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CHAPTER 5

Protection of Triplet Excited State Materials from Oxygen Quenching and Photooxidation in Optical Sensing Applications

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5.1 Introduction

Molecular oxygen is known to interact with triplet excited states in an energy transfer process resulting in non-emissive deactivation of the phosphor.[1–3] The effect of oxygen on the phosphorescence intensity and triplet state lifetimes is used for the quantification of oxygen concentration using the Stern–Volmer equation.[4–6] Biomedical applications of oxygen-dependent phosphorescence quenching are currently under active development.[7–15] Compared to other techniques, this method provides high selectivity and sensitivity, excellent temporal and spatial resolution, while being relatively simple in implementation.

In oxygen-saturated conditions, intensive deactivation of triplet excited states results in very short decay times and weak emission intensity that is

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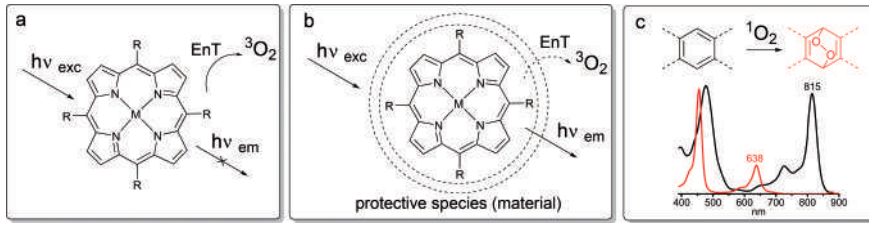


Figure 5.1 (a) Complete deactivation of triplet excited states in oxygen-saturated conditions. (b) Protection from quenching by encapsulation of the phosphor into a protective material or adding oxygen scavengers. (c) Possible photooxidation reaction mediated by singlet oxygen and corresponding change in the absorption spectra.

deemed to result in incorrect estimation of oxygen concentration (Figure 5.1a). In this case, a physical barrier, preventing oxygen diffusion, or a protective material, reacting with the excess of oxygen, is required to attenuate quenching constants in order to provide reliable response of the sensor (Figure 5.1b). A straightforward solution being applied for most phosphorescent probes is based on encapsulation into a polymer, to achieve the desired sensitivity towards quenching in specific conditions. However, non-exponential phosphorescence decays and nonlinear Stern–Volmer plots are often observed for such materials due to heterogeneity of binding sites in the polymer and differential oxygen quenching between dyes localized in different binding sites.[16] These effects are particularly pronounced for the dyes possessing only limited solubility in a polymer matrix that possibly cause phase separation in a solid state. Moreover, for *in vivo* applications, it is preferable to use water-soluble molecular probes which can be directly delivered into the tissue, rather than polymeric optodes, which can cause mechanical damage and decrease measurement accuracy.

Another important issue in the application of the phosphorescent dyes is the possibility of photooxidation processes in oxygen-saturated conditions. Energy transfer between the triplet excited state and molecular oxygen leads to singlet oxygen ($^1O_2$), which is a high energy state of oxygen and possesses profound reactivity towards organic molecules.[17] As many optical probes rely on molecules with an extended conjugated π-system, the reactions with self-generated $^1O_2$ deteriorate the operating performance of the corresponding devices. In particular, singlet oxygen typically takes part in [4 + 2] cycloaddition reactions with aromatic compounds, breaking the conjugation in the π-systems and resulting in a blue-shift of the absorption and emission wavelengths (Figure 5.1c). Such processes are responsible for the photobleaching of various chromophores, including phosphorescent metal complexes.[18] Thus, in addition to the restriction of oxygen access to triplet excited states, approaches towards scavenging of singlet oxygen are required for the development of robust sensor materials.

Due to the growing interest in applications of phosphorescent dyes as biomedical probes, the specific problem of controlling the sensitivity of the

triplet excited states towards oxygen has recently attracted broad attention. Apart from oxygen sensing applications, phosphorescent dyes possess huge potential in biophotonics, due to the large Stokes shift of the phosphorescence emission that allows for imaging in the "optical transparency window" of biological tissues.[19] In these applications, oxygen quenching of the triplet states is an unwanted complication, reducing the efficiency of corresponding probes. To date, a few reviews have been devoted to the protection of triplet excited state materials against quenching and photooxidation.[20,21] This chapter focuses more specifically on the approaches towards phosphorescent compositions with controllable quenching rates and enhanced stability against singlet oxygen. Two general solutions for this challenging problem that have been proposed to date are discussed: (i) active protection of the triplet states, based on the application of oxygen scavenging species and (ii) passive protection, based on barrier materials to reduce physical contact between oxygen and excited states.

5.2 Phosphorescent Probes with Appended Protective Groups

5.2.1 Phosphorescent Dendrimers

Accuracy of quantification of the dissolved molecular oxygen based on the phosphorescence quenching critically depends on quenching rates k_q and excited-state lifetimes τ. For typical phosphors, such as metalloporphyrins and transition metal complexes, the phosphorescence lifetime and intensity are already rather low at intermediate physiological O_2 levels due to high k_q. In order to keep the accuracy and dynamic range of the method the k_q values in water must be in the range of 0.5–2.5 × 10^8 M^{-1} s^{-1}.[22] One way to tune k_q values is based on the introduction of steric congestion around the phosphorescent center, which can be caused by bulky peripheral substituents. A comparison of platinum tetraphenylporphyrin (PtTPP) and porphyrin **1**, containing bulky 2,4,6-triethylphenyl groups (Figure 5.2), demonstrates the role of the substituents in suppressing quenching of the triplet excited states: k_q of **1** and PtTPP were found to be 4.1 × 10^8 M^{-1} s^{-1} and 14.8 × 10^8 M^{-1} s^{-1}, respectively.[23] The difference was attributed to the steric effects of the ethyl groups, which "screen" the core from interacting with oxygen and thus protect the excited states from quenching.

Dendrimers surrounding luminescent centers have been shown to reduce deactivation of the excited states by oxygen and other quenchers due to a diffusion barrier.[24–27] Balzani and co-workers[28,29] studied tris(bipyridine) ruthenium(II) complexes ($[Ru(bpy)_3]^{2+}$) bearing lengthy dendritic branches attached in the 4,4′-positions of the ligands. Dendritic complexes, showed longer triplet state lifetimes in aerated solutions due to the shielding effect of the dendrimer branches on the central core. The rate constants for oxygen quenching were found to depend on the length of the dendritic branches. For higher generation dendrimer **2** (Figure 5.2), k_q = 0.22 × 10^9 M^{-1} s^{-1} in

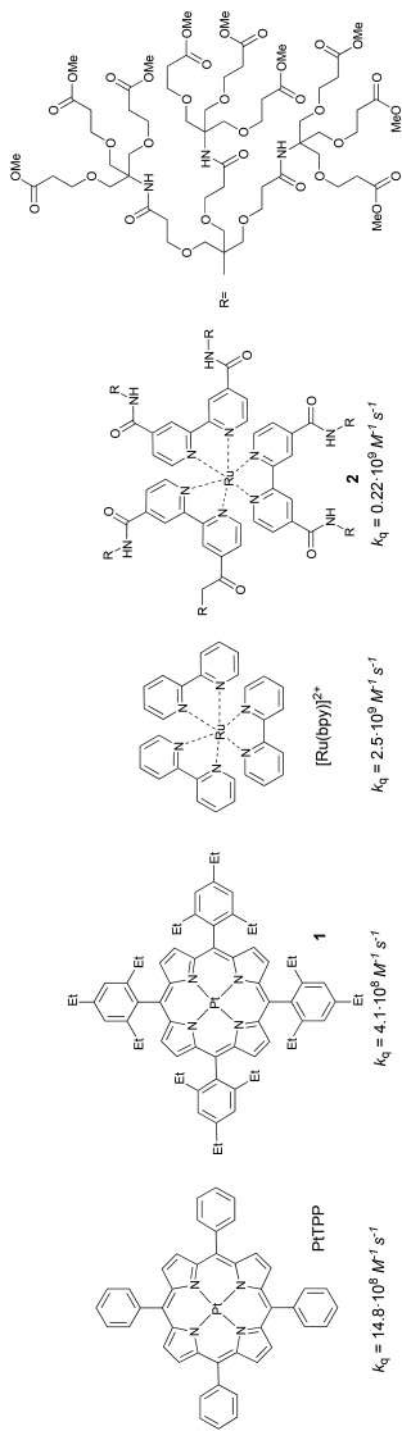


Figure 5.2 Sterically shielded phosphorescent porphyrin and ruthenium bpy complexes.

air-saturated acetonitrile solution is 12-fold lower, compared to the parent $[Ru(bpy)_3]^{2+}$. This approach for the design of the phosphorescent probes has recently attracted much attention. Dendritic branches provide biological compatibility of the probe, in particular, good solubility in water and protection from interactions with biomolecules. On the other hand, the length and chemical nature of the branches allow control of the values of oxygen quenching rates. Particularly, folding of hydrophobic dendrimers strongly depends on the media polarity and in a water environment enhances the oxygen diffusion barrier.[30] Furthermore, multiple peripheral functionalities on the dendrimer make possible further synthetic modifications to minimize interactions with proteins and other biomolecules.

Biocompatible oxygen probes with variable oxygen quenching rates based on Pd(II) and Pt(II) porphyrin complexes equipped with a protective dendrimer layer have been developed by Vinogradov and co-workers. Poly-*l*-glutamic acid[31] and poly(arylglycine) (AG) dendrons[32] were found to be especially well-suited for the construction of the phosphorescent oxygen probes (Figure 5.3). The resulting molecules closely resemble natural heme proteins ensuring low toxicity and immunoreactivity. In water the values of k_q are decreased 3–4 times due to the lower oxygen diffusion through the dendrimer layer, that is significantly less than the similar effect observed for the dendritic $[Ru(bpy)_3]^{2+}$. This was accounted for by the size of the phosphorescent metalloporphyrin core, which is considerably larger than the Ru^{2+} complexes, leading to much less restricted O_2 access in a similar dendrimer. For *in vivo* applications, probes possessing absorption bands in the NIR region were developed based on π-extended porphyrins with external aromatic rings annelated to the central macrocycle, such as tetraaryltetrabenzoporphyrins (TBP) **5–6** and tetraaryltetranaphthoporphyrins (TNP) **7**.[33–36] The absorption bands of these molecules lie in the region of 630–950 nm, where the absorption of natural chromophores is negligible. Peripheral poly(ethylene glycol) (PEG) groups on the dendrimers eliminate interactions with biomacromolecules, while keeping the probes highly hydrophilic. Notably, dendrimers derived from porphyrins with *meso*-3,5-dicarboxyphenyl groups exhibited strongly reduced k_q rates since the diffusion of O_2 through such dense dendrimer layers is much more restricted, compared to *meso*-4-carboxyphenyl derivatives. The potential of these probes was demonstrated in the physiological studies, where they allowed for the determination of the absolute blood pO_2.[37–40]

A critical limitation for applications of dendrimer-appended phosphorescent probes is the rather complicated synthesis of these materials, which involves stepwise construction of the dendrimer layers. Modification of the porphyrin precursors with pre-organized dendritic building blocks using the "click chemistry" approach represents an attractive alternative route to such protected probes. A new family of "clickable" phosphorescent molecules incorporating dendritic residues bound *via* Huisgen reaction has recently been reported by Evans and co-workers.[41] Following this approach, an alkynyl-substituted platinum(II) tetrabenzoporphyrin precursor was

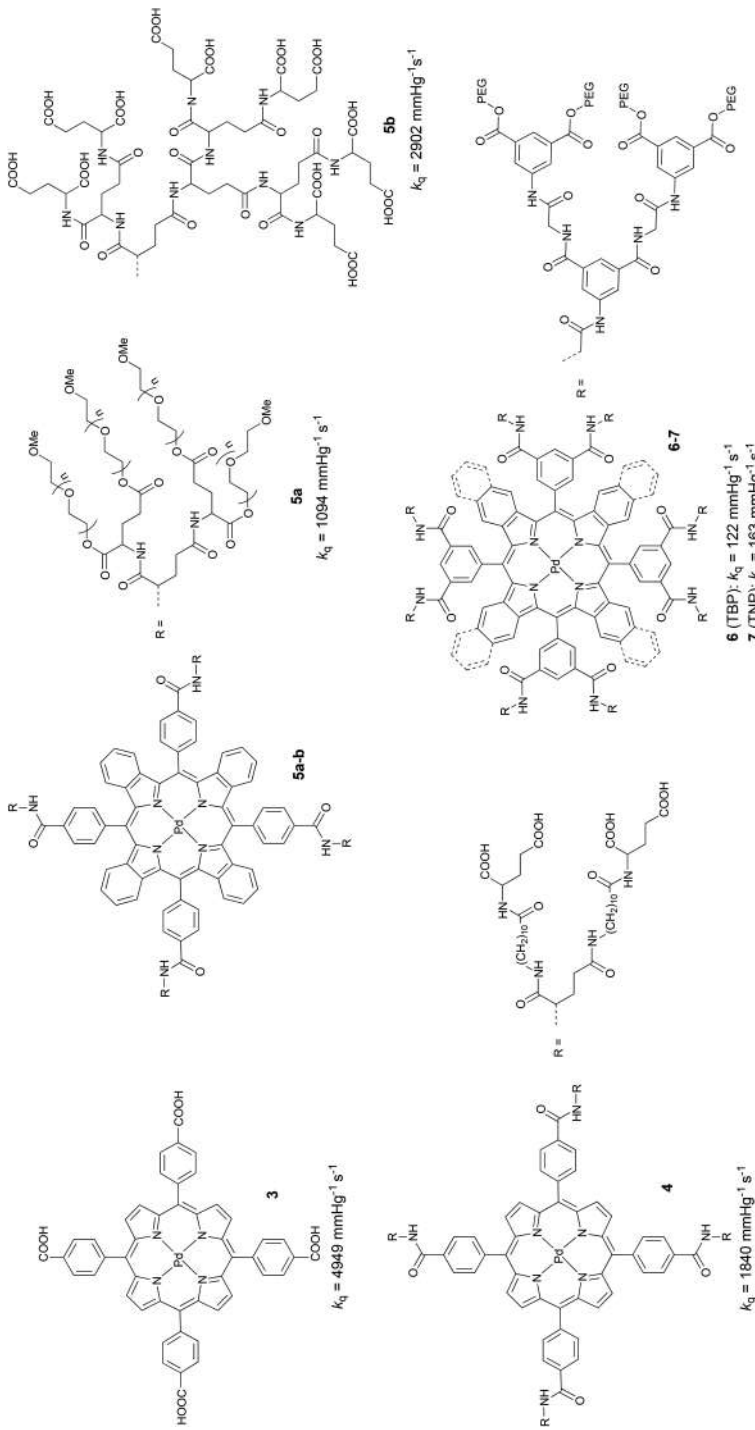


Figure 5.3 Palladium(II) porphyrin-based phosphorescent dendrimers probes.

converted into a glutamic dendrimer in one synthetic step through the reaction with eight azido-terminated glutamic dendrons. The starting porphyrin was found to be completely non-emissive in air saturated conditions due to the quenching, which was substantially attenuated in the dendrimer. The probe was further incorporated into a wound dressing bandage and applied for visualization and quantification of the skin burns oxygenation by the naked eye under room lighting conditions, providing a clinical diagnostic tool for mapping oxygen consumption in wounds.[42]

5.2.2 "Self-healing" Phosphorescent Complexes

In phosphorescent dendrimers, the modulation of quenching rates is achieved by the restriction of physical contact between oxygen molecules and excited state cores. Such an approach can be referred to as "passive protection". An alternative "active protection" is based on surrounding a dye molecule with special subunits able to react with oxygen and thus reduce the quenching rates. This approach has not been explored in the design of the phosphorescent systems until very recently due to a lack of suitable molecular building blocks, which could provide chemical "trapping" of oxygen. In its ground state, which is of triplet character ($^3\Sigma_g$), reactivity of oxygen is rather low. However, the transition of an oxygen molecule into the higher energy singlet state ($^1\Delta_g$) significantly increases its reactivity. Taking into account that such a transition takes place during the interaction between oxygen and triplet excited states of organic molecules, phosphorescence dyes capable of binding oxygen molecules can be developed. Importantly, the processes of oxygen binding can be reversible, as certain types of organic molecules are known to release oxygen back from initially formed adduct *via* thermal dissociation even at ambient temperature.[43,44] Such protection is of sacrificial character and limited in timescale. However, the thermal release of bound oxygen upon heating allows for regeneration of the protective moieties. Anthracene-appended porphyrin **8** was shown to bind up to four molecules of oxygen in solution under irradiation, forming corresponding endoperoxide (Figure 5.4a).[45] Anthracene groups do not affect photophysical properties of the porphyrin either before or after the reaction with oxygen due to a lack of conjugation between the aromatic systems. This process was demonstrated for enhancement of the porphyrin phosphorescence intensity in (i) oxygen contaminated (100 ppm) and (ii) oxygen-saturated solutions. In the first case, increase of the phosphorescence was obtained after irradiation of the whole sample with 633 nm light for 10 min. During the irradiation, dissolved oxygen, which quenches triplet excited states, is being converted into singlet oxygen, reacting with anthracene groups to form endoperoxide that leads to reduction of the overall oxygen concentration in the sample and enhancement of the phosphorescence intensity (Figure 5.4b). Alternatively, the process was performed under oxygen-saturated conditions with excitation of the sample in a local area (400 μm diameter). The excitation pulse of rather low intensity

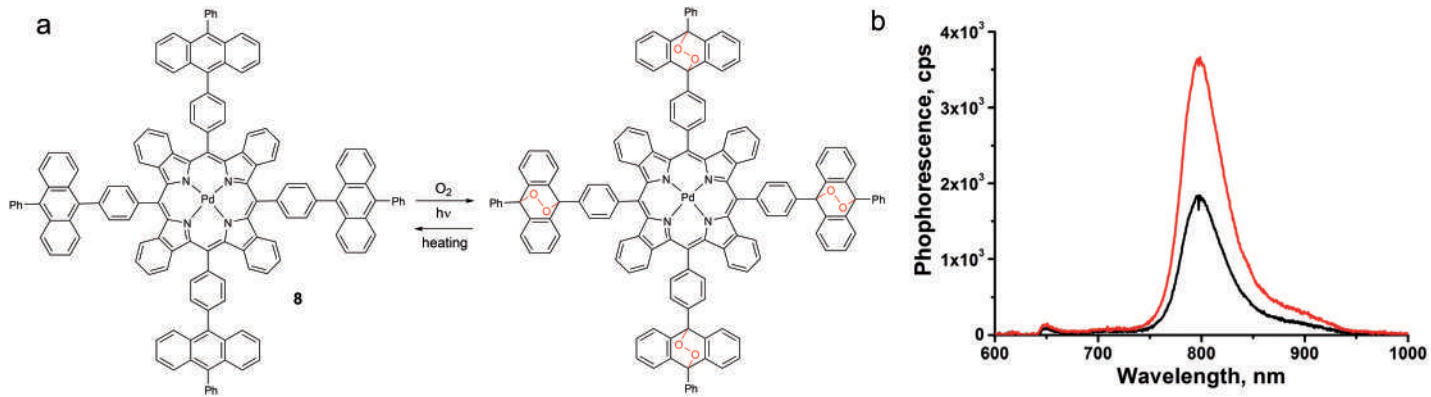


Figure 5.4 (a) Photosensitized oxygen addition to porphyrin **8**. (b) Increase of the phosphorescence intensity in toluene solution prepared in the atmosphere containing 100 ppm of oxygen (black line) after 10 minutes of continuous irradiation (633 nm, 250 μWcm^{-2}) (red line).

(500 μWcm^{-2}) caused real-time "deoxygenation" of the local area, resulting in up to 60% intensity increase of the phosphorescence signal within 10–20 s. Endoperoxide product, formed in the process, regenerated parent sensitizer molecules upon heating.

Chemical binding of oxygen to the phosphorescent dyes represents an interesting alternative to currently known approaches towards oxygen sensing based on the phosphorescence quenching. Further development of such probes requires efforts on molecular design, particularly access to water-soluble biocompatible derivatives. Systems based on the anthracene as oxygen trapping subunits are not suitable for *in vivo* studies, due to their hydrophobic character. Porphyrins with four and eight 2-pyridone groups appended at the periphery of the macrocycle have recently been demonstrated for the reversible oxygen binding on *in vitro* cell models.[46] These systems are well suited for biomedical applications due to their facile synthesis and good solubility in water, enhanced by intrinsic polarity of 2-pyridone substituents.

5.3 Host-guest Complexes and Aggregates

5.3.1 Tryptophan Phosphorescence in Proteins

Incorporation of the phosphorescent molecules into the rigid environment, which confines the molecular diffusion, allows the reduce quenching of the excited states even in oxygen-saturated conditions due to keeping the phosphor and the quencher out of the collision distance. Development of practical methods based on this principle was initially stimulated by the studies of tryptophan phosphorescence in proteins.[47] Normally the phosphorescence of proteins is hard to detect due to high sensitivity towards quenching by low levels of dissolved oxygen in solution. However, in certain cases the quenching constants were found to be reduced due to the steric hindrance of tryptophan residues resulting from the folding of the polypeptide chains. The observations of protein phosphorescence with increasing temperature revealed that the folding of the polypeptide chains in protein molecules is hindering the diffusion of O_2 to this region of the macromolecule and reducing the quenching rates of the triplet states. As was shown by Galley and co-workers, tryptophan phosphorescence in horse liver alcohol dehydrogenase, can be observed even in aerated solutions at room temperature due the low quenching values (6.5×10^4 1 M^{-1} s^{-1}).[48] It has been shown that certain protein conformations strongly inhibit quenching of tryptophan triplets by dissolved oxygen and that k_q is directly correlated to the rigidity of the protein matrix surrounding the chromophore.[49] Oxygen-dependent quenching of tryptophan phosphorescence in apoazurin, liver alcohol dehydrogenase, and alkaline phosphatase as a function of temperature (0−50 °C) and applied pressure (up to 3 kbar) has been applied by Strambini and Cioni to study structural dynamics of these proteins.[50] This method was further used in the studies of the small molecules migration inside protein structures.[51]

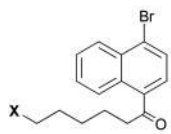
5.3.2 Phosphorescence of Cyclodextrin Complexes in the Presence of Oxygen

Turro and co-workers first described inhibition of oxygen quenching upon complexation of the phosphorescent molecules with cyclodextrins (CD). A series of substituted naphthalene derivatives in aqueous solutions containing γ-CD was found to display two distinct excited state decays: the fast decay and the slow decay (Figure 5.5). The oxygen completely quenched the fast decay component, while the slow decay was not influenced. It was further shown that the fast decay originates from the 1 : 1 complex of the phosphor and γ-CD, while the slow component corresponds to the 1 : 2 complex.[52] At the same time, no phosphorescence in the presence of oxygen was observed for the complexes of the same naphthalenes with α- and β-CD.

Phosphorescence under oxygen-saturated conditions can be observed if cyclodextrin–phosphor inclusion complex interacts with other molecules, which serve as space-filling components (Figure 5.6).[53] In the resulting complexes, the motion of the phosphor and diffusion of the oxygen into the cavity are largely restricted, inhibiting both the non-radiative decays of the triplet state and oxygen quenching. The enhancement of the phosphorescence can be very strong, in certain cases up to a 10^5 increase in intensity has been observed, and depends on the fit between the sizes of the phosphor, spacer regulator and CD cavity.[54]

Several types of space regulation in phosphorescent CD inclusion complexes have been described.[55] 6-Bromo-2-naphthol the 1 : 1 inclusion complex with α-CD is not phosphorescent in aerated solution, while the 2 : 1 complex showed intense emission under similar conditions due to protection of the chromophore in the cavity formed by two CD molecules.[56,57] A similar effect was observed for β-CD which forms the 2 : 2 complex with naphthalene stabilized by intramolecular hydrogen bonding between the hydroxy groups of two CDs.[58]

Protic solvent molecules can play the role of space-filling component: due to the formation of H-bonds with hydroxyls of CD, the entrance of the CD cavity can become hindered (Figure 5.7) that prevents oxygen molecule from entering the cavity.[59] Phosphorescence enhancement strongly depends on the fit of the alcohol lid on top of the CD cup. The rate constants for oxygen quenching generally decreases with the increase in the alcohol bulkiness.



	Lifetime, τ (ms)		
	Fast decay	Slow decay (N_2)	Slow decay (O_2)
X = Br	0.506	3.9	3.8
X = -NMe_3Br	0.645	3.3	2.5
X = -O-CO-Ph-CO-Ph	-	3.4	2.8

Figure 5.5 Structures and excited state lifetimes of the phosphorescent naphthalene derivatives in γ–CD aqueous solution.

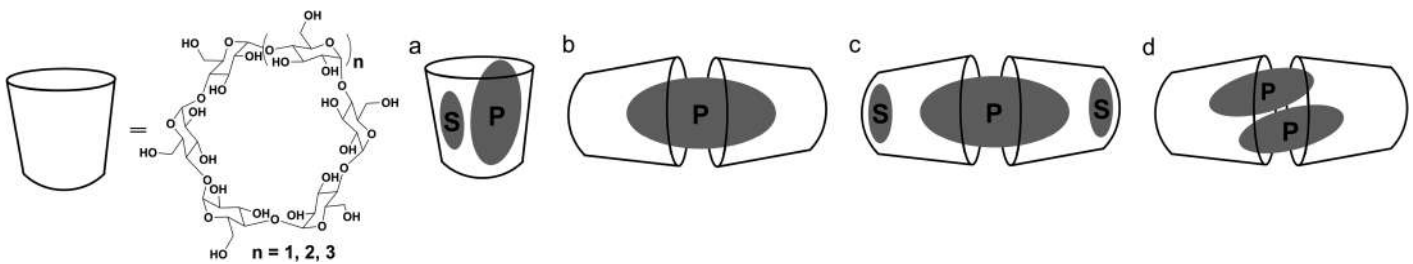


Figure 5.6 Cyclodextrin inclusion complexes (S is a space regulator and P is a phosphorescent molecule). a – α-CD/P/S, b – α-CD2/P, c – β-CD2/P2/S2, d – β-CD2/P2.

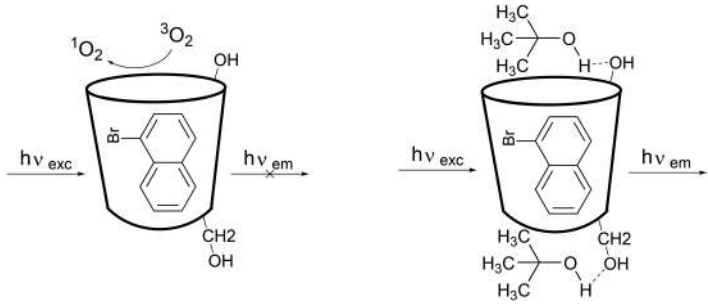


Figure 5.7 "Covering" effect of the solvent on the quenching of the phosphorescent inclusion complex.

Table 5.1 Phosphorescence quantum yields in air-saturated solutions and oxygen quenching rates of the complex between 1-bromonaphthalene and glucosyl-β-cyclodextrin (Gβ-CD) in different alcohols.[54]

Alcohol	$\Phi_{phos}/10^{-4}$	$k_q/10^5$, $M^{-1}\ s^{-1}$
tert-Butanol	340	8.9
Neopentanol	1149	0.3
3,3-Dimethyl-1-butanol	789	1.6
Cyclohexanol	350	1.2
Cyclohexylmethanol	771	0.2
Cyclohexylethanol	364	0.8
cis-1,2-Cyclohexanediol	883	2
1,3-Cyclohexanediol	51	32
1,4-Cyclohexanediol	<0.01	$>10^4$

Accordingly, neopentanol and 3,3-dimethyl-1-butanol strongly reduce oxygen quenching rates and provide the highest phosphorescence quantum yields (Table 5.1).

Based on this approach towards phosphorescence protection, Zhang and Johnson developed a technique for measuring over-saturated dissolved oxygen concentrations up to 40.2 mM (14 500% saturation with respect to air-equilibrated water).[60,61] *tert*-Butanol was used as a lid for Gβ-CD complex with 1-bromonaphthalene prepared in aqueous solution. Phosphorescence of the complex decreases with increasing concentrations of oxygen. The phosphorescence lifetime measurements were found to be in good agreement with the Stern–Volmer equation even for very high dissolved oxygen levels.

Phosphorescence protection through complexation with CD can be anticipated to provide new developments in the biological oxygen sensing techniques in the near future. Compared to other approaches towards triplet state protection it has the following advantages: (i) it does not require efforts on the synthesis and purification, as the complexes readily form upon mixing the components and possess high stability constants; (ii) the availability of various CDs and space-filling molecules allows to tune the size of the cavity for a specific phosphor molecule; (iii) oxygen quenching rates can be tuned

e.g. via changing the lid for the CD complex; (iv) other hosts molecules which have structures similar to cyclodextrins and can act as protective media are available, *e.g.* cucurbit[n]uril[62] and its analogues;[63] (v) complexes with CD can be formed not only in solution but also in solid films or nanoparticles.

5.3.3 Steroids as Protective Matrixes

Reduced oxygen quenching of the phosphorescence in molecular aggregates based on sodium deoxycholate (SDC) was first described by Jin and co-workers.[64] 1-Bromo-4-(bromoacetyl)naphthalene (**9**) was found to exhibit phosphorescence in aqueous solution without deoxygenation upon addition of SDS. The oxygen quenching rate constant was measured to be 4.15×10^4 M^{-1} s^{-1} in air-saturated SDS solution at 1 atm. The effect of SDC on the phosphorescence was interpreted by assuming a formation of "sandwich" type dimers in which the dye is trapped between two steroid scaffolds (Figure 5.8). Although such dimers could not be isolated, molecular modelling study allows the conclusion that the naphthalene molecule is stacked between two SDS molecules by the apolar faces. The effect was much less pronounced for other aggregate forming molecules, such as sodium dodecylsulfate (SDS) and DNA. A similar effect of SDS phosphorescence protection was later reported for water-soluble palladium(II) *meso*-tetraarylporphyrins.[65] The phosphorescence of air-saturated porphyrin solution showed a gradual increase of the intensity upon addition of SDC along with increase of the lifetime. However, at SDC concentrations higher than 4×10^{-3} M the emission gradually decays to negligible values. It suggests that the "protective dimer", which is formed at low concentrations of SDC, can further transform into larger aggregates possessing less rigid structure and allowing oxygen diffusion.[66]

Based on this strategy, phosphorescent materials with oxygen-persistent emission and long lifetimes (>1 s) have been recently developed by Adachi and co-workers.[67] β-Estradiol and cholesterol have been applied as host matrixes for a series of organic phosphors based on aromatic hydrocarbons. An advantage of a steroid matrix is the possibility for oxygen removal by repetitive heating above the melting point and cooling. Due to slow diffusion of oxygen in a solid matrix the resulting materials did not show any drop of the phosphorescence intensity over long periods of time.

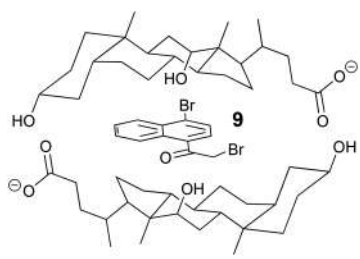


Figure 5.8 Phosphorescent aggregate of SDC and **9**.

5.3.4 Gel Matrixes

The possibility to apply organogels and hydrogels as quenching protective matrixes for phosphorescent materials was recognized very recently and few reports on the incorporation of phosphors into protective gel matrixes have been made. Shirakawa and co-workers developed phosphorescent gels based on copper, palladium and platinum complexes with 8-quinolinol **10** bearing 3,4,5-tris(n-dodecyloxy)benzoylamide substituents (Figure 5.9a).[68,69] Theses complexes were found to form gels in various organic solvents at very low critical gelation concentrations of 0.10 mg L^{-1} (0.05 mM). Due to the formation of a crystal-like structure in the gel state, up to a 3-fold increase of the emission intensity in aerated conditions was observed compared to reference complexes having no gel-forming ligands. It was shown that the packing structure is responsible for reduced oxygen diffusion into the gel phase. De Cola and co-workers reported phosphorescent hydrogels based on host–guest interactions between water-soluble Pt (II) complex **11** (Figure 5.9b) with attached tetraethylene glycol chains and cyclodextrins (α- and β-CD). The materials were found to be strongly emissive and not sensitive towards quenching, although the behavior of the system has not been studied in detail.[70] Further, Yang and co-workers reported 1,3 : 2,4-di-*O*-benzylidene-D-sorbitol (DBS) as a gelator for phosphorescent 3-bromoquinoline. The supramolecular gels were prepared by self-assembly of DBS in DMF-water mixture. It was found that deoxygenation of the samples is not required to observe phosphorescence. Moreover, the emission intensities of a pre-deoxygenated sample showed similar values compared to air-saturated.[71]

Organogel matrixes offer several advantages for oxygen sensor techniques. Various phosphorescent molecules can be modified with gel-forming functionalities or entrapped into pre-formed organogels. The matrix does not affect optical properties of the dye and restricts the access of the quencher. The resulting materials can be obtained in any shape (films, fibers, beads). Oxygen sensing *via* phosphorescence quenching of $[Ru(bpy)_3]^{2+}$, entrapped in organogels films, has been demonstrated by Díaz García and co-workers.[72]

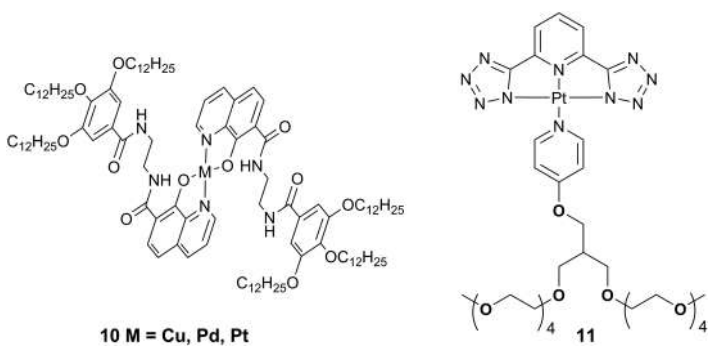


Figure 5.9 Gel-forming phosphorescent complexes.

Gelatine gels prepared from reverse micellar solutions were employed for the immobilization of the complex. The resulting films possess long-term stability and showed no swelling or mechanical stability problems if contacted with organic solvent. Phosphorescence emission of the entrapped complex at 580 nm is quenched by oxygen, allowing oxygen detection with a detection limit of 0.2 ppm. An attractive feature of the developed organogel is its high photochemical stability: no substantial fading of the phosphorescence intensity was observed after more than 20 h of continuous illumination.

5.4 Application of Oxygen Scavengers

In optical oxygen sensing methods, a calibration of the sensor response in a range of concentrations is usually required. In particular, depletion of oxygen, for oxygen-free measurements has to be performed. Deoxygenation of liquid photoactive compositions *via* bubbling of inert gases or freeze–thaw cycles leaves residual amounts of dissolved oxygen (nM concentration range), which can still affect the phosphorescence intensity. Moreover, in certain cases, such methods lead to serious complications. For instance, with colloidal or micellar systems, gas bubbling results in mechanical damaging of the material and in the formation of bubbles, which can scatter excitation and emission light.[73] For this reason, chemical deoxygenation of the phosphorescent samples *via* the addition of oxygen scavengers is preferable.

On the other hand, formation of singlet oxygen during triplet excited state quenching can lead to fast oxidation of dye molecules incorporated into oxygen sensor material. Additions of compounds, which selectively react with singlet oxygen in the samples or their incorporation into the probe, provide extended lifetimes and reliable operation of the corresponding materials. This can be achieved only if scavenger and products of its oxidation are inert with respect to photoactive components of the sample.

5.4.1 Inorganic Oxygen Scavengers

Sodium sulfite was the first demonstrated suitable oxygen scavenger for solution deoxygenation based on the redox reaction (5.1).

$$2SO_3^{2-} + O_2 \rightarrow 2SO_4^{2-} \tag{5.1}$$

Díaz-García and Sanz-Medel first applied sodium sulfite as an alternative to nitrogen bubbling for the phosphorescence measurements in the SDS micellar solutions.[74] Addition of sodium sulfite leads to a gradual increase of the phosphorescence intensity, since a diffusion rate of oxygen limits the efficiency of the phosphorescence quenching coming from the solubilized phosphor molecules. Due to the dynamic nature of the micellar equilibrium, the molar concentration of negatively charged sulfite ion is higher in the bulk solution compared to the micelle surface. This accounts for more rapid oxygen consumption in the bulk phase than inside the micelle. At high

concentration of the surfactant, it takes more time to reduce the dissolved oxygen as it is more soluble in the micellar phase than in water and quenches the phosphorescence emission. Increase of sulfite concentration progressively heightens phosphorescence intensity and shortens signal stabilization time to <1 s at concentration of 1.0 mmol.[75]

This technique allows for the protection of corresponding samples on a timescale of hours in the case the sample is open to air or for a longer time, if the sample is sealed. Sulfite-based O_2 scavenging to observe phosphorescence emission in aqueous solutions was applied for the development of analytical methods for the detection of a variety of organic compounds (including agricultural, pharmaceutical, petroleum, and biological-related samples) and extended the technique to facile metal ion determinations in solution using room temperature phosphorescence (RTP).[76–80]

In phosphorescence quenching oxygen sensing, measurement of the phosphorescence and decay times in sodium sulfite solution is commonly applied for the calibrations of the phosphor response in anoxic conditions.[81]

5.4.2 Application of Natural Antioxidants

Although the addition of inorganic salts into the solutions provides convenient protection of the phosphorescence, it causes certain side effects, such as a change of pH and a formation of solid deposits resulting from oxidation products. In addition, application of sodium sulfite is limited only to non-biological samples. To reduce oxygen concentration in the photoactive materials for application in bio-imaging and sensing, many groups applied the enzymatic oxygen-scavenging system,[82] which utilizes a mixture of glucose oxidase and catalase. Glucose oxidase catalyzes the oxidation of D-glucose according to the reaction (5.2) that results in the net loss of O_2 in solution.

$$\text{D-glucose} + O_2 + H_2O \rightarrow H_2O_2 + \text{D-gluconic acid} \qquad (5.2)$$

In the samples without glucose oxidase, the O_2 level is in equilibrium with the medium. Upon addition of glucose oxidase, O_2 level is decreased, depending on glucose concentration. The relationship between glucose and oxygen concentration is not linear because the reaction follows typical enzyme kinetics. This approach allows for "gradual" deoxygenation of the sample by varying the amount of added glucose.[83]

Liu and co-workers showed that natural lipid compounds could be applied as oxygen scavengers.[84] The main components of soybean oil are linoleic acid and oleic acid, which contain double bonds, capable of undergoing oxidation reaction with singlet oxygen (Figure 5.10), providing photo-initiated deoxygenation of the samples. The particular reaction depends on the nature of the double bonds and different types of reaction products (epoxides, dioxetanes, alcohols, *etc.*) can be generated.[85] The effect was studied for platinum(II) tetraphenyltetrabenzoporphyrin (PtTPBP) in aerated soybean oil. After 635 nm irradiation for 30 s, the phosphorescent lifetime of PtTPBP in soybean oil in the

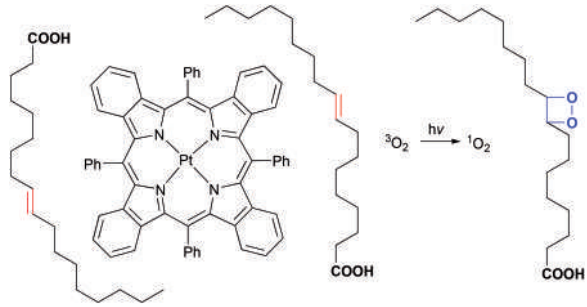


Figure 5.10 Platinum tetrabenzoporphyrin phosphorescence protection by a component of soybean oil.

presence of oxygen was found to be ~39 μs, similar to that of PtTPBP in toluene under an argon environment (40 μs). For comparison, the effect of ascorbyl palmitate, an efficient reducing agent, was studied. The phosphorescence of PtTPBP in toluene with prolonged exposure to 635 nm laser for 240 s was increased to ~32 μs at 2.0×10^{-3} M concentration of the scavenger. These results indicate that soybean oil can act as a protective solvent to prevent oxygen quenching and photooxidation. Linoleic acid and oleic acid of high purity showed similar effect.

5.4.3 Scavengers of Singlet Oxygen

Singlet oxygen generation by a phosphorescent probe can significantly influence the measurements and result in incorrect values due to degradation of dye molecules or matrix material, especially if UV radiation or powerful lasers are used as excitation sources. For instance, long-term aging of Pd(II) tetraphenylporphyrin probes encapsulated into poly(methylmethacrylate) (PMMA) films revealed 4-fold decrease of the quenching rates and oxygen diffusion constants.[86] Similarly, Ru(II) complexes with phenanthroline ligands exhibited a significant photodegradation, manifested by decay time decrease and specific absorption spectral changes.[87,88]

Photodegradation process can be reduced by addition of certain amines. Singlet oxygen is known to be susceptible to physical quenching through collisions with solvents or other molecules, causing decay back to the ground triplet state ($^3\Sigma_g$) on a timescale of few microseconds.[89] Quenching of singlet oxygen by amines involves the formation of a charge–transfer complex *via* electron transfer from the amine. It could be expected that phosphorescent materials containing amine additives possess higher photostability. However, not any type of amine is suitable for this purpose, as triplets excited states of the phosphor can also be quenched *via* electron transfer from the amine. Hartman studied the effect of different amines on the phosphorescence of Ru(II) complexes and showed and 1,4-diazabicyclo[2.2.2]octane (DABCO) is one of the most suitable scavengers as it is photochemically stable, does not

significantly quench triplets of the phosphor in contrast to other amines and does not react chemically with singlet oxygen, requiring only 1% (w/w) of the additive for efficient protection.[87] Ricketts and Douglas applied DABCO as a stabiliser for oxygen-sensitive Pt (II) octaethylporphyrin (PtOEP) in ethyl cellulose containing TiO_2 or ZnO as scattering agents.[90] The resulting material showed stability of the emission signal in the presence of oxygen during continuous use for over an hour. Klimant and co-workers studied the effect of DABCO on the photodegradation processes of Pd(II) and Pt(II) tetra-(pentafluorophenyl)porphyrin and PtOEP in different polymers.[91] It has been shown that in addition to dye photooxidation, singlet oxygen is capable of reacting with polymer matrix that ultimately alters its physical properties and influences mechanical stability, hydrophobicity, and solubility. Polystyrene and poly(phenylsilesquioxane) were found to have the highest stability in the presence of singlet oxygen, while different poly(methylmethacrylates) have the lowest stability. Addition of DABCO into the matrix inhibited photooxidation in most polymers, except for PMMA and its derivatives. *N*-Alkylated derivative of DABCO and the polystyrene-containing covalently grafted DABCO have been prepared in order to overcome the volatility and water solubility of the quencher. However, these derivatives did not show any positive effect on the photostability of the photosensitizer and polymer matrix.

5.5 Encapsulation of the Phosphorescent Molecules into Polymers

Polymers are widely used as carriers for phosphorescent dyes and selection of the specific material depends on the target application. The polymer provides the necessary protection for the phosphorescent dye from the environment and helps to tune oxygen sensitivity. The use of various polymers in oxygen sensing and phosphorescence imaging applications has been discussed in several reviews.[92–94]

Permeation rate of oxygen into a polymer film is characterized by the oxygen permeability coefficient *P*. Since the permeation of oxygen into the polymer layer includes the steps of its dissolution and further diffusion, the permeability coefficient is given by $P = D \cdot S$, where *D* and *S* are the diffusion constant and the oxygen solubility coefficient, respectively. Corresponding parameters for various polymers are given in Table 5.2.

Polysiloxanes possess exceptionally high oxygen solubility and diffusion constants compared to most other polymers, as reflected in Table 5.2. Matrices based on polysilicons were among the first applied in the development of optical oxygen sensors and are still used quite often.[95–99] Organic glassy polymers such as polystyrene, poly(methyl methacrylate), poly(isobutyl methacrylate), poly(vinyl chloride) have lower permeability but secure mechanical strength to thin-films. Among these polymers, polystyrene has been one of the most commonly used polymer matrixes for the preparation of oxygen

Table 5.2 Permeation rate, solubility and diffusion constants of oxygen in different polymers at 20 °C.[124]

Polymer	P($\times10^{13}$) cm^3(STP) cm^{-2} s cmHg	D($\times10^6$) cm^2 s^{-1}	S ($\times10^6$) cm^3(STP) cm^{-3} $cmHg^{-1}$
Poly(dimethylsiloxane)	695	40	24
Poly(1-trimethylsily-1-propyne)	7700	47	170
Polyethylene (low density)	0.7–0.9	46–60	21
Polyethylene (high density)	0.13–2.3	17	8
Polystyrene	2.63	–	–
Poly(methylmethacrylate)	9	10	8.5
Poly(vinylchloride)	0.34	1.2	2.9
Poly(vinylacetate)	0.4	5.5	6.2
Poly(isobutylmethacrylate)	20	–	–
Poly(2,2,2-trifluoroethyl-methacrylate)	32	15	0.27
Ethylcellulose	11.0	0.639	1.73
Teflon	1.65	15	1.3–2.5
Cellulose acetobutyrate	3.56	–	–
Cellulose acetate	5.85	–	–
Water	–	0.025	–

sensors due its good oxygen permeability, but complete impermeability to ions, low cost, and easiness in material manufacturing.[100–102] The dynamic range and sensitivity of the probes can be fine-tuned by employing differently substituted polystyrenes, *e.g.* poly(2,6-dichlorostyrene), poly(4-*tert*-butylstyrene) or poly(2,6-fluorostyrene).[103]

Introduction of fluorine atoms into the polymer backbone improves the sensor stability against photooxidation upon prolonged light irradiation. The C–F chemical bond is short and strong (binding energy of 116 kcal mol^{-1}), providing high resistance towards photooxidation. These polymers have high *D* and *S* values and are useful matrixes of optical oxygen sensor based on the phosphorescent metalloporphyrins.[104–107]

In the applications where oxygen quenching is an unwanted complication, *e.g.* in phosphorescence bio-imaging, low oxygen permeability matrixes are required. Wolfbeis and co-workers showed that ruthenium(II) complexes in films of poly(acrylonitrile) (PAN) are not sensitive to quenching by oxygen and applied this to develop luminescent temperature sensors.[108] Such behavior was observed for other phosphorescent complexes in beads made of PAN and various copolymers due to the low oxygen permeability. Their phosphorescence intensity and lifetime have been reported to be similar to the corresponding phosphorescent molecules in an oxygen-free environment.[109] PAN nanoparticles loaded with Ru(II) phosphorescent complexes were obtained by precipitating them from solutions in organic solvents.[110] Such particles were found to be impermeable to oxygen and negligible quenching of the phosphorescence was observed, making them suitable as phosphorescent labels or as oxygen-insensitive probes for temperature.

However, many phosphorescent dyes possess only limited solubility in PAN and related co-polymers. Halogen-containing polymers encapsulation matrix for organic and organometallic phosphorescent molecules, based on poly(vinylfluoride) (PVF), poly(vinylchloride) (PVC) and their co-polymers, were developed by Song and co-workers[111] to produce phosphorescent materials which are substantially unaffected by oxygen at ambient conditions. In contrast to PAN, these polymers provide a particularly high loading of the phosphorescent molecules. The phosphorescence intensity of Pt(II) tetra-*meso*-fluorophenylporphine (PtTMPFP) encapsulated into PAN particles was observed to drop at >1% loading due to the dye aggregation. For PVF and PVC the phosphorescence increases with loading of PtTMPFT up to 3%. With the same loading (1%) of the dye, PVF and PVC showed 4 and 2 times stronger phosphorescence than PAN particles, respectively. Nanoparticles based on these halogen-containing polymer matrixes and PtTMPFT have been prepared and covalently bound to antibodies to perform C-reactive protein phosphorescent assay.

5.6 Inorganic Matrix Materials

Inorganic materials for the fabrication of optical oxygen sensors attracted much attention due their high mechanical strength, thermal and oxidative stability. Various glasses, silica, sol–gels, organically modified silicates and metal oxides have been employed for this purpose.[94] The development of specific types of nanosensors for imaging applications, such as silica nanoparticles and hydrogel nanosensors, has been the subject of comprehensive recent reviews.[112,113] Specific aspects of such materials design related to the control of the phosphorescence quenching rates are discussed here.

It has been demonstrated that silicon oxide possesses excellent gas barrier properties due to very tight interstitial spaces of the Si–O lattice, resulting in oxygen diffusivities in the range 10^{-5}–10^{-9} cm^2 s^{-1}. Even nanometer-thick silica layers can act as an oxygen barrier in silica-coated polymer films.[114]

MacCraith and co-workers reported that oxygen quenching rates of the phosphorescent Ru(II) complexes in sol–gel silica films can be tuned to fit different oxygen concentration ranges in different environments.[115] These materials were prepared through hydrolysis of tetraethylorthosilicate precursor in acidic solution.[116] Quenching rates of the immobilized phosphor strongly depend on the porosity of the material and average pore size which, in turn, is determined by such parameters as the dip speed, water-to-precursor ratio, pH value and sol aging time. By adjusting these parameters, film properties can be tailored to optimise oxygen quenching sensitivity. Using these matrixes, optical oxygen sensors with controllable sensitivity towards quenching were fabricated based on PtOEP as a phosphor.[117–119]

The use of silanes modified with hydrophobic groups (*i.e.* compound of general formula $R–Si(OR)_3$, where R is alkyl or phenyl) as sol–gel precursors gives so-called ormosils, which were found to provide certain advantages as matrixes, particularly long-term quenching stability and good reproducibility

within a batch of films.[120] Xerogel ormosils doped with platinum(II) porphyrins provide widely adjustable sensitivity towards quenching, which can be readily tuned by adjusting the xerogel composition and the phosphor.[121]

Winnik *et al.* have studied oxygen quenching of PtOEP in the hybrid polymers obtained from polydimethylsiloxane (PDMS), poly(n-butylaminothionylphosphazene) (C4PATP) and 10 nm silica particles.[122] Silica particles were shown to improve the mechanical properties of the matrix and affect oxygen diffusion and permeation. The quenching constants depended on the fraction of silica particles, which are acting as obstacles to oxygen diffusion.

Mirenda and co-workers reported phosphorescence properties of tris (bipyridine)ruthenium(II)-doped silica nanoparticles.[123] While the emission of the complex in solution is strongly quenched by oxygen, the emission of immobilized complex remains the same in O_2-free and O_2-saturated conditions. A similar effect has been observed by Zhang and co-workers for encapsulated $[Ru(phen)_3]^{2+}$, that was attributed to the low oxygen permeability of the silica shell.[124,125] In addition, encapsulated complexes showed higher photostability compared to free solutions. Leakage of dye molecules from the silica particles is negligible, due to strong electrostatic attractions between the positively charged ruthenium complex and the negatively charged silica allowing the use of such materials in bioanalytical assays.

5.7 Conclusions and Perspectives

A significant advance in applications of triplet excited state chromophores has been achieved during the last two decades. In particular, oxygen sensing techniques based on the phosphorescence quenching attracted much attention and efforts which included multistep synthesis of new probe molecules, design of nano-carriers, development of specific instrumentation and biological studies. These activities did not only enable impressive applications, such as 2D or 3D oxygen imaging in tissue, but also delivered new fundamental knowledge of the interactions between optically excited molecules and oxygen.

An ultimate requirement for accurate quantification of oxygen *via* phosphorescence quenching method is the possibility for tuning quenching rates and preventing photooxidation of sensor components. In this chapter, various approaches to control these processes are described. These can be generally divided into two categories referred to as “active” and “passive” protection. Attenuation of triplet state quenching in most of the probes reported so far is achieved through incorporation of the photoactive components into organic polymers or inorganic materials with defined oxygen permeability. However, applicability of this method in certain cases is limited, especially for sensing in living objects. Alternatively, chemical modification of the phosphorescent molecules with special groups allows both limiting oxygen diffusion and ensuring the desired localization of the probe or its interactions with biomolecules. Due to the fact that access to such tailor-made probes usually requires significant synthetic efforts, supramolecular chemistry approaches for the phosphor’s design are currently attracting attention.

On the other hand, the limited lifetime of sensor probes due to singlet oxygen-mediated photooxidation is another important issue in practical applications. Further efforts are needed to better understand the consequences of such deteriorative processes and to improve current protection approaches. An appealing solution is based on the introduction of "self-healing" phosphorescent molecules and matrix materials capable of reversible binding of singlet oxygen or its deactivation into ground state. Chemical toolkits that enable such transformations are under active development.

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References

1. H. Kautsky, A. Hirsch and W. Baumeister, *Ber. Dtsch. Chem. Ges.*, 1931, **64**, 2053.
2. D. R. Kearns and A. U. Khan, *Photochem. Photobiol.*, 1969, **10**, 193.
3. C. Grewer and H.-D. Brauer, *J. Phys. Chem.*, 1994, **98**, 4230.
4. J. M. Vanderkooi, G. Maniara, T. J. Green and D. F. Wilson, *J. Biol. Chem.*, 1987, **262**, 5476.
5. D. F. Wilson, W. L. Rumsey, T. J. Green and J. M. Vanderkooi, *J. Biol. Chem.*, 1988, **263**, 2712.
6. D. B. Papkovsky and R. I. Dmitriev, *Chem. Soc. Rev.*, 2013, **42**, 8700.
7. T. V. Esipova, A. Karagodov, J. Miller, D. F. Wilson, T. M. Busch and S. A. Vinogradov, *Anal. Chem.*, 2011, **83**, 8756.
8. O. S. Finikova, A. Galkin, V. Rozhkov, M. Cordero, C. Hagerhall and S. A. Vinogradov, *J. Am. Chem. Soc.*, 2003, **125**, 4882.
9. D. F. Wilson, W. M. F. Lee, S. Makonnen, O. S. Finikova, S. Apreleva and S. A. Vinogradov, *J. Appl. Physiol.*, 2006, **101**, 1648.
10. Q. Zhao, M. X. Yu, L. X. Shi, S. J. Liu, C. Y. Li, M. Shi, Z. G. Zhou, C. H. Huang and F. Y. Li, *Organometallics*, 2010, **29**, 1085.
11. Q. Liu, B. R. Yin, T. S. Yang, Y. C. Yang, Z. Shen, P. Yao and F. Y. Li, *J. Am. Chem. Soc.*, 2013, **135**, 5029.
12. M. Chen, Z. Lei, W. Feng, C. Y. Li, Q. M. Wang and F. Y. Li, *Biomaterials*, 2013, **34**, 4284.
13. K. Koren, R. Dmitriev, S. Borisov, D. Papkovsky and I. Klimant, *ChemBioChem*, 2012, **13**, 1184.
14. A. V. Kondrashina, R. I. Dmitriev, S. Borisov, I. Klimant, I. O'Brien, Y. M. Nolan, A. Zdanov and D. B. Papkovsky, *Adv. Funct. Mater.*, 2012, **22**, 4931.
15. A. Fercher, S. Borisov, A. Zdanov, I. Klimant and D. B. Papkovsky, *ACS Nano*, 2011, **5**, 5499.
16. S. Draxler, in *Advanced Concepts in Fluorescence Sensing*, ed. C. D. Geddes and J. R. Lakowicz, Springer, Norwell, 2005, pp. 241–274.

17. P. R. Ogilby, *Chem. Soc. Rev.*, 2010, **39**, 3181.
18. M. A. Filatov, S. Baluschev, I. Z. Ilieva, V. Enkelman, T. Miteva, K. Landfester, S. E. Aleshchenkov and A. V. Cheprakov, *J. Org. Chem.*, 2012, **77**, 11119.
19. P. N. Prasad, *Introduction to Biophotonics*, Wiley-Interscience, New York, 2004, p. 162.
20. M. A. Filatov, S. Baluschev and K. Landfester, *Chem. Soc. Rev.*, 2016, **45**, 4668.
21. S. Baluschev, K. Katta, Y. Avlasevich and K. Landfester, *Mater. Horiz.*, 2016, **3**, 478.
22. I. B. Rietveld, E. Kim and S. A. Vinogradov, *Tetrahedron*, 2003, **59**, 3821.
23. A. G. Moiseev, E. A. Margulies, J. A. Schneider, F. Beanger-Garieyb and D. F. Perepichka, *Dalton Trans.*, 2014, **43**, 2676.
24. R. H. Jin, T. Aida and S. Inoue, *Chem. Commun.*, 1993, 1260.
25. R. Sadamoto, N. Tomioka and T. Aida, *J. Am. Chem. Soc.*, 1996, **118**, 3978.
26. M. Kimura, K. Nakada, Y. Yamaguchi, K. Hanabusa, H. Shirai and N. Kobayashi, *Chem. Commun.*, 1997, 1215.
27. J. M. Riley, S. Alkan, A. D. Chen, M. Shapiro, W. A. Khan, W. R. Murphy and J. E. Hanson, *Macromolecules*, 2001, **34**, 1797.
28. J. Issberner, F. Vogtle, L. DeCola and V. Balzani, *Chem.–Eur. J.*, 1997, **3**, 706.
29. F. Vogtle, M. Plevoets, M. Nieger, G. C. Azzellini, A. Credi, L. De Cola, V. De Marchis, M. Venturi and V. Balzani, *J. Am. Chem. Soc.*, 1999, **121**, 6290.
30. V. Rozhkov, D. Wilson and S. Vinogradov, *Macromolecules*, 2002, **35**, 1991.
31. S. A. Vinogradov, L.-W. Lo and D. F. Wilson, *Chem.–Eur. J.*, 1999, **5**, 1338.
32. S. A. Vinogradov, *Org. Lett.*, 2005, **7**, 1761.
33. O. S. Finikova, S. Y. Chernov, A. V. Cheprakov, M. A. Filatov, S. A. Vinogradov and I. P. Beletskaya, *Dokl. Chem.*, 2003, **391**, 222.
34. A. Y. Lebedev, A. V. Cheprakov, S. Sakadzic, D. A. Boas, D. F. Wilson and S. A. Vinogradov, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1292.
35. A. V. Cheprakov and M. A. Filatov, *J. Porphyrins Phthalocyanines*, 2009, **13**, 291.
36. M. A. Filatov and A. V. Cheprakov, *Tetrahedron*, 2011, **67**, 3559.
37. I. Dunphy, S. A. Vinogradov and D. F. Wilson, *Anal. Biochem.*, 2002, **310**, 191.
38. M. A. Yaseen, V. J. Srinivasan, S. Sakadžić, W. Wu, S. Ruvinskaya, S. A. Vinogradov and D. A. Boas, *Opt. Express*, 2009, **17**, 22341.
39. D. F. Wilson, W. M. Lee, S. Makonnen, O. Finikova, S. Apreleva and S. A. Vinogradov, *J. Appl. Physiol.*, 2006, **101**, 1648.
40. S. V. Apreleva, D. F. Wilson and S. A. Vinogradov, *Appl. Opt.*, 2006, **45**, 8547.
41. E. Roussakis, Z. Li, N. H. Nowell, A. J. Nichols and C. L. Evans, *Angew. Chem., Int. Ed.*, 2015, **54**, 14728.
42. P. G. L. Koolen, Z. Li, E. Roussakis, M. A. Paul, A. M. S. Ibrahim, R. Matyal, T. Huang, C. L. Evans and S. J. Lin, *Plast. Reconstr. Surg.*, 2017, **140**, 89.

43. J.-M. Aubry, C. Pierlot, J. Rigaudy and R. Schmidt, *Acc. Chem. Res.*, 2003, **36**, 668.
44. M. A. Filatov and M. O. Senge, *Mol. Syst. Des. Eng.*, 2016, **1**, 258.
45. M. A. Filatov, E. Heinrich, D. Busko, I. Z. Ilieva, K. Landfester and S. Baluschev, *Phys. Chem. Chem. Phys.*, 2015, **17**, 6501.
46. S. Callaghan, M. A. Filatov, E. Sitte, H. Savoie, R. W. Boyle, K. J. Flanagan and M. O. Senge, *Photochem. Photobiol. Sci.*, 2017, **16**, 1371.
47. Y. Kai and K. Imakubo, *Photochem. Photobiol.*, 1979, **29**, 261.
48. M. L. Saviotti and W. C. Galley, *Proc. Natl. Acad. Sci. U. S. A.*, 1974, **71**, 4154.
49. M. W. Geiger and N. J. Turro, *Photochem. Photobiol.*, 1975, **22**, 273.
50. G. B. Strambini and P. Cioni, *J. Am. Chem. Soc.*, 1999, **121**, 8337.
51. P. Cioni, *Biophys. Chem.*, 2000, **87**, 15.
52. N. J. Turro, G. Sidney Cox and X. Li, *Photochem. Photobiol.*, 1983, **37**, 149.
53. Y. L. Peng, Y. T. Wang, Y. Wang and W. J. Jin, *J. Photochem. Photobiol., A*, 2005, **173**, 301.
54. W. K. Hartmann, M. H. B. Gray, A. Ponce, D. G. Nocera and P. A. Wong, *Inorg. Chim. Acta*, 1996, **243**, 239.
55. J. Kuijt, F. Ariese, U. A. Th. Brinkman and C. Gooijer, *Anal. Chim. Acta*, 2003, **488**, 135.
56. S. Hamai, *J. Phys. Chem.*, 1995, **99**, 12109.
57. S. Hamai, *Chem. Commun.*, 1994, **19**, 2243.
58. G. Grabner, K. Rechthaler, B. Mayer and G. Kohler, *J. Phys. Chem. A*, 2000, **104**, 1365.
59. Y. Zhao, L. D. Li and A. J. Tong, *Chin. J. Anal. Chem.*, 1997, **25**, 1016.
60. Y. Zhang and D. Johnson, *Sens. Actuators, B*, 2003, **96**, 379.
61. Y. Zhang and D. Johnson, *Anal. Chim. Acta*, 2004, **511**, 333.
62. Z.-W. Gao, X. Feng, L. Mu, X.-L. Ni, L.-L. Liang, S.-F. Xue, Z. Tao, X. Zeng, B. E. Chapman, P. W. Kuchel, L. F. Lindoy and G. Wei, *Dalton Trans.*, 2013, **42**, 2608.
63. N. Jayaraj, M. V. Maddipatla, R. Prabhakar, S. Jockusch, N. J. Turro and V. Ramamurthy, *J. Phys. Chem. B*, 2010, **114**, 14320.
64. Y. Wang, W.-J. Jin, J.-B. Chao and L.-P. Qin, *Supramol. Chem.*, 2003, **15**, 459.
65. G.-R. Li, J.-J. Wu, W.-J. Jin and J.-W. Xie, *Talanta*, 2003, **60**, 555.
66. Y.-T. Wang, X.-W. Wang and Y. Zhang, *Photochem. Photobiol.*, 2011, **87**, 772.
67. S. Hirata, K. Totani, J. Zhang, T. Yamashita, H. Kaji, S. R. Marder, T. Watanabe and C. Adachi, *Adv. Funct. Mater.*, 2013, **23**, 3386.
68. M. Shirakawa, N. Fujita, T. Tani, K. Kanekob and S. Shinkai, *Chem. Commun.*, 2005, 4149.
69. M. Shirakawa, N. Fujita, T. Tani, K. Kaneko, M. Ojima, A. Fujii, M. Ozaki and S. Shinkai, *Chem.–Eur. J.*, 2007, **13**, 4155.
70. N. Kumar Allampally, M. Bredol, C. A. Strassert and L. De Cola, *Chem.–Eur. J.*, 2014, **20**, 16863.
71. H. Wang, H. Wang, X. Yang, Q. Wang and Y. Yang, *Langmuir*, 2015, **31**, 486.

72. N. Velasco-Garcia, M. J. Valencia-Gonzalez and M. E. Díaz García, *Analyst*, 1997, **122**, 1405.
73. S. H. C. Askes, N. Lopez Mora, R. Harkes, R. I. Koning, B. Koster, T. Schmidt and S. Bonnet, *Chem. Commun.*, 2015, **51**, 9137.
74. M. E. Díaz García and A. Sanz-Medel, *Anal. Chem.*, 1986, **58**, 1436.
75. B. Cañabate-Díaz, A. Segura Carretero, C. Cruces-Blanco and A. Fernández-Gutiérrez, *Analyst*, 2003, **128**, 411.
76. M. R. Fernández de la Campa, Y. Ming Liu, M. E. Díaz García and A. Sanz Medel, *Anal. Chim. Acta*, 1990, **238**, 297.
77. A. Segura-Carretero, C. Cruces-Blanco and A. Fernández-Gutiérrez, *Talanta*, 1996, **43**, 1001.
78. A. Segura Carretero, C. Cruces Blanco and A. Fernández Gutiérrez, *Anal. Chim. Acta*, 1997, **353**, 337.
79. A. Segura-Carretero, C. Cruces-Blanco, B. Cañabate-Diaz, J. F. Fernández-Sánchez and A. Fernández-Gutiérrez, *Anal. Chim. Acta*, 2000, **417**, 19.
80. J. A. Arancibia and G. M. Escandar, *Analyst*, 2001, **126**, 917.
81. C. O'Donovan, J. Hynes, D. Yashunski and D. B. Papkovsky, *J. Mater. Chem.*, 2005, **15**, 2946.
82. R. E. Benesch and R. Benesch, *Science*, 1953, **118**, 447.
83. R. L. Plant and D. H. Burns, *Appl. Spectrosc.*, 1993, **47**, 1594.
84. Q. Liu, B. R. Yin, T. S. Yang, Y. Yang, Z. Shen, P. Yao and F. Y. Li, *J. Am. Chem. Soc.*, 2013, **135**, 5029.
85. D. P. Jason, G. O. Brian, P. B. Justin, K. H. Mohammad, R. Jo Ann, S. W. Jeffrey, W. R. James and N. Sergei, *High-oxygen Barrier Materials Based on Hyperbranched Aliphatic Polyesters. Polymer Degradation and Performance*, American Chemical Society, Washington, 2009, pp. 17–30.
86. K. Õige, T. Avarmaa, A. Suisalu and R. Jaaniso, *Sens. Actuators, B*, 2005, **106**, 424.
87. P. Hartmann, M. J. P. Leiner and P. Kohlbacher, *Sens. Actuators, B*, 1998, **51**, 196.
88. P. Hartmann, *Anal. Chem.*, 2000, **72**, 2828.
89. C. Schweitzer and R. Schmidt, *Chem. Rev.*, 2003, **103**, 1685.
90. S. R. Ricketts and P. Douglas, *Sens. Actuators, B*, 2008, **135**, 46.
91. B. Enko, S. M. Borisov, J. Regensburger, W. Bäumler, G. Gescheidt and I. Klimant, *J. Phys. Chem. A*, 2013, **117**, 8873.
92. B. Adhikari and S. Majumdar, *Prog. Polym. Sci.*, 2004, **29**, 699.
93. G. Korotcenkov, *Handbook of Gas Sensor Materials*, Springer, New York, 2014, pp. 249–263.
94. X.-D. Wang and O. S. Wolfbeis, *Chem. Soc. Rev.*, 2014, **43**, 3666.
95. J. R. Bacon and J. N. Demas, *Anal. Chem.*, 1987, **59**, 2780.
96. M. E. Cox and B. Dunn, *Appl. Opt.*, 1985, **24**, 2114.
97. T. Ishiji and M. Kaneko, *Analyst*, 1995, **120**, 1633.
98. E. R. Carraway, J. N. Demas and B. A. DeGraff, *Langmuir*, 1991, **7**, 2991.
99. S. B. Bambot, R. Holavanahali, J. R. Lakowicz, G. M. Carter and G. Rao, *Biotechnol. Bioeng.*, 1994, **43**, 1139.
100. S.-K. Lee and I. Okura, *Anal. Commun.*, 1997, **34**, 185.

101. E. Schmälzlin, J. T. van Dongen, I. Klimant, B. Marmodee, M. Steup, J. Fisahn, P. Geigenberger and H. G. Löhmannsröben, *Biophys. J.*, 2005, **89**, 1339.
102. A. Bizzarri, H. Koehler, M. Cajlakovic, A. Pasic, L. Schaupp, I. Klimant and V. Ribitsch, *Anal. Chim. Acta*, 2006, **573**, 48.
103. K. Koren, L. Hutter, B. Enko, A. Pein, S. M. Borisov and I. Klimant, *Sens. Actuators, B*, 2013, **176**, 344.
104. Y. Amao, I. Okura, H. Shinohara and H. Nishide, *Polym. J.*, 2002, **34**, 411.
105. Y. Amao, K. Asai, I. Okura, H. Shinohara and H. Nishide, *Analyst*, 2000, **125**, 1911.
106. N. Yi-Yan, R. M. Felder and W. J. Koros, *J. Appl. Polym. Sci.*, 1980, **25**, 1755.
107. W. A. Zisma, *Adv. Chem. Ser.*, 1964, **43**, 1.
108. G. Liebsch, I. Klimant and O. S. Wolfbeis, *Adv. Mater.*, 1999, **11**, 1296.
109. J. M. Kürner, I. Klimant, C. Krause, H. Preu, W. Kunz and O. S. Wolfbeis, *Bioconjugate Chem.*, 2001, **12**, 883.
110. O. S. Wolfbeis, *Chem. Soc. Rev.*, 2015, **44**, 4743.
111. X. Song, L. Huang and B. Wu, *Anal. Chem.*, 2008, **80**, 5501.
112. B. Korzeniowska, R. Nooney, D. Wencel and C. McDonagh, *Nanotechnology*, 2013, **24**, 442002.
113. A. Ray and R. Kopelman, *Nanomedicine*, 2013, **8**, 1829.
114. Y. Leterrier, *Prog. Mater. Sci.*, 2003, **48**, 1.
115. A. K. McEvoy, C. McDonagh and B. D. MacCraith, *J. Sol–Gel Sci. Technol.*, 1997, **8**, 1121.
116. B. D. MacCraith, C. M. McDonagh, G. O'Keeffe, E. T. Keyes, J. G. Vos, B. O'Kelly and J. F. McGilp, *Analyst*, 1993, **118**, 385.
117. S. K. Lee and I. Okura, *Analyst*, 1997, **122**, 81.
118. T.-S. Yeh, C.-S. Chu and Y.-L. Lo, *Sens. Actuators, B*, 2006, **119**, 701.
119. C.-S. Chu and Y.-L. Lo, *Sens. Actuators, B*, 2007, **124**, 376.
120. C. McDonagh, B. D. MacCraith and A. K. McEvoy, *Anal. Chem.*, 1998, **70**, 45.
121. V. S. Tripathi, G. Lakshminarayana and M. Nogami, *Sens. Actuators, B*, 2010, **147**, 741.
122. X. Lu, I. Manners and M. A. Winnik, *Macromolecules*, 2001, **34**, 1917.
123. M. Mirenda, V. Levi, M. L. Bossi, L. Bruno, A. V. Bordoni, A. E. Regazzoni and A. Wolosiuk, *J. Colloid Interface Sci.*, 2013, **392**, 96.
124. D. Zhang, Z. Wu, J. Xu, J. Liang, J. Li and W. Yang, *Langmuir*, 2010, **26**, 6657.
125. L. M. Rossi, L. Shi, F. H. Quina and Z. Rosenzweig, *Langmuir*, 2005, **21**, 4277.