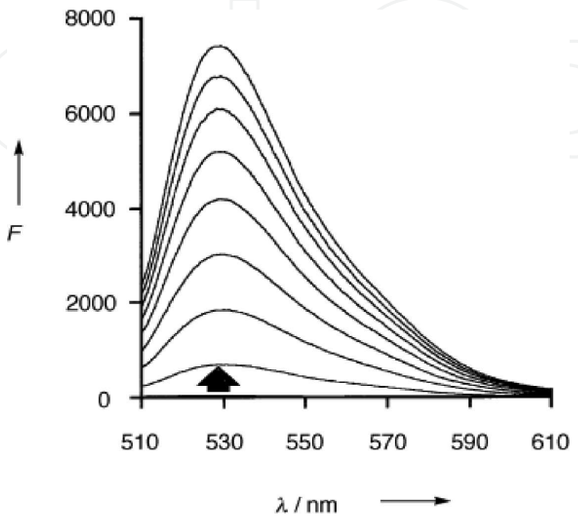


Figure 7.
Emission spectra of DPAX-2 at 505 nm in reaction with $^1O_2$, generated from MnO_4^-/H_2O_2 system. The reaction was performed in 0.1 M sodium phosphate buffer medium of pH 10.5 containing 0.1 mM EDTA at 25°C (adapted from [34]).

3.3 9-[2-(3-Carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX)

Following the same approach of Umezawa group, Tanaka et al. synthesised another fluorescence probe molecule for the faster and efficient detection of $^1O_2$, 9-[2-(3-carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX) (**Figure 8**), targeting to achieve great sensitivity and rapid rate of formation of endoperoxide compare to already reported one i.e. DPAX (**Figure 8**) [27]. The crucial point of DMA compound is that it reacts rapidly with $^1O_2$ to give the 9,10 endoperoxide, DMA-EP with rate constant k = 9.1 × 10^8 M^{-1} s^{-1} in water. This observation clearly indicates that DMAX shows much greater sensitivity for $^1O_2$ than DPAX. Comparing to this reaction, the classical singlet oxygen trap 1,3-diphenylisobenzophuran (DPBF) reacts with $^1O_2$ with a comparable rate constant k = 9.6 × 10^8 M^{-1} s^{-1} but DPBF reacts with other reactive oxygen species like hypochlorite, hydroxyl radical to generate the same products.

Both DMAX and its endoperoxide DMAX-EP have similar excitation and emission wavelength (λ_{ex} = 492 nm and λ_{em} = 515 nm) but DMAX-EP is highly fluorescent whereas DMAX itself is practically non-fluorescent (**Figure 8**). From their study Tanaka et al. confirmed that fluorescence intensity of DMAX increases with

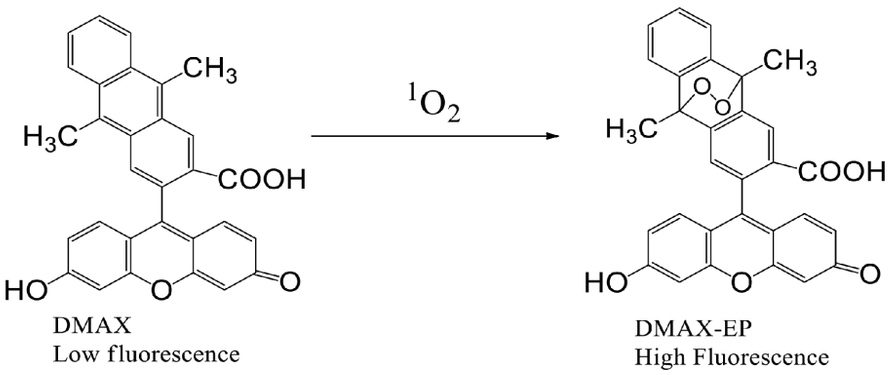


Figure 8.
Reaction of 9-[2-(3-carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX) with $^1O_2$ to produce DMAX-EP (adapted from [27]). λ_{ex} = 492 nm and λ_{em} = 515 nm.

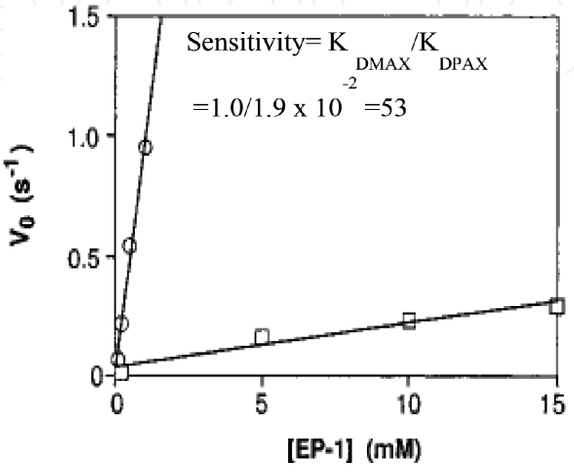


Figure 9.
Initial rate of fluorescence increase of DMAX (in hollow sphere) and DPAX-1(in hollow square) with increasing concentration of EP-1 [adapted from [27]].

concentration dependent manner of singlet oxygen generator, 3-(1,4-Dihydro-1,4-epidioxy-4-methyl-1-naphthyl)propionic acid (EP-1) and a good linear relationship has been observed for fluorescence intensity and concentration of EP-1. This enables DMAX to use as a quantitative detection probe for $^1O_2$. **Figure 9** demonstrate the change of gradient of fluorescence intensity of DMAX and DPAX-1 with increasing concentration of EP-1 having gradient 1.0 and 1.9×10^{-2} (arbitrary unit)s^{-1}[EP-1 (mM)]$^{-1}$ for DMAX and DPAX respectively. This result suggests that DMAX reacts with $^1O_2$ more rapidly and sensitivity is 53 times more than that of DPAX [27].

Tanaka et al. further confirmed that DMAX did not show any change in fluorescence intensity upon reaction with 1.0 mM H_2O_2, 0.1 mM nitric oxide and 0.2 mM superoxide suggesting the specificity towards $^1O_2$. Further the hydrophobicity of DMAX is less than that of DPAXs making it suitable to use for assays in biological sample [27].

4. Rare earth chelate based luminescence probes

Rare-earth based chelate luminescence probe can also serve significant role in the detection of singlet oxygen. In comparison to organic fluorescence probe rare earth based chelate probes exhibits markedly different advantages including long luminescence lifetime, sharp emission profile, large Stokes shift, which makes them suitable to use in time-resolved luminescence detection of singlet oxygen [36]. Large Stokes shift helps to reduce the influence caused by excitation light effectively. Time resolved luminescence measurement using rare-earth-chelated probe can efficiently reduce the interference originated from the background noise associated with biological sample, scattering lights (Tyndall, Rayleigh and Raman scattering) and the optical components [16]. Rare earth chelate probes which are successfully applied for the detection of singlet oxygen including Eu, Tb and Re based chelate complexes are well-known [16, 31, 37].

4.1 [4′-(9-Anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+})

Bo Song et al. first synthesised the Eu^{3+} cheleate based phosphorescence probe, [4′ -(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl] bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+}) (shown in **Figure 9**) for the time resolved luminescence study. The probe is highly sensitive, selective for $^1O_2$ and water soluble for time resolved luminescence detection of $^1O_2$ with a detection limit of 2.8 nM/L [16]. The almost non luminescent ATTA-Eu^{3+} specifically reacts with $^1O_2$ to yield its endoperoxide (**Figure 10**) (EP- ATTA-Eu^{3+}) resulting a great enhancement of luminescence intensity (luminescence quantum yield of EP- ATTA-Eu^{3+} is 17 fold greater than that of ATTA-Eu^{3+}) as the population of the excited state of Eu^{3+} was increased enormously after formation of endoperoxide [16, 27]. The endoperoxide compound EP-ATTA-Eu^{3+} exhibit favourable chemical stability with a conditional stability constant was measured to be 10^{20} level. Apart from that no decrease in phosphorescence intensity was observed even after storage of EP-ATTA-Eu^{3+} for several days at room temperature.

Another advantage of EP-ATTA-Eu^{3+} is that the phosphorescence intensity of EP-ATTA-Eu^{3+} is stable even at very low pH of 3 whereas in case of fluorescein based probe (DPAXs and DMAXs) the rapid decrease of fluorescence intensity is reported below pH 7 [16, 27].

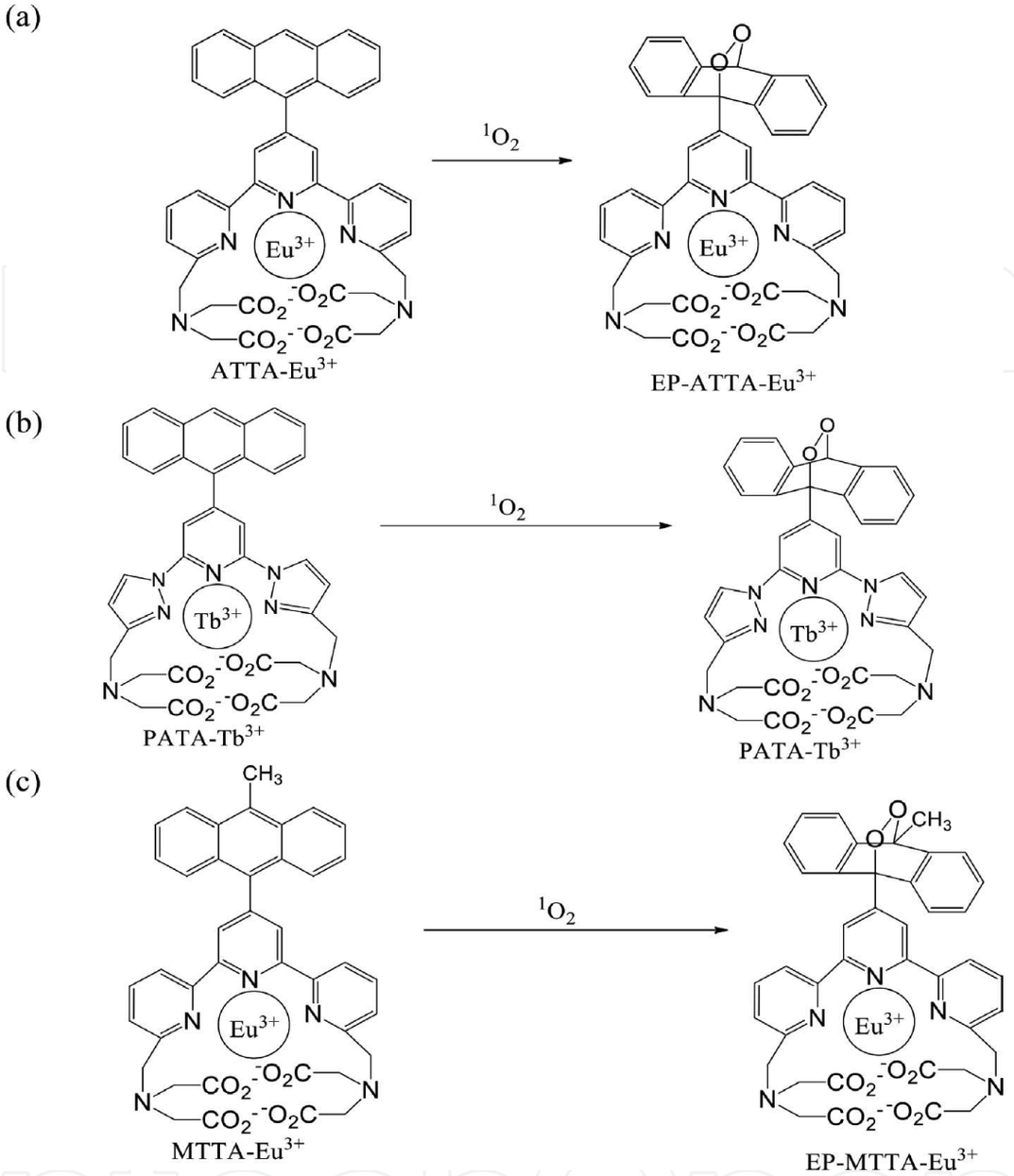


Figure 10.
The chemical structure and the reaction scheme of (a) ATTA-Eu^{3+}, (b) PATA-Tb^{3+} and (c) MTTA-Eu^{3+} with singlet oxygen (adapted from [16]).

4.2 N,N,N′,N′-[2,6-Bis-(3′-aminomethyl-1′-pyrazolyl)-4-(9″-anthryl)pyridine] tetrakis(acetate)-Tb^{3+} (PATA-Tb^{3+})

Mingqian Tan et al. developed another chelate complex of Tb^{3+}, N,N,N′,N′-[2,6-bis-(3′-aminomethyl-1′-pyrazolyl)-4-(9″-anthryl)pyridine] tetrakis(acetate) Tb^{3+} (PATA-Tb^{3+}), (shown in **Figure 10b**) is known to be effective fluorescent probe for the detection of $^1O_2$ [38]. Because of the presence of 9-anthryl moiety within the ligand the compound, PATA-Tb^{3+} is almost non fluorescent but once it reacts with $^1O_2$ the corresponding endoperoxide, EP-PATA-Tb^{3+} becomes strongly fluorescent with almost 23 fold enhancement of fluorescent quantum yield [39]. PATA- Tb^{3+} is known to be an excellent fluorescent probe for $^1O_2$ due to the characteristics, including high water solubility, wide applicable pH range and long fluorescence lifetime of

endoperoxide (2.76 ms) which makes it suitable for time resolved fluorescent detection with a detection limit as low as 10.8 nM/L [27, 38]. The specificity of PATA- Tb^{3+} towards $^1O_2$ is also confirmed upon reaction with some other reactive oxygen species, including hydroxyl radical, superoxide ion, peroxynitrite and hydrogen peroxide, the no significant change of fluorescence intensity support its specificity for $^1O_2$.

4.3 [4′-(10-Methyl-9-anthryl)-2,2′:6′,2′′-terpyridine-6,6′′-diyl] bis(methylenenitrilo)tetrakis(acetate)-Eu^{3+}, abbreviated as MTTA-Eu^{3+}

To improve the selectivity, sensitivity and rapid reaction with singlet oxygen species, Bo Song et al. introduced a new chelate complex of Eu^{3+}, [4′-(10-methyl-9-anthryl)-2,2′:6′,2′′-terpyridine-6,6′′-diyl]bis(methylenenitrilo) tetrakis(acetate)-Eu^{3+}, abbreviated as MTTA- Eu^{3+} as a $^1O_2$ probe [39]. MTTA- Eu^{3+} complex is highly water soluble and the reaction rate with $^1O_2$ is much faster in comparison to previously mentioned ATTA-Eu^{3+} (**Figure 10a** and **c**) with reaction rate constant of 10^{10} $M^{-1}S^{-1}$. Apart from that this complex can also be applied for a wide pH range from 3 to 10 accompanied by the large enhancement of luminescence quantum yield from 0.90% to 13.8%. The complex is almost non luminescent and when specifically reacts with $^1O_2$ to form the endoperoxide it becomes highly fluorescent with luminescent lifetime changes from 0.8 to 1.29 ms which makes the complex suitable for time gated luminescent measurement. The quantitative detection limit of $^1O_2$ using MTTA- Eu^{3+} complex is found to be 3.8 nM/L which is very much comparable to the detection limit of ATTA- Eu^{3+} complex [31, 39].

5. Transition metal based singlet oxygen probe

Though lanthanide based fluorescent probes possess many advantages including low background interference, widely applicable pH range, and excellent water solubility; these probes can be expected to utilise for visualising spatial and temporal distribution of $^1O_2$ in aqueous system but a major drawback of these probes is that they need ultraviolet light for excitation which might cause cell damage, thus limiting their application in biological system. Liu et al. demonstrated a Re^I complex, $Re(CO)_3Cl(aeip)${aeip = 2-(anthracen-9-yl)-1-ethyl-imidazo[4,5-f] [1, 10], phenanthroline, which can be excited via visible light of 410 nm wavelength in aqueous medium thus minimising the effect of cell damage (shown in **Figure 11**) [31]. The Re^I complex is non-luminescent in the native state probably due to quenching of luminescence of Re → aeip (M → L) charge transfer transition in the excited state through exchange triplet-triplet intramolecular energy transfer by anthryl moiety

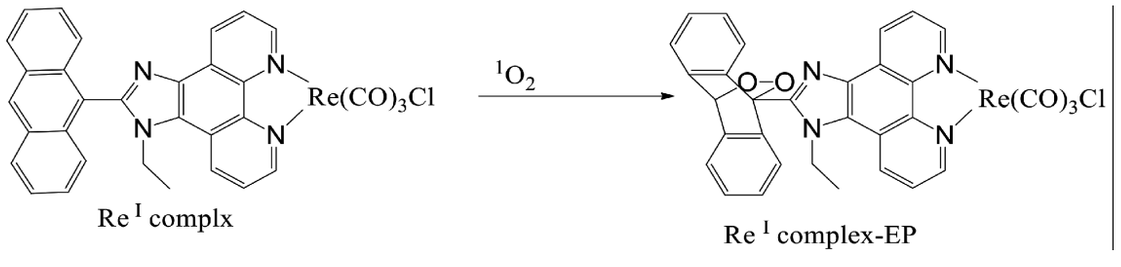


Figure 11.
The Re^I complex and the formation of endoperoxide via reaction with $^1O_2$ (adapted from [37]).

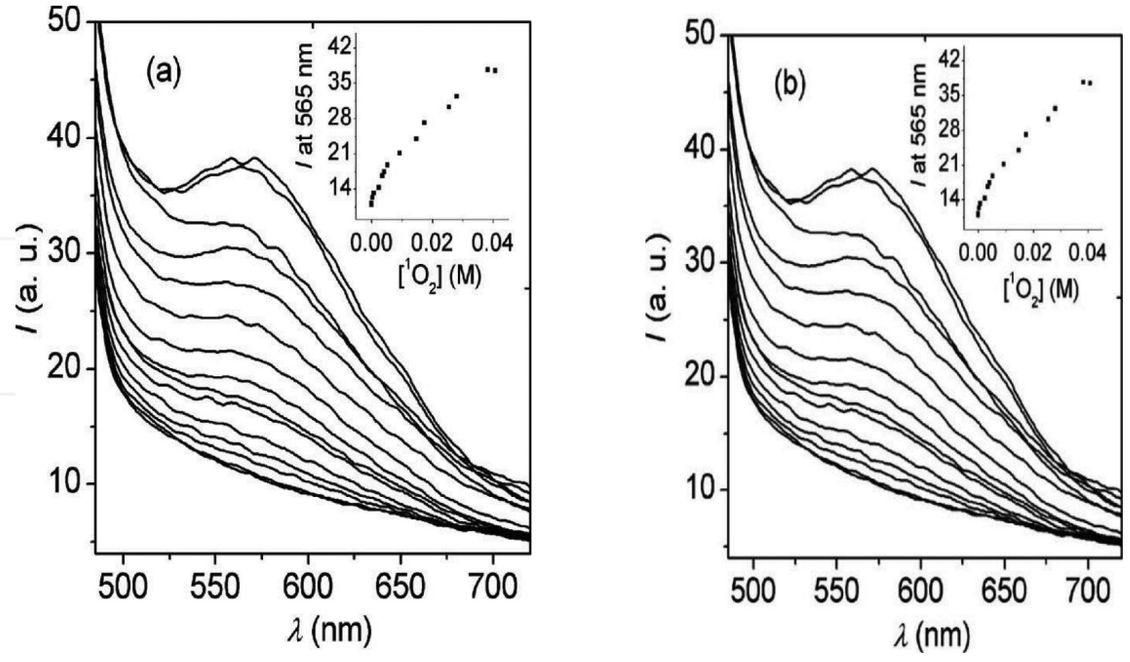


Figure 12.
Changes in the luminescence intensity of Re^I complex (λ_{ex} = 410 nm) with increasing concentration of $^1O_2$ in (a) in neutral and (b) in alkaline medium. The inset of these figures show that the sharp change in luminescence intensity at 565 nm with gradually increasing concentration of $^1O_2$ (adapted from [40]).

as mentioned in similar type Re^I complexes [37]. The strong enhancement of luminescence of Re^I complex due to the formation of endoperoxide in both neutral and alkaline medium is perceived, that due to the termination of electronic coupling between the anthryl and the parent Re^I complex (**Figure 11**). In comparison to other fluorescent probes of Eu^{3+} and Tb^{3+} complexes, Re^I complex exhibits higher molar absorption at the visible wavelength of 410 nm and fluorescence can also be initiated with this wavelength of light [31].

With increasing concentration of $^1O_2$ the luminescence intensity increases as shown in **Figure 12** and the luminescence quantum yield changes from 8.9×10^{-5} to 7.1×10^{-4} and 4.7×10^{-5} to 8.7×10^{-4} in neutral and alkaline media [37]. The limit of detection obtained using Re^I complex is found to be 4.9 nM/L and 10.5 nM/L in neutral and alkaline medium respectively, which are very much comparable with that obtained for Eu^{3+} and Tb^{3+} complex [31, 37, 39].

The additional benefit of visible light excitation and long lifetime enable Re^I complex to be used in biological systems.

6. Chemiluminescence singlet oxygen probe

In the previous discussion, the detection of $^1O_2$ was based upon either decrease in the absorbance of singlet oxygen probe or enhancement of luminescence signal of fluorescein based probe in presence of $^1O_2$. In this respect Chemiluminescence is supposed to be one of the most suitable methods for singlet oxygen detection as it does not require any excitation light source, so background fluorescence and interference caused by background light can be eliminated. At the same time the signal to noise ratio can be improved and the possible damage of living cell caused by UV irradiation during fluorescence measurement can be eliminated.

6.1 TTF substituted singlet oxygen probe: 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-α]pyrazine-3-one (CLA), MCLA and FCLA

A number of Chemiluminescence probes have been developed in recent years for $^1O_2$ detection. Among the mostly used Chemiluminescence probes for $^1O_2$ includes 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-α] pyrazine-3-one (CLA), and its derivatives MCLA and FCLA [41–43] (shown in **Figure 13**). These compounds are very good in detecting $^1O_2$ and spontaneously emit light but the major drawback of these compound is that they not only reacts with singlet oxygen but also with superoxide anion; thus lacks the selectivity for $^1O_2$.

Chemiluminescence probe with a strong electron donor tetra thiafulvalenem (TTF) and anthracene as a luminophore possess excellent selectivity and sensitivity for $^1O_2$ detection. As TTF moiety is a strong electron donor, it enhances the reaction between anthracene skeleton to react specifically with $^1O_2$ to form highly luminescent endoperoxide.

Guanxin Zhang et al. reported 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio] tetrathiafulvalene (as shown in **Figure 14**) as selective and sensitive probe for $^1O_2$ with a much better response for $^1O_2$, representing better selectivity than CLA [40, 44]. 4,5-dimethylthio-4′-[2-(9-anthryloxy) ethylthio] tetrathiafulvalene was another Chemiluminescence probe with similar functionality [40, 45]. A linear relationships between the Chemiluminescence intensity and the amount of $^1O_2$ was found for H_2O_2/ClO^- system and the LOD of 76 nM/L was reported for $^1O_2$. In mixed solvent of

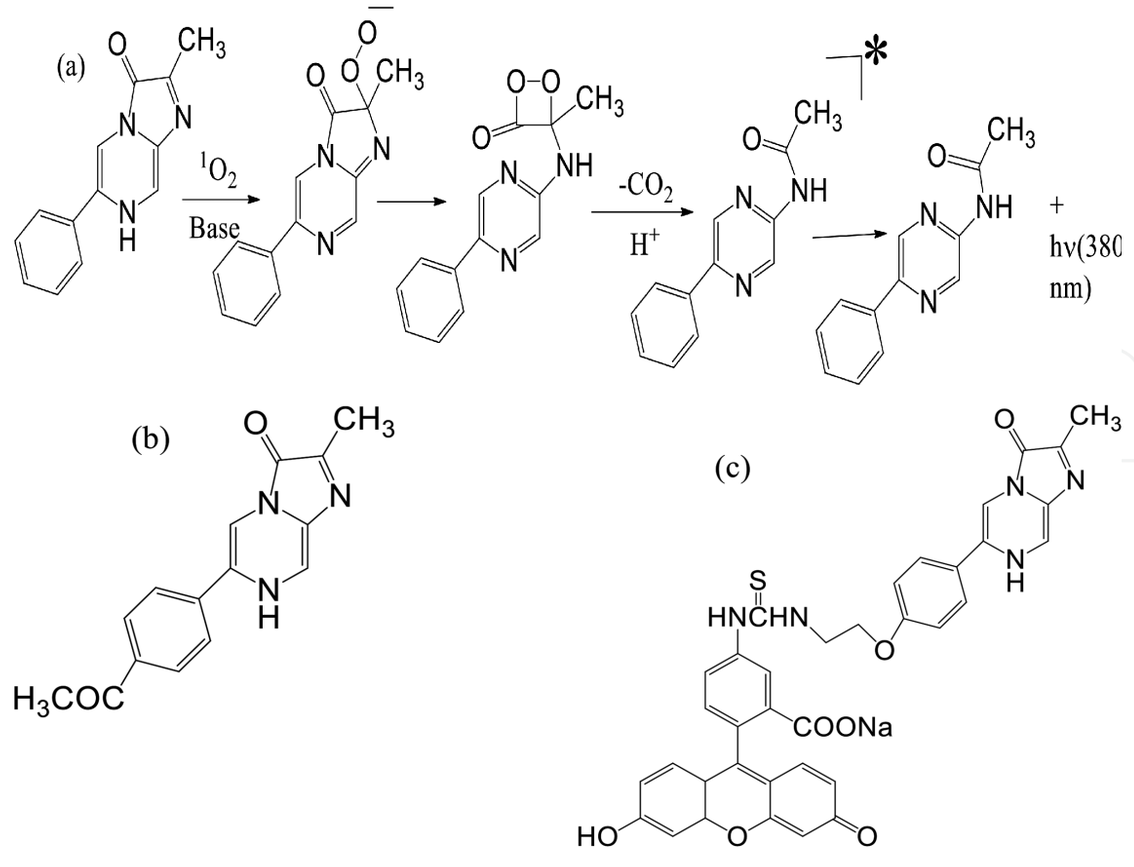


Figure 13.
(a) The chemical structure of CLA and its reaction with singlet oxygen; (b) the chemical structure of MCLA and (c) the chemical structure of FCLA (adapted from [41–43]).

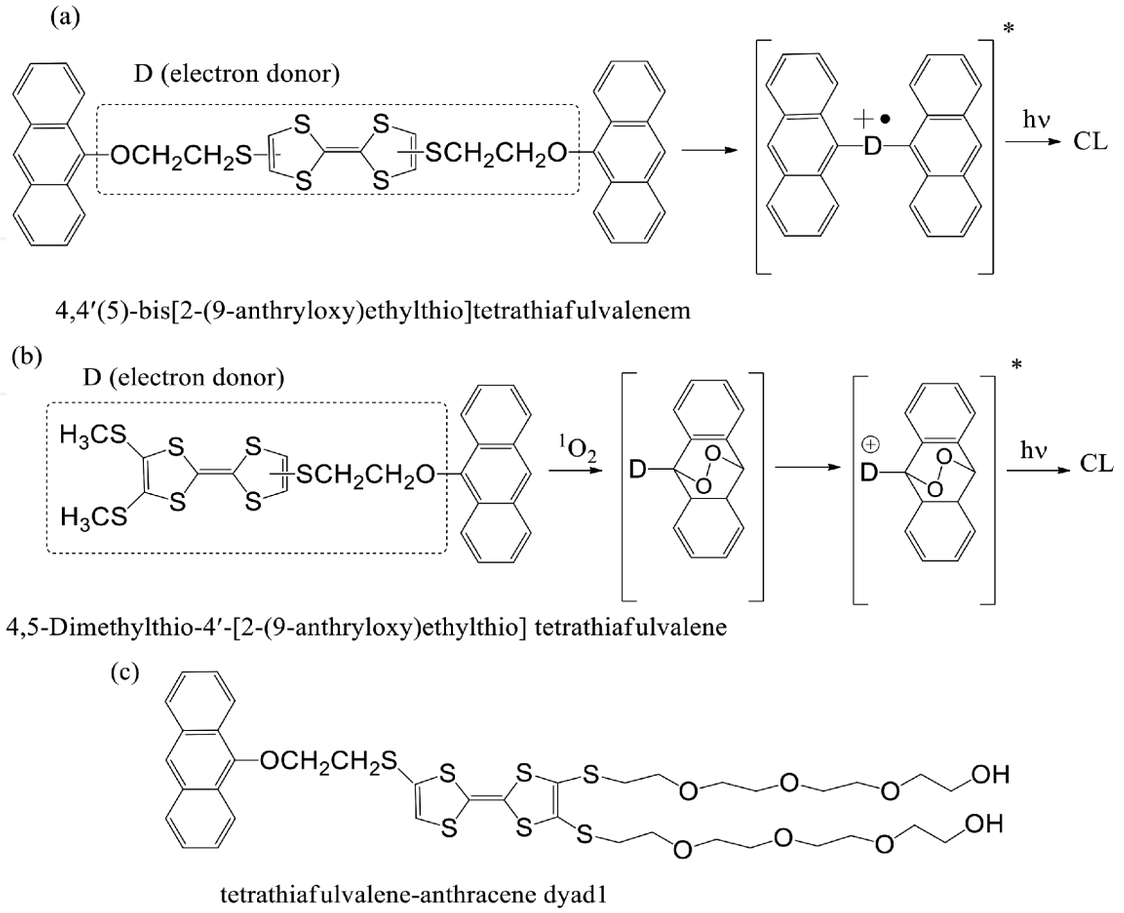


Figure 14.
The chemical structure and reaction scheme of (a) 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio] tetrathiafulvalene, (b) 4,5-dimethylthio-4′-[2-(9-anthryloxy)ethylthio] tetrathiafulvalene with with $^1O_2$ and (c) the chemical structure of tetrathiafulvalene-anthracence dyad1 (adapted from [40, 44, 45]).

tetrahydrofuran and H_2O, both the probes cannot be applied. However, tetrathiafulvalene-anthracence dyad1 (as shown in **Figure 13c**) dissolve easily in methanol and ethanol and permits detection of $^1O_2$ under relatively low polarity solvent.

6.2 Stable dioxetene chemiluminescence probe

Dioxetene chemistry offers potential for selective and sensitive detection of $^1O_2$. McNeill and co-workers reported "trap and trigger" Chemiluminescence probe for $^1O_2$ [46] using Schaap's dioxetene [47] enol ether precursor to trap the $^1O_2$ in the first step and in the second step Chemiluminescence was triggered by adding fluoride ion. This probe was limited application in organic solvents due to the quenching mechanism of the emitting species in water [48]. Nir Hananya et al. developed a new Chemiluminescence probe by incorporating an electron withdrawing substituent at the ortho position of phenol group of Schaap's dioxetene, namely SOCL-CPP [15] (**Figure 15**). SOCL-CPP reacts with $^1O_2$ to generate a phenol-dioxetene species that spontaneously decompose in aqueous medium to generate corresponding electronically excited benzoate ester [15].

A green light emission is obtained from excited benzoate ester. The incorporation of acrylic acid substituent at the ortho position of phenol is to generate a donor-acceptor

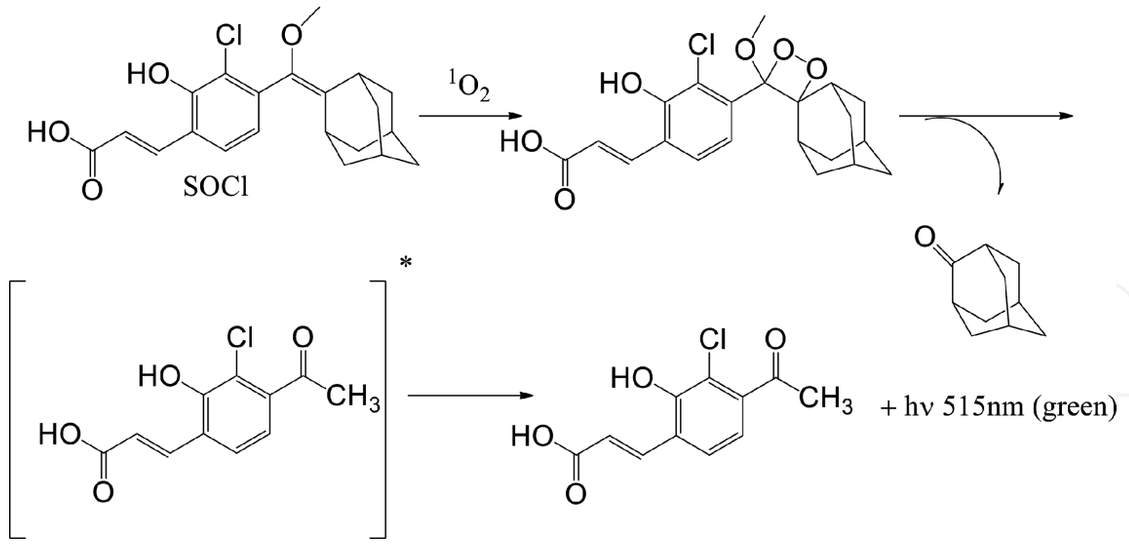


Figure 15.
Structure of SO chemiluminescent probe **SOCL** *and its chemiexcitation pathway upon reaction with $^1O_2$ (adapted from [15]).*

pair that enhances the emissive nature of benzoate intermediate [15]. Addition of a chlorine substituents at the ortho position reduces the pKa of the phenol and thus enriches the percentage of phenoxy ion that eventually accelerate the chemiexcitation kinetics of the phenol-dioxitene species to monitor in real time.

7. Conclusions

Singlet oxygen as a highly reactive form of molecular oxygen plays a vital role in many environmental and biomedical processes. Selective and sensitive detection and quantification of singlet oxygen species provides crucial information for understanding its involvement and mechanism in various processes. EPR method for the detection of $^1O_2$ has major disadvantage of requiring an expensive instrument and complicated operating procedures. Direct photoluminescence measurement from $^1O_2$ at about 1270 nm is useful for singlet oxygen detection but that also suffers drawback due to very low quantum efficiency. UV–Vis absorbance probes for selective detection of $^1O_2$ is significant but low sensitivity put some limitation for spectrophotometric method. Molecular fluorescence or lanthanide based fluorescence probe and Chemiluminescence probe provides high sensitivity and desirable selectivity, therefore ensure great potential for singlet oxygen detection. Additional benefits of fluorescence probe including the capability of detection of $^1O_2$ among various other reactive oxygen species. The temporal and spatial resolution of these probes can provide detailed information on site and the kinetics of singlet oxygen production or decay. In comparison to organic fluorescence probe, lanthanide complex based time gated luminescence probe possess many advantages such as long luminescence lifetime, large Stokes shift and sharp emission profile that makes them suitable for time gated detection mode for minimising background luminescence interference. Chemiluminescence probes does not require any excitation light sources, can be applied in certain cases where background fluorescence and various light scattering phenomena lower the signal to noise ratio. Furthermore, due to high sensitivity

of Chemiluminescence detection, only low concentration of probe is necessary eventually decreasing the occurrence of artifactual interference of secondary reactions. Although, much has been conferred in this concise chapter, perhaps these are not all, but the future will invoke more questions and thus newer and emerging methods, would help expand the level of our understanding.

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