



Reactive oxygen species in biological media are they friend or foe? Major *In vivo* and *In vitro* sensing challenges

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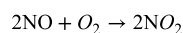
ABSTRACT

The role of Reactive Oxygen Species (ROS) on biological media has been shifting over the years, as the knowledge on the complex mechanism that lies underneath their production and overall results has been growing. It has been known for some time that these species are associated with a number of health conditions. However, they also participate in the immunoactivation cascade process, and can have an active role in theranostics. Macrophages, for example, react to the presence of pathogens through ROS production, potentially allowing the development of new therapeutic strategies. However, their short lifetime and limited spatial distribution of ROS have been limiting factors to the development and understanding of this phenomenon. Even though, ROS have shown successful theranostic applications, e.g., photodynamic therapy, their wide applicability has been hampered by the lack of effective tools for monitoring these processes in real time. Thus the development of innovative sensing strategies for *in vivo* monitoring of the balance between ROS concentration and the resultant immune response is of the utmost relevance. Such knowledge could lead to major breakthroughs towards the development of more effective treatments for neurodegenerative diseases. Within this review we will present the current understanding on the interaction mechanisms of ROS with biological systems and their overall effect. Additionally, the most promising sensing tools developed so far, for both *in vivo* and *in vitro* tracking will be presented along with their main limitations and advantages. This review focuses on the four main ROS that have been studied these are: singlet oxygen species, hydrogen peroxide, hydroxyl radical and superoxide anion.

1. Introduction

Reactive Oxygen Species (ROS) is actually a collective term that is used to describe oxygen-derived small and highly reactive molecules. Those can be divided into two main categories. The first group is free radicals and include superoxide anion (O_2^-), hydroxyl radical (OH^\cdot), peroxy radical (ROO^\cdot) and alkoxy radical (RO^\cdot). These species are free radicals that contain one or more unpaired electrons. The second group is the nonradicals which do not contain unpaired electrons. These include singlet oxygen, (1O_2), hydrogen peroxide (H_2O_2), organic peroxides ($ROOH$) and Ozone (O_3) (Fig. 1). These non-radical species can directly induce oxidation and/or can be converted into free radicals according to their environmental conditions [1]. ROS lifetime in biological conditions is different for each species and dependent on external conditions, namely, temperature; light; reducing agents and metal ions, among others [2–4] (Fig. 1).

The presence of radicals in biological media is not synonymous with the presence of ROS. For example, the presence of Reactive Nitrogen Species (RNS) should not be neglected [5]. Nitrogen monoxide (NO^\cdot), is a RNS radical that is capable of diffusion through cell membrane systems. In a similar manner to its oxygen based counterparts, (ROS), this specie can be biologically synthesised from the amino acid L-arginine by a family of different nitrogen monoxide synthases. Its effect on the body is largely dependent on the local environment. In body fluids, for example, small percentages of NO^\cdot reacts with O_2 to form nitrogen dioxide (NO_2^\cdot), which is a more unstable radical:



However, the majority of NO^\cdot is oxidised to nitrite ion (NO_2^-) or reacts with heme groups of oxyhemoglobin to form metahemoglobin and nitrate (NO_3). In fact, one of the most common methods for assessing

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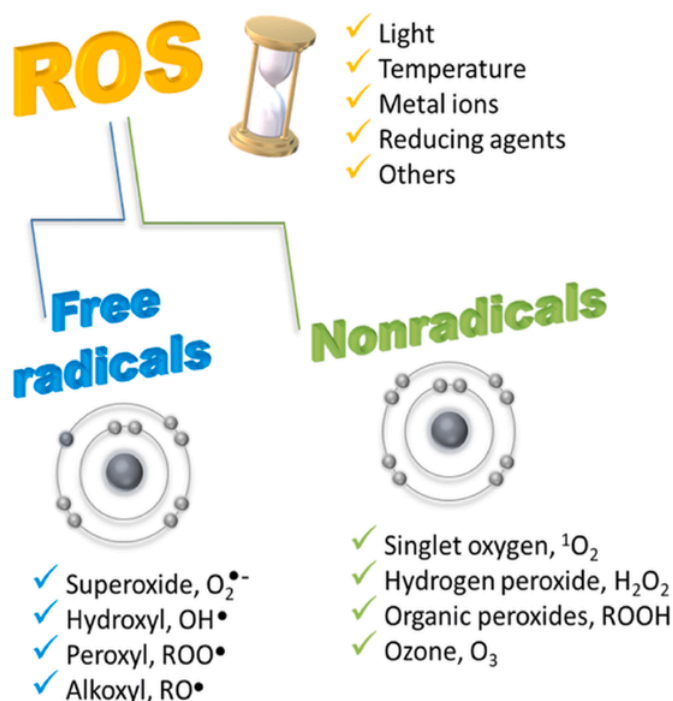


Fig. 1. Schematic representation of the two main categories of ROS and the biological factors that affect their lifetime.

the formation/synthesis of nitrogen monoxide *in vivo* is the measurement of free nitrite and nitrate levels [5].

The high reactivity of ROS is simultaneously its major hazard and advantage. ROS over expression is commonly associated with a number of harmful effects linked to oxidative stress such as neurodegeneration, cancer, inflammation and apoptosis, amongst others [5,6]. ROS are common by-products of cellular metabolism and depending on their concentration the effects of these species can lead to cellular death. Nonetheless, there is also a beneficial role associated with the production of these species. They are known to participate in numerous highly relevant physiological processes as secondary messengers, such as, signaling and cell proliferation during cell adhesion and gene expression [7–9]. Phagocytes, for example, use superoxide radical along with other ROS species to kill bacteria. Additionally, they also contribute actively to the immune system through fibroblast proliferation and differentiation control, as well as, ensuring the proper folding and maturation of immunoglobulins [10,11].

Their role as secondary messengers in cellular signaling has been attracting much attention. The ROS messenger role is related to oxygen sensing systems such as carotid body chemoreceptor cells, pulmonary artery smooth muscle cells and erythropoietin-producing cells. These unique cells comprise essential parts of homeostatic loops required to maintain oxygen levels in multicellular organisms in hypoxia situations [12]. Since ROS plays such an important role in living organisms it is not surprising that the development of sensors/biosensors for its detection has attracted so much attention over recent years [13,14].

In this work we will present a structured review beginning with a brief introduction to the known ROS biological roles in living organisms. A brief discussion on the main challenges faced in the development of sensing technologies for the most commonly studied ROS: singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical will follow. Then an overview on the commonest sensing techniques used for each ROS with the best outcomes will be presented. The intention is not to present an exhaustive review but one where the main focus is on the most recent innovative and promising solutions towards the development of ROS sensing. Therefore, the most relevant sensing technologies for *in vitro* and *in vivo* ROS sensing will be presented. Their

advantages and drawbacks will be highlighted to allow greater critical understanding of the ROS sensing field.

2. ROS biological production cascade-mechanism

In biological systems, ROS can be produced by different types of cells, namely, phagocytes which include neutrophils, monocytes and macrophages amongst others [1]. The production of ROS by these types of cells is closely related to their function in the immune system. Indeed, superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radical are the most prominent ROS produced by phagocytes and play an essential role in the host defense against pathogens [15–17]. Therefore, it is not surprising that a range of sensors for these species have been developed over recent years (Fig. 2).

The impact that ROS have in host immune defense is clearly demonstrated by what occurs for people with chronic granulomatous disease (CGD). This is a genetic disease where the patients have recurrent infections due to their phagocytes incapacity to produce ROS. The lack of ROS hinders the killing and elimination of pathogens that usually pose a much lesser problem to healthy people [18,19]. In normal biological conditions, the phagocyte NADPH oxidase is responsible for the production of the superoxide anion which, in the presence of protons, forms hydrogen peroxide. Furthermore, hydrogen peroxide also reacts with superoxide anion forming hydroxyl radicals that is utilised by myeloperoxidase in the production of a powerful killing agent, hypochlorous acid (HOCl) [1,20,21].

Despite being produced by a range of cell types, within each cell they can be formed within a number of cellular structures, ranging from the endoplasmic reticulum to the cell nucleus [22]. In some particular cases, ROS can also be formed upon oxidation of different compounds, such as, lipids proteins or DNA [10,11]. Nonetheless the most relevant source of ROS production is within the mitochondria [22,23] (Fig. 3). During the generation of adenosine triphosphate (ATP), electrons are transported through the mitochondria via the electron transport chain. In the mitochondria inner membrane, complex I and III cause some electrons to “leak” from the transport chain. These can react with free diatomic oxygen to form superoxide anions. When the oxygenation conditions are normal, about 1–2% of the electrons transported within the mitochondria, participate in this secondary process [24]. These superoxide anions are rapidly converted into hydrogen peroxide upon bind-

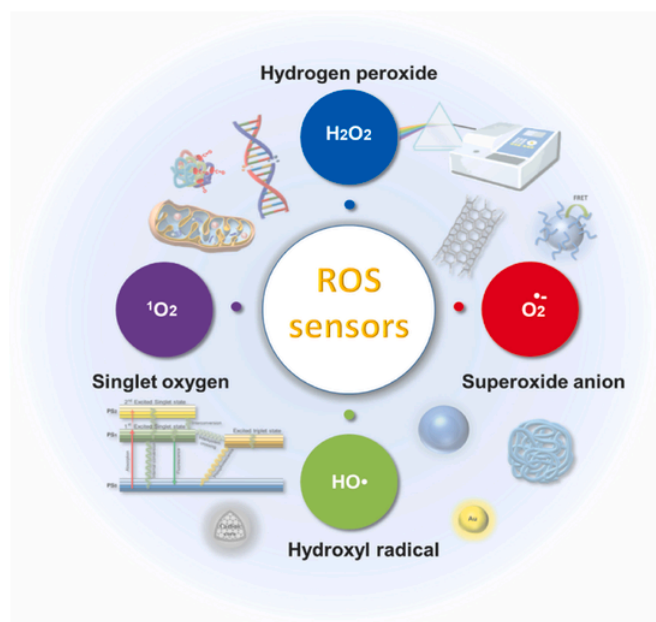


Fig. 2. Determination of the most studied ROS.

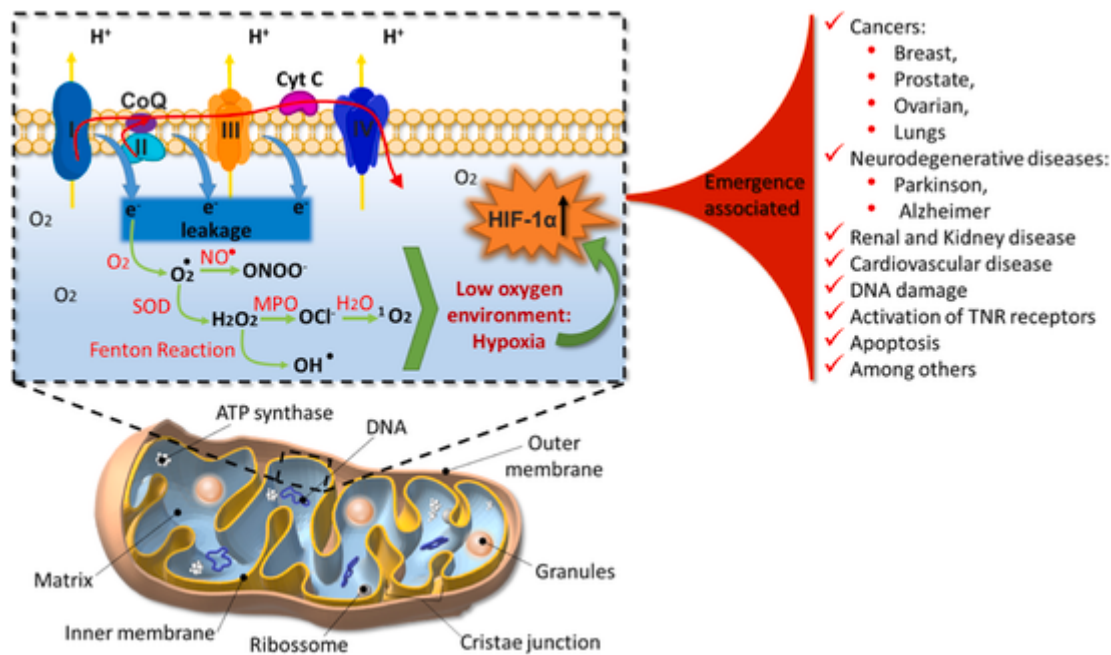


Fig. 3. Schematic representation of the mechanism for ROS biological production on the mitochondria and their known association with several diseases.

ing to superoxide dismutase 1 or 2 (SOD₁ or SOD₂) meaning they only have a short lifetime. However, in low oxygen conditions the ROS production actually increases in complex III at the QO site [25]. This initiates a cascade-like event where the number of superoxide species increases and are released into the mitochondria intermembrane space, where they are then reduced to hydrogen peroxide and released into the cytosol [26]. In order to help mediate the cell survival and activity, transcription factors known as hypoxia-inducible factors (HIF) are released (Fig. 3) [27]. Under hypoxic conditions, HIF-1α expression increases in an attempt to stabilise the cell; however, overexpression of this transcription factor has been associated with numerous cancers, including breast, prostate, lung and ovarian cancers. Moreover, overexpression is also related to several neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases [23,28] (Fig. 3).

Elevated levels of ROS and related transcription factors have also been linked to: DNA damage at the transcriptome level by way of DNA methylation, apoptosis induction through cell signaling, the activation of tumor necrosis factor (TNF) receptors and protein oxidation/These can all lead to denaturation occurring [23,29–31]. Hence the oxygen abundance and the subsequent ROS generation cascade have a relevant role in many human diseases, including renal/kidney disease and cardiovascular conditions, amongst others [32,33]. Therefore, the number of studies performed to better ascertain the link between these factors and the mechanism by which these reactions occur have been recently increasing in number [5,6,23,34–37]. This has led to a subsequent

growing increase in knowledge on ROS production, concentration and specific roles in biological media [22].

Cellular ROS are tightly controlled since their concentration can dictate the difference between cell differentiation and cell death. This appears to suggest that inside the cells there is a “ROS rheostat” that controls cellular survival [8]. However, their high reactivity which leads to their short lifetime, relatively low spatial abundance, poor photostability and rapid off-site diffusion has been hampering studies into their presence and the biological mechanisms lying beneath the production and effects associated with ROS [23]. A number of innovative solutions have been developed in order to overcome the shortcomings of the current ROS detecting systems, namely their lack of stability, specificity and sensitivity, among others. Amperometric sensors, chemiluminescence, fluorescence and their combination with nanomaterials have been used to determine appealing results for imaging probes and theranostics products, since they allow ratiometric sensing that is relatively unaffected by environmental drift, which is one of the major difficulties on *in vitro* and *in vivo* ROS sensing [23] (Table 1).

3. ROS sensing in biological media

To date, the non-destructive and selective intracellular analysis of ROS has remained one of the biggest challenges in molecular bioimaging. The most conventional methods have advantages and drawbacks (Table 1). In many cases although they are widely used, they are either applied in cells post-mortem or require labeling. Studying cells post-

Table 1
Common direct sensing methods for ROS: Limitations and advantages.

	Sensing Method	Techniques	Limitations	Advantages	Refs
Direct ROS Sensing	Traditional	Electron Spin Resonance Spin Trapping Pulse Radiolysis	✓ Expensive; ✓ Time Consuming; ✓ Specialised personnel and equipment	✓ Specific; ✓ Sensitive;	[50–52]
	Current Innovations	Nanotechnology Chemiluminescence Fluorescence Amperometric Association between 2 techniques	✓ Requires the development of specific probes	✓ Highly Specific ✓ Highly Sensitive; ✓ Low LODs; ✓ Adequate for routine analysis; ✓ Applicable for <i>in vivo</i> and <i>in vitro</i> .	

mortem is quite limiting, since it does not allow the exploration of the ROS signaling mechanism and alters ROS physiology [7]. The use of probes that require labeling is also challenging, since the reaction mechanism of fluorophores is often unclear. Moreover, organic fluorophores are prone to photobleaching and blinking which restricts the analysis timeline. Additionally, most commercially available probes are not specific to one ROS molecule [38]. Another recognised drawback of organic dyes is their tendency to form aggregates at high loading concentrations, thereby substantially reducing the fluorescence intensity. This phenomenon is known as aggregation-caused quenching [39,40] and is responsible for reductions in brightness, which results in poor performance in biosensing and bioimaging applications [41]. An alternative to these traditional organic dyes is the use of aggregation-induced emission (AEI) fluorophores. These demonstrate low fluorescence in the isolated state due to intramolecular motions, but exhibit high fluorescence intensity in the aggregated state [39]. AEI fluorophores also have high brightness in their aggregated state thereby enabling them to be encapsulated within biocompatible polymers for biological applications [40,42–49].

Nanoparticles have also helped surpass the common drawbacks associated with the use of traditional organic dyes. Indeed, the versatility of nanoparticles, since they can be functionalised with a variety of functional groups to confer specificity onto the sensor, is one of their most appealing features [63]. Moreover, their high photo-stability and brightness has made them highly appealing for bioimaging applications [64]. Carbon dots are fluorescent carbon-based nanoparticles that due to their biocompatibility and ease of functionalization to develop specific sensors have become appealing over recent years [5]. Gong et al. [53], reported a novel sensing platform using biocompatible carbon dots that displayed high stability against extreme pH's and high ionic strengths, making them suitable for *in vivo* applications. These Cdots

were successfully applied towards live, rapid, cell imaging of hypochlorite in macrophages along with other nitrogen radical species (RNS).

Chemiluminescence is another interesting alternative to organic dyes. Since this method does not require external excitation, the background noise and photobleaching associated with fluorescence are no longer substantial drawbacks towards wide application. The association between chemiluminescence and nanoparticles is gathering much attention towards the development of innovative ROS sensing methodologies [54,55,65].

Another interesting application of chemiluminescence and nanoparticles is the use of semiconductor polymer nanoparticles (SPNs). These nanoparticles were initially designed for optoelectronic devices therefore they have intrinsic high photostability and brightness [56–58], which makes them particularly appealing for cell imaging and tracking, tumour, homodynamic and photoacoustic imaging [59,66–68].

Nonetheless, the dynamic and real-time monitoring of the relationship between hypoxia, ROS concentration and their overall consequences *in vivo* and *in vitro* is still a challenge. To improve the understanding of these systems, several complex techniques have been employed. Amongst them, electron spin resonance, spin trapping and pulse radiolysis [50–52] have been utilised. However, although these methods produce accurate results, they are both labor-intensive and time-consuming. Moreover, they usually require costly equipment and specialised personnel, which limit widespread application [69]. The indirect measurement of ROS was an alternative proposed by some researchers that can overcome the limitations associated with the short lifetime and spatial distribution of ROS in biological media (Fig. 4) [22]. Here the ROS production can be estimated through the by-products generated during the damage that these reactive species induce (Table 2) [70]. Malondialdehyde (MDA), ROOH derived from lipids (LOOH), e.g., phosphatidyl choline hydroperoxide (PcOOH), F2-isoprostanes or 8-hydroxy-2'-deoxyguanosine that are produced in con-

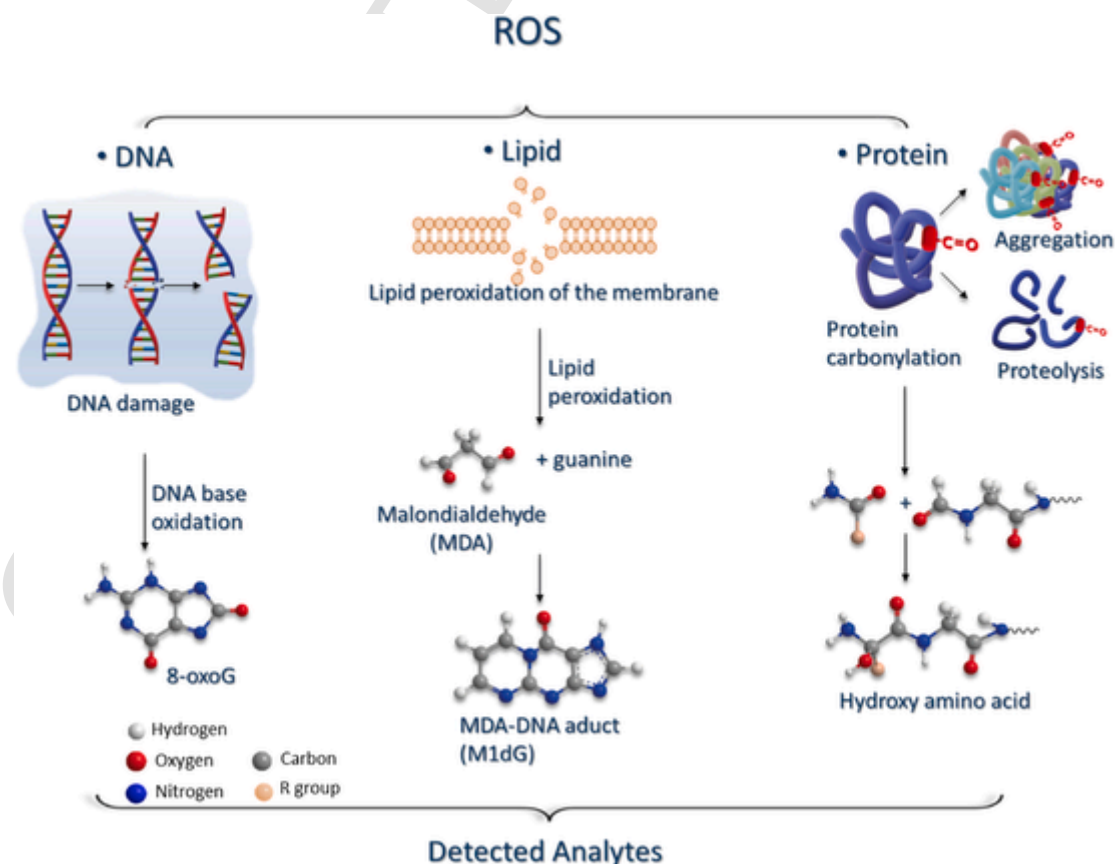


Fig. 4. Schematic representation of the indirect methods for ROS sensing.

Table 2
Common indirect sensing methods for ROS: Limitations and advantages.

	Analytes Detected	Techniques	Limitations	Advantages	Refs
Indirect ROS Sensing	Malondialdehyde (MDA)	Low or high-performance liquid chromatography techniques with the following post-treatments: <ul style="list-style-type: none">• Post-column Chemiluminescence detection• Reductive-mode electrochemical detection• Coupling to a Tandem Mass Spectrometry Elisa Kits	✓ Time Consuming; ✓ Difficult to implement in a regular basis	✓ Specific; ✓ Sensitive;	[22, 70–73]
	Thiobarbituric acid reactive substances (TBARS)	Lipid oxidation; Reactive substract Protein oxidation; Protein products	Possible interference with other common biomolecules that can lead to false results	✓ Simpler than previous techniques. ✓ Easy to implement	[22, 72–76]

junction with DNA damage are some of the commonest resulting by-products due to ROS cytotoxicity that can be utilised within this indirect approach [22]. Measurement of these species can be performed, with high accuracy, through gas or high-performance liquid chromatography (HPLC) techniques, involving post-column chemiluminescence detection, reductive-mode electrochemical detection or by coupling to a tandem mass spectrometry. Alternatively, there are also some ELISA kits available that provide estimation values for these targets [71]. Nonetheless, these techniques are still very time consuming and difficult to implement in a routine basis.

An alternative approach has been proposed and utilised with some degree of success, namely spectrophotometric assays [72,73]. These are easier to implement and less complex than the techniques previously mentioned. The best known are the thiobarbituric acid reactive substances (TBARS) and the advanced oxidation protein products (AOPP) that are able to provide an estimate of ROS levels by analyzing the by-products formed during lipid and protein oxidation, respectively [22]. The simplicity of these assays is its most appealing feature. However, there are drawbacks, such as the possibility of false results obtained for the TBARS assay in the MDA measurement and the presence of interference due to a variety of other biological compounds such as bile pigments, saturated and unsaturated aldehydes, sucrose, amino acids and urea [72–78]. These are all factors that undermine this techniques possible wider use. When choosing the technique that best serves our purposes it is appropriate to balance factors such as simplicity, cost, the time-efficiency and also the nature of the sample required. For example, the measurement of ROS in serum or plasma is quite complex due to the short lifetime of these reactive species. When using these samples, the most common ROS detected are only the most stable ones, e.g. hydrogen peroxide or ROOH [22]. Therefore, the data must be treated with proper care, taking into consideration these known limitations. It is also relevant to highlight that this indirect approach is very limiting, despite

the relative simplicity of this technique. Also this method does not allow determination of the mechanism underlying the ROS toxicity, nor the identification of the species responsible for such damage.

For more sensitive and specific measurement of ROS in biological the use of fluorescence and phosphorescence molecular probes are smart choices. These have a well-established chemistry and have been utilised successfully for some time, both *in vitro* and *in vivo* [79–81]. All these techniques demonstrate high analyte sensitivity, relative low cost, fast response time and allow ROS quantification. The resultant signal can be associated with changes in the luminescence intensity or display a red/blue shift in the emission spectra [82,83]. However, there are some challenges, fluorescence, for example, is limited by the range of available dyes. The most common dyes used fluoresce within the visible light region (400–500 nm). This can be quite restrictive since both cells and tissues have autofluorescence in the same wavelength range. As such, research has been focused towards the development of new dyes, both organic and nanoparticle based, that emit within the red/near-infrared region of the spectra (>700 nm) [23,84], as well as, developing alternative techniques. More recently, researchers have described ROS monitoring in biological systems using Fluorescence Lifetime Microscopy (FLIM) [85]. This is a powerful technique, because in steady-state fluorescence detection methods the dye concentration and the light source intensity can influence the target quantification. On the contrary, FLIM allows a time-resolved monitoring of the fluorophores fluorescence decay and it is not affected by any of these steady-state fluorescence drawbacks [23,86].

Hence, over the years several models have been used for ROS detection, namely; human, animal and plant cells, serum, cell cultures, tissues for *in vitro*; and Drosophila melanogaster, mice and zebra fish for *in vivo* sensing (Fig. 5). Despite the overall interest in all ROS determination, studies have been focusing mainly in four families of species, two

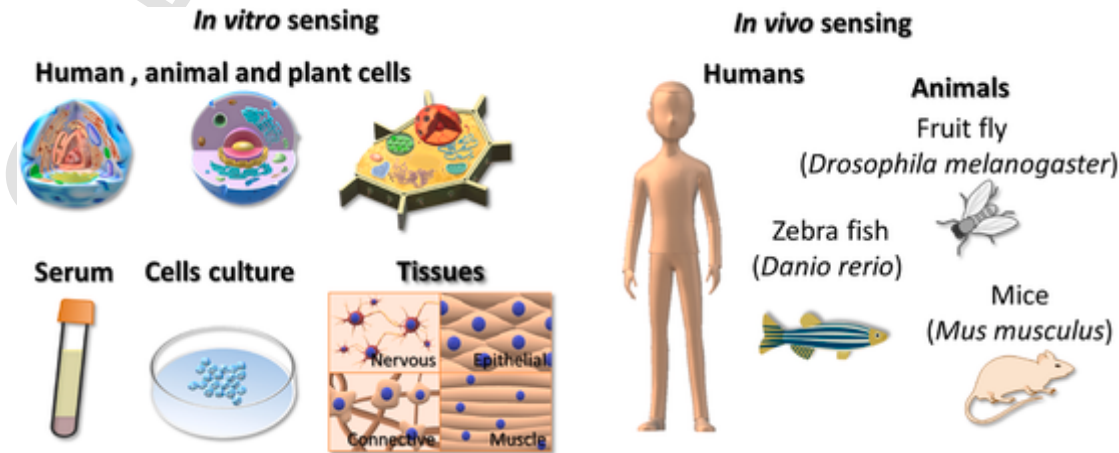


Fig. 5. Most studied biological models for ROS sensing.

non-radical ROS: hydrogen peroxide and singlet oxygen; and two free radical ROS: hydroxyl radical and superoxide anion.

3.1. Hydrogen peroxide (H_2O_2) sensing: relevance and challenges

Hydrogen Peroxide is one of the most studied ROS. It is produced in biological media as a protection mechanism against invading pathogens and also for cellular signaling. However, the over expression of this molecule has been closely associated with the onset and progression of many diseases, such as arthritis and chronic obstructive pulmonary disease, amongst others [87]. This hydrogen peroxide dual function makes it extremely important to develop new methodologies to directly quantify intracellular H_2O_2 and thus contribute to improving the knowledge on the progression of these diseases [7]. Moreover, the total effects that H_2O_2 presence and concentration has on the overall health of living organisms remains elusive, in part due to the limited number of methods available for studying this transient molecule *in vivo* [88]. Some methods do exist, the oxidation biological mechanism of hydrogen peroxide can be followed using for example: boronated fluorophores, fluorescent protein receptors, gold nanodots and carbon nanotubes (Fig. 6) [87,89–91]. However, most of these probes are only useful in cultured cells, mainly due to their short excitation wavelength that leads to strong autofluorescence signals in living mammalian animals [65]. To overcome these drawbacks, some recent advances have been obtained using two different techniques combined: bioluminescence and chemiluminescence [65]. Both approaches are promising, since they do not require external light excitation, thus removing the issues associated with using short excitation and emission wavelengths. Nonetheless, they are also somewhat limited. The use of bioluminescence, such as the method reported by Van de Bittner et al., 2010 [88], where the detection was performed using a boronic-cage firefly luciferin is limited to firefly luciferase expressed transgenic in mouse models since it requires the presence of the enzyme to trigger the response signal [92]. Alternatively, chemiluminescence is a non-enzymatic approach, thus it does not have this issue, but it still relies on small-molecule dyes as reporters. These dyes are often low emissive and can be unstable in the presence of highly oxidative ROS, such as, hypochlo-

rite and hydroxyl radical [93,94]. This ROS-bleaching of the organic dyes can potentially lead to false negative results, limiting the applicability of this technique. In an attempt to overcome this problem the use of semiconductor polymer nanoparticles (SPNs) has emerged [95,96]. Due to their excellent photostability and high brightness they have been utilised with success in biological media for: cell imaging and tracking, target tumor imaging, hemodynamic imaging, photoacoustic imaging and switchable probes for ROS imaging in living mice, amongst other applications [66–68,97]. Amperometric approaches have also been applied to hydrogen peroxide sensing. Their high sensitivity, low response time and the possibility of high resolution mapping of analyte distributions have made this sensing strategy appealing [98]. Indeed, amperometric sensors based on micro- or nanoelectrodes allow real-time investigation of biological processes at the single-cell level [99]. Initially, microsensors were based on carbon microfibers or metal wire electrodes [100–103]. However, this requires the use of tedious manual manipulation techniques. Moreover, these methods do not allow further miniaturisation of the sensor and can only be applied to extracellular monitoring, since the probes are too large to be located entirely within the cell and there are also stability issues [98]. In order to overcome these sensors lack of stability, additional coatings steps were inserted, but the coating material itself is subject to the same limitations.

3.1.1. Hydrogen peroxide (H_2O_2) *In vivo* and *In vitro* sensing

The most promising techniques used so far for *in vivo* and *in vitro* sensing of hydrogen peroxide are: chemiluminescence, chemiluminescence associated with nanoparticles and a new age of amperometric sensors.

Lee et al. [54], reported the development of a multimode integrated nanoprobe with enhanced chemiluminescence response to hydrogen peroxide for *in vitro* and *in vivo* measurement. Here, the association between a nanoscopic co-aggregation AEI dye with a hydrogen peroxide responsive peroxalate, which can convert chemical reaction energy into electronic excitation, and resulted in the self-assembly and construction of concentrated hydrophobic dye/peroxalate nanoparticles. This system was successfully tested for the *in vivo* visualisation of intracellular

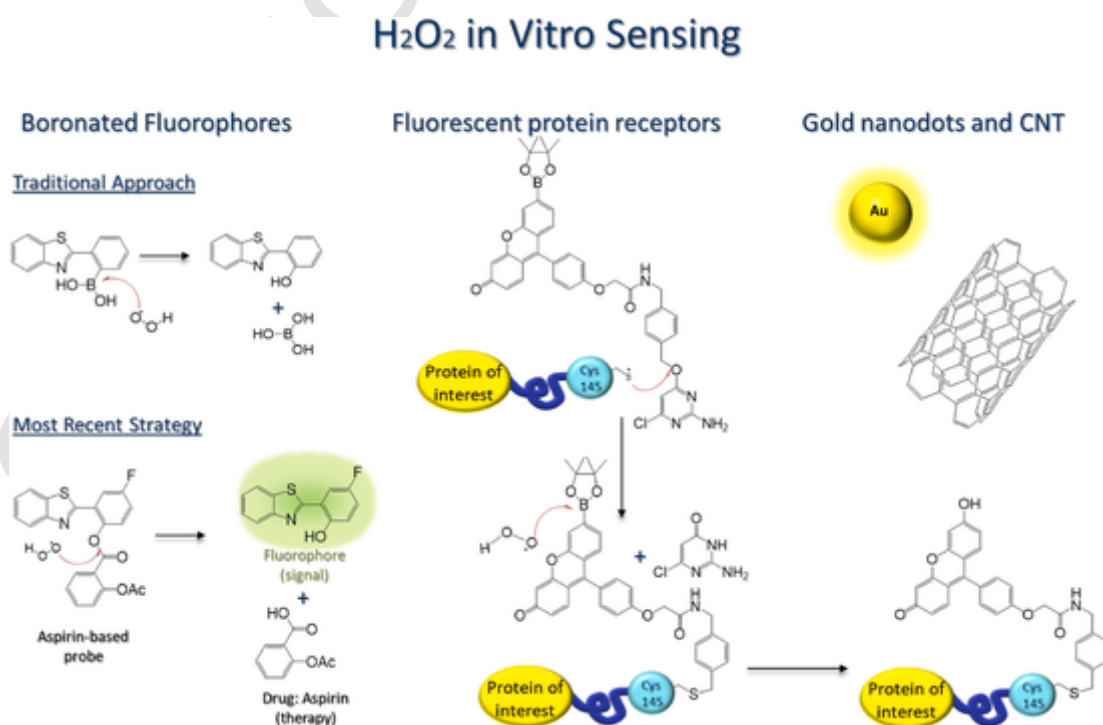


Fig. 6. Schematic representation of the most common methods for hydrogen peroxide *in vivo* and *in vitro* sensing.

overproduced hydrogen peroxide during early stages of inflammation [54].

Making use of the knowledge that hydrogen peroxide has an important role in biological resistance to infectious pathogens within the respiratory tract. Kim et al. [55] attempted the *in situ*, real-time, analysis of hydrogen peroxide generation and used this information to evaluate the immune response. According to the authors, the application of a chemiluminescent nanoparticle-based real-time imaging technique allowed the evaluation of the hydrogen peroxide generation after influenza A (WSN/33/H₁N₁) viral infection using a non-invasive approach. Indeed, they determined that the nasal epithelial cells respond to viral infection through the production of hydrogen peroxide and that the *in vivo* cumulative hydrogen peroxide level in the nasal mucosa peaks at day 3 post-infection [55]. This *in vivo* concentration evaluation was possible using a chemiluminescence nanoparticle-based sensor and provided relevant information on the immune response of the respiratory tract to viral infections.

Shuhendler et al. [62], also reported a chemiluminescence sensor that enabled simultaneous and differential imaging of peroxynitrite and hydrogen peroxide for drug toxicity evaluation using SPNs. It is a known fact that ROS production acts as a strong indicator of drug-related hepatotoxicity [62]. Therefore, in this work, the authors were able to monitor the *in vivo* hepatotoxicity caused by two widely used drugs: the analgesic and anti-pyretic acetaminophen and the anti-tuberculosis agent isoniazid. Within previous work, these authors were also able to demonstrate deep tissue tumor imaging of RNS *in vivo* in the particular context of systemic bacterial infection [60], thereby enhancing the applicability of these SPNs.

The integration of SPNs with chemiluminescence provides a potential advance in, for example, the detection and monitoring of H₂O₂ in biological media. However, the mechanism that controls the chemiluminescent process is still poorly understood, not only for SPNs-based probes, but also for other small organic molecules [65]. This may limit both the technique sensitivity, as well as, the imaging applications. In an attempt to surpass this issue, Zhen et al. [65], performed a study that determined that in order to increase the hydrogen peroxide sensitivity *in vivo* it is necessary to analyse the energy level alignment between the semiconductor polymer and peroxalate (a common probe used in chemiluminescence processes). By adjusting these two parameters it was possible to increase the quantum yield up to 2.30×10^{-2} E/mol allowing detection of H₂O₂ at levels as low as 5 nM, a substantial improvement over previously reported probes [65]. Additionally, it was also possible to image this analyte in peritonitis and neuroinflammation mouse models. This is a fast and efficient process, where just 1 min after application of 2 μ L of SPNs it was possible to specifically detect endogenous hydrogen peroxide in the mouse brain in real-time with no need for external excitation [65]. This methodology seems to be highly promising for *in vivo* imaging of hydrogen peroxide in neurodegenerative diseases, e.g. Parkinsons and Alzheimers.

An appealing alternative to chemiluminescence approaches is the use of amperometric sensors based on ultramicro or nanoelectrodes. Their small size and needle-like shape, along with the concurrent spatial and temporal resolution makes them suitable for measurement in tissues, as well as within single cell analysis [85,99,101,104–109]. Amatore and co-workers performed a ground-breaking study using ultramicroelectrodes where they demonstrated successful detection of ROS and RNS release from single cells to the extracellular environment after stimulation of an immune response [110–113]. These nanoprobes are particularly suitable for probing the intracellular environment without leading to leakage between the extra- and intracellular media. Ultramicroelectrodes could be utilised to detect hydrogen peroxide during penetration-induced oxidative outbursts in murine macrophages, where the temporal evolution of the transient intracellular hydrogen peroxide levels were monitored and used to evaluate the anti-oxidative capabilities of the cells. Furthermore, it was also possible to quantify the intra-

cellular concentration of hydrogen peroxide and monitor its permeation through the cell membrane [7].

Clausmeyer et al. [98] reported a way to overcome not only the lack of stability but also the miniaturisation problems seen in previous amperometric sensors. According to the authors, it is possible to bypass these issues by electrodeposition of Prussian Blue (PB) on the nanoelectrode. The carbon electrode was fabricated by pyrolytic decomposition of butane within a quartz nanopipette and further etched in order to construct nanocavities on the electrode surface onto which the PB would be deposited. By using this approach the authors reported a selective quantification of hydrogen peroxide in a concentration range from 10 μ M to 3 mM that could be useful for intracellular oxidative stress in living cells. The possible *in vivo* use of this sensor was also demonstrated by the Marquitan et al. [7], where it was possible to detect hydrogen peroxide produced during penetration-induced oxidative outburst in murine macrophages. Moreover, the temporal evolution of the transient intracellular hydrogen peroxide levels can be resolved and may be used to evaluate the anti-oxidative capacities of the cells. Additionally, it was also possible to quantify the hydrogen peroxide intracellular concentration and monitor its permeation through the cell membrane [7], thus improving the understanding of the interaction mechanisms between hydrogen peroxides and mammalian cells.

3.2. Singlet oxygen (¹O₂) sensing: relevance and challenges

Dioxygen species play important biological roles since they can mutually transform into each other as well as forming oxygen containing radicals including hydroxyl and nitric oxide radicals, amongst others [114]. These structurally identical oxygen-based species have distinct physicochemical properties and biological reactivity due to minor differences in their outer electron distributions [115]. In biological media, dioxygen species dominate cellular respiration through a series of metabolic pathways. They can also affect intracellular energy supplies, redox homeostasis, signal transduction and immunological defense [116,117]. However, most oxygen-derived species need to be carefully monitored, since they are commonly associated with a high potential to inflict oxidative damage in biological organisms [118]. The most relevant dioxygen species in biological media are: molecular oxygen (³O₂) singlet oxygen, (¹O₂) and superoxide anion, (O₂^{•-}). Singlet oxygen is formed from molecular oxygen through triplet photosensitisation, thus it has a set of paired electrons of opposite spins, which grants it a higher oxidising ability than the ground-state, molecular oxygen.

Singlet oxygen has a very low lifetime, 3×10^{-6} s and a relatively low spatial distribution of 100 nm [119]. These factors, along with the structural resemblance to other oxygen species, have led to major obstacles to the development of new sensing systems to track and quantify this ROS. Over the years several physical and chemical methods have been reported for singlet oxygen sensing [120–124]. The method considered the gold standard for singlet oxygen detection is a luminescence-based technique. This method is based on the detection of the near infrared emission of this radical, which corresponds to the transition from the singlet oxygen state to molecular oxygen (Fig. 7) [125]. This technique is particularly used in PDT dosimetry; since it is a direct sensing method that does not depend on secondary reporters [120]. However, and despite the recent advances in the development of highly sensitive near infrared photomultiplier tubes and in single photon counting instrumentation, this technique is still challenging due to the radicals intrinsic properties (low concentration and biological lifetime). In order to overcome these drawbacks, research efforts have been employed towards the development of new probes for indirect sensing [126–128]. In 2004 the fluorescent probe known as Single Oxygen Sensor Green reagent (SOSG), has become commercially available. Since then several authors have reported a successful application of this probe towards monitoring singlet oxygen in a variety of biological systems, namely light-activated plant defenses, plasmonic engineering,

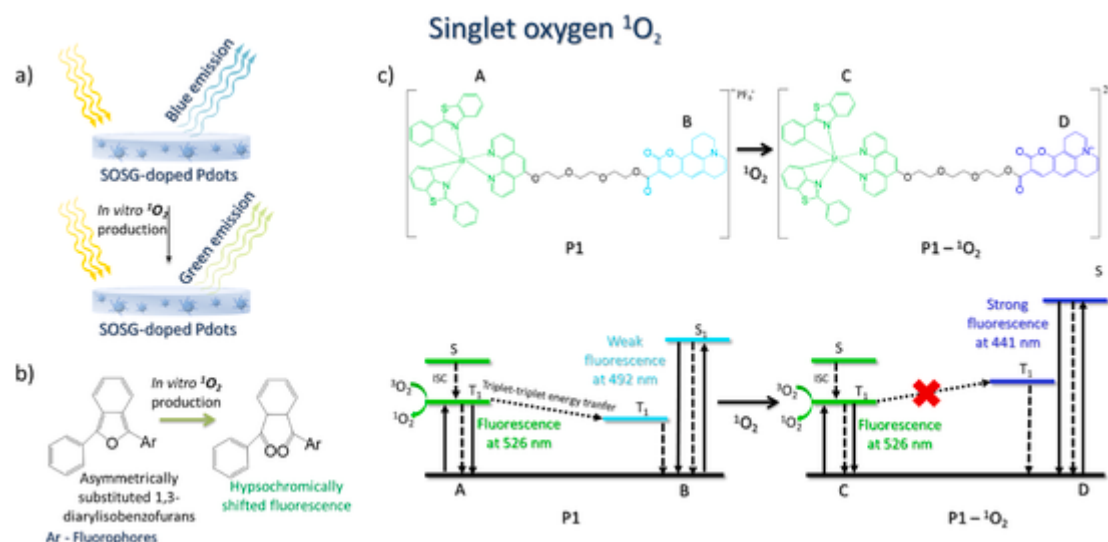


Fig. 7. Schematic representation of the most common methods for singlet oxygen *in vivo* and *in vitro* sensing.

photoinactivation of viruses, photosensitizer-based PDT, among others [129–132]. However, the feasibility of using this probe for effective quantification of singlet oxygen generation was still lacking. This was accomplished by Lin et al. [125]. These authors successfully demonstrated that it is possible to use SOSG for quantitative measurement of singlet oxygen production providing an appropriate measurement protocol is designed that allows the determination of the reaction rate.

From this range of strategies, the one that seems to present the best results in terms of specificity, higher penetration depth and routine applicability, is the two-channel responsive luminescence sensing technique, since it allows not only high spatial and temporal resolution, but also self-calibration. Hence there have been major efforts towards the development of new probes for the application of this technique. These probes, chemosensors, are based on small molecules, macromolecules and also nanocomposites [133–137].

3.2.1. Singlet oxygen ($^1\text{O}_2$) *In vivo* and *In vitro* sensing

The detection and follow-up of this radical in both *in vitro* and *in vivo* is very important, but as found for the other short-term radicals, a difficult task to accomplish. Hou et al. [138], demonstrated in 2018 that by using semiconductor polymer dots doped with SOSG dye it is possible to develop a ratiometric singlet oxygen sensing method for *in vitro* applications (Fig. 7a). Indeed, the SOSG-semiconductor polymer dots system exhibits a constant blue fluorescence owing to the nanoparticles that changes to green in the presence of singlet oxygen due to the SOSG moiety. This methodology was successfully tested for *in situ* evaluation of singlet oxygen generation during PDT using both spectroscopic and confocal imaging techniques.

The use of (iso)benzofuran and anthracene derivatives that produces endoperoxides in the presence of singlet oxygen has also produced interesting results (Fig. 7b). In 2013, Song et al. [139], devised three different probes that consist in: an asymmetrical isobenzofuran derivative and a fluorophore moiety. They have determined that the use of pyrene as the fluorophore attached to the isobenzofuran moiety provides the best results in terms of biocompatibility and sensitivity. Through the use of this probe it was possible to detect intracellular singlet oxygen in macrophage cells using dual channel two-photon fluorescence.

Flores-Cruz et al. [140], also described a dual emissive fluorescent probe for singlet oxygen *in vitro* sensing (Fig. 7c). These authors developed a probe based on chromenium and coumarin 334 couple by C=C double bonds (RC334). Immediately upon cell internalization RC334 has two separate emission bands in the visible and near-infrared range,

respectively. When in contact with singlet oxygen, the coumarin moiety is released from the probe, which allows a ratiometric detection and monitoring of the radical species. This strategy allows the dynamic tracking of singlet oxygen in the presence of agents that both disturb the membrane potential and also under photodynamic therapy. The use of coumarin 314 along with an Ir(III) complex led to the production of another singlet oxygen sensing system successfully applied for ratiometric bioimaging in lipopolysaccharide/interferon/PMA-stressed macrophage cells and also in therapeutics by You et al. [141].

3.3. Superoxide anion ($\text{O}_2^{\cdot-}$) sensing: relevance and challenges

Superoxide anion, $\text{O}_2^{\cdot-}$, is produced by the single electron reduction of oxygen. It is one of the shortest lifetime ROS and is closely related to many inflammatory diseases [142]; however, its biological role is much more than this. Indeed, this ROS can react with reducing substances or biological macromolecules within the cell, thus playing a relevant part in the control of redox-related substances expression. Over the years it has become clearer the biological relevance that superoxide has and its overall impact. It is now known that it can produce hydrogen peroxide; regulate the expression of the NOX family proteins and consequently cell apoptosis or proliferation, as well as, performing other relevant roles in several metabolic mechanisms [142]. The relationship between the presence of superoxide anion and its biological consequences has been a major drive towards the development of new sensing methods in the biomedicine area. The most traditional method for superoxide anion determination is through electron paramagnetic resonance (EPR) [143,144]. However, its high equipment cost and requirements for specialised personnel mean it is not suitable for routine analysis. Over the years several other sensing techniques have been developed, amongst them the most popular are: electrochemical biosensors, fluorescence imaging, chemiluminescence, enzymatic techniques and surface enhanced Raman spectroscopy (SERS) [142]. Each of these techniques has its own advantages and drawbacks. Electrochemical biosensors are non-destructive in nature and are easy to implement, with high sensitivity, good selectivity and easy real-time *in situ* detection [145]. Over the years several new materials have been reported for selective superoxide anion detection with varying degrees of success [146–150]. However, despite these breakthroughs there is still much to uncover on the dynamics between superoxide anion generation and cell death under physiological conditions [142].

3.3.1. Superoxide anion ($O_2^{\cdot-}$) *In vivo* and *In vitro* sensing

The behaviour of this radical species *in vivo* is of great relevance, but the development of specific sensing methods to monitor it is not an easy task. Its low intracellular concentration, along with its short stability makes the development of effective and sensitive methods for following the superoxide anion *in vivo* an extremely difficult task. To overcome these limitations it is necessary to develop strategies that are suited to these characteristics. The use of fluorescent probes has been suggested as a way to gain a better insight on superoxide presence and role in biological systems and subsequent consequences. When compared with EPR and electrochemical sensors, fluorescence imaging is a highly attractive alternative. Its non-invasive nature and the fact that it allows tracking superoxide anion in living or isolated tissues and organs are some of its most appealing features. This method for ROS direct detection has been gathering much attention over recent years. The most traditional methods require single photon excitation, where a probe is designed for specific detection and real-time follow-up of a ROS. The main factor to be considered in choosing the probe is the evaluation of the complex environment where the measurement will take place. Hence it is highly relevant to tailor it according to the pH in which it will be used, the photostability, the sensitivity and the selectivity towards a specific ROS. It must be capable of performing these measurements in biological media or abiotic conditions [142]. A number of probes have been developed and can be used according to the desired application [151–155]. However, and despite the great efforts that have been employed in the development of effective probes, most still suffer interference from other ROS, undergo irreversible reactions and demonstrate a delayed response time [156,157]. Indeed, for ideal monitoring of superoxide anion fluxes, it is necessary that the fluorescence probe demonstrates a suitable penetration depth, minimal photodamage, rapid response and reversibility. If such a probe is available then it is possible to use the two-photon excitation technique, which allows the use of more benign wavelengths and at the same time provides superior fluorescence imaging features [158].

Recently, Pu et al. [60], determined that SNPs were highly appealing for the development of switchable probes for ROS imaging in living mice as they are inert towards ROS. Thus it was possible to use SNPs with a ROS inert core as an energy donor and a SPN functionalised surface containing ROS sensitive fluorophore molecules as energy acceptors. When in the presence of superoxide anion the FRET phenomenon between the SNPs core and surface is disrupted and leads to a consequent emission shift. Moreover, since the functionalising molecule is based on polyethylene glycol (PEG), it is also possible to passively target tissues with leaky vasculature through the enhanced permeability and retention (EPR) effect [60]. Since this leaky vasculature is a hallmark of inflammation [61], the SPN ROS sensor can effectively accumulate at inflammatory regions, thereby allowing ROS imaging following systemic administration (Fig. 8a) [60].

Zhang et al. [158], developed a reversible fluorescent probe which can be applicable using traditional one-photon and the two-photon excitation techniques. According to these authors the turn-on probe based on (N,N')-di ((2E)-3-(3,4-dihydroxyphenyl)acrylicacyl)-1,3,5-triazine-2,4,6-triamine (TCA), which is involved in the interaction between superoxide anion, caffeic acid and phenethyl ester, is capable of providing accurate intracellular monitoring of superoxide anion in a dynamic, reversible way during reperfusion injury in hepatocytes, zebrafish and mice (Fig. 8b). Thus, it is a suitable approach for both *in vitro* and *in vivo* following of this highly unstable ROS [158].

Another interesting technique for superoxide detection is based on luminescent proteins. The overall process is fairly simple; there is a class of proteins, photoproteins, which emit light upon catalysis by a number of specific substances. This light emission can be used to both detect and quantify ROS [142]. Rahmani et al. [159], reported an appealing system, where a photoprotein, aequorin, was successfully used

for superoxide anion sensing with a very low detection limit (1.2 pM) and high sensitivity (Fig. 8).

When the detection limit and the specificity are relevant factors, researchers often turn to enzymatic methods. In relation to superoxide anion, the most used enzyme is superoxide dismutase (SOD) and its derivatives (Fig. 8c). Over the years several papers have been produced describing the association of this enzyme with nanoparticles, e.g., multi-walled carbon nanotubes, and other composite structures [146,160–169]. These sensors are usually highly sensitive and have low detection limits, however; enzymes are usually expensive and several precautions need to be undertaken during their use in order to assure the assay overall viability.

Another novel approach is the use of chemiluminescence. Here the most commonly employed is luminol-amplified chemiluminescence. However, this traditional method is limited by its non-specificity. Indeed, even though it reacts with ROS, in most systems it is not possible to determine the species that induced the reaction [1]. Since superoxide anion has such a short lifetime and rapidly forms the more stable hydrogen peroxide, it is to be expected that hydrogen peroxide would be the most common interferent in superoxide anion measurements. Bedouhène et al. [1], attempted to shed some light on this subject and analysed this dichotomy using common ROS producing systems. According to their findings, the luminol chemiluminescence reaction can be used for specific superoxide anion detection only in the absence of peroxidases. However, when in the presence of peroxidases, the same system responds to the presence of both species.

3.4. Hydroxyl radical (HO^{\cdot}) sensing: relevance and challenges

Hydroxyl radical, HO^{\cdot} , is the most easily oxidised among all ROS [12]. Thus it is associated with deleterious effects towards several groups of biomolecules such as DNA, lipid and proteins, through the induction of oxidative hydroxylation of their bases, lipid peroxidation and oxidative protein damages, respectively [170–172]. Hydroxyl radical high concentrations are commonly linked to several oxidative-stress related pathophysiological processes, e.g., inflammation, cardiovascular disorders and cancer [173–175]. However, the mechanism through which these alterations occur is still poorly understood. Like other ROS, hydroxyl radical has a very high reactivity associated with a short lifetime and low distribution in biological systems, which makes it difficult to evaluate and follow. Over recent decades several techniques have been developed in order to attempt to gain a better insight on this radicals overall effects towards biological media. The most traditional, which is used for several ROS is electron spin resonance (ESR) spectroscopy, but as stated before; it has several disadvantages that restrict its wide application. Other methods ranging from, electrochemical [176], hydrogen abstraction [177,178], recombination of nitroxide radicals [179,180], aromatic hydroxylation [181,182], oxidative dearylation [183,184] and oxidation of electron-rich heteroatoms [185,186] amongst others, have also been proposed. However, these methods have been hampered by limited selectivity, sensitivity and sometimes their inability to be used for *in vivo* sensing [187–189].

3.4.1. Hydroxyl radical (HO^{\cdot}) *In vivo* and *In vitro* sensing

On the previous two decades there has been a wider application of fluorescence associated detection methods for *in vivo* hydroxyl radical tracking [190–192]. Indeed, fluorescence-based sensing presents several appealing features. Its main limitation is associated with low photostability and lack of specificity that is associated with small organic dyes [193,194]. The main revolution in this field, which has been applied with much success in hydroxyl radical sensing, is the use of nanoparticles [192]. Nanoparticle synthesis and application is an ever growing field; hence, it is not surprising that the number of different nanoplateforms applied for hydroxyl radical sensing is constantly increasing. The most common systems until now are polymeric nanopar-

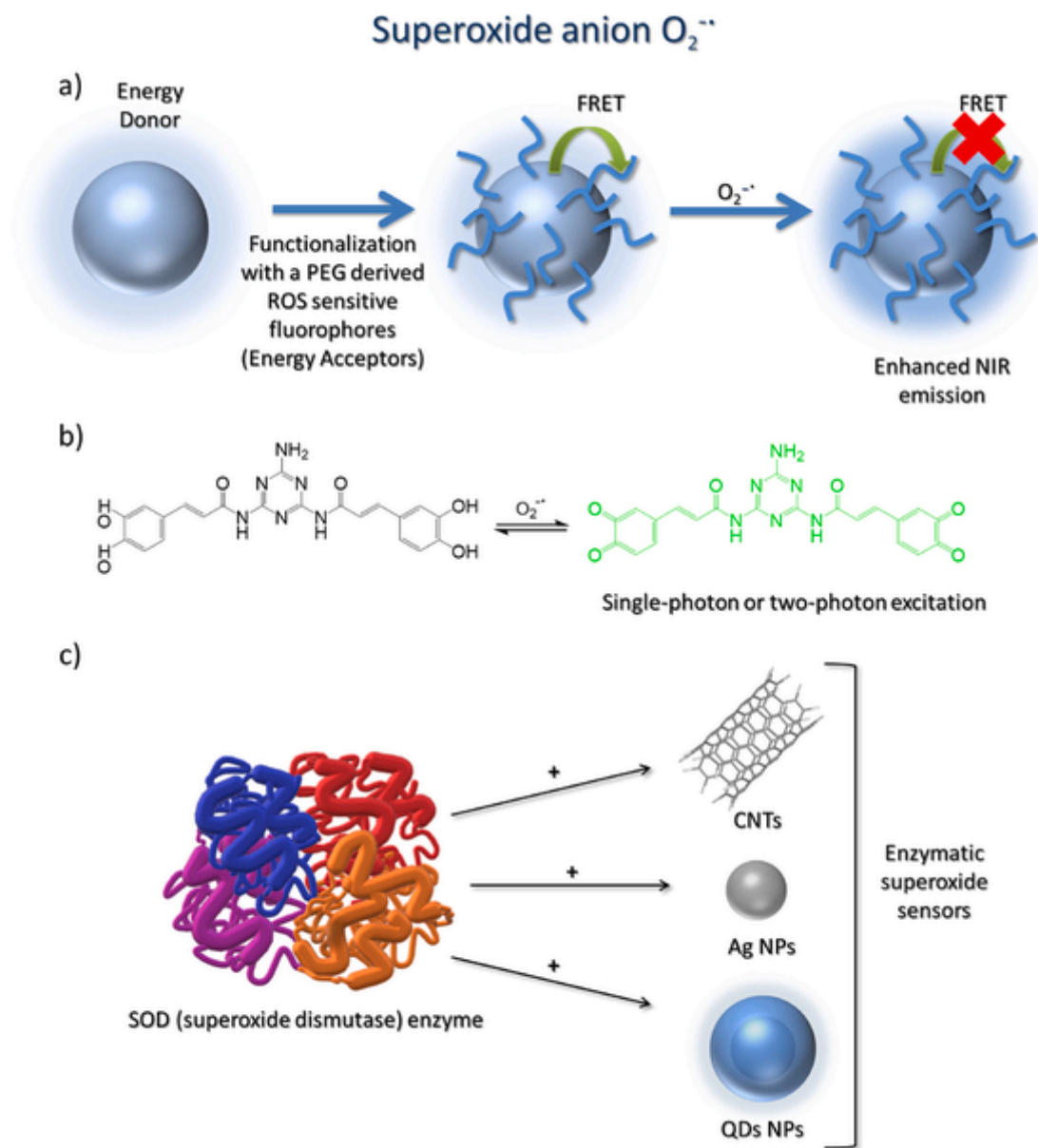


Fig. 8. Schematic representation of the most common methods for superoxide anion *in vivo* and *in vitro* sensing.

ticles, quantum dots, metal nanoclusters and up-conversion nanoparticles (Fig. 9) [195–203]. Nonetheless, this situation is changing constantly.

The polymeric nanoparticles are quite versatile in terms of size and composition. Ganea et al., for example [204], reported a ratiometric nanoprobe that was moderately selective towards hydroxyl radical when compared to other ROS, with a low detection limit (LOD = 0.73 μ M). Another novel system was developed by Tong et al. [205], where it was possible to successfully detect the analyte of interest in human proximal renal tubular epithelial HK-2 cells. Quantum dots are also appealing, since they are relatively easy to functionalise in order to develop more specific biosensors. Moreover, their optoelectronic properties make them also suitable for bioimaging applications using single or multiple systems, through ratiometric data treatment. Hai et al. [196], reported graphene oxide-derived quantum dot composite that was able to selectively detect hydroxyl radical through fluorescence enhancement. This system did not suffer interference from other common ROS and had a very low detection limit (LOD = 12 nM), which is similar to the low concentration values found in biological sys-

tem. Alternative systems are metal nanoclusters, amongst which the most appealing are functionalised gold nanoparticles. The use of gold nanoparticles for biological applications is intensively researched and over the past five decades their application as theranostic agent in pre-clinical and clinical studies has shown great promise [206,207]. Indeed, in clinical trials they have been used as drug carriers for late stage cancer treatments and as PDT agents in prostate cancer and acne treatments, which further highlights their versatility in biological applications [207–209]. In relation to hydroxyl radical sensing some interesting results have also been obtained. Zhang et al. [197], developed a gold nanocluster for hydroxyl radical detection and determined that the developed nanoprobe displayed a linear range from 50 nM to 5 μ M. Moreover, the sensing system did not suffer from interference in the presence of common species, namely H_2O_2 , Fe^{2+} , Na^+ , K^+ , Ca^{2+} , Mg^{2+} , lysine, serine, histidine and glucose. This nanoprobe was successfully applied *in vitro* using human bone marrow neuroblastoma cells. Zhuang et al. [198], reported a non-toxic bioimaging hydroxyl radical sensor with a linear response to the analyte in the 1–150 μ M range and a detection limit of 0.68 μ M. This novel sensing platform was

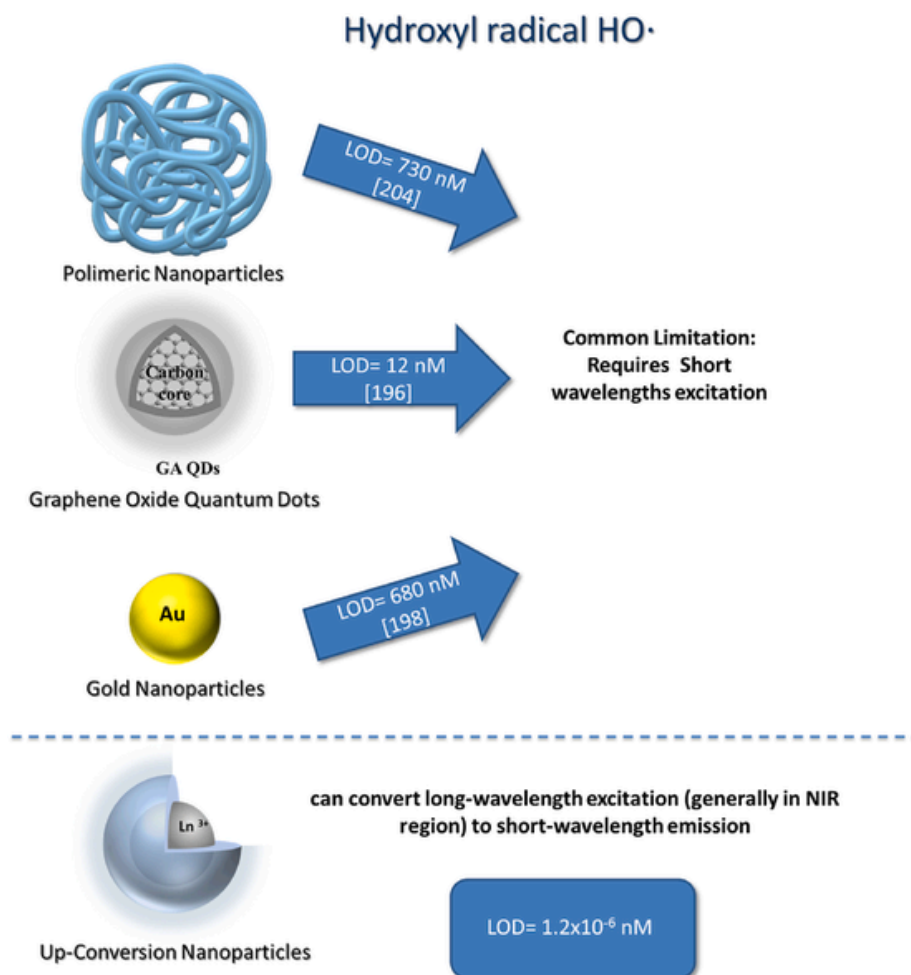


Fig. 9. Schematic representation of the most common methods for hydroxyl radical *in vivo* and *in vitro* sensing [196,198,204].

also reasonably stable in the presence of other known ROS interferents and allowed accurate detection of hydroxyl radical's *in vitro* using HeLa cells.

Even though these sensors and the ones based on quantum dots are interesting, when applied in a biological context they have a common drawback. They usually require short wavelength excitation, which does not allow high tissue penetration for bioimaging analyte tracking [210]. In order to overcome this issue, researchers have been focusing on the use of up-conversion nanoparticles that can convert long-wavelength excitation (generally in NIR region) to short-wavelength emission. This is usually accomplished by the use of trivalent lanthanide ions, such as, Er³⁺ and Yb³⁺, which are doped into an appropriate dielectric host lattice, e.g., NaYF₄ that has at least one dimension less than 100 nm [211]. Li et al. [199], for example, developed a sandwich structured up-conversion nanoparticle system for hydroxyl detection that had a linear concentration range of 1.2–194.6 fM and a quantification limit of 1.2 fM. Moreover, this sensing system was successfully applied towards *in vitro* hydroxyl detection using PMA-treated HeLa cells and liver slices from mice.

4. Therapeutic application of ROS

In biological systems, ROS assures that cellular functions are properly conducted through cell signaling pathways. When overexpressed, these species have been associated with cell death. Initially cell death was considered to be a passive event that occurs when cells become damaged or injured to a point that they disintegrate into cellular debris, often called necrotic cell death [8]. However, genetic studies performed

on *C. elegans* by Lettre and Hengartner [212] showed a much more complex situation. These author's genetic studies proved that many genes could control the amount of cell death and the physiological changes that occurs within dying cells. The different types of cell death induce specific cell signaling pathways, where ROS are pivotal [8]. Indeed, the ROS concentration in cells can determine whether they survive or not and also the manner of cell death mechanism they undergo. This “ROS rheostat” role has become more than evident for *in vitro* studies, but its application needs to be further explored *in vivo*. If this mechanism can more thoroughly understood, particularly under pathological conditions, it would be possible to utilise this cell “ROS rheostat” in therapeutics [8]. Indeed, even though they are used externally in photodynamic therapy [213], the tight control over the ROS internal “rheostat” would create a myriad of other new applications.

The evaluation of the mechanism through which the ROS is involved in immune responses has been one of the major areas of interest in the development of specific sensors for use within biological media. In an innate immune response cells such as neutrophils and macrophages spontaneously increase their ROS production as a method to fight infectious agents [214,215]. In an adaptive immune response, the T cell receptor activation triggers ROS production inside T cells this activates them and is responsible for cytokine secretion [54,216–218]. In theory in this manner ROS can be used to evaluate the immunoactivation process which would be particularly relevant in immunotherapy. This method of therapy aims to “train” the host immune cells to destroy malignant cells and is a promising approach towards cancer treatment [219]. However, to fully explore the advantages of this therapeutic approach it is necessary to monitor the immunoactivation in real-time

[220–222] by a non-invasive technique. Cui et al. [219], reported the successful imaging of *in vivo* immunoactivation during cancer treatments by utilising a turn-on superoxide anion sensor based on chemiluminescent SNPs. Here, the superoxide anion acts as a trigger for the activation of CD4⁺ and CD8⁺ T cells [54,216–218]. This sensor emits in the near-infrared spectrum, making it suitable for therapeutic use in biological systems optical “window”.

Within another approach, macrophages may also enable the prediction of the immune system response to pathogens and cell debris. Indeed, macrophages are responsible for the formation of complex atherosclerotic plaques, where pro-inflammatory and pro-atherogenic M1 macrophages induce the production of ROS and unstable plaques by secreting pro-inflammatory mediators, i.e., interleukin-1b, interleukin-6 and tumour necrosis factor- α [223,224]. Therefore, the study of the relationship between ROS and macrophages is one of the most appealing research areas in ROS sensing [225]. Within this particular field, fluorescent dyes have been proven to be applicable [53,225,226]. Park et al. [227], reported a near-infrared fluorescent nanosensor based on hyaluronic acid, chlorin e6 and a thioether linker for ROS sensing, capable of specifically targeting pro-inflammatory M1 macrophages while simultaneously imaging ROS in M1 macrophages in high-risk plaques. This turn-on sensor emits strongly in the presence of ROS and provides a good comparison to sensors that rely on the quenching effect. Indeed, quenching can be a problematic phenomenon to follow in biological conditions, since it relies on a predictable decrease of fluorescence intensity when exposed to the analyte. Unfortunately, there is usually a high level of background noise which limits the sensors sensitivity ranges and detection limits. The successful application of this turn-on sensor appears to be promising for imaging atherosclerotic tissues and evaluation of the antioxidants effects [227].

The application of ROS in therapeutics is not a new topic. Indeed, the use of superoxide anion and RNS has proven promising and a useful technique in guided-photodynamic therapy (PDT) [5]. This emerging therapeutic tool is appealing since it is minimally invasive and demonstrates low side effects [228]. This technique uses photosensitiser, materials that upon irradiation can induce the production of ROS or RNS, with subsequent cell apoptosis, necrosis and/or tissue destruction [229]. Until recently PDT relied on the highly destructive superoxide anion; however, in hypoxia [230] environments such as inside solid tumours, this technique is limited. Moreover, the glutathione present in the intracellular medium acts as a natural scavenger of ROS, thereby protecting cancer cells from the therapeutic agents [231]. To overcome these issues some authors have reported the use of RNS, namely nitric oxide that can both accelerate the intracellular glutathione metabolism and is applicable in hypoxia environments [232].

Another novel approach towards utilising the therapeutics properties of ROS is that of image-guided therapy. Mao et al. [233], for example, reported a tumour targeted strategy in which the authors used a chemically excited far/near infrared emission nanomaterial coupled to a specially designed photosensitiser for singlet oxygen *in situ* production. This system displayed potential wide applicability since the organic nanoparticles are effective hydrogen peroxide sensors triggering chemiluminescence imaging and at the same time, they react with the hydrogen peroxide produced by tumours to form singlet oxygen, which is a powerful apoptosis inducing agent [233]. Furthermore, both the hydrogen peroxide detection and subsequent singlet oxygen production can be enhanced in the presence of an anti-tumour drug, FEITC, which was capable of significantly increasing the hydrogen peroxide concentration at the tumour, thereby enhancing the therapeutic effect in a non-invasive manner. This chemiluminescence imaging and therapeutic approach offers a number of advantages when compared to the traditional bioluminescence method, namely the higher penetration depth and higher signal-to-noise ratio which are key parameters for *in vivo* imaging [234,235].

Singlet oxygen has a high oxidising ability that can be considered harmful and beneficial, depending on both its location and concentration. Even though, traditionally the ROS presence was seen only as a potentially damaging factor, this high reactivity and “destruction power” was put to good use, with the development of several therapeutic strategies [236]. Fu et al. [237], for example, used upregulation of oxidative stress as a way to destroy hypoxic solid tumours. This technique has been attracting much attention and it is based on photodynamic therapy (PDT) for cancer treatments. Since this technique relies on ROS production through a sensitiser, which usually converts molecular oxygen into singlet oxygen, the efficient detection and tracking, *in vivo*, of these species has become a pressing need.

5. Conclusions

Reactive Oxygen Species play key roles in biological media. Their high reactivity and short lifetime are two of their innate characteristics. Until recently, ROS were mainly associated with detrimental effects, namely their association with many diseases, e.g., cancer, neurological and cardiac conditions, kidney and other organ disorders. However, they also have a relevant contribution to the immune system as they are responsible for controlling fibroblast proliferation and differentiation, as well as, ensuring the proper folding of immunoglobulins and their maturation. Furthermore, immune cells when exposed to a pathogen produce ROS which acts to help assure cell survival and pathogen destruction. Within biological media they can be produced by a number of cell types and, within each cell, they can be produced in numerous structures. However, their main production site is within the mitochondria.

In the electron transport chain, under normal oxygen conditions, about 2% of all electrons participate in a secondary process that leads to ROS production. In fact, they “leak” from the transport chain and react with molecular oxygen to form superoxide anion, which initiates a cascade reaction culminating in the production of ROS that demonstrate a longer biological lifetime, such as hydrogen peroxide amongst others. In hypoxia conditions, the reaction is exacerbated and the ROS concentration increases to a point that can lead to cell apoptosis. It has been deduced that these reactive species are not only responsible for cell survival, but also the type of cell death mechanism they undergo. Using this valuable information, it is potentially possible to use ROS in therapeutically applications; however, in order to do so, it will be necessary to develop new sensing approaches that allow direct, real-time *in vivo* monitoring the production and concentration of these species.

Fluorescence assays seem to be the most promising for ROS biosensing in terms of simplicity, cost and specificity, particularly when associated with nanoparticles. The major revolution in nanotechnology, ranging from synthesis to functionalization and targeting made the biosensing fluorescence a highly appealing technique. Nanoparticles can be functionalised for high sensitivity and specificity towards ROS; moreover, it is possible to discriminate between ROS by using these nanoprobe. Additionally, they can be applied with success in one-photon and two-photon excitation technique for *in vitro* and *in vivo* imaging of ROS. A more recent trend is the use of up-conversion nanoparticles that can convert long-wavelength excitation (generally in NIR region) to short-wavelength emission and, thus, avoid the autofluorescence of tissues and cells associated with short-excitation wavelengths. Another fluorescence-based promising alternative is FLIM that has all of fluorescence technique advantages along with the fact that it is not affected by the steady-state fluorescence drawbacks.

ROS high resolution detection has also boosted their application in therapeutics, such as, immunoactivation during cancer treatments, in a more effective way. Thus, a more detailed insight into the mechanism that is the foundation of the immune system ROS production will be of major relevance in using these species as a biological ally. ROS can be

considered as both friend and foe, it simply depends on the perspective and the increase knowledge on its biological role and pathways.

Author contributions

M. A Cardoso, H.M.R. Gonçalves and F. Davis conceived the project and contributed to writing the manuscript.

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Declaration of competing interest

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Data availability

No data was used for the research described in the article.

References

- [1] S. Bedouhène, F. Mouliti-Mati, M. Hurtado-Nedelec, P. My-Chan Dang, J. El-Benna, Luminol-amplified chemiluminescence detects mainly superoxide anion produced by human neutrophils, *Am J Blood Res* 7 (2017) 41–48.
- [2] S. Fu, S. Gebicki, W. Jessup, J.M. Gebicki, R.T. Dean, Biological fate of amino acid, peptide and protein hydroperoxides, *Biochem. J.* 311 (1995) 821–827, <https://doi.org/10.1042/bj3110821>.
- [3] J.A. Simpson, S. Narita, S. Gieseg, S. Gebicki, J.M. Gebicki, R.T. Dean, Long-lived reactive species on free-radical-damaged proteins, *Biochem. J.* 282 (1992) 621–624, <https://doi.org/10.1042/bj2820621>.
- [4] H.A. Headlam, M.J. Davies, Cell-mediated reduction of protein and peptide hydroperoxides to reactive free radicals, *Free Radic. Biol. Med.* 34 (2003) 44–55, [https://doi.org/10.1016/S0891-5849\(02\)01181-4](https://doi.org/10.1016/S0891-5849(02)01181-4).
- [5] M.A. Cardoso, A.J. Duarte, H.M.R. Gonçalves, Carbon dots as reactive nitrogen species nanosensors, *Anal. Chim. Acta* 1202 (2022) 339654, <https://doi.org/10.1016/j.aca.2022.339654>.
- [6] J. Garthwaite, Concepts of neural nitric oxide-mediated transmission, *Eur. J. Neurosci.* 27 (2008) 2783–2802, <https://doi.org/10.1111/j.1460-9568.2008.06285.x>.
- [7] M. Marquitan, J. Clausmeyer, P. Actis, A.L. Córdoba, Y. Korchev, M.D. Mark, S. Herlitze, W. Schuhmann, Intracellular hydrogen peroxide detection with functionalised nanoelectrodes, *Chemoelectrochem* 3 (2016) 2125–2129, <https://doi.org/10.1002/celc.201600390>.
- [8] G.E. Villalpando-Rodriguez, S.B. Gibson, Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat, *Oxid. Med. Cell. Longev.* 2021 (2021) 1–17, <https://doi.org/10.1155/2021/9912436>.
- [9] R. Mittler, S.I. Zandalinas, Y. Fichman, F. Van Breusegem, Reactive oxygen species signalling in plant stress responses, *Nat. Rev. Mol. Cell Biol.* 23 (2022) 663–679, <https://doi.org/10.1038/s41580-022-00499-2>.
- [10] H. Sies, D.P. Jones, Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, *Nat. Rev. Mol. Cell Biol.* 21 (2020) 363–383, <https://doi.org/10.1038/s41580-020-0230-3>.
- [11] M.D. Brand, Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling, *Free Radic. Biol. Med.* 100 (2016) 14–31, <https://doi.org/10.1016/j.freeradbiomed.2016.04.001>.
- [12] J.-T. Hou, M. Zhang, Y. Liu, X. Ma, R. Duan, X. Cao, F. Yuan, Y.-X. Liao, S. Wang, W. Xiu Ren, Fluorescent detectors for hydroxyl radical and their applications in bioimaging: a review, *Coord. Chem. Rev.* 421 (2020) 213457, <https://doi.org/10.1016/j.ccr.2020.213457>.
- [13] J.-T. Hou, K.-K. Yu, K. Sunwoo, W.Y. Kim, S. Koo, J. Wang, W.X. Ren, S. Wang, X.-Q. Yu, J.S. Kim, Fluorescent imaging of reactive oxygen and nitrogen species associated with pathophysiological processes, *Chem* 6 (2020) 832–866, <https://doi.org/10.1016/j.chempr.2019.12.005>.
- [14] X. Bai, K.K.-H. Ng, J.J. Hu, S. Ye, D. Yang, Small-molecule-based fluorescent sensors for selective detection of reactive oxygen species in biological systems, *Annu. Rev. Biochem.* 88 (2019) 605–633, <https://doi.org/10.1146/annurev-biochem-013118-111754>.
- [15] W.M. Nauseef, How human neutrophils kill and degrade microbes: an integrated view, *Immunol. Rev.* 219 (2007) 88–102, <https://doi.org/10.1111/j.1600-065X.2007.00550.x>.
- [16] W.M. Nauseef, N. Borregaard, Neutrophils at work, *Nat. Immunol.* 15 (2014) 602–611, <https://doi.org/10.1038/ni.2921>.
- [17] B. Halliwell, Reactive oxygen species (ROS), oxygen radicals and antioxidants: where are we now, where is the field going and where should we go? *Biochem. Biophys. Res. Commun.* 633 (2022) 17–19, <https://doi.org/10.1016/j.bbrc.2022.08.098>.
- [18] D. Roos, D.B. Kuhns, A. Maddalena, J. Roesler, J.A. Lopez, T. Ariga, T. Avcin, M. de Boer, J. Bustamante, A. Condino-Neto, G. Di Matteo, J. He, H.R. Hill, S.M. Holland, C. Kannengiesser, M.Y. Köker, I. Kondratenko, K. van Leeuwen, H.L. Malech, L. Marodi, H. Nunoí, M.-J. Stasia, A.M. Ventura, C.T. Witwer, B. Wolach, J.I. Gallin, Hematologically important mutations: X-linked chronic granulomatous disease (third update), *Blood Cells, Mol. Dis.* 45 (2010) 246–265, <https://doi.org/10.1016/j.bcmd.2010.07.012>.
- [19] C. Kannengiesser, B. Gérard, J. El Benna, D. Henri, Y. Kroviarski, S. Chollet-Martin, M.-A. Gougerot-Pocidallo, C. Elbim, B. Grandchamp, Molecular epidemiology of chronic granulomatous disease in a series of 80 kindreds: identification of 31 novel mutations, *Hum. Mutat.* 29 (2008) E132 <https://doi.org/10.1002/humu.20820>, –E149.
- [20] J. El-Benna, P.M.-C. Dang, M.-A. Gougerot-Pocidallo, C. Elbim, Phagocyte NADPH oxidase: a multicomponent enzyme essential for host defenses, *Arch. Immunol. Ther. Exp.* 53 (2005) 199–206, <http://www.ncbi.nlm.nih.gov/pubmed/15995580>.
- [21] B.M. Babior, NADPH oxidase: an update, *Blood* 93 (1999) 1464–1476, <https://doi.org/10.1182/blood.V93.5.1464>.
- [22] C.P. Rubio, J.J. Cerón, Spectrophotometric assays for evaluation of Reactive Oxygen Species (ROS) in serum: general concepts and applications in dogs and humans, *BMC Vet. Res.* 17 (2021) 226, <https://doi.org/10.1186/s12917-021-02924-8>.
- [23] G.T. Huynh, V. Kesarwani, J.A. Walker, J.E. Frith, L. Meagher, S.R. Corrie, Review: nanomaterials for reactive oxygen species detection and monitoring in biological environments, *Front. Chem.* 9 (2021), <https://doi.org/10.3389/fchem.2021.728717>.
- [24] G. Solaini, A. Baracca, G. Lenaz, G. Sgarbi, Hypoxia and mitochondrial oxidative metabolism, *Biochim. Biophys. Acta, Bioenerg.* 1797 (2010) 1171–1177, <https://doi.org/10.1016/j.bbabi.2010.02.011>.
- [25] E.L. Bell, T.A. Klimova, J. Eisenbart, C.T. Moraes, M.P. Murphy, G.R.S. Budinger, N.S. Chandel, The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production, *J. Cell Biol.* 177 (2007) 1029–1036, <https://doi.org/10.1083/jcb.200609074>.
- [26] R.D. Guzy, B. Hoyos, E. Robin, H. Chen, L. Liu, K.D. Mansfield, M.C. Simon, U. Hammerling, P.T. Schumacker, Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing, *Cell Metabol.* 1 (2005) 401–408, <https://doi.org/10.1016/j.cmet.2005.05.001>.
- [27] L. Xie, Z. Yang, Y. Wang, J. Hu, Y. Lu, H. Zhang, S. Jiang, W. Li, 1-O-acetylbrutinilactone ameliorates alcohol-induced hepatotoxicity through regulation of ROS/akt/NF-κB-mediated apoptosis and inflammation, *ACS Omega* 7 (2022) 18122–18130, <https://doi.org/10.1021/acsomega.2c01681>.
- [28] M.I. Anik, N. Mahmud, A. Al Masud, M.I. Khan, M.N. Islam, S. Uddin, M.K. Hossain, Role of reactive oxygen species in aging and age-related diseases: a review, *ACS Appl. Bio Mater.* (2022), <https://doi.org/10.1021/acsbam.2c00411>.
- [29] M. Redza-Dutordoir, D.A. Averill-Bates, Activation of apoptosis signalling pathways by reactive oxygen species, *Biochim. Biophys. Acta Mol. Cell Res.* 1863 (2016) 2977–2992, <https://doi.org/10.1016/j.bbamcr.2016.09.012>.
- [30] T. Kietzmann, A. Petry, A. Shvetsova, J.M. Gerhold, A. Görlach, The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system, *Br. J. Pharmacol.* 174 (2017) 1533–1554, <https://doi.org/10.1111/bph.13792>.
- [31] P.R. Angelova, A.Y. Abramov, Role of mitochondrial ROS in the brain: from physiology to neurodegeneration, *FEBS Lett.* 592 (2018) 692–702, <https://doi.org/10.1002/1873-3468.12964>.
- [32] T. Honda, Y. Hirakawa, M. Nangaku, The role of oxidative stress and hypoxia in renal disease, *Kidney Res. Clin. Pract.* 38 (2019) 414–426, <https://doi.org/10.23876/j.krccp.19.063>.
- [33] Y. Tan, M.S. Cheong, W.S. Cheang, Roles of reactive oxygen species in vascular complications of diabetes: therapeutic properties of medicinal plants and food, *Oxygen* 2 (2022) 246–268, <https://doi.org/10.3390/oxygen2030018>.
- [34] G. Waris, H. Ahsan, Reactive oxygen species: role in the development of cancer and various chronic conditions, *J. Carcinog.* 5 (2006) 14, <https://doi.org/10.1186/1477-3163-5-14>.
- [35] D.A. Patten, M. Germain, M.A. Kelly, R.S. Slack, Reactive oxygen species: stuck in the middle of neurodegeneration, *J. Alzheim. Dis.* 20 (2010) <https://doi.org/10.3233/JAD-2010-100498>, S357–S367.
- [36] M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, J. Telsner, Free radicals and antioxidants in normal physiological functions and human disease, *Int. J. Biochem. Cell Biol.* 39 (2007) 44–84, <https://doi.org/10.1016/j.biocel.2006.07.001>.
- [37] T. Finkel, N.J. Holbrook, Oxidants, Oxidative stress and the biology of ageing, *Nature* 408 (2000) 239–247, <https://doi.org/10.1038/35041687>.

- [38] S. Escada-Rebello, F. Mora, A. Sousa, T. Almeida-Santos, A. Paiva, J. Ramalho-Santos, Fluorescent probes for the detection of reactive oxygen species in human spermatozoa, *Asian J. Androl.* 22 (2020) 465, https://doi.org/10.4103/aja.aja_132_19.
- [39] Y. Hong, J.W.Y. Lam, B.Z. Tang, Aggregation-induced emission: phenomenon, mechanism and applications, *Chem. Commun.* (2009) 4332, <https://doi.org/10.1039/b904665h>.
- [40] D. Ding, C.C. Goh, G. Feng, Z. Zhao, J. Liu, R. Liu, N. Tomczak, J. Geng, B.Z. Tang, L.G. Ng, B. Liu, Ultrabright organic dots with aggregation-induced emission characteristics for real-time two-photon intravital vasculature imaging, *Adv. Mater.* 25 (2013) 6083–6088, <https://doi.org/10.1002/adma.201301938>.
- [41] J. Geng, K. Li, W. Qin, B.Z. Tang, B. Liu, Red-emissive chemiluminescent nanoparticles with aggregation-induced emission characteristics for in vivo hydrogen peroxide imaging, *Part. Part. Syst. Char.* 31 (2014) 1238–1243, <https://doi.org/10.1002/ppsc.201400156>.
- [42] K. Li, W. Qin, D. Ding, N. Tomczak, J. Geng, R. Liu, J. Liu, X. Zhang, H. Liu, B. Liu, B.Z. Tang, Photostable fluorescent organic dots with aggregation-induced emission (AIE dots) for noninvasive long-term cell tracing, *Sci. Rep.* 3 (2013) 1150, <https://doi.org/10.1038/srep01150>.
- [43] W. Qin, D. Ding, J. Liu, W.Z. Yuan, Y. Hu, B. Liu, B.Z. Tang, Biocompatible nanoparticles with aggregation-induced emission characteristics as far-red/near-infrared fluorescent bioprobes for in vitro and in vivo imaging applications, *Adv. Funct. Mater.* 22 (2012) 771–779, <https://doi.org/10.1002/adfm.201102191>.
- [44] Y. Wang, Y. Cui, R. Liu, Y. Wei, X. Jiang, H. Zhu, L. Gao, Y. Zhao, Z. Chai, X. Gao, Blue two-photon fluorescence metal cluster probe precisely marking cell nuclei of two cell lines, *Chem. Commun.* 49 (2013) 10724, <https://doi.org/10.1039/c3cc46690f>.
- [45] J. Geng, Z. Zhu, W. Qin, L. Ma, Y. Hu, G.G. Gurzadyan, B.Z. Tang, B. Liu, Near-infrared fluorescence amplified organic nanoparticles with aggregation-induced emission characteristics for in vivo imaging, *Nanoscale* 6 (2014) 939–945, <https://doi.org/10.1039/C3NR04243J>.
- [46] J. Geng, K. Li, W. Qin, L. Ma, G.G. Gurzadyan, B.Z. Tang, B. Liu, Eccentric loading of fluorogen with aggregation-induced emission in PLGA matrix increases nanoparticle fluorescence quantum yield for targeted cellular imaging, *Small* 9 (2013) 2012–2019, <https://doi.org/10.1002/sml.201202505>.
- [47] W.-C. Wu, C.-Y. Chen, Y. Tian, S.-H. Jang, Y. Hong, Y. Liu, R. Hu, B.Z. Tang, Y.-T. Lee, C.-T. Chen, W.-C. Chen, A.K.-Y. Jen, Enhancement of aggregation-induced emission in dye-encapsulating polymeric micelles for bioimaging, *Adv. Funct. Mater.* 20 (2010) 1413–1423, <https://doi.org/10.1002/adfm.200902043>.
- [48] Z. Zhu, X. Zhao, W. Qin, G. Chen, J. Qian, Z. Xu, Fluorescent AIE dots encapsulated organically modified silica (ORMOSIL) nanoparticles for two-photon cellular imaging, *Sci. China Chem.* 56 (2013) 1247–1252, <https://doi.org/10.1007/s11426-013-4943-4>.
- [49] Z. Wang, B. Xu, L. Zhang, J. Zhang, T. Ma, J. Zhang, X. Fu, W. Tian, Folic acid-functionalized mesoporous silica nanospheres hybridized with AIE luminogens for targeted cancer cell imaging, *Nanoscale* 5 (2013) 2065, <https://doi.org/10.1039/c2nr33685e>.
- [50] C.A. Rice-Evans, A.T. Diplock, M.C.R. in B., M.B. Symons (Eds.), Chapter 1 Introduction to free radicals, *Tech. Free Radic. Res.*, Elsevier, 1991, pp. 1–18, [https://doi.org/10.1016/S0075-7535\(08\)70041-X](https://doi.org/10.1016/S0075-7535(08)70041-X).
- [51] T. Frischer, A. Pullwitt, J. Kühr, R. Meinert, N. Haschke, M. Studnicka, G. Lubec, Aromatic hydroxylation in nasal lavage fluid following ambient Ozone exposure, *Free Radic. Biol. Med.* 22 (1997) 201–207, [https://doi.org/10.1016/S0891-5849\(96\)00292-4](https://doi.org/10.1016/S0891-5849(96)00292-4).
- [52] H. Utsumi, K. Yamada, In vivo electron spin resonance-computed tomography/nitroxyl probe technique for non-invasive analysis of oxidative injuries, *Arch. Biochem. Biophys.* 416 (2003) 1–8, [https://doi.org/10.1016/S0003-9861\(03\)00285-6](https://doi.org/10.1016/S0003-9861(03)00285-6).
- [53] Y. Gong, B. Yu, W. Yang, X. Zhang, Phosphorus, and nitrogen co-doped carbon dots as a fluorescent probe for real-time measurement of reactive oxygen and nitrogen species inside macrophages, *Biosens. Bioelectron.* 79 (2016) 822–828, <https://doi.org/10.1016/j.bios.2016.01.022>.
- [54] Y.-D. Lee, C.-K. Lim, A. Singh, J. Koh, J. Kim, I.C. Kwon, S. Kim, Dye/peroxalate aggregated nanoparticles with enhanced and tunable chemiluminescence for biomedical imaging of hydrogen peroxide, *ACS Nano* 6 (2012) 6759–6766, <https://doi.org/10.1021/nn3014905>.
- [55] H.J. Kim, Y.H. Seo, S. An, A. Jo, I.C. Kwon, S. Kim, Chemiluminescence imaging of Duox2-derived hydrogen peroxide for longitudinal visualization of biological response to viral infection in nasal mucosa, *Theranostics* 8 (2018) 1798–1807, <https://doi.org/10.7150/thno.22481>.
- [56] M. Zhang, M. Zuo, C. Wang, Z. Li, Q. Cheng, J. Huang, Z. Wang, Z. Liu, Monitoring neuroinflammation with an HOCl-activatable and blood-brain barrier permeable upconversion nanoprobe, *Anal. Chem.* 92 (2020) 5569–5576, <https://doi.org/10.1021/acs.analchem.0c00526>.
- [57] Y. Wang, L. Shi, Z. Ye, K. Guan, L. Teng, J. Wu, X. Yin, G. Song, X.-B. Zhang, Reactive oxygen correlated chemiluminescent imaging of a semiconducting polymer nanoplateform for monitoring chemodynamic therapy, *Nano Lett.* 20 (2020) 176–183, <https://doi.org/10.1021/acs.nanolett.9b03556>.
- [58] Q. Chen, J. Zhou, Z. Chen, Q. Luo, J. Xu, G. Song, Tumor-specific expansion of oxidative stress by glutathione depletion and use of a fenton nanoagent for enhanced chemodynamic therapy, *ACS Appl. Mater. Interfaces* 11 (2019) 30551–30565, <https://doi.org/10.1021/acsami.9b09323>.
- [59] M. Bouché, M. Pühringer, A. Iturmendi, A. Amirshaghghi, A. Tsourkas, I. Teasdale, D.P. Cormode, Activatable hybrid polyphosphazene-AuNP nanoprobe for ROS detection by bimodal PA/CT imaging, *ACS Appl. Mater. Interfaces* 11 (2019) 28648–28656, <https://doi.org/10.1021/acsami.9b08386>.
- [60] K. Pu, A.J. Shuhendler, J. Rao, Semiconducting polymer nanoprobe for in vivo imaging of reactive oxygen and nitrogen species, *Angew. Chem. Int. Ed.* 52 (2013) 10325–10329, <https://doi.org/10.1002/anie.201303420>.
- [61] N.M. Goldenberg, B.E. Steinberg, A.S. Slutsky, W.L. Lee, Broken barriers: a new take on sepsis pathogenesis, *Sci. Transl. Med.* 3 (2011), <https://doi.org/10.1126/scitranslmed.3002011>.
- [62] A.J. Shuhendler, K. Pu, L. Cui, J.P. Uetrecht, J. Rao, Real-time imaging of oxidative and nitrosative stress in the liver of live animals for drug-toxicity testing, *Nat. Biotechnol.* 32 (2014) 373–380, <https://doi.org/10.1038/nbt.2838>.
- [63] H.M.R. Gonçalves, J.C.G. Esteves da Silva, A new insight on silicon dots, *Curr. Anal. Chem.* 8 (2012) 67–77, <https://doi.org/10.2174/157341112798472260>.
- [64] J.C.G. Esteves da Silva, H.M.R. Gonçalves, Analytical and bioanalytical applications of carbon dots, *TrAC, Trends Anal. Chem.* 30 (2011) 1327–1336, <https://doi.org/10.1016/j.trac.2011.04.009>.
- [65] X. Zhen, C. Zhang, C. Xie, Q. Miao, K.L. Lim, K. Pu, Intraparticle energy level alignment of semiconducting polymer nanoparticles to amplify chemiluminescence for ultrasensitive in vivo imaging of reactive oxygen species, *ACS Nano* 10 (2016) 6400–6409, <https://doi.org/10.1021/acsnano.6b02908>.
- [66] H. An, C. Guo, D. Li, R. Liu, X. Xu, J. Guo, J. Ding, J. Li, W. Chen, J. Zhang, Hydrogen peroxide-activatable nanoparticles for luminescence imaging and in situ triggerable photodynamic therapy of cancer, *ACS Appl. Mater. Interfaces* 12 (2020) 17230–17243, <https://doi.org/10.1021/acsami.0c01413>.
- [67] P. Cheng, Q. Miao, J. Li, J. Huang, C. Xie, K. Pu, Unimolecular chemo-fluoro-luminescent reporter for crosstalk-free duplex imaging of hepatotoxicity, *J. Am. Chem. Soc.* 141 (2019) 10581–10584, <https://doi.org/10.1021/jacs.9b02580>.
- [68] J. Song, L. Lin, Z. Yang, R. Zhu, Z. Zhou, Z.-W. Li, F. Wang, J. Chen, H. Yang, X. Chen, Self-assembled responsive bilayered vesicles with adjustable oxidative stress for enhanced cancer imaging and therapy, *J. Am. Chem. Soc.* 141 (2019) 8158–8170, <https://doi.org/10.1021/jacs.8b13902>.
- [69] O. Erel, A new automated colorimetric method for measuring total oxidant status, *Clin. Biochem.* 38 (2005) 1103–1111, <https://doi.org/10.1016/j.clinbiochem.2005.08.008>.
- [70] J.W.M. Yuen, I.F.F. Benzie, Hydrogen peroxide in urine as a potential biomarker of whole body oxidative stress, *Free Radic. Res.* 37 (2003) 1209–1213, <https://doi.org/10.1080/10715760310001616032>.
- [71] S. Miyamoto, G.E. Ronsein, F.M. Prado, M. Uemi, T.C. Corrêa, I.N. Toma, A. Bertolucci, M.C.B. Oliveira, F.D. Motta, M.H.G. Medeiros, P. Di Mascio, Biological hydroperoxides and singlet molecular oxygen generation, *IUBMB Life* 59 (2007) 322–331, <https://doi.org/10.1080/15216540701242508>.
- [72] W. Wasowicz, J. Nève, A. Peretz, Optimized steps in fluorimetric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage, *Clin. Chem.* 39 (1993) 2522–2526, <https://doi.org/10.1093/clinchem/39.12.2522>.
- [73] J.A. Buege, S.D. Aust, [30] Microsomal lipid peroxidation, in: *Biomembr. - Part C Biol. Oxidations*, 1978, pp. 302–310, [https://doi.org/10.1016/S0076-6879\(78\)52032-6](https://doi.org/10.1016/S0076-6879(78)52032-6).
- [74] A.M. Jentzsch, H. Bachmann, P. Fürst, H.K. Biesalski, Improved analysis of malondialdehyde in human body fluids, *Free Radic. Biol. Med.* 20 (1996) 251–256, [https://doi.org/10.1016/0891-5849\(95\)02043-8](https://doi.org/10.1016/0891-5849(95)02043-8).
- [75] J.A. Knight, R.K. Pieper, L. McClellan, Specificity of the thiobarbituric acid reaction: its use in studies of lipid peroxidation, *Clin. Chem.* 34 (1988) 2433–2438, <https://doi.org/10.1093/clinchem/34.12.2433>.
- [76] T. Tug, F. Karatas, S.M. Terzi, N. Ozdemir, Comparison of serum malondialdehyde levels determined by two different methods in patients with COPD: HPLC or TBARS methods, *Lab. Med.* 36 (2005) 41–44, <https://doi.org/10.1309/WTEET9TJ2LUMB3C3>.
- [77] E. Seljeskog, T. Hervig, M.A. Mansoor, A novel HPLC method for the measurement of thiobarbituric acid reactive substances (TBARS). A comparison with a commercially available kit, *Clin. Biochem.* 39 (2006) 947–954, <https://doi.org/10.1016/j.clinbiochem.2006.03.012>.
- [78] V. Nair, C.S. Cooper, D.E. Vietti, G.A. Turner, The chemistry of lipid peroxidation metabolites: crosslinking reactions of malondialdehyde, *Lipids* 21 (1986) 6–10, <https://doi.org/10.1007/BF02534294>.
- [79] T. Yoshihara, Y. Hirakawa, M. Hosaka, M. Nangaku, S. Tobita, Oxygen imaging of living cells and tissues using luminescent molecular probes, *J. Photochem. Photobiol. C Photochem. Rev.* 30 (2017) 71–95, <https://doi.org/10.1016/j.jphotochemrev.2017.01.001>.
- [80] Y. Zhang, M. Dai, Z. Yuan, Methods for the detection of reactive oxygen species, *Anal. Methods* 10 (2018) 4625–4638, <https://doi.org/10.1039/C8AY01339J>.
- [81] V. Huntosova, E. Gerelli, D. Horvath, G. Wagnieres, Measurement of pO₂ by luminescence lifetime spectroscopy: a comparative study of the phototoxicity and sensitivity of [Ru(Phen) 3]²⁺ and Pd(TCPP) in vivo, *J. Biophot.* 10 (2017) 708–717, <https://doi.org/10.1002/jbio.201600127>.
- [82] C. Liu, C. Shao, H. Wu, B. Guo, B. Zhu, X. Zhang, A fast-response, highly sensitive and selective fluorescent probe for the ratiometric imaging of hydrogen peroxide with a 100 nm red-shifted emission, *RSC Adv.* 4 (2014) 16055, <https://doi.org/10.1039/C4RA01039F>.
- [83] Y. Zhang, J. Clausmeyer, B. Babakinejad, A. López Córdoba, T. Ali, A. Shevchuk, Y. Takahashi, P. Novak, C. Edwards, M. Lab, S. Gopal, C. Chiappini, U. Anand, L. Magnani, R.C. Coombes, J. Gorelik, T. Matsue, W. Schuhmann, D. Klennerman, E.V. Sviderskaya, Y. Korchev, Spearhead nanometric field-effect transistor sensors for single-cell analysis, *ACS Nano* 10 (2016) 3214–3221, <https://doi.org/10.1021/acsnano.5b05211>.
- [84] C. Li, Q. Wang, Challenges and opportunities for intravital near-infrared fluorescence imaging Technology in the second transparency window, *ACS Nano* 12 (2018) 9654–9659, <https://doi.org/10.1021/acsnano.8b07536>.

- [85] R. Datta, T.M. Heaster, J.T. Sharick, A.A. Gillette, M.C. Skala, Fluorescence lifetime imaging microscopy: fundamentals and advances in instrumentation, analysis, and applications, *J. Biomed. Opt.* 25 (2020) 1, <https://doi.org/10.1117/1.JBO.25.7.071203>.
- [86] J. Balke, P. Volz, F. Neumann, R. Brodwin, A. Wolf, H. Pischon, M. Radbruch, L. Mundhenk, A.D. Gruber, N. Ma, U. Alexiev, Visualizing oxidative cellular stress induced by nanoparticles in the subcytotoxic range using fluorescence lifetime imaging, *Small* 14 (2018) 1800310, <https://doi.org/10.1002/smll.201800310>.
- [87] X. Wang, W. Yuan, M. Xu, X. Su, F. Li, Visualization of acute inflammation through a macrophage-camouflaged afterglow nanocomplex, *ACS Appl. Mater. Interfaces* 14 (2022) 259–267, <https://doi.org/10.1021/acsami.1c19388>.
- [88] G.C. Van de Bittner, E.A. Dubikovskaya, C.R. Bertozzi, C.J. Chang, In vivo imaging of hydrogen peroxide production in a murine tumor model with a chemoselective bioluminescent reporter, *Proc. Natl. Acad. Sci. USA* 107 (2010) 21316–21321, <https://doi.org/10.1073/pnas.1012864107>.
- [89] W. Fan, L. Li, J. Yuan, X. Ma, J. Xia, X. Zhang, Aggregation-induced emission effect within peroxyoxalate-loaded mesoporous silica nanoparticles for efficient harvest of chemiluminescence energy in aqueous solutions, *Anal. Chem.* 93 (2021) 17043–17050, <https://doi.org/10.1021/acs.analchem.1c03798>.
- [90] Y. Jiang, K. Pu, Molecular probes for autofluorescence-free optical imaging, *Chem. Rev.* 121 (2021) 13086–13131, <https://doi.org/10.1021/acs.chemrev.1c00506>.
- [91] J. Li, Y. Hu, Z. Li, W. Liu, T. Deng, J. Li, Photoactivatable red chemiluminescent AIEgen probe for in vitro/vivo imaging assay of hydrazine, *Anal. Chem.* 93 (2021) 10601–10610, <https://doi.org/10.1021/acs.analchem.1c01804>.
- [92] X. Yu, S. Du, Y. Yang, Z. Di, M. Wu, Two pyrene-based metal-organic frameworks for chemiluminescence, *Inorg. Chem.* 60 (2021) 1320–1324, <https://doi.org/10.1021/acs.inorgchem.0c03627>.
- [93] Y.-J. Zhang, L. Guo, Y.-L. Yu, J.-H. Wang, Photoacoustic-based miniature device with smartphone readout for point-of-care testing of uric acid, *Anal. Chem.* 92 (2020) 15699–15704, <https://doi.org/10.1021/acs.analchem.0c03470>.
- [94] J. Li, X. Wang, M. Shen, X. Shi, Polyethylenimine-assisted generation of optical nanopores for biosensing applications, *ACS Appl. Bio Mater.* 3 (2020) 3935–3955, <https://doi.org/10.1021/acsabm.0c00536>.
- [95] Y. Liu, M.A. Haghighatbin, W. Shen, H. Cui, Functionalized polydopamine nanospheres with chemiluminescence and immunoactivity for label-free copeptin immunosensing, *ACS Appl. Nano Mater.* 3 (2020) 4681–4689, <https://doi.org/10.1021/acsanm.0c00682>.
- [96] Y. Gao, R. Sun, M. Zhao, J. Ding, A. Wang, S. Ye, Y. Zhang, Q. Mao, W. Xie, G. Ma, H. Shi, Sulfenic acid-mediated on-site-specific immobilization of mitochondrial-targeted NIR fluorescent probe for prolonged tumor imaging, *Anal. Chem.* 92 (2020) 6977–6983, <https://doi.org/10.1021/acs.analchem.9b05855>.
- [97] X. Zhu, J.-X. Wang, L.-Y. Niu, Q.-Z. Yang, Aggregation-induced emission materials with narrowed emission band by light-harvesting strategy: fluorescence and chemiluminescence imaging, *Chem. Mater.* 31 (2019) 3573–3581, <https://doi.org/10.1021/acs.chemmater.9b01338>.
- [98] J. Clausmeyer, P. Actis, A. López Córdoba, Y. Korchev, W. Schuhmann, Nanosensors for the detection of hydrogen peroxide, *Electrochem. Commun.* 40 (2014) 28–30, <https://doi.org/10.1016/j.elecom.2013.12.015>.
- [99] A. Schulte, W. Schuhmann, Single-cell microelectrochemistry, *Angew. Chem. Int. Ed.* 46 (2007) 8760–8777, <https://doi.org/10.1002/anie.200604851>.
- [100] S. Dong, G. Che, Electrocatalytic oxidation of ascorbic acid at a prussian blue film modified microdisk electrode, *J. Electroanal. Chem. Interfacial Electrochem.* 315 (1991) 191–199, [https://doi.org/10.1016/0022-0728\(91\)80069-3](https://doi.org/10.1016/0022-0728(91)80069-3).
- [101] P. Salazar, M. Martín, R. Roche, R.D. O'Neill, J.L. González-Mora, Prussian Blue-modified microelectrodes for selective transduction in enzyme-based amperometric microbiosensors for in vivo neurochemical monitoring, *Electrochim. Acta* 55 (2010) 6476–6484, <https://doi.org/10.1016/j.electacta.2010.06.036>.
- [102] M.G. Garguilo, A.C. Michael, An enzyme-modified microelectrode that detects choline injected locally into brain tissue, *J. Am. Chem. Soc.* 115 (1993) 12218–12219, <https://doi.org/10.1021/ja00078a092>.
- [103] J. Li, J. Yu, Fabrication of Prussian Blue modified ultramicroelectrode for GOD imaging using scanning electrochemical microscopy, *Bioelectrochemistry* 72 (2008) 102–106, <https://doi.org/10.1016/j.bioelechem.2007.11.013>.
- [104] N.V. Kulagina, A.C. Michael, Monitoring hydrogen peroxide in the extracellular space of the brain with amperometric microbiosensors, *Anal. Chem.* 75 (2003) 4875–4881, <https://doi.org/10.1021/ac034573g>.
- [105] P. Salazar, M. Martín, R.D. O'Neill, J. González-Mora, Biosensors based on prussian blue modified carbon fibers electrodes for monitoring lactate in the extracellular space of brain tissue, *Int. J. Electrochem. Sci.* 7 (2012) 5910–5926.
- [106] R. Li, X. Liu, W. Qiu, M. Zhang, In vivo monitoring of H₂O₂ with polydopamine and prussian blue-coated microelectrode, *Anal. Chem.* 88 (2016) 7769–7776, <https://doi.org/10.1021/acs.analchem.6b01765>.
- [107] X.T. Zheng, W. Hu, H. Wang, H. Yang, W. Zhou, C.M. Li, Bifunctional electro-optical nanoprobe to real-time detect local biochemical processes in single cells, *Biosens. Bioelectron.* 26 (2011) 4484–4490, <https://doi.org/10.1016/j.bios.2011.05.007>.
- [108] M. Nebel, S. Grütke, N. Diab, A. Schulte, W. Schuhmann, Visualization of oxygen consumption of single living cells by scanning electrochemical microscopy: the influence of the faradaic tip reaction, *Angew. Chem. Int. Ed.* 52 (2013) 6335–6338, <https://doi.org/10.1002/anie.201301098>.
- [109] J. Clausmeyer, W. Schuhmann, Nanoelectrodes: applications in electrocatalysis, single-cell analysis and high-resolution electrochemical imaging, *TrAC, Trends Anal. Chem.* 79 (2016) 46–59, <https://doi.org/10.1016/j.trac.2016.01.018>.
- [110] C. Amatore, S. Arbault, C. Bouton, K. Coffi, J.-C. Drapier, H. Ghandour, Y. Tong, Monitoring in real time with a microelectrode the release of reactive oxygen and nitrogen species by a single macrophage stimulated by its membrane mechanical depolarization, *Chembiochem* 7 (2006) 653–661, <https://doi.org/10.1002/cbic.200500359>.
- [111] S. Arbault, P. Pantano, J.A. Jankowski, M. Vuillaume, C. Amatore, Monitoring an oxidative stress mechanism at a single human fibroblast, *Anal. Chem.* 67 (1995) 3382–3390, <https://doi.org/10.1021/ac00115a004>.
- [112] S. Arbault, Activation of the NADPH oxidase in human fibroblasts by mechanical intrusion of a single cell with an ultramicroelectrode, *Carcinogenesis* 18 (1997) 569–574, <https://doi.org/10.1093/carcin/18.3.569>.
- [113] C. Amatore, S. Arbault, A.C.W. Koh, Simultaneous detection of reactive oxygen and nitrogen species released by a single macrophage by triple potential-step chronoamperometry, *Anal. Chem.* 82 (2010) 1411–1419, <https://doi.org/10.1021/ac902486x>.
- [114] G.R. Buettner, The pecking order of free radicals and antioxidants: lipid peroxidation, α -tocopherol, and ascorbate, *Arch. Biochem. Biophys.* 300 (1993) 535–543, <https://doi.org/10.1006/abbi.1993.1074>.
- [115] Z. Zhang, J. Fan, J. Du, X. Peng, Two-channel responsive luminescent chemosensors for dioxygen species: molecular oxygen, singlet oxygen and superoxide anion, *Coord. Chem. Rev.* 427 (2021) 213575, <https://doi.org/10.1016/j.ccr.2020.213575>.
- [116] T. Finkel, Signal transduction by reactive oxygen species, *J. Cell Biol.* 194 (2011) 7–15, <https://doi.org/10.1083/jcb.201102095>.
- [117] R.B. Hamanaka, N.S. Chandel, Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes, *Trends Biochem. Sci.* 35 (2010) 505–513, <https://doi.org/10.1016/j.tibs.2010.04.002>.
- [118] J.M. Matés, J.A. Segura, F.J. Alonso, J. Márquez, Oxidative stress in apoptosis and cancer: an update, *Arch. Toxicol.* 86 (2012) 1649–1665, <https://doi.org/10.1007/s00204-012-0906-3>.
- [119] P.R. Ogilby, Singlet oxygen: there is indeed something new under the sun, *Chem. Soc. Rev.* 39 (2010) 3181, <https://doi.org/10.1039/b926014p>.
- [120] M.T. Jarvi, M.J. Niedre, M.S. Patterson, B.C. Wilson, Singlet oxygen luminescence dosimetry (SOLD) for photodynamic therapy: current status, challenges and future prospects, *Photochem. Photobiol.* 82 (2006) 1198, <https://doi.org/10.1562/2006-05-03-IR-891>.
- [121] B.H. Li, B.C. Wilson, Direct and indirect measurements of singlet oxygen for photodynamic therapy, in: *Proc. 13th Int. Photodyn. Assoc. World*, 2011, pp. 43–47.
- [122] D. Kessel, M. Price, Evaluation of diethyl-3,3'-(9,10-anthracenediyl)bis acrylate as a probe for singlet oxygen formation during photodynamic therapy, *Photochem. Photobiol.* 88 (2012) 717–720, <https://doi.org/10.1111/j.1751-1097.2012.01106.x>.
- [123] X. Chen, X. Tian, I. Shin, J. Yoon, Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species, *Chem. Soc. Rev.* 40 (2011) 4783, <https://doi.org/10.1039/c1cs15037e>.
- [124] M.S. Oliveira, D. Severino, F.M. Prado, J.P.F. Angeli, F.D. Motta, M.S. Baptista, M.H.G. Medeiros, P. Di Mascio, Singlet molecular oxygen trapping by the fluorescent probe diethyl-3,3'-(9,10-anthracenediyl)bisacrylate synthesized by the Heck reaction, *Photochem. Photobiol. Sci.* 10 (2011) 1546–1555, <https://doi.org/10.1039/c1pp05120b>.
- [125] H. Lin, Y. Shen, D. Chen, L. Lin, B.C. Wilson, B. Li, S. Xie, Feasibility study on quantitative measurements of singlet oxygen generation using singlet oxygen sensor green, *J. Fluoresc.* 23 (2013) 41–47, <https://doi.org/10.1007/s10895-012-1114-5>.
- [126] N. Umezawa, K. Tanaka, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, Novel fluorescent probes for singlet oxygen, *Angew. Chem. Int. Ed.* 38 (1999) 2899–2901, [https://doi.org/10.1002/\(SICI\)1521-3773\(19991004\)38:19<2899::AID-ANGE2899>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1521-3773(19991004)38:19<2899::AID-ANGE2899>3.0.CO;2-M).
- [127] K. Tanaka, T. Miura, N. Umezawa, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, Rational design of fluorescein-based fluorescence probes. Mechanism-based design of a maximum fluorescence probe for singlet oxygen, *J. Am. Chem. Soc.* 123 (2001) 2530–2536, <https://doi.org/10.1021/ja0035708>.
- [128] B. Song, G. Wang, M. Tan, J. Yuan, A europium(III) complex as an efficient singlet oxygen luminescence probe, *J. Am. Chem. Soc.* 128 (2006) 13442–13450, <https://doi.org/10.1021/ja062990f>.
- [129] C. Flors, Imaging the production of singlet oxygen in vivo using a new fluorescent sensor, Singlet Oxygen Sensor Green(R), *J. Exp. Bot.* 57 (2006) 1725–1734, <https://doi.org/10.1093/jxb/erj181>.
- [130] Y. Zhang, K. Aslan, M.J.R. Previte, C.D. Geddes, Metal-enhanced singlet oxygen generation: a consequence of plasmon enhanced triplet yields, *J. Fluoresc.* 17 (2007) 345–349, <https://doi.org/10.1007/s10895-007-0196-y>.
- [131] E.M. Hotze, A.R. Badireddy, S. Chellam, M.R. Wiesner, Mechanisms of bacteriophage inactivation via singlet oxygen generation in UV illuminated fullerol suspensions, *Environ. Sci. Technol.* 43 (2009) 6639–6645, <https://doi.org/10.1021/es901110m>.
- [132] F. Yan, Y. Zhang, H. Yuan, M.K. Gregas, T. Vo-Dinh, Apoferritin protein cages: a novel drug nanocarrier for photodynamic therapy, *Chem. Commun.* (2008) 4579, <https://doi.org/10.1039/b810949d>.
- [133] S. Onitsuka, H. Nishino, K. Kurosawa, Photooxygenation of 3-acetyl-5-aryl-2-methylfurans via endoperoxide intermediate and the following reactions, *Tetrahedron* 57 (2001) 6003–6009, [https://doi.org/10.1016/S0040-4020\(01\)00559-2](https://doi.org/10.1016/S0040-4020(01)00559-2).
- [134] L. Yang, N.S. Finney, A mechanistically-distinct approach to fluorescence visualization of singlet oxygen, *Chem. Commun.* 53 (2017) 11449–11452, <https://doi.org/10.1039/C7CC06214A>.
- [135] A.M.L. Hossion, M. Bio, G. Nkepan, S.G. Awuah, Y. You, Visible light

- controlled release of anticancer drug through double activation of prodrug, *ACS Med. Chem. Lett.* 4 (2013) 124–127, <https://doi.org/10.1021/ml3003617>.
- [136] E.I. Zenkevich, E.I. Sagun, V.N. Knyuksho, A.S. Stasheuski, V.A. Galievsky, A.P. Stupak, T. Blaudeck, C. von Borzyskowski, Quantitative analysis of singlet oxygen (1O_2) generation via energy transfer in nanocomposites based on semiconductor quantum dots and porphyrin ligands, *J. Phys. Chem. C* 115 (2011) 21535–21545, <https://doi.org/10.1021/jp203987r>.
- [137] M. Buchalska, P. Labuz, L. Bujak, G. Szwedczyk, T. Sarna, S. Maćkowski, W. Macyk, New insight into singlet oxygen generation at surface modified nanocrystalline TiO_2 – the effect of near-infrared irradiation, *Dalton Trans.* 42 (2013) 9468, <https://doi.org/10.1039/c3dt50399b>.
- [138] W. Hou, Y. Yuan, Z. Sun, S. Guo, H. Dong, C. Wu, Ratiometric fluorescent detection of intracellular singlet oxygen by semiconducting polymer dots, *Anal. Chem.* 90 (2018) 14629–14634, <https://doi.org/10.1021/acs.analchem.8b04859>.
- [139] D. Song, S. Cho, Y. Han, Y. You, W. Nam, Ratiometric fluorescent probes for detection of intracellular singlet oxygen, *Org. Lett.* 15 (2013) 3582–3585, <https://doi.org/10.1021/ol401421r>.
- [140] R. Flores-Cruz, A. Jiménez-Sánchez, Tracking mitochondrial 1O_2 -ROS production through a differential mitochondria-nucleoli fluorescent probe, *Chem. Commun.* 54 (2018) 13997–14000, <https://doi.org/10.1039/C8CC08289H>.
- [141] Y. You, E.J. Cho, H. Kwon, J. Hwang, S.E. Lee, A singlet oxygen photosensitizer enables photoluminescent monitoring of singlet oxygen doses, *Chem. Commun.* 52 (2016) 780–783, <https://doi.org/10.1039/C5CC08411C>.
- [142] Z. Jie, J. Liu, M. Shu, Y. Ying, H. Yang, Detection strategies for superoxide anion: a review, *Talanta* 236 (2022) 122892, <https://doi.org/10.1016/j.talanta.2021.122892>.
- [143] S. Scheinok, B. Driesschaert, D. D'Hose, P. Sonveaux, R. Robiette, B. Gallez, Synthesis and characterization of a 5-membered ring cyclic hydroxylamine coupled to triphenylphosphonium to detect mitochondrial superoxide by EPR spectrometry, *Free Radic. Res.* 53 (2019) 1135–1143, <https://doi.org/10.1080/10715762.2019.1692137>.
- [144] E. Besson, S. Gastaldi, E. Bloch, J. Zielonka, M. Zielonka, B. Kalyanaraman, S. Aslan, H. Karoui, A. Rockenbauer, O. Ouari, M. Hardy, Embedding cyclic nitron in mesoporous silica particles for EPR spin trapping of superoxide and other radicals, *Analyst* 144 (2019) 4194–4203, <https://doi.org/10.1039/C9AN00468H>.
- [145] P. Manning, C.J. McNeil, Electrochemical and optical sensing of reactive oxygen species: pathway to an integrated intracellular and extracellular measurement platform, *Biochem. Soc. Trans.* 39 (2011) 1288–1292, <https://doi.org/10.1042/BST0391288>.
- [146] P. Santhosh, K.M. Manesh, S.-H. Lee, S. Uthayakumar, A.I. Gopalan, K.-P. Lee, Sensitive electrochemical detection of superoxide anion using gold nanoparticles distributed poly(methyl methacrylate)-polyaniline core-shell electrospun composite electrode, *Analyst* 136 (2011) 1557, <https://doi.org/10.1039/c0an00616e>.
- [147] F.X. Hu, C. Guo, H. Bin Yang, Z. Shi, M. Wang, Y.H. Xue, L. Zhu, T. Chen, L. Dai, C.M. Li, 3D Pt/Graphene foam bioplateform for highly sensitive and selective in-situ adsorption and detection of superoxide anions released from living cells, *Sensor. Actuator. B Chem.* 287 (2019) 209–217, <https://doi.org/10.1016/j.snb.2019.02.037>.
- [148] C. Wu, M. Cui, X. Chen, M. Zhai, J. Ren, C. Yu, X. Yu, X. Ji, Electrochemical determination of the superoxide anion radical using a gold nanoparticle poly(3,4-ethylenedioxythiophene) ferrocyanide multiwalled carbon nanotube glassy carbon electrode, *Anal. Lett.* 51 (2018) 1529–1543, <https://doi.org/10.1080/00032719.2017.1381107>.
- [149] Y. Li, L. Shi, X. Cai, H. Zhao, X. Niu, M. Lan, Construction of non-enzymatic sensor based on porous carbon matrix loaded with Pt and Co nanoparticles for real-time monitoring of cellular superoxide anions, *Electrochim. Acta* 294 (2019) 304–311, <https://doi.org/10.1016/j.electacta.2018.10.105>.
- [150] A. Olean-Oliveira, J.C. Pacheco, P.M. Seraphim, M.F.S. Teixeira, Synergistic effect of reduced graphene oxide/azo-polymer layers on electrochemical performance and application as nonenzymatic chemiresistor sensors for detecting superoxide anion radicals, *J. Electroanal. Chem.* 852 (2019) 113520, <https://doi.org/10.1016/j.jelechem.2019.113520>.
- [151] J.J. Hu, N.-K. Wong, S. Ye, X. Chen, M.-Y. Lu, A.Q. Zhao, Y. Guo, A.C.-H. Ma, A.Y.-H. Leung, J. Shen, D. Yang, Fluorescent probe HKSOX-1 for imaging and detection of endogenous superoxide in live cells and in vivo, *J. Am. Chem. Soc.* 137 (2015) 6837–6843, <https://doi.org/10.1021/jacs.5b01881>.
- [152] J. Wang, L. Liu, W. Xu, Z. Yang, Y. Yan, X. Xie, Y. Wang, T. Yi, C. Wang, J. Hua, Mitochondria-targeted ratiometric fluorescent probe based on diketopyrrolopyrrole for detecting and imaging of endogenous superoxide anion in vitro and in vivo, *Anal. Chem.* 91 (2019) 5786–5793, <https://doi.org/10.1021/acs.analchem.9b00014>.
- [153] H. Ma, M. Yang, C. Zhang, Y. Ma, Y. Qin, Z. Lei, L. Chang, L. Lei, T. Wang, Y. Yang, Aggregation-induced emission (AIE)-active fluorescent probes with multiple binding sites toward ATP sensing and live cell imaging, *J. Mater. Chem. B* 5 (2017) 8525–8531, <https://doi.org/10.1039/C7TB02399E>.
- [154] J. Zhang, C. Li, R. Zhang, F. Zhang, W. Liu, X. Liu, S.M.-Y. Lee, H. Zhang, A phosphinate-based near-infrared fluorescence probe for imaging the superoxide radical anion in vitro and in vivo, *Chem. Commun.* 52 (2016) 2679–2682, <https://doi.org/10.1039/C5CC09976E>.
- [155] X. Lu, Z. Chen, X. Dong, W. Zhao, Water-soluble fluorescent probe with dual mitochondria/lysosome targetability for selective superoxide detection in live cells and in zebrafish embryos, *ACS Sens.* 3 (2018) 59–64, <https://doi.org/10.1021/acssensors.7b00831>.
- [156] M.P. Murphy, A. Holmgren, N.-G. Larsson, B. Halliwell, C.J. Chang, B. Kalyanaraman, S.G. Rhee, P.J. Thornalley, L. Partridge, D. Gems, T. Nyström, V. Belousov, P.T. Schumacker, C.C. Winterbourn, Unraveling the biological roles of reactive oxygen species, *Cell Metabol.* 13 (2011) 361–366, <https://doi.org/10.1016/j.cmet.2011.03.010>.
- [157] J.J. Gao, K.H. Xu, B. Tang, L.L. Yin, G.W. Yang, L.G. An, Selective detection of superoxide anion radicals generated from macrophages by using a novel fluorescent probe, *FEBS J.* 274 (2007) 1725–1733, <https://doi.org/10.1111/j.1742-4658.2007.05720.x>.
- [158] W. Zhang, P. Li, F. Yang, X. Hu, C. Sun, W. Zhang, D. Chen, B. Tang, Dynamic and reversible fluorescence imaging of superoxide anion fluctuations in live cells and in vivo, *J. Am. Chem. Soc.* 135 (2013), 14956 <https://doi.org/10.1021/ja408524j>, –14959.
- [159] H. Rahmani, F. Ghavamipour, R.H. Sajedi, Bioluminescence detection of superoxide anion using aequorin, *Anal. Chem.* 91 (2019) 12768–12774, <https://doi.org/10.1021/acs.analchem.9b02293>.
- [160] K. Thandavan, S. Gandhi, S. Sethuraman, J.B.B. Rayappan, U.M. Krishnan, A novel nano-interfaced superoxide biosensor, *Sensor. Actuator. B Chem.* 176 (2013) 884–892, <https://doi.org/10.1016/j.snb.2012.09.031>.
- [161] J. Di, S. Peng, C. Shen, Y. Gao, Y. Tu, One-step method embedding superoxide dismutase and gold nanoparticles in silica sol-gel network in the presence of cysteine for construction of third-generation biosensor, *Biosens. Bioelectron.* 23 (2007) 88–94, <https://doi.org/10.1016/j.bios.2007.03.011>.
- [162] S. Rajesh, A.K. Kanugula, K. Bhargava, G. Ilavazhagan, S. Kotamraju, C. Karunakaran, Simultaneous electrochemical determination of superoxide anion radical and nitrite using Cu,ZnSOD immobilized on carbon nanotube in polypyrrole matrix, *Biosens. Bioelectron.* 26 (2010) 689–695, <https://doi.org/10.1016/j.bios.2010.06.063>.
- [163] L. Wang, W. Wen, H. Xiong, X. Zhang, H. Gu, S. Wang, A novel amperometric biosensor for superoxide anion based on superoxide dismutase immobilized on gold nanoparticle-chitosan-ionic liquid biocomposite film, *Anal. Chim. Acta* 758 (2013) 66–71, <https://doi.org/10.1016/j.aca.2012.10.050>.
- [164] X. Zhu, X. Niu, H. Zhao, J. Tang, M. Lan, Immobilization of superoxide dismutase on Pt-Pd/MWCNTs hybrid modified electrode surface for superoxide anion detection, *Biosens. Bioelectron.* 67 (2015) 79–85, <https://doi.org/10.1016/j.bios.2014.07.004>.
- [165] J. Tang, X. Zhu, X. Niu, T. Liu, H. Zhao, M. Lan, Amperometric superoxide anion radical biosensor based on SOD/PtPd-PDARG modified electrode, *Talanta* 137 (2015) 18–24, <https://doi.org/10.1016/j.talanta.2015.01.009>.
- [166] R.E. Ozel, G. Bulbul, J. Perez, N. Pourmand, Functionalized quartz nanopipette for intracellular superoxide sensing: a tool for monitoring reactive oxygen species levels in single living cell, *ACS Sens.* 3 (2018) 1316–1321, <https://doi.org/10.1021/acssensors.8b00185>.
- [167] M.M. Doran, N.J. Finnerty, J.P. Lowry, In-vitro development and characterisation of a superoxide dismutase-based biosensor, *ChemistrySelect* 2 (2017) 4157–4164, <https://doi.org/10.1002/slct.201700793>.
- [168] Q. Ye, W. Li, Z. Wang, L. Zhang, X. Tan, Y. Tian, Direct electrochemistry of superoxide dismutases (Mn-, Fe-, and Ni-) from human pathogen *Clostridium difficile*: toward application to superoxide biosensor, *J. Electroanal. Chem.* 729 (2014) 21–26, <https://doi.org/10.1016/j.jelechem.2014.06.029>.
- [169] M. Braik, M.M. Barsan, C. Dridi, M. Ben Ali, C.M.A. Brett, Highly sensitive amperometric enzyme biosensor for detection of superoxide based on conducting polymer/CNT modified electrodes and superoxide dismutase, *Sensor. Actuator. B Chem.* 236 (2016) 574–582, <https://doi.org/10.1016/j.snb.2016.06.032>.
- [170] M.D. Evans, M. Dizdaroğlu, M.S. Cooke, Oxidative DNA damage and disease: induction, repair and significance, *Mutat. Res. Mutat. Res.* 567 (2004) 1–61, <https://doi.org/10.1016/j.mrrev.2003.11.001>.
- [171] A. Catalá, A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids, *Biochem. Biophys. Res. Commun.* 399 (2010) 318–323, <https://doi.org/10.1016/j.bbrc.2010.07.087>.
- [172] E. Cabiscol, J. Tamarit, J. Ros, Oxidative stress in bacteria and protein damage by reactive oxygen species, *Int. Microbiol.* 3 (2000) 3–8, <http://www.ncbi.nlm.nih.gov/pubmed/10963327>.
- [173] J.M. McCord, Free radicals and inflammation: protection of synovial fluid by superoxide dismutase, *Science* 185 (1974) 529–531 <https://doi.org/10.1126/science.185.4150.529>, 80–.
- [174] J.-G. Ren, H.-L. Xia, T. Just, Y.-R. Dai, Hydroxyl radical-induced apoptosis in human tumor cells is associated with telomere shortening but not telomerase inhibition and caspase activation, *FEBS Lett.* 488 (2001) 123–132, [https://doi.org/10.1016/S0014-5793\(00\)002377-2](https://doi.org/10.1016/S0014-5793(00)002377-2).
- [175] S. Pennathur, J.D. Wagner, C. Leeuwenburgh, K.N. Litwak, J.W. Heinecke, A hydroxyl radical-like species oxidizes cynomolgus monkey artery wall proteins in early diabetic vascular disease, *J. Clin. Invest.* 107 (2001) 853–860, <https://doi.org/10.1172/JCI11194>.
- [176] L. Fu, A. Wang, F. Lyu, G. Lai, J. Yu, C.-T. Lin, Z. Liu, A. Yu, W. Su, A solid-state electrochemical sensing platform based on a supramolecular hydrogel, *Sensor. Actuator. B Chem.* 262 (2018) 326–333, <https://doi.org/10.1016/j.snb.2018.02.029>.
- [177] L. Yuan, W. Lin, J. Song, Ratiometric fluorescent detection of intracellular hydroxyl radicals based on a hybrid coumarin–cyanine platform, *Chem. Commun.* 46 (2010) 7930, <https://doi.org/10.1039/c0cc02390f>.
- [178] J.-Y. Wang, Z.-R. Liu, M. Ren, X. Kong, K. Liu, B. Deng, W. Lin, A fast-responsive turn on fluorescent probe for detecting endogenous hydroxyl radicals based on a hybrid carbazole–cyanine platform, *Sensor. Actuator. B Chem.* 236 (2016) 60–66, <https://doi.org/10.1016/j.snb.2016.04.163>.
- [179] P. Li, T. Xie, X. Duan, F. Yu, X. Wang, B. Tang, A new highly selective and sensitive assay for fluorescence imaging of $\cdot OH$ in living cells: effectively avoiding

- the interference of peroxyxynitrite, *Chem. Eur. J.* 16 (2010) 1834–1840, <https://doi.org/10.1002/chem.200901514>.
- [180] N.B. Yapici, S. Jockusch, A. Moscatelli, S.R. Mandalapu, Y. Itagaki, D.K. Bates, S. Wiseman, K.M. Gibson, N.J. Turro, L. Bi, New rhodamine nitroxide based fluorescent probes for intracellular hydroxyl radical identification in living cells, *org. Lett.* 14 (2012) 50–53, <https://doi.org/10.1021/ol202816m>.
- [181] Y. Manevich, K.D. Held, J.E. Biaglow, Coumarin-3-Carboxylic acid as a detector for hydroxyl radicals generated chemically and by gamma radiation, *Radiat. Res.* 148 (1997) 580, <https://doi.org/10.2307/3579734>.
- [182] H. Li, X. Li, W. Shi, Y. Xu, H. Ma, Rationally designed fluorescence . OH probe with high sensitivity and selectivity for monitoring the generation of . OH in iron autoxidation without addition of H₂O₂, *Angew. Chem. Int. Ed.* 57 (2018) 12830–12834, <https://doi.org/10.1002/anie.201808400>.
- [183] G. Cui, Z. Ye, J. Chen, G. Wang, J. Yuan, Development of a novel terbium(III) chelate-based luminescent probe for highly sensitive time-resolved luminescence detection of hydroxyl radical, *Talanta* 84 (2011) 971–976, <https://doi.org/10.1016/j.talanta.2011.02.051>.
- [184] K. Setskinali, Y. Urano, K. Kakinuma, H.J. Majima, T. Nagano, Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species, *J. Biol. Chem.* 278 (2003) 3170–3175, <https://doi.org/10.1074/jbc.M209264200>.
- [185] F. Liu, J. Du, D. Song, M. Xu, G. Sun, A sensitive fluorescent sensor for the detection of endogenous hydroxyl radicals in living cells and bacteria and direct imaging with respect to its ecotoxicity in living zebra fish, *Chem. Commun.* 52 (2016) 4636–4639, <https://doi.org/10.1039/C5CC10658C>.
- [186] X. Qu, W. Song, Z. Shen, A highly selective NIR fluorescent turn-on probe for hydroxyl radical and its application in living cell images, *Front. Chem.* 7 (2019), <https://doi.org/10.3389/fchem.2019.00598>.
- [187] T. Oka, S. Yamashita, M. Midorikawa, S. Saiki, Y. Muroya, M. Kamibayashi, M. Yamashita, K. Anzai, Y. Katsumura, Spin-trapping reactions of a novel gauchetype radical trapper G-CYPMPO, *Anal. Chem.* 83 (2011) 9600–9604, <https://doi.org/10.1021/ac2023926>.
- [188] E. Jabeen, N.K. Janjua, S. Ahmed, E. Domínguez-Álvarez, C. Jacob, A selective and sensitive monitoring of the OH radical using flavonoid-modified electrodes, *Electrochim. Acta* 258 (2017) 228–235, <https://doi.org/10.1016/j.electacta.2017.10.065>.
- [189] G. Stein, J. Weiss, Detection of free hydroxyl radicals by hydroxylation of aromatic compounds, *Nature* 166 (1950) 1104–1105, <https://doi.org/10.1038/1661104a0>.
- [190] Y. Wu, W. Huang, D. Peng, X. Huang, J. Gu, S. Wu, T. Deng, F. Liu, Synthesis of dihydroquinolines as scaffolds for fluorescence sensing of hydroxyl radical, *Org. Lett.* 23 (2021) 135–139, <https://doi.org/10.1021/acs.orglett.0c03852>.
- [191] S. Jindal Garima, S. Garg, I. Matai, G. Packirisamy, A. Sachdev, Dual-emission copper nanoclusters-based ratiometric fluorescent probe for intracellular detection of hydroxyl and superoxide anion species, *Microchim. Acta* 188 (2021) 13, <https://doi.org/10.1007/s00604-020-04683-z>.
- [192] Q. Li, T. Zhang, J. Chen, W. Ji, Y. Wei, In situ synthesis of fluorescent polydopamine polymer dots based on Fenton reaction for a multi-sensing platform, *J. Mater. Chem. B* 9 (2021) 5503–5513, <https://doi.org/10.1039/D1TB00764E>.
- [193] X. Jiao, Y. Li, J. Niu, X. Xie, X. Wang, B. Tang, Small-molecule fluorescent probes for imaging and detection of reactive oxygen, nitrogen, and sulfur species in biological systems, *Anal. Chem.* 90 (2018) 533–555, <https://doi.org/10.1021/acs.analchem.7b04234>.
- [194] X. Chen, F. Wang, J.Y. Hyun, T. Wei, J. Qiang, X. Ren, I. Shin, J. Yoon, Recent progress in the development of fluorescent, luminescent and colorimetric probes for detection of reactive oxygen and nitrogen species, *Chem. Soc. Rev.* 45 (2016) 2976–3016, <https://doi.org/10.1039/C6CS00192K>.
- [195] K.E. Uhrich, S.M. Cannizzaro, R.S. Langer, K.M. Shakesheff, Polymeric systems for controlled drug release, *Chem. Rev.* 99 (1999) 3181–3198, <https://doi.org/10.1021/cr940351u>.
- [196] X. Hai, Z. Guo, X. Lin, X. Chen, J. Wang, Fluorescent TPA@GQDs probe for sensitive assay and quantitative imaging of hydroxyl radicals in living cells, *ACS Appl. Mater. Interfaces* 10 (2018) 5853–5861, <https://doi.org/10.1021/acsami.7b16094>.
- [197] L. Zhang, R.-P. Liang, S.-J. Xiao, J.-M. Bai, L.-L. Zheng, L. Zhan, X.-J. Zhao, J.-D. Qiu, C.-Z. Huang, DNA-templated Ag nanoclusters as fluorescent probes for sensing and intracellular imaging of hydroxyl radicals, *Talanta* 118 (2014) 339–347, <https://doi.org/10.1016/j.talanta.2013.09.021>.
- [198] M. Zhuang, C. Ding, A. Zhu, Y. Tian, Ratiometric fluorescence probe for monitoring hydroxyl radical in live cells based on gold nanoclusters, *Anal. Chem.* 86 (2014) 1829–1836, <https://doi.org/10.1021/ac403810g>.
- [199] Z. Li, T. Liang, S. Lv, Q. Zhuang, Z. Liu, A rationally designed upconversion nanoprobe for in vivo detection of hydroxyl radical, *J. Am. Chem. Soc.* 137 (2015) 11179–11185, <https://doi.org/10.1021/jacs.5b06972>.
- [200] Y. Liu, Q. Jia, Q. Guo, A. Jiang, J. Zhou, In vivo oxidative stress monitoring through intracellular hydroxyl radicals detection by recyclable upconversion nanoprobe, *Anal. Chem.* 89 (2017) 12299–12305, <https://doi.org/10.1021/acs.analchem.7b03270>.
- [201] S. Liu, J. Zhao, K. Zhang, L. Yang, M. Sun, H. Yu, Y. Yan, Y. Zhang, L. Wu, S. Wang, Dual-emissive silica nanohybrid measurements of hydroxyl radicals using a coumarin-activated silica nanohybrid probe, *Analyst* 141 (2016) 2296–2302, <https://doi.org/10.1039/C5AN02261D>.
- [202] B. Ma, M. Lu, B.-Y. Yu, J. Tian, A galactose-mediated targeting nanoprobe for intracellular hydroxyl radical imaging to predict drug-induced liver injury, *RSC Adv.* 8 (2018) 22062–22068, <https://doi.org/10.1039/C8RA01424H>.
- [203] D. Zhou, H. Huang, Y. Wang, Y. Wang, Z. Hu, X. Li, A yellow-emissive carbon nanodot-based ratiometric fluorescent nanosensor for visualization of exogenous and endogenous hydroxyl radicals in the mitochondria of live cells, *J. Mater. Chem. B* 7 (2019) 3737–3744, <https://doi.org/10.1039/C9TB00289H>.
- [204] G.M. Ganea, P.E. Kolic, B. El-Zahab, I.M. Warner, Ratiometric Coumarin–Neutral red (CONER) nanoprobe for detection of hydroxyl radicals, *Anal. Chem.* 83 (2011) 2576–2581, <https://doi.org/10.1021/ac102874x>.
- [205] Y. Tong, X. Huang, M. Lu, B.-Y. Yu, J. Tian, Prediction of drug-induced nephrotoxicity with a hydroxyl radical and caspase light-up dual-signal nanoprobe, *Anal. Chem.* 90 (2018) 3556–3562, <https://doi.org/10.1021/acs.analchem.7b05454>.
- [206] M. Khoobchandani, K.K. Katti, A.R. Karikachery, V.C. Thiye, D. Srisimal, D.K. Dhurvas Mohandoss, R.D. Darshakumar, C.M. Joshi, K. V. Katti, New approaches in breast cancer therapy through green nanotechnology and nano-ayurvedic medicine – pre-clinical and pilot human clinical investigations, *Int. J. Nanomed.* 15 (2020) 181–197, <https://doi.org/10.2147/IJN.S219042>.
- [207] E.R. Evans, P. Bugba, V. Asthana, R. Drezek, Metallic nanoparticles for cancer immunotherapy, *Mater. Today Off.* 21 (2018) 673–685, <https://doi.org/10.1016/j.mattod.2017.11.022>.
- [208] D.Y. Paithankar, F.H. Sakamoto, W.A. Farinelli, G. Kosiratna, R.D. Blomgren, T.J. Meyer, L.J. Faupel, A.N.B. Kauvar, J.R. Lloyd, W.L. Cheung, W.D. Owczarek, A.M. Suwalska, K.B. Kochanska, A.K. Nawrocka, E.B. Paluchowska, K.M. Podolec, M.M. Pirowska, A.B. Wojas-Pelc, R.R. Anderson, Acne treatment based on selective photothermolysis of sebaceous follicles with topically delivered light-absorbing gold microparticles, *J. Invest. Dermatol.* 135 (2015) 1727–1734, <https://doi.org/10.1038/jid.2015.89>.
- [209] A.C. Anselmo, S. Mitragotri, A review of clinical translation of inorganic nanoparticles, *AAPS J.* 17 (2015) 1041–1054, <https://doi.org/10.1208/s12248-015-9780-2>.
- [210] N.R.S. Sibuyi, K.L. Moabelo, A.O. Fadaka, S. Meyer, M.O. Onani, A.M. Madiehe, M. Meyer, Multifunctional gold nanoparticles for improved diagnostic and therapeutic applications: a review, *Nanoscale Res. Lett.* 16 (2021) 174, <https://doi.org/10.1186/s11671-021-03632-w>.
- [211] Y. Wu, M.R.K. Ali, K. Chen, N. Fang, M.A. El-Sayed, Gold nanoparticles in biological optical imaging, *Nano Today* 24 (2019) 120–140, <https://doi.org/10.1016/j.nantod.2018.12.006>.
- [212] G. Lettre, M.O. Hengartner, Developmental apoptosis in *C. elegans*: a complex CEDnario, *Nat. Rev. Mol. Cell Biol.* 7 (2006) 97–108, <https://doi.org/10.1038/nrm1836>.
- [213] L. Ming, K. Cheng, Y. Chen, R. Yang, D. Chen, Enhancement of tumor lethality of ROS in photodynamic therapy, *Cancer Med.* 10 (2021) 257–268, <https://doi.org/10.1002/cam4.3592>.
- [214] P. Wang, J. Geng, J. Gao, H. Zhao, J. Li, Y. Shi, B. Yang, C. Xiao, Y. Linghu, X. Sun, X. Chen, L. Hong, F. Qin, X. Li, J.-S. Yu, H. You, Z. Yuan, D. Zhou, R.L. Johnson, L. Chen, Macrophage achieves self-protection against oxidative stress-induced ageing through the Mst1Rf2 axis, *Nat. Commun.* 10 (2019) 755, <https://doi.org/10.1038/s41467-019-08680-6>.
- [215] W.J. Sander, C. Fourie, S. Sabiu, F.H. O'Neill, C.H. Pohl, H.G. O'Neill, Reactive oxygen species as potential antiviral targets, *Rev. Med. Virol.* 32 (2022), <https://doi.org/10.1002/rmv.2240>.
- [216] S. Cho, O. Hwang, I. Lee, G. Lee, D. Yoo, G. Khang, P.M. Kang, D. Lee, Chemiluminescent and antioxidant micelles as theranostic agents for hydrogen peroxide associated-inflammatory diseases, *Adv. Funct. Mater.* 22 (2012) 4038–4043, <https://doi.org/10.1002/adfm.201200773>.
- [217] C.-K. Lim, Y.-D. Lee, J. Na, J.M. Oh, S. Her, K. Kim, K. Choi, S. Kim, I.C. Kwon, Chemiluminescence-Generating nanoreactor formulation for near-infrared imaging of hydrogen peroxide and glucose level in vivo, *Adv. Funct. Mater.* 20 (2010) 2644–2648, <https://doi.org/10.1002/adfm.201000780>.
- [218] D. Lee, S. Khajia, J. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. Taylor, N. Murthy, Organic nanoparticle bioimaging agents, *Synfacts* (2008) <https://doi.org/10.1055/s-2007-992400> (2007) 0042–0042.
- [219] D. Cui, J. Li, X. Zhao, K. Pu, R. Zhang, Semiconducting polymer nanoreporters for near-infrared chemiluminescence imaging of immunoactivation, *Adv. Mater.* 32 (2020) 1906314, <https://doi.org/10.1002/adma.201906314>.
- [220] V. Ntziachristos, J. Ripoll, L. V. Wang, R. Weissleder, Looking and listening to light: the evolution of whole-body photonic imaging, *Nat. Biotechnol.* 23 (2005) 313–320, <https://doi.org/10.1038/nbt1074>.
- [221] A.M. Smith, M.C. Mancini, S. Nie, Second window for in vivo imaging, *Nat. Nanotechnol.* 4 (2009) 710–711, <https://doi.org/10.1038/nnano.2009.326>.
- [222] J. Chan, S.C. Dodani, C.J. Chang, Reaction-based small-molecule fluorescent probes for chemoselective bioimaging, *Nat. Chem.* 4 (2012) 973–984, <https://doi.org/10.1038/nchem.1500>.
- [223] D. Soreescu, D. Weiss, B. Lassegue, R.E. Clempus, K. Szöcs, G.P. Soreescu, L. Valppu, M.T. Quinn, J.D. Lambeth, J.D. Vega, W.R. Taylor, K.K. Griendling, Superoxide production and expression of nox family proteins in human atherosclerosis, *Circulation* 105 (2002) 1429–1435, <https://doi.org/10.1161/01.CIR.0000012917.74432.66>.
- [224] T.J. Barrett, Macrophages in atherosclerosis regression, *Arterioscler. Thromb. Vasc. Biol.* 40 (2020) 20–33, <https://doi.org/10.1161/ATVBAHA.119.312802>.
- [225] Y. Zhang, S. Choksi, K. Chen, Y. Pobezinskaya, I. Linnoila, Z.-G. Liu, ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages, *Cell Res.* 23 (2013) 898–914, <https://doi.org/10.1038/cr.2013.75>.
- [226] M. Weber, N. Yamada, X. Tian, S.D. Bull, M. Minoshima, K. Kikuchi, A.B. Mackenzie, T.D. James, Sensing peroxyxynitrite in different organelles of murine RAW264.7 macrophages with coumarin-based fluorescent probes, *Front. Chem.* 8

- (2020), <https://doi.org/10.3389/fchem.2020.00039>.
- [227] E.J. Park, J.W. Song, H.J. Kim, C.-S. Kim, Y.J. Song, D.H. Yang, H. Yoo, J.W. Kim, K. Park, In vivo imaging of reactive oxygen species (ROS)-producing pro-inflammatory macrophages in murine carotid atheromas using a CD44-targetable and ROS-responsive nanosensor, *J. Ind. Eng. Chem.* 92 (2020) 158–166, <https://doi.org/10.1016/j.jiec.2020.08.034>.
- [228] P. Agostinis, K. Berg, K.A. Cengel, T.H. Foster, A.W. Girotti, S.O. Gollnick, S.M. Hahn, M.R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B.C. Wilson, J. Golab, Photodynamic therapy of cancer: an update, *CA, Cancer J. Clin.* 61 (2011) 250–281, <https://doi.org/10.3322/caac.20114>.
- [229] J. Ge, M. Lan, B. Zhou, W. Liu, L. Guo, H. Wang, Q. Jia, G. Niu, X. Huang, H. Zhou, X. Meng, P. Wang, C.-S. Lee, W. Zhang, X. Han, A graphene quantum dot photodynamic therapy agent with high singlet oxygen generation, *Nat. Commun.* 5 (2014) 4596, <https://doi.org/10.1038/ncomms5596>.
- [230] Q. Chen, L. Feng, J. Liu, W. Zhu, Z. Dong, Y. Wu, Z. Liu, Intelligent albumin-MnO₂ nanoparticles as pH-/H₂O₂-responsive dissociable nanocarriers to modulate tumor hypoxia for effective combination therapy, *Adv. Mater.* 28 (2016) 7129–7136, <https://doi.org/10.1002/adma.201601902>.
- [231] H. Fan, G. Yan, Z. Zhao, X. Hu, W. Zhang, H. Liu, X. Fu, T. Fu, X. Zhang, W. Tan, A smart photosensitizer–manganese dioxide nanosystem for enhanced photodynamic therapy by reducing glutathione levels in cancer cells, *Angew. Chem. Int. Ed.* 55 (2016) 5477–5482, <https://doi.org/10.1002/anie.201510748>.
- [232] P.N. Coneski, M.H. Schoenfish, Nitric oxide release: Part III. Measurement and reporting, *Chem. Soc. Rev.* 41 (2012) 3753–3758, <https://doi.org/10.1039/c2cs15271a>.
- [233] D. Mao, W. Wu, S. Ji, C. Chen, F. Hu, D. Kong, D. Ding, B. Liu, Chemiluminescence-guided cancer therapy using a chemiexcited photosensitizer, *Chem* 3 (2017) 991–1007, <https://doi.org/10.1016/j.chempr.2017.10.002>.
- [234] L. Kricka, Clinical applications of chemiluminescence, *Anal. Chim. Acta* 500 (2003) 279–286, [https://doi.org/10.1016/S0003-2670\(03\)00809-2](https://doi.org/10.1016/S0003-2670(03)00809-2).
- [235] L. Hu, G. Xu, Applications and trends in electrochemiluminescence, *Chem. Soc. Rev.* 39 (2010) 3275, <https://doi.org/10.1039/b923679c>.
- [236] Z. Zhou, J. Song, L. Nie, X. Chen, Reactive oxygen species generating systems meeting challenges of photodynamic cancer therapy, *Chem. Soc. Rev.* 45 (2016) 6597–6626, <https://doi.org/10.1039/C6CS00271D>.
- [237] J. Fu, T. Li, Y. Zhu, Y. Hao, Ultrasound-activated oxygen and ROS generation nanosystem systematically modulates tumor microenvironment and sensitizes sonodynamic therapy for hypoxic solid tumors, *Adv. Funct. Mater.* 29 (2019) 1906195, <https://doi.org/10.1002/adfm.201906195>.