Czech Technical University in Prague
Faculty of Electrical Engineering

Kateřina Červinková ~ *Doctoral Thesis*

September 2024

Czech Technical University in Prague
Faculty of Electrical Engineering ~ Department of Electromagnetic Field

Signal Modulators of the Ultra-Weak Photon Emission from Biological Cells

Doctoral Thesis

Ing. Kateřina Červinková

Prague, September 2024

Ph.D. Programme: Electrical Engineering and Information Technology P 2612
Branch of Study: Radioelectronics 2601V010

Supervisor: Prof. Ing. Jan Vrba, CSc.
Supervisor specialist: Ing. Michal Cifra, Ph.D.

Thesis Supervisor:

Prof. Ing. Jan Vrba, CSc.
Department of Electromagnetic Field
Faculty of Electrical Engineering
Czech Technical University in Prague
Technická 2
160 00, Prague 6
Czechia

Thesis Supervisor Specialist:

Ing. Michal Cifra, Ph.D.
Bioelectrodynamics Research Team
Institute of Photonics and Electronics
The Czech Academy of Sciences
Chaberská 1014/57
182 00, Praha 8
Czechia

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Prof. Jan Vrba and Dr. Michal Cifra, for their professional and scientific guidance, as well as their personal mentorship and encouragement throughout my research journey. I am also thankful to the Faculty of Electrical Engineering at the Czech Technical University in Prague and the Institute of Photonics and Electronics of the Czech Academy of Sciences for providing me with the opportunity to conduct research, gather knowledge, and develop my skills. I am deeply appreciative of the collaboration with the outstanding scientists and remarkable individuals at both institutions. I also express my big gratitude to Dr. Viliam Kolivoška for his excellent suggestions and contributions to this research. Furthermore, I am grateful for the opportunities to visit foreign institutions, specifically Technion - Israel Institute of Technology under the kind supervision of Prof. Ayelet Fishman, and Leiden University, where I was invited by Dr. Eduard van Wijk. I would like to extend my thanks Michaela, Jakub, Viktor, and Lukáš for being persistive friends and for sharing the challenges of our PhD studies together. Lastly, I would like to acknowledge and thank my family for their unwavering support.

Declaration of originality

I, the undersigned, hereby declare that this doctoral thesis is the result of my research in our research team and my contribution corresponds to that specified at the beginning of each research chapter. The thesis was written under the professional supervision of Dr. Michal Cifra and Prof. Jan Vrba, using the literature and resources listed in the Bibliography. I used AI technologies for proofreading and text rephrasing in this thesis.

In Prague, 1 September 2024 .....

Kateřina Červinková

Abstract

This thesis presents a comprehensive study of biological autoluminescence (BAL), exploring the mechanisms of its generation, the factors that modulate its properties, and the methods for its detection. Through a multidisciplinary approach that incorporates chemistry, biology, and physics, the thesis aims to deepen understanding of the oxidative processes within cells and the potential applications of BAL monitoring in various industrial and medical fields. The thesis is structured around several core investigations: it begins by establishing the theoretical foundations for BAL, detailing the kinetic models that describe the generation and modulation of reactive oxygen species (ROS) within biological systems. The rate constants critical to these models are compiled in this extend for the first time, providing a valuable resource for further research in this area. Experimental work forms the crux of the thesis, with studies conducted on different biological models, including yeast and human promyelocytic leukemia cells (HL-60), proteins and germinating seeds. These experiments assess the effects of various chemical, physical, biological, and environmental modulators on BAL. The ability of BAL to provide real-time, non-invasive insights into the oxidative status of cell, tissues and organisms presents a promising tool for early diagnosis and the evaluation of therapeutic strategies aimed at mitigating oxidative damage. In conclusion, the thesis not only advances the scientific understanding of BAL but also demonstrates its potential as a diagnostic and monitoring tool in diverse applications. The development of improved experimental protocols and the integration of BAL with kinetic modeling offer robust methodologies for future research, potentially transforming the way we detect and manage oxidative stress-related conditions.

Keywords

biological autoluminescence, ultra-weak photon emission, reactive oxygen species, oxidative stress, pulsed electric field, photomultiplier

Abstrakt

Tato práce představuje komplexní studii biologické autoluminiscence (BAL), zkoumá mechanismy jejího vzniku, faktory, které modulují její vlastnosti, a metody její detekce. Prostřednictvím multidisciplinárního přístupu, který zahrnuje chemii, biologii a fyziku, si práce klade za cíl prohloubit pochopení oxidačních procesů v buňkách a potenciálních aplikací monitorování BAL v různých průmyslových a lékařských oborech. Práce začíná stanovením teoretických základů pro BAL, popisuje kinetický model, který popisuje tvorbu a modulaci reaktivních forem kyslíku (ROS) v biologických systémech. Rychlostní konstanty kritické pro tento model jsou v tomto rozsahu sestaveny poprvé, což poskytuje cenný zdroj pro další výzkum v této oblasti. Experimentální práce tvoří jádro práce se studiemi prováděnými na různých biologických modelech, včetně kvasinkových a lidských promyelocytárních leukemických buněk (HL-60), proteinů a klíčících semen. Tyto experimenty hodnotí účinky různých chemických, fyzikálních, biologických a environmentálních modulátorů na BAL. Schopnost BAL poskytovat v reálném čase neinvazivní pohled na oxidační stav buněk, tkání a organismů představuje slibný nástroj pro včasnou diagnostiku a hodnocení terapeutických strategií zaměřených na zmírnění oxidačního poškození. Práce nejen posouvá vědecké chápání BAL, ale také demonstruje její potenciál jako diagnostického a monitorovacího nástroje v různých aplikacích. Vývoj vylepšených experimentálních protokolů a integrace BAL s kinetickým modelováním nabízí robustní metodiky pro budoucí výzkum a potenciálně může měnit způsob, jakými se detekují a ovlivňujípodmínky související s oxidačním stresem.

Klíčová slova

biologická autoluminiscence, ultra-slabá emise fotonů, reaktivní formy kyslíku, oxidativní stres, pulzní elektrické pole, fotonásobič

CHEMICAL ABBREVIATIONS

4-HNE	4-hydroxynonenal
8-oxo-Gua	8-hydroxyguanine
ANS	8-anilino-1-naphthalenesulfonic acid
APX	ascorbate peroxidase
$AscH^-$	ascorbate
ATP	adenosine triphosphate
ATRA	all-trans retinoic acid
BSA	bovine serum albumin
$^1C^*$	singlet excited chromophore
$^3C^*$	triplet excited chromophore
Ca	calcium
CAT	catalase
Cd	cadmium
$^1Chl^*$	singlet excited chlorophyll
CoA	coenzyme A
Cu	copper
DNA	deoxyribonucleic acid
DUOX	dual oxidase
EDTA	ethylenediaminetetraacetic acid
EPO	eosinophyl peroxidase
Fe	iron
GPx	glutathione peroxidase
HClO	hypochlorous acid
H_2O_2	hydrogen peroxide
$HO^\bullet$	hydroxyl radical
$HO_2^\bullet$	hydroperoxyl radical
HOCl	hypochlorous acid
LH	lipid
$^3(L = O)^*$	lipid-derived triplet carbonyl
MDA	malondialdehyde
Mg	magnesium
Mn	manganese

MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate
Ni	nickel
NO	nitric oxide
NOS	nitric oxide synthase
NOX	nicotinamide adenine dinucleotide phosphate oxidase
$^{1}O_2$	singlet oxygen
$^{3}O_2$	molecular oxygen
O_3	ozone
$O_2^{\bullet -}$	superoxide anion radical
$ONOO^-$	peroxynitrite
PI	propidium iodide
PMA	phorbol 12-myristate 13-acetate
RNA	ribonucleic acid
$^{3}(R = O)^*$	triplet excited carbonyl
$R^\bullet$	alkyl radical
RH	biomolecule
$RO^\bullet$	alkoxyl radical
$ROO^\bullet$	peroxyl radical
ROOH	hydroperoxide
ROOR	dioxetane
ROOOOR	tetraoxide
ROS	reactive oxygen species
-SH	thiol group
SOD	superoxide dismutase
XO	xantine oxidase
Zn	zinc

ABBREVIATIONS

BAL	biological autoluminescence
CCD	charge-coupled device
CMV	cucumber mosaic virus
CMV-L	cucumber mosaic virus, yellow strain
CMV-Y	cucumber mosaic virus, legume strain
DLS	dynamic light scattering
EMCCD	electron multiplying charge-coupled device
EPR	electron paramagnetic resonance
ETC	electron transport chain
FBS	fetal bovine serum
GMP	good manufacturing practices
HEPA	high-efficiency particulate air filter
HL-60	human promyelocytic leukemia
ICP-OES	inductively coupled plasma optical emission spectroscopy
IR	infrared
LCR	inductance, capacitance, resistance
PB	phosphate buffer
PEF	pulsed electric field
pI	isoelectric point
PMNL	polymorphonuclear leukocytes
PMT	photomultiplier
QE	quantum efficiency
RF	radio-frequency
RPMI	medium developed at Roswell Park Memorial Institute
UPE	ultra-weak photon emission
UV	ultra-violet
YPD	Yeast extract-Peptone-Dextrose

Contents

1 Introduction: Electromagnetic activity of cells

The study of electromagnetic activity in living cells is a dynamic and evolving field that intersects with various branches of biology, physics, and medicine. Electromagnetic phenomena in cells encompass a broad spectrum, from low-frequency ionic currents essential for cellular communication and function, to the more speculative and less understood high-frequency activities in the radio-frequency and millimeter-wave ranges. Kučera et al. [1] provided a comprehensive overview of this multifaceted topic, highlighting the well-established mechanisms, such as those governing action potentials in neurons and muscle cells, and delving into more contentious areas where experimental evidence remains sparse and theoretical models are still under development.

Electromagnetic activity in cells is pivotal for various physiological processes, including signaling [2], and transport [3]. At low frequencies, below 1 kHz, the generation and propagation of ionic currents across cellular membranes are well-documented, underpinned by established biophysical principles. These low-frequency activities are crucial for maintaining cellular homeostasis and enabling rapid communication within and between cells. Conversely, the potential roles of electromagnetic processes in the radio-frequency and millimeter-wave ranges are less clear, with ongoing debates about their existence and implications. Despite these uncertainties, there is significant interest in understanding these higher frequency phenomena, driven by their potential to revolutionize diagnostic and therapeutic approaches in medicine.

In addition to low-frequency and high-frequency electromagnetic phenomena, visible electromagnetic radiation also plays a significant role in cellular activities. Cells can emit and interact with visible light, a fact that underpins various biological and medical technologies. Bioluminescence, for instance, is a well-known phenomenon where certain cells produce visible light through chemical reactions. This natural emission of light is harnessed in scientific research, particularly in the study of gene expression and cellular processes through the use of luminescent markers.

Moreover, cells can respond to visible light, a property utilized in techniques such as optogenetics. Optogenetics involves the use of light to control cells within living tissue, typically neurons, that have been genetically modified to express light-sensitive ion channels. This method has revolutionized neuroscience by allowing precise control over neuronal activity, thereby enabling the detailed study of neural circuits and behavior.

Understanding the full spectrum of electromagnetic activity in cells could unveil new diagnostic tools and therapeutic methods, leveraging both endogenous electromagnetic fields and externally applied electromagnetic stimuli. Such advancements could lead to innovative treatments that modulate cellular functions in ways previously unimagined. Kučera et al. [1] emphasize the importance of continued research in this field, advocating for rigorous experimental studies and the development of robust theoretical frameworks to elucidate these complex and often elusive electromagnetic processes within living cells.

1.1 Sub kHz range

The bioelectromagnetic phenomena in cells, particularly in the range up to hundreds of Hz, are well understood and result from electrochemical potentials across biological membranes. These potentials are established by ion concentration differences, maintained by ion channels and pumps within the lipid bilayer. Nonexcitable cells keep a stable electrostatic potential, while excitable cells like neurons can generate action potentials for signaling. Although fluctuations in membrane potential may generate low-power electromagnetic radiation, it is usually attenuated by physiological buffers, being considered as low-frequency electric fields [4, 5]

1.2 Radio-frequency and lower THz range

The exploration of electromagnetic activity in living cells extends into the radio-frequency (RF) and lower terahertz (THz) range, an area that remains highly speculative and controversial within the scientific community. This frequency range, which lies between 3 kHz and 3 THz, encompasses a variety of electromagnetic waves that are less understood compared to their low-frequency and optical counterparts. The limited experimental evidence and the absence of a universally accepted theoretical framework make the study of RF and lower THz bio-electromagnetic processes particularly challenging. Despite these challenges, the potential applications of understanding these high-frequency phenomena are significant. There is ongoing research into the effects of RF and THz radiation on cellular functions, with implications for both diagnostic and therapeutic technologies. For instance, RF and THz waves could potentially influence cellular activities such as gene expression [6, 7], protein folding [8], and have notable effects on microtubules, which are crucial for cell division. This interaction is particularly relevant in cancer research, where disrupting microtubule function could hinder the proliferation of cancer cells [9, 10]. However, the precise mechanisms by which these frequencies interact with biological tissues remain largely unknown, necessitating further experimental validation and theoretical development.

1.3 Infrared radiation

Infrared (IR) radiation, spanning wavelengths from approximately 700 nm to 1 mm, originates primarily from the thermal energy produced by cellular metabolic and biochemical processes. Cellular processes, such as respiration and energy production, generate heat, which in turn emits IR radiation. Mitochondria, play a crucial role in this context as they are involved in ATP production through oxidative phosphorylation, a process that releases energy in the form of heat. This heat emission can be detected as IR radiation, providing a window into cellular energy dynamics and metabolic states. It plays a critical role in monitoring the physiological state of organisms, such as tracking body temperature for fever screening. This natural emission of IR can be harnessed for various biomedical applications including imaging [11, 12] and therapy [13, 14].

1.4 Visible range

Visible light is the portion of the electromagnetic spectrum that can be seen by the human eye. It ranges in wavelength from approximately 380 to 700 nm. This spectrum of light is essential for life on Earth, as it provides the energy that drives many biological processes.

For organisms, visible light plays a critical role. In plants, it powers photosynthesis, the process by which they convert light energy into chemical energy, producing oxygen and food. For animals, including humans, light regulates circadian rhythms, and is necessary for vision, enabling us to perceive the world around us. Additionally, many organisms rely on light for navigation, communication, and even mating behaviors.

Thus, visible light is not only vital for energy production but also influences the behavior and survival of organisms across ecosystems. Beside that, some organisms have a remarkable ability to produce light through chemical reactions within their bodies, known as bioluminescence and all living organisms emit a faint form of light known as biological autoluminescence.

1.4.1 Bioluminescence

In a process known as bioluminescence, some organisms produce light as a results from a chemical reaction that converts chemical energy directly into light energy. The key components of bioluminescence are specific organic molecules called luciferins, which are light-emitting compounds, and enzymes known as luciferases, which catalyze the reaction. When luciferin interacts with oxygen in the presence of luciferase, this chemical reaction releases energy in the form of photons, producing light without significant heat. This natural phenomenon occurs in a wide range of organisms, including certain species of bacteria, fungi, insects (like fireflies), and marine animals (such as jellyfish and deep-sea fish).

Bioluminescence plays well-defined roles in communication and adaptation among various species. For instance, it enables organisms to signal each other in the dark, providing advantages for mating [15,16], warning off predators [17], or even locating and attracting prey [18].

1.4.2 Biological auto-luminescence

Biological auto-luminescence (BAL) represents a phenomenon that has garnered considerable interest across diverse scientific fields, including biophysics, biochemistry, biomedical research, food science, agriculture, and environmental studies. BAL is characterized by the spontaneous emission of photons from biological cells and tissues without external light stimulation, typically at rates as low as a few photons $\cdot$ s^{-1} $\cdot$ cm^{-2}. In case of induced BAL the intensity can be up to thousands photons $\cdot$ s^{-1} $\cdot$ cm^{-2} [19]. This emission is intrinsically linked to oxidative metabolic processes and the presence of reactive oxygen species (ROS) within cells.

Unlike emissions from specialized structures or processes, such as the luciferin-luciferase protein complex that generates the intense bioluminescence, as described higher 1.4.1, BAL results from a broad array of general

biochemical reactions. These reactions are both universal and relatively rare, contributing to the exceedingly low intensity of the emitted light. Understanding the processes behind BAL and the factors that modulate its signal—such as chemical, physical, environmental, and biological influences—is paramount for advancing both basic and applied sciences, although its specific functions within the cell are not yet confirmed [20].

Insights into these underlying mechanisms can reveal how variations in cellular oxidative stress, metabolic states, and external conditions impact photon emission. By elucidating the factors that affect the intensity and spectral properties of BAL, researchers can better interpret the subtle signals emitted by cells and tissues. BAL has promising applications in monitoring the oxidative state of organic systems. By detecting the low-level light emission inherent in these systems, BAL can provide real-time insights into oxidative stress and related metabolic processes. Understanding how BAL relates to oxidative stress and metabolic changes can provide valuable insights into the pathogenesis of various diseases, including cancer, neurodegenerative disorders, and cardiovascular conditions. Furthermore, knowledge of how different factors modulate BAL can lead to innovations in fields ranging from environmental monitoring to food safety, ultimately contributing to a more comprehensive grasp of biological processes and their broader implications. A significant advantage of BAL is that it has no phototoxicity, making it a non-invasive and safe method for continuous monitoring of living organisms without causing damage or interfering with their natural states. This makes BAL particularly valuable in medical diagnostics and practices in the basic research where maintaining the integrity of the sample is crucial.

1.5 Extremely high frequencies

Extremely high frequencies, including ultra-violet (UV) radiation and beyond, are generally not associated with biological electromagnetic activity due to the high energy, which can damage biological molecules such as DNA. While UV photons can cause molecular excitation and rearrangement, leading to the production of reactive oxygen species and potential cellular damage, frequencies higher than visible light, such as X-rays and gamma rays, are known as ionizing radiation because they carry enough energy to ionize atoms, causing significant molecular damage. However, the energy associated with these high-frequency photons makes them unlikely to be produced by biological systems, which typically operate at much lower energy levels.

The introductory chapter drew upon the published paper:

Kučera, O., Červinková, K., Nerudová, M., and Cifra, M.

Spectral perspective on the electromagnetic activity of cells

Current topics in medicinal chemistry, 15(6), 513-522, 2015.

DOI:10.2174/1568026615666150225103105

2 State of the art

2.1 Terminology

Over the years of research on this topic, various terms have been used to describe the phenomenon. The title of this thesis contains the term "ultra-weak photon emission" (UPE), which emphasizes the extremely low intensity and photon-like nature of the emitted light. However, it does not specify the light's origin. This term still remains widely used in research conducted by other groups and appears frequently in scientific literature [19, 21–29]. However, with the progress made by our research group over the years, we have introduced the term "biological autoluminescence" (BAL), which we find more suitable and informative for our scientific perspective. The term "luminescence" indicates that the light emission is not due to thermal processes, "auto" signifies that the luminescence arises from substances within the biological sample itself without the need for external luminescence probes, and "biological" denotes that the emission originates from biological systems or samples containing biomolecules.

Other terms used to describe this phenomenon include "endogenous chemiluminescence [30]," "low-level chemiluminescence [31–34]," "spontaneous chemiluminescence [35]," "auto-luminescence [36, 37]," " and "biophoton emission [38–45]," with each term reflecting the perspective of the research group using it. The choice of terminology often conveys the specific viewpoint and emphasis of the researchers on the nature and origin of the light emission. During different stages of PhD studies, the candidate also used some these various terms.

In this thesis, the candidate consistently uses the term "biological autoluminescence" (BAL), although she acknowledges that this terminology may not always be entirely appropriate. Specifically, this issue arises when photons are detected from non-biological samples, such as buffers, or when the origin of detected photons lies in electrogenerated reactive oxygen species (ROS) and their reactions with proteins, as described in chapter 6. In such cases, the more accurate term would be "chemiluminescence," or "biochemiluminescence." The decision to use BAL throughout the thesis, despite its inaccuracy, is driven by the need for consistency and clarity. The primary focus of this work is on biological systems, where BAL is the relevant and commonly understood term. By maintaining a single term, the candidate aims to create a cohesive narrative and avoid potential confusion that might arise from switching between different terminologies. The candidate hopes the reader will understand that this choice was made to facilitate a more focused and coherent exploration of the topic, even if it occasionally sacrifices terminological precision.

2.2 Mechanisms of biological autoluminescence

The phenomenon of electromagnetic radiation emission in the visible spectrum from biological systems was first described by Fritz-Albert Popp, who coined the term "biophotons" in the 1970s. Initially, Popp believed this radiation originated from DNA. However, photon emission is now considered to be associated with biochemical reactions within cells [44].

Biological autoluminescence (BAL) is the release of light that occurs during oxidation-reduction reactions within the metabolic processes of living organisms and during the oxidation of biomolecules [46]. BAL can be classified into two types: spontaneous and induced. Spontaneous BAL occurs naturally during oxidative metabolism, without any external influence, and is a normal part of the metabolic processes that maintain cellular function. In contrast, induced BAL is triggered by external stressors and oxidative factors [19]. These stressors can include biological agents (Chapter 2.6), such as bacteria or viruses, which activate the immune system and increase BAL emission. Chemical compounds (Chapter 2.4) that initiate or enhance oxidation-reduction reactions, as well as environmental factors (Chapter 2.4) like pollutants and salinity, can also cause induced BAL. Additionally, physical stressors (Chapter 2.5) such as radiation, magnetic fields, and electric fields can disrupt cellular homeostasis and lead to oxidative stress, resulting in higher BAL levels. Understanding the mechanisms behind BAL and the triggers that influence it is essential for studying cellular processes and evaluating the effects of external factors on biological systems.

The current understanding of BAL highlights its close association with reactive oxygen species (ROS) [46]. As the therm clearly indicates, ROS are intimately connected to oxygen. Oxygen is essential for organisms because it is a critical component of cellular respiration, the process by which cells generate energy. In aerobic organisms, oxygen serves as the final electron acceptor in the electron transport chain, a series of reactions that produce adenosine triphosphate (ATP), the primary energy "currency" of the cell. This process occurs in the mitochondria and is significantly more efficient at generating ATP compared to anaerobic pathways. Oxygen's ability to support high energy production enables complex multicellular life forms to perform various biological functions, from muscle contraction to neural activity. Moreover, oxygen is involved in the synthesis of essential biomolecules and the detoxification of harmful substances. However, its reactive nature means that organisms must also have mechanisms to mitigate potential oxidative damage, underscoring oxygen as both a vital resource and a potential threat to cellular integrity [47].

In biological systems, the reaction described in equation 2.1 illustrates the reduction of molecular oxygen ($^3O_2$) to the superoxide anion radical ($O_2^{\bullet-}$), a crucial step in the formation of ROS. Molecular oxygen, denoted as $^3O_2$ exists in its ground state as triplet oxygen, which is the most stable form under standard conditions. This form of oxygen is characterized by two unpaired electrons in its outermost molecular orbitals, making it a diradical. Because of this, triplet oxygen is relatively unreactive compared to other oxygen species.

The one-electron reduction of molecular oxygen $^3O_2$ to superoxide anion ($O_2^{\bullet-}$) is a fundamental process in biological redox reactions and is pivotal in generating ROS. $O_2^{\bullet-}$ itself is not only reactive but also serves

as a precursor for other ROS, such as hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and peroxynitrite ($ONOO^-$). These reactive species are involved in various cellular processes, including cell signaling and immune responses. However, excessive production of ROS can lead to oxidative stress, which causes damage to biomolecules like DNA, proteins, and lipids, contributing to diseases and aging. The reaction outlined in equation 2.1 thus represents a key step in both normal cellular function and pathological processes driven by oxidative stress.

$$^3O_2 + e^- \rightarrow O_2^{\bullet -} \tag{2.1}$$

2.2.1 Reactive oxygen species

As touched on earlier (Chapter 2.2), ROS are chemically reactive molecules that serve dual functions in cellular physiology, acting both as essential signaling molecules under normal conditions and as damaging agents under oxidative stress. These molecules are produced within cells through both enzymatic and non-enzymatic pathways. Enzymatic sources include various cellular enzymes involved in metabolic activities, such as mitochondrial respiration and NADPH oxidase activity. Non-enzymatic production occurs through spontaneous reactions and interactions with environmental factors, such as UV radiation and pollutants. ROS can be generated at multiple sites within the cell, utilizing available oxygen.

ROS can be classified into two groups: radical ROS and non-radical ROS. Radical ROS molecules always contain at least one unpaired electron, making them highly reactive and capable of initiating chain reactions that can lead to cellular damage. Examples of radical ROS include superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^\bullet$), hydroperoxyl radical ($HO_2^\bullet$), peroxyl radical ($ROO^\bullet$), and alkoxyl radical ($RO^\bullet$). These radicals are known for their high reactivity and potential to cause significant cellular damage. Non-radical ROS, on the other hand, do not contain unpaired electrons but can still be highly reactive and contribute to oxidative stress. Examples include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and ozone (O_3). Both types of ROS play significant roles in cellular signaling and homeostasis.

Enzymatic production of ROS

Enzymatic activity is a fundamental source of ROS. Enzymes such as NADPH oxidases, xanthine oxidase, and mitochondrial electron transport chain (ETC) complexes are primary contributors to ROS generation, producing these reactive molecules as by-products of their catalytic activities.

Mitochondria primarily produce $O_2^{\bullet -}$ through **electron transport chain** during cellular respiration, that is essential for energy production in eukaryotic cells, generating ATP in the process. It involves a series of transmembrane protein complexes (I-IV) and the freely mobile electron transfer carriers ubiquinone and cytochrome c [48, 49]. It must be assembled into a specifically configured supercomplex to function properly [50]. During normal ATP generation, the ETC produces $O_2^{\bullet -}$ as natural byproducts. However, under stressful conditions, the

over-reduction of the ETC can lead to excessive ROS production, which can trigger oxidative damage and even programmed cell death [51, 52].

NADPH oxidases (NOX/DUOX enzymes) are membrane-bound enzyme complexes that catalyze the transfer of electrons from NADPH to molecular oxygen, resulting in the reduction of oxygen to the primary ROS, superoxide anion radical ($O_2^{\bullet -}$) as described in Equation 2.1. The NOX family comprises several isoforms, each with distinct tissue distributions and functions. The primary members of this family are NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, and DUOX2 [53, 54]. NOX2 is predominantly expressed in neutrophils [55], macrophages [56], other phagocytic cells [57–60] and it plays a critical role in the immune response by generating $O_2^{\bullet -}$ during the respiratory burst. This $O_2^{\bullet -}$ is subsequently converted to other ROS, such as H_2O_2 and hypochlorous acid (HClO), which are employed to eliminate invading pathogens [55]. Other NADPH oxidase isoforms are involved in cell signaling and regulation. NOX1, found in colon epithelial cells [61], vascular smooth muscle cells [62, 63], and other tissues [64–67], plays a crucial role in cell proliferation [68, 69], angiogenesis [70, 71], and responses to inflammatory cytokines [72, 73]. In addition it has been reported that NOX1 are also expressed in macrophages [74]. NOX4, expressed in a variety of cells including renal [75, 76], vascular [77], and cardiac cells [78]. NOX4 is implicated in processes such as cellular differentiation [79–81], oxygen sensing [76, 82] and immune response [83]. NOX5, regulated by intracellular calcium levels [84], is involved in cellular functions within the cardiovascular system [85], testis [84], and lymphoid tissues [75]. DUOX1 and DUOX2 are primarily expressed in the thyroid gland. They generate ROS necessary for the synthesis of thyroid hormones [86].

Cytochrome P450 enzymes are known to produce ROS during their catalytic cycles. Cytochrome P450 enzymes, a large and diverse group, are attached to the membranes of either the mitochondria or the endoplasmic reticulum. They play a crucial role in metabolizing a wide variety of substances, including drugs, toxins, and endogenous compounds [87]. In their catalytic cycle, as illustrated in Eq. 2.2, one atom of O_2 is incorporated into the substrate (XH), while the other atom from O_2 is reduced to water. In these reactions NADPH donates two electrons which are transferred to P450 via two electron transfer proteins, during this electron transfer there is a potential for electron leakage to electron acceptor (O_2) resulting in the formation of $O_2^{\bullet -}$ [88]. This process is represented previously by Eq. 2.1.

$$\mathrm{NADPH} + \mathrm{O_2} + \mathrm{XH} + \mathrm{H^+} \xrightarrow{\mathrm{P450}} \mathrm{NADP^+} + \mathrm{XOH} + \mathrm{H_2O} \tag{2.2}$$

Xanthine oxidase (XO) utilizes molecular oxygen to oxidize substrates, producing $O_2^{\bullet -}$ as byproduct. It catalyzes the oxidation of hypoxanthine to xanthine and subsequently to uric acid, which are the final steps in the catabolism of purine nucleotides [89, 90].

$$\mathrm{hypoxantine} + 2\mathrm{O_2} + \mathrm{H_2O} \xrightarrow{\mathrm{XO}} \mathrm{xantine} + \mathrm{O_2^{\bullet -}} + 2\mathrm{H^+} \tag{2.3}$$

$$\text{xantine} + 2O_2 + H_2O \xrightarrow{\text{XO}} \text{uric acid} + O_2^{\bullet -} + 2H^+ \tag{2.4}$$

Nitric oxide synthases (NOS) include three distinct isoforms—endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), each responsible for producing nitric oxide (NO), is a essential signaling molecule in cellular processes [91, 92]. Both eNOS and nNOS play roles in normal cellular signaling and are constitutively expressed to contribute to physiological functions such as vasodilation [93], neurotransmission [94, 95], and immune response regulation. In contrast, iNOS is typically activated in response to inflammatory stimuli, such as cytokines [96] and bacterial endotoxins [97]. Besides the production of NO, all three isoforms of NOS can generate $O_2^{\bullet -}$ and H_2O_2, this happens during NADPH oxidation especially in the absence of L-arginine [98]. The rate of $O_2^{\bullet -}$ and H_2O_2 production is relatively slow [99, 100]. Subsequent reaction of NO and $O_2^{\bullet -}$ leads to the formation of peroxynitrite ($ONOO^-$), a potent oxidant implicated in oxidative stress and cellular damage [101].

As described earlier, $O_2^{\bullet -}$ is produced as a byproduct of normal cellular processes, particularly during mitochondrial respiration. **Superoxide dismutase** (SOD) is a vital antioxidant enzyme that protects cells from oxidative damage by catalyzing the conversion of $O_2^{\bullet -}$ into O_2 and H_2O_2 (Eq. 2.5). Although the H_2O_2 produced by SOD requires further detoxification by other enzymes, such as catalase or glutathione peroxidase, SOD plays a critical role in cellular defense against oxidative stress by converting $O_2^{\bullet -}$ into less reactive species. This helps maintain the balance of ROS and prevents cellular damage. SOD exists in multiple forms, each located in different cellular compartments: SOD1 (Cu/Zn-SOD) is found in the cytoplasm and contains copper and zinc as cofactors; SOD2 (Mn-SOD) is located in the mitochondria and uses manganese as a cofactor; and SOD3 (extracellular SOD) is present in the extracellular space, also containing copper and zinc.

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \tag{2.5}$$

Peroxisomal enzymes are integral to numerous metabolic processes vital for the proper functioning of eukaryotic cells. One of the key enzymes, **Acyl-CoA oxidase**, catalyzes the first step of fatty acid β-oxidation within peroxisomes, generating H_2O_2 as a by-product [102, 103]. This hydrogen peroxide is subsequently broken down by **catalase** (CAT), another critical peroxisomal enzyme, into water and O_2 (Eq. 2.6), thereby preventing oxidative damage. Beyond that, peroxisomal enzymes are involved in the metabolism of amino acids such as D-amino acid oxidase that catalyzes the stereoselective oxidative deamination of d-amino acids. During this oxidation reaction, molecular oxygen is used as an electron acceptor, and H_2O_2 is generated [104, 105].

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2 \tag{2.6}$$

Singlet oxygen ($^1O_2$) is a highly reactive form of molecular oxygen ($^3O_2$) where both electrons in the oxygen molecule's highest occupied molecular orbital are paired in the same orbital, resulting in a unique electronic configuration. Unlike the more stable triplet oxygen ($^3O_2$), which is the ground state of molecular

oxygen and has two unpaired electrons, $^1O_2$ exists in an excited state and exhibits distinctive chemical reactivity. This makes it a crucial player in various chemical and biological processes. $^1O_2$ can be generated through various mechanisms, including enzymatic reactions - myeloperoxidase (MPO) [106], eosinophil peroxidase (EPO) [107].

Non-enzymatic production of ROS

Non-enzymatic production of ROS is a significant contributor to cellular oxidative stress and damage. Unlike enzymatic pathways, non-enzymatic ROS generation occurs through chemical reactions that do not involve enzymes and is often triggered by external factors such as ultra-violet radiation [108], pollutants [109–111], heavy metals [112], and certain drugs and toxins [113]. Additionally, endogenous processes like the autoxidation of molecules, such as catecholamines [114, 115], flavins, and thiol compounds, and the Fenton reaction involving transition metals [47, 116], contribute to non-enzymatic ROS production.

The Fenton reaction is particularly important in this context, as it involves the reaction of H_2O_2 with ferrous ions (Fe^{2+}) to produce highly reactive hydroxyl radicals ($HO^\bullet$), which is the most reacting and damaging ROS. This reaction can lead to extensive oxidative damage to cellular components [46].

$$H_2O_2 + Fe^{2+} \rightarrow HO^\bullet + HO^- + Fe^{3+} \tag{2.7}$$

In addition to enzymatic production, $^1O_2$ can also be generated through energy transfer, photoexcitation and chemical reactions, such as the interaction between H_2O_2 and hypochlorous acid (HOCl) [117].

$$H_2O_2 + ClO^- \rightarrow {}^1O_2 + H_2O + Cl^- \tag{2.8}$$

2.2.2 Biological targets of ROS

ROS, especially the highly reactive $HO^\bullet$, interact with a wide range of biomolecules, including saturated and unsaturated hydrocarbons, polymers, lipids, proteins, carbohydrates, and DNA. The $HO^\bullet$ is known for its high reactivity, which enables it to abstract hydrogen atoms from organic molecules (Eq. 2.8), initiating chain reactions that propagate oxidative damage [118].

For example, when ROS react with **lipids** (LH) by abstracting hydrogen atoms from polyunsaturated fatty acids in cell membranes, they can initiate lipid peroxidation, resulting in the formation of lipid radicals ($L^\bullet$) and ultimately leading to cell membrane damage [119, 120]. This process generates secondary products such as malondialdehyde (MDA) and 4-hydroxynonenalNOS (4-HNE), which can further react with proteins and DNA, exacerbating cellular damage [121, 122].

Proteins are the most abundant macromolecules in cells and are particularly vulnerable to oxidative damage due to their high concentration and proximity to ROS production sites, such as mitochondria (where ROS are

generated by the ETC) and peroxisomes [123]. The reactivity of ROS leads to significant modifications in proteins. Hydroxyl radicals ($HO^{\bullet}$) primarily target the polypeptide backbone and the aliphatic side chains of hydrophobic amino acid residues. The abstraction of a hydrogen atom results in the formation of carbon-centered radicals (Eq. 2.9), which rapidly convert to peroxyl radicals ($ROO^{\bullet}$) in the presence of oxygen [123, 124] (Eq. 2.10). Certain amino acids are more susceptible to oxidative modifications than others. For instance, cysteine and methionine residues, both containing sulfur atoms, are highly prone to oxidation. Other amino acids such as histidine, lysine, arginine, and tryptophan are also vulnerable. Notably, cysteine is the only amino acid that is oxidized in an uncatalyzed reaction with H_2O_2 due to its reactive thiol (-SH) group [125]. Peroxyl radicals ($ROO^{\bullet}$) can perpetuate oxidative damage by abstracting hydrogen atoms from other biomolecules, leading to the formation of hydroperoxides (ROOH), Eq. 2.11. Protein hydroperoxides are relatively stable compared to other oxidized species but can decompose under certain conditions (eg. in the presence of transition metals, Eq. 2.12), producing additional radicals that drive further oxidative damage. Unlike some oxidative modifications, protein hydroperoxides are not easily repaired by cellular mechanisms, leading to their accumulation in cells.

$$RH + HO^{\bullet} \rightarrow R^{\bullet} + H_2O \tag{2.9}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \tag{2.10}$$

$$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH \tag{2.11}$$

$$ROOH + Fe^{2}+ \rightarrow RO^{\bullet} + HO^{-} + Fe^{3+} \tag{2.12}$$

DNA is also a critical target for ROS. Hydroxyl radicals react with nucleic acids by attacking both the sugar-phosphate backbone and the nucleotide bases, leading to significant DNA damage. For instance, the reaction of hydroxyl radicals with guanine results in the formation of 8-hydroxyguanine (8-oxo-Gua). Additionally, the sugar-phosphate backbone can be damaged, resulting in strand breaks and cross-linking between strands [126, 127].

2.2.3 High-energy intermediates

Peroxyl radicals ($ROO^{\bullet}$) are more stable than highly reactive hydroxyl radicals ($OH^{\bullet}$). This increased stability allows peroxyl radicals to recombine with another peroxyl radical (Eq. 2.13), to form high-energy linear intermediate called tetroxides (ROOOOR) [128–130]. Peroxyl radical can also undergo cyclization to form dioxetane (ROOR) [131–133], Eq. 2.14. When $^1O_2$ reacts with unsaturated bonds in amino acids or polyunsaturated fatty acids, it can produce ROOR through a [2+2] cycloaddition reaction [46], Eq. 2.15.

The intermediates, dioxetanes and tetroxides, are usually prone to decomposition.

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOOOR \tag{2.13}$$

$$ROO^{\bullet} \rightarrow ROOR^{\bullet} \tag{2.14}$$

$$RH + {}^{1}O_2 \rightarrow ROOR \tag{2.15}$$

2.2.4 Electronically excited states

The decomposition of high-energy intermediates, such as tetroxides (ROOOOR), as shown in Eq. 2.16 and 2.17, and dioxetanes (ROOR), as represented in Eq. 2.18, can lead to the formation of electronically excited species, including excited triplet carbonyls $^{3}(R{=}O)^{*}$ and $^{1}O_2$ [134–136]. BAL has been attributed to both $^{3}(R{=}O)^{*}$ and $^{1}O_2$ [137, 138] Eq. 2.19, 2.20, 2.21.

Triplet excited carbonyls

The quantum yield of triplet excited carbonyl $^{3}(R{=}O)^{*}$ generation from dioxetanes (ROOR), can vary dramatically, ranging from 0.01% to as high as 60% [135, 139]. This variation in yield reflects the efficiency of different activation methods and the specific reaction conditions. In typical biological environments, the quantum yield for $^{3}(R{=}O)^{*}$ formation from ROOR (Eq. 2.16) tends to be on the lower end of the range because the energy available under normal physiological conditions is usually insufficient to activate these intermediates efficiently. Without sufficient energy, the decomposition of ROOR into $^{3}(R{=}O)^{*}$ is thermodynamically unfavorable [46]. However, in scenarios where external energy sources such as heat (thermolysis) or light (photolysis) are applied, the reaction can proceed with much higher efficiency.

$$ROOR \rightarrow {}^{3}(R{=}O)^{*} + R{=}O \tag{2.16}$$

The decomposition of the high-energy intermediate ROOOOR releases enough energy to generate $^{3}(R{=}O)^{*}$ (Eq. 2.17), which require a significant energy input to form. However, despite the energy being sufficient [46], the efficiency of generating these $^{3}(R{=}O)^{*}$ is very low, with a yield of less than 0.1 % [139]. This means that although the reaction has the potential to produce excited states, only a small fraction of the reaction actually results in their formation.

$$ROOOOR \rightarrow {}^{3}(R{=}O)^{*} + ROH + {}^{3}O_2 \tag{2.17}$$

The electronic transition from the triplet energy level of $^{3}(R{=}O)^{*}$ to the ground state $^{1}(R{=}O)$ is accompanied by the emission of light, with its wavelengths ranging from 350 to 550 nm [46], Eq. 2.18. This emission occurs particularly under anaerobic conditions and in the absence of chromophores. Otherwise, $^{3}(R{=}O)^{*}$ tend to transfer their energy to molecular oxygen Eq. 2.20 or chromophores Eq. 2.23 and 2.24.

$$^3(\mathrm{R{=}O})^* \rightarrow \mathrm{R{=}O} + h\nu_{(350-550\ \mathrm{nm})} \tag{2.18}$$

Singlet oxygen

The breakdown of ROOOOR can produce $^1O_2$, and this process is thermodynamically possible [46], Eq. 2.19. The amount of $^1O_2$ generated varies depending on the type of alkylperoxyl involved. For primary alkylperoxyl termination, the yield ranges from 3.4 % to 6.0 %, while for secondary alkylperoxyl termination, it ranges from 3.9 % to 14 % [140].

$$\mathrm{ROOOOR} \rightarrow {}^1O_2 + \mathrm{ROH} + \mathrm{R{=}O} \tag{2.19}$$

$^1O_2$ can be also formed through triplet-singlet energy transfer from an $^3(\mathrm{R{=}O})^*$ molecule to molecular oxygen [141], Eq. 2.20.

$$^3(\mathrm{R{=}O})^* + {}^3O_2 \rightarrow \mathrm{R{=}O} + {}^1O_2 \tag{2.20}$$

The emission of light from $^1O_2$ can occur via two distinct pathways: dimolar and monomolar emission. In the monomolar pathway, individual $^1O_2$ molecule return to the ground state by releasing energy in the form of a single photon. This emission typically occurs in the near-infrared region, around 1270 nm [141], Eq 2.21.

$$^1O_2 \rightarrow {}^3O_2 + h\nu_{(1270\ \mathrm{nm})} \tag{2.21}$$

Dimolar emission occurs when two $^1O_2$ molecules interact simultaneously as they return to their ground state. In this case, the energy from two $^1O_2$ molecules is released together, resulting in a different emission spectrum [142], Eq. 2.22.

$$^1O_2 + {}^1O_2 \rightarrow {}^3O_2 + {}^3O_2 + h\nu_{(634\ \mathrm{nm},703\ \mathrm{nm})} \tag{2.22}$$

Chromophores

Chromophores, biomolecules such as tetrapyrroles, flavins, and melanin, can accept energy from $^3(\mathrm{R{=}O})^*$ [46], Eq. Eq. 2.23 and 2.25. The process involves the formation of excited states of chromophores, which can exist in two forms: singlet ($^1C^*$) or triplet excited state ($^3C^*$), and both electronic transition from singlet or triplet state to the ground state can results in photon emission. A singlet excited state ($^1C^*$) occurs when an electron in a chromophore is excited by energy absorption Eq. 2.23, and the electron's spin remains paired with another electron in the ground state. This singlet state can emit photons as the electron returns to the ground state accompanied by photon emission in the green-red region of the spectrum (550–750 nm) [46], Eq. 2.24.

$$^3(\mathrm{R{=}O})^* + \mathrm{C} \rightarrow \mathrm{R{=}O} + {}^1\mathrm{C}^* \tag{2.23}$$

$$^1\mathrm{C}^* \rightarrow \mathrm{C} + h\nu_{(550-750\mathrm{nm})} \tag{2.24}$$

A triplet excited state (^{3}C*) is a higher-energy state in which the excited electron has an unpaired spin, making it parallel to the spin of another electron. Although triplet states are typically lower in energy than singlet states, they have longer lifetimes, allowing them to persist for a longer period before returning to the ground state. This transition from the triplet excited state to the ground state is accompanied by photon emission in the near-infrared region of the spectrum (750–1000 nm) [46], Eq. 2.26.

$$^3(\mathrm{R{=}O})^* + \mathrm{C} \rightarrow \mathrm{R{=}O} + {}^3\mathrm{C}^* \tag{2.25}$$

$$^3\mathrm{C}^* \rightarrow \mathrm{C} + h\nu_{(750-1000\mathrm{nm})} \tag{2.26}$$

The process by which a molecule transitions between singlet and triplet states is called intersystem crossing. This non-radiative process involves a change in the spin orientation of the electron, allowing a singlet excited chromophore (^{1}C*) to convert into a triplet excited state (^{3}C*), or vice versa [46], Eq. 2.27.

$$^1\mathrm{C}^* \leftrightarrow {}^3\mathrm{C}^* \tag{2.27}$$

A simplified diagram depicting the complex chemical reactions that lead to photon emission is shown in Fig. 2.1.

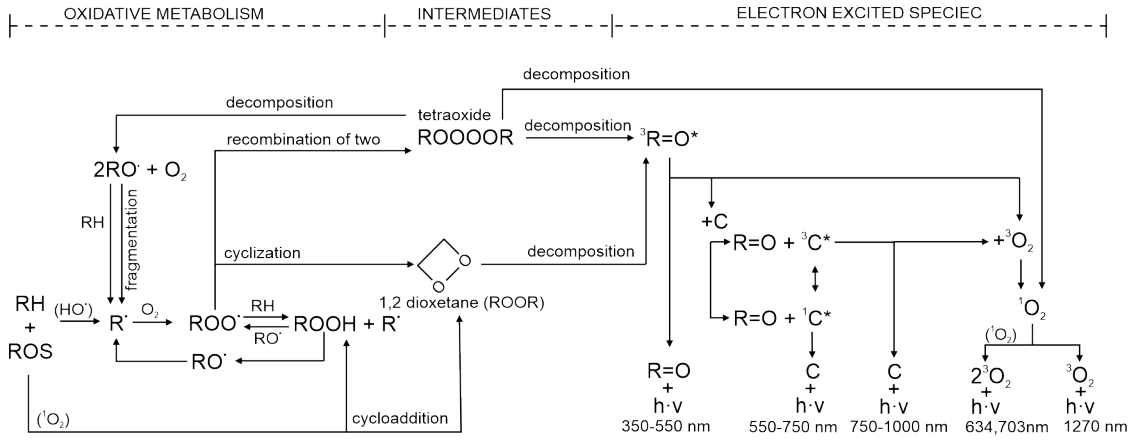

Figure 2.1: A simplified diagram of the complex chemical reactions leading to the photon emission, addapted from [143], based on [46]

2.3 Detection of BAL

BAL offers insights into fundamental oxidative processes and has potential applications in biomedical research. The advancements in detection technologies, such as photomultiplier tubes and CCD cameras, have significantly enhanced our ability to study and understand this phenomenon. The detection of BAL requires highly sensitive instrumentation due to the extremely low intensity of the emitted light. Two primary types of detectors are used: photomultiplier tubes (PMTs) and charge-coupled devices (CCDs).

2.3.1 Photomultipliers

Photomultiplier tubes (PMTs) are highly sensitive photon detectors that are integral to the detection of BAL in biological research. PMTs operate on the principle of the photoelectric effect, where incident photons strike a photocathode, causing the emission of electrons. These primary electrons are then directed towards a series of dynodes, each of which amplifies the signal by releasing additional electrons upon impact. This cascading multiplication process results in a substantial amplification of the original photon signal, enabling the detection of extremely low levels of light, down to the single-photon level. PMTs are renowned for their rapid response times, high gain, and excellent sensitivity, making them indispensable tools for real-time monitoring of dynamic biological processes. Their ability to detect faint light emissions with high temporal resolution makes them particularly suitable for studies involving oxidative stress, cell viability, and other physiological processes where BAL serves as a key indicator. However, PMTs require careful shielding from ambient light and electronic noise to maintain their high sensitivity, and they are typically used in conjunction with sophisticated signal processing and cooling systems to enhance performance [144].

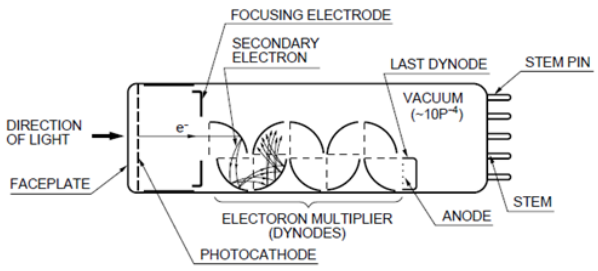

Figure 2.2: Construction scheme of a photomultiplier tube [144]

2.3.2 Charge-coupled devices

Charge-coupled devices (CCDs) are advanced semiconductor devices widely used for detecting and BAL in biological systems. CCDs convert incident photons into electronic signals through a process in which photons striking the semiconductor material generate electron-hole pairs. These charges are then transferred through the device and collected at an output node, where they are converted into a voltage signal that can be digi-

tized and processed. One of the standout features of CCDs is their high quantum efficiency, allowing them to effectively capture a significant proportion of incoming photons, making them highly sensitive to low light levels [145–147]. Additionally, CCDs provide high spatial resolution, enabling detailed imaging of photon emission patterns across biological samples. This imaging capability is particularly valuable for mapping cellular processes and understanding the spatial dynamics of phenomena such as oxidative stress and metabolic activity. To further enhance sensitivity and reduce thermal noise, CCDs are often cooled to very low temperatures during operation. Despite their complexity, the high resolution, and efficiency of CCDs make them essential tools in BAL research, offering insights into the intricate light-emitting activities of living organisms.

2.4 Chemical modulators

Since the mechanisms of BAL production are rooted in complex chemical interactions, chemical modulators play a crucial role in influencing the emission process. These modulators, which can either enhance or suppress BAL, have a strong impact by altering the dynamics of key intermediates involved in photon generation. For instance, the presence of specific antioxidants, prooxidants, or metal ions can significantly influence the redox reactions and the formation of ROS, thereby modulating BAL intensity. By directly interacting with intermediates like free radicals, $^1O_2$, and lipid peroxides, chemical modulators can either facilitate or inhibit the electron excitation and relaxation processes that lead to photon emission [148].

2.4.1 Pro-oxidants

Chemical prooxidant modulators, which promote the generation of reactive oxygen species (ROS), play a significant role in influencing BAL. **Molecular oxygen** (O_2) is a critical component in BAL, as it serves as a substrate for various oxidative reactions. Under standard aerobic conditions, molecular oxygen facilitates the formation of ROS, such as $O_2^{\bullet-}$ and $^1O_2$, which contribute to oxidative damage and the resulting photon emission. Conversely, under anaerobic or anoxic conditions, the lack of oxygen limits the generation of ROS, leading to a significant reduction in BAL [149–152].

In hyperoxic conditions, however, the production of ROS and oxidized matter is enhanced. For example, the concentration of peroxyl radicals $ROO^{\bullet}$, formed through the interaction of alkyl radicals ($R^{\bullet}$) increases in hyperoxic environments. This leads to a higher probability of two $ROO^{\bullet}$ radicals colliding and generating the high-energy intermediate ROOOOR. The increase in BAL intensity under hyperoxic conditions has been successfully demonstrated in studies such as [149, 153]. This highlights the dependence of BAL on oxygen availability and its crucial role in driving redox reactions.

Among the most potent pro-oxidant modulators is **hydrogen peroxide** (H_2O_2). The addition of H_2O_2 to biomolecules can lead to the formation of hydroxyl radicals ($HO^{\bullet}$) as it reacts with endogenous **transition metal ions**, such as ferrous ions (Fe^{2+}) and copper ions (Cu^{+}). This reaction, known as the Fenton reaction,

is a key process that generates highly reactive $HO^{\bullet}$ [154], as described previously by Eq. 2.7. The Fenton reaction, driven by the interaction of transition metals ions with H_2O_2, is a powerful source of oxidative stress, leading to elevated BAL due to the increased production of excited molecular states [22, 155–159]. Besides that, in proteins H_2O_2 can directly react with cysteine's thiol (-SH) groups, leading to BAL [125].

Overall, chemical prooxidant modulators are key determinants of BAL intensity, as they drive the formation of ROS and other reactive intermediates that contribute to oxidative stress.

2.4.2 Anti-oxidants and quenchers

Antioxidants are crucial players in cellular systems, as they effectively neutralize reactive oxygen species (ROS) and reduce the concentration of free radicals that would otherwise be available to damage biomolecules. By donating electrons to these unstable radicals, antioxidants stabilize them and prevent the chain reactions that lead to cellular damage. Given that the generation of biological autoluminescence (BAL) depends on the presence of ROS, it is unsurprising that antioxidants typically limit the available sources for generating high-energy intermediates, thereby reducing BAL.

In addition to chemically neutralizing ROS, antioxidants can also physically quench excited molecular states. This physical quenching involves absorbing excess energy from excited molecules or dissipating it without undergoing chemical changes. By both chemically and physically quenching reactive species, antioxidants play a vital role in modulating BAL and protecting cells from oxidative stress.

Ascorbic acid, commonly known as vitamin C, primarily exists in its deprotonated form as ascorbate ($AscH^-$) under physiological conditions (pH = 7.4). It is a well-known non-enzymatic antioxidant with the ability to scavenge various ROS, including $HO^{\bullet}$, $R^{\bullet}$, and $ROO^{\bullet}$, Eq. 2.28, 2.29, and 2.30, respectively. By donating electrons, ascorbate neutralizes these free radicals, reducing their concentration and thereby lowering oxidative stress within the cell. This reduction in ROS levels subsequently diminishes BAL, as fewer oxidative reactions occur that would otherwise generate electronically excited states responsible for photon emission. Additionally, ascorbate can regenerate other antioxidants, such as vitamin E, thereby enhancing the overall antioxidant capacity of the cell.

$$HO^{\bullet} + AscH^- \rightarrow H_2O + Asc^{\bullet -} \tag{2.28}$$

$$R^{\bullet} + AscH^- \rightarrow RH + Asc^{\bullet -} \tag{2.29}$$

$$ROO^{\bullet} + AscH^- \rightarrow ROOH + Asc^{\bullet -} \tag{2.30}$$

However, ascorbate can also exhibit prooxidant effects under certain conditions. In the presence of transition metal ions, particularly iron and copper, ascorbate can reduce Fe^{3+} and Cu^{2+} metal ions, leading to the regeneration of Fe^{2+} (Eq. 2.31) and Cu^+, that can lately parcitipate in Fenton-type reactions, leading to forma-

tion of $HO^{\bullet}$ [160]. This prooxidant activity of ascorbate can increase BAL by promoting additional oxidative reactions, highlighting its dual role as both an antioxidant [149, 156, 159, 161–163] and a prooxidant [164], depending on the cellular environment.

$$Fe^{3+} + AscH^{-} \rightarrow Fe^{2+} + Asc^{\bullet -} + H^{+} \tag{2.31}$$

Among other non-enzymatic antioxidants that can modulate the BAL signal are **glutathione**, which directly neutralizes ROS and serves as a substrate for glutathione peroxidase in the detoxification of hydrogen peroxide [32, 165], **mannitol** [166], α**-tocopherol** (vitamin E) which protects lipid membranes from oxidative damage by preventing lipid peroxidation [167], β-carotene [167], and licopene [167].

Enzymatic antioxidants like **superoxide dismutase** (SOD) and **catalase** (CAT) play critical roles in controlling oxidative stress and modulating BAL. SOD catalyzes the conversion of $O_2^{\bullet -}$ into hydrogen peroxide (Eq. 2.5), which is a less reactive species. However, hydrogen peroxide can still contribute to oxidative damage if not promptly neutralized. CAT addresses this by breaking down hydrogen peroxide into water and oxygen (Eq. 2.6), effectively reducing the overall oxidative load and further decreasing BAL [166, 168].

Overall, the interaction between antioxidants and prooxidants is a delicate balance that profoundly influences BAL. While antioxidants reduce the concentration of free radicals and mitigate oxidative stress, conditions that promote the prooxidant activity of compounds like H_2O_2 can have the opposite effect, increasing ROS production and enhancing BAL. This duality emphasizes the complex regulatory role of antioxidants in cellular oxidative balance and their impact on BAL as a marker of cellular health and oxidative stress.

This topic was further explored and discused by the candidate in the chapter 5.

2.4.3 pH

One of the most influential factors in chemical interactions is the pH. The pH value affects the ionization states of various molecules, influencing their reactivity. For instance, the protonation of the $O_2^{\bullet -}$ is pH-dependent, as protonation involves the addition of a proton (H^+) to a molecule, Eq. 2.32:

$$O_2^{\bullet -} + H^{+} \leftrightarrow HO_2^{\bullet} \tag{2.32}$$

The equilibrium between the superoxide anion radical ($O_2^{\bullet -}$) and the hydroperoxyl radical ($HO_2^{\bullet}$) is pH-dependent. The pKa of this equilibrium is approximately 4.7 [169], meaning that at a pH of 4.7, there is an equal concentration of $O_2^{\bullet -}$ and $HO_2^{\bullet}$. At lower pH then 4.7, $O_2^{\bullet -}$ is more likely to be protonated to form the $HO_2^{\bullet}$, which is more lipid-soluble and can diffuse across membranes more easily [170]. In contrast, at higher pH, the superoxide anion radical remains in its deprotonated form $O_2^{\bullet -}$. This also affects efficiency of SOD, as it is a specific enzyme for the dismutation of $O_2^{\bullet -}$ but is ineffective in detoxifying $HO_2^{\bullet}$.

The internal pH of cells significantly influences the activity of key enzymes involved in cellular respiration,

which directly affects oxygen consumption. Cellular respiration, particularly aerobic respiration, relies on oxygen as the final electron acceptor in the ETC. Enzymes involved in the Krebs cycle, oxidative phosphorylation, and ATP synthesis are all sensitive to pH fluctuations, and any deviation from their optimal pH can hinder these processes, reducing the efficiency of oxygen utilization. Additionally, pH impacts overall oxidative processes, including oxygen uptake [171–173].

The Fenton reaction (Eq. 2.7) is an important redox process in the context of BAL. The efficiency of the Fenton reaction is strongly dependent on pH, as pH influences the availability and reactivity of Fe^{2+} and Fe^{3+} ions. Optimal Fenton chemistry typically occurs in mildly acidic environments (around pH 3-4) [174], where Fe^{2+} can be more readily available and reactive. This is particularly relevant for acidic organelles such as lysosomes [175] or some specific peroxisomes [176], where the Fenton reaction can occur efficiently due to the acidic pH and the presence of iron ions. Under highly acidic or alkaline conditions, the reaction's efficiency decreases, leading to reduced $HO^{\bullet}$ production and altered BAL dynamics.

Alkaline conditions can enhance certain oxidative processes, which may lead to an increase in photon emission, as observed in previous studies [177, 178].

2.5 Physical modulators

BAL can be influenced by a variety of physical factors that alter the biochemical processes involved in light production. Mechanical damage can trigger BAL by disrupting cellular structures, leading to oxidative stress and ROS production, which in turn stimulates BAL production. Ionizing radiation and UV exposure can also enhance BAL by inducing DNA damage, ROS generation, and cellular stress responses. Additionally, external factors such as magnetic fields and pulsed electric fields can influence cellular redox states and membrane status, affecting BAL. These physical factors collectively modulate the intensity and dynamics of BAL, reflecting the organism's response to stress.

2.5.1 Mechanical damage

Mechanical damage to plants, such as cutting or bruising, induces a significant biological response that includes the generation of ROS and subsequent BAL. The study by Slawinska [179] examines BAL from plants subjected to mechanical and chemical stress. It was observed that while native plants emit low-intensity radiation associated with metabolic processes, the intensity of BAL increases when the plants are stressed by mechanical damage.

Upon mechanical injury, plants initiate a defense response characterized by the rapid production of ROS, such as $O_2^{\bullet-}$ and $^1O_2$ [180]. The formation of $^1O_2$ during this process is hypothesized to have signaling relevance [181]. $^1O_2$ can be produced through various pathways, including the lipid peroxidation process catalyzed by lipoxygenases. Wounding triggers the release of polyunsaturated fatty acids from cell membranes, leading

to their accumulation at the site of injury [182]. These polyunsaturated fatty acids act as substrates for lipoxygenase, resulting in the production of lipid hydroperoxides (LOOH) and subsequently generates electronically excited species, such as lipid-derived triplet carbonyls (^{3}(L=O)*), singlet chlorophyll (^{1}Chl*), and $^1O_2$, which contribute to the enhanced BAL observed at wounded parts of the leaves. This phenomenon has been extensively studied in *Arabidopsis thaliana* [180, 181], providing valuable insights into the underlying biochemical processes and their physiological implications. The wounding also stimulates an influx of ions, particularly calcium ions (Ca^{2+}), into the cytoplasm [183]. This ionic change activates NADPH oxidases, that catalyze the reduction O_2 to $O_2^{\bullet-}$. This happens rapidly following mechanical injury and contributes to the plant's local and systemic responses to stress [180].

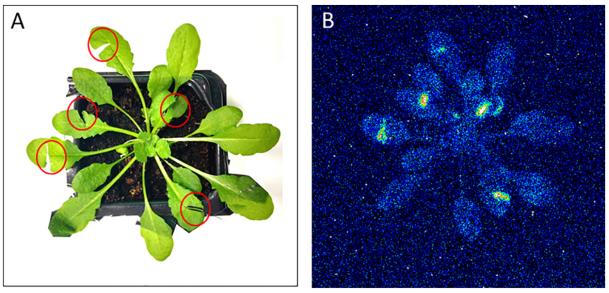

Figure 2.3: Two-dimensional imaging of the BAL from the plant of *Arabidopsis thaliana*. The figure shows photographs (A) and the corresponding two-dimensional images of BAL recorded by a highly sensitive CCD camera (B). [180]

The generation of BAL in response to mechanical damage has significant physiological implications. The production of ROS is a part of the plant's defense mechanism, signaling damage and initiating repair processes. As the localized production of $^1O_2$ and other ROS at the wound site can activate defense genes, promote the accumulation of defensive compounds, and lead to the restructuring of cell walls to prevent pathogen entry and further injury. In summary, the study of BAL in mechanically damaged plants provides valuable insights into the complex signaling and defense mechanisms that plants employ in response to physical injury.

2.5.2 Temperature

Temperature is a versatile factor due to its wide-ranging effects. While primarily a physical modulator, temperature changes influence chemical reaction rates and biological processes, such as enzyme activity and metabolic rates, ultimately leading to physiological stress responses. As a result, temperature affects organisms across physical, chemical, and biological domains. At the molecular level, temperature changes can alter the kinetic energy of molecules, disrupt membrane fluidity, and influence the rate of biochemical reactions. Heat can accelerate reactions and destabilize proteins, while cold can slow down metabolic processes and decrease membrane fluidity, both of which can trigger stress responses and increase ROS production. This, in turn, contributes to variations in BAL.

Research has extensively explored the influence of temperature on BAL across various biological systems, highlighting its critical role in modulating the underlying biochemical and biophysical processes. Temperature's direct impact on molecular and cellular dynamics makes it a key factor in understanding how organisms respond to environmental stressors through changes in BAL.

Nakamura and Hiramatsu [184] demonstrated that BAL from the human hand increases with temperature, suggesting a direct correlation between thermal energy and the intensity of BAL emissions. This temperature dependence implies that BAL is primarily a result of chemiluminescent reactions, where the activation energy required for photon emission can be influenced by thermal conditions. Further supporting this, Roschger et al. [185] observed distinct phase transitions in plant leaves through intrinsic BAL, which are indicative of thermotropic changes in membrane lipids. These findings suggest that the structural integrity and function of biological membranes are temperature-sensitive, thereby affecting the emission of BAL.

2.5.3 Ultraviolet irradiation

Ultraviolet (UV) radiation is a potent environmental stressor that significantly impacts biological systems, particularly at the molecular level. As a high-energy component of sunlight, UV radiation can penetrate cells and tissues, leading to both direct and indirect damage to biomolecules such as DNA, proteins, and lipids. In addition to direct damage, UV radiation indirectly affects biomolecules by generating ROS.

Exposure of organic matter typically results in a higher BAL signal. This was demonstrated in the study by Rastogi et al. [24], where *Arabidopsis thaliana* plants exposed to UVA radiation showed a significant increase in BAL compared to non-exposed plants, indicating elevated oxidative stress in the UVA-exposed plants. The study identified two kinetically distinct phases of BAL decay: a fast decay phase and a slow decay phase. The fast decay phase involved contributions from $^3(R{=}O)^*$ formed in chloroplasts, while the slow decay phase was primarily associated with $^3(R{=}O)^*$ formed in other parts of the cell, such as mitochondria and the plasma membrane [24].

The study by Prasad an Pospíšil [186] found that exposure of human skin to UVA radiation leads to significantly higher oxidative stress and BAL. This was particularly evident on the palmar side of the hand, where lower melanin content resulted in reduced antioxidant defense against ROS. In comparison, visible light exposure also increased BAL, but to a lesser degree. The study suggests that chromophores in the skin, such as melanins and porphyrins, absorb UVA and visible light, initiating photosensitization reactions that generate ROS through electron transfer and energy transfer mechanisms. The findings indicate that UVA radiation poses a greater risk of oxidative damage to the skin than visible light, highlighting the need for protective measures against UVA exposure [186].

The study by Evelson [187] also confirmed that UVA exposure significantly increases BAL in mouse skin, indicating heightened oxidative stress. The spectral analysis of the emitted light revealed that the main emission occurred in the 400-500 nm range, indicating the involvement of $^3(R{=}O)^*$ as the primary emitting species.

In addition to BAL, other markers of oxidative stress were measured. Thiobarbituric acid reactive substances, which indicate lipid peroxidation, increased by 130 % after 2 hours of UVA exposure. The activities of antioxidant enzymes, specifically SOD and CAT, were significantly reduced after irradiation. These findings suggest that UVA radiation not only induces oxidative damage, but also disrups the skin's antioxidant defenses and leading to lipid peroxidation and other oxidative modifications.

In summary, UV irradiation can significantly impact BAL, altering the intensity and spectral properties. BAL has various potential applications, including dermatology and cosmetology, where it is used to evaluate the protective effects of sunscreens and anti-aging skincare products.

2.5.4 Ionizing radiation

When high-energy ionizing radiation penetrates an organism's tissues, it transfers energy to the cellular atoms and molecules. This energy absorption can excite electrons to higher energy states or eject them from atoms and molecules, leading to the formation of ions and free radicals. Ionizing radiation can directly interact with critical biomolecules, such as DNA, proteins, and lipids. The energy from the radiation causes ionization, which can lead to the breaking of chemical bonds and structural changes. In biological systems, water is the most abundant molecule, so a significant portion of the radiation energy is absorbed by water, making its water radiolysis a major source of radicals. The interaction of radiation with water molecules produces ROS, including $H^{\bullet}$, $HO^{\bullet}$, or $HO_2^{\bullet}$.

Studies on BAL from the liver of a living mouse revealed a significant increase in photon emission intensity following gamma ray irradiation. This effect was similarly observed in extracts of liver lipids, where photon emission also increased [188, 189].

Zaqaryan and Badalyan [190] explored the effects of X-ray and gamma (γ) radiation on BAL in rats' whole blood with added H_2O_2. The study focused on how radiation-induced oxidative processes trigger free radical reactions, which can be detected through the measurement of BAL. Exposure to X-rays led to an intensification of oxidative processes in biological materials, particularly those containing lipid membranes. This increase in oxidative activity resulted in heightened BAL. The effects of γ-radiation differed from X-rays. At lower doses, γ-irradiation increased BAL, indicating initial oxidative damage. However, at higher doses, BAL decreased, which was attributed by the authors to the degradation of biomolecules, leading to reduced levels of detectable BAL [190].

2.5.5 Magnetic field

Magnetic fields can influence ROS formation in organisms through various mechanisms, including altering the spin dynamics of radical pairs [191, 192], modulating the activity of ROS-producing [193] and antioxidant enzymes [194], affecting mitochondrial properties [195], and impacting gene expression [196, 197] and cell

proliferation [198]. The specific effects depend on the characteristics of the magnetic field (e.g., strength, frequency, duration) and the biological context.

In his work, Bereta [158] demonstrated that a disturbance in BAL kinetics was detected in yeast cell cultures under the influence of a low-frequency magnetic field, while measurements of cell concentration showed no significant difference between the magnetic field-exposed samples and the control samples. This finding suggests that BAL can be used to detect subtle effects of magnetic fields on cells, which are not observable using standard cell culture characterization techniques.

2.5.6 Pulsed electric field

Pulsed electric field (PEF) technology represents a versatile tool, which can be tailored to specific needs across various fields, such as biotechnology, food preservation, and medical therapy, offering a non-thermal method of altering cellular structures and functions. This chapter delves into the fundamental mechanisms of PEF, and the pivotal role of sensing PEF effects by BAL.

PEF technology involves the application of short bursts of high-voltage electric pulses, which can permeabilize cell membranes, a process primarily utilized in electroporation. Electroporation creates transient nanopores in cellular membranes, enabling the introduction of foreign molecules into cells or the extraction of intracellular components [199]. This capability is pivotal for various applications, including gene [200] therapy, non-thermal tissue ablation [201], cancer treatment through electrochemotherapy [202,203], and microbial inactivation in food products [204, 205].

The fundamental physical mechanism of PEF is based on manipulating biomolecular structures, such as membranes or proteins, by acting on mobile or bound electric charges, thereby inducing functional changes in biomolecules. However, alongside these electrophysical effects, PEF also triggers electrochemical effects [206], such as the dissolution of electrode materials into the sample [207] or the generation of ROS [208], which are often underappreciated. These electrochemical effects can influence both the efficiency and safety of PEF-based applications, underscoring the importance of considering both electrophysical and electrochemical impacts in the design and optimization of PEF technologies.

By understanding and controlling the role of PEF in modifying proteins, it is possible to optimize processes in food production, enhance drug delivery systems in medicine, and innovate biotechnological applications. This manipulation of protein properties through electrical fields opens new avenues for research and industrial innovation. PEF can induce structural changes in proteins [209], including denaturation, unfolding, and aggregation [210, 211]. The extent of these modifications depends on the field strength, pulse duration, and the specific properties of the protein. Monitoring BAL from proteins subjected to PEF treatment can significantly enhance our understanding of the underlying molecular processes impacted by PEF. Given the complexity of PEF's interactions with biological samples, particularly how it influences proteins at the molecular level, the deployment of sensitive luminescence techniques is particularly valuable. Understanding the relations between

PEF parameters (like pulse duration and intensity) and biological outcomes (such as protein oxidation) can aid in optimizing PEF applications in both medical therapies and industrial processes.

For instance, the study by Bereta et al. [212] highlighted the application of BAL as a novel, non-invasive method for real-time monitoring of yeast cell electroporation induced by PEF. The study demonstrates cell electroporation can be effectively monitored through BAL in real time, providing immediate feedback on the electroporation process. This method was validated by comparing BAL data with more traditional approaches, such as propidium iodide (PI) uptake assays, impedance measurements, and cell growth assays. These methods confirmed that BAL not only correlates with electroporation efficiency but also offers a non-contact, label-free alternative to the more invasive and labor-intensive techniques. The increase in BAL observed during electroporation reflects the ROS activity, which is heightened as the cell membranes become permeable and more biomolecules are exposed to oxidative reactions. The mechanisms behind this involve two key processes: first, the increased electric conductivity of the cell suspensions during electroporation leads to higher Faradaic currents, producing more electrochemical products, including ROS. Second, the permeabilized cell membranes release intracellular biomolecules into the extracellular environment, which are subsequently oxidized by ROS, further boosting the BAL signal. This dual mechanism results in a measurable increase in BAL that corresponds with the extent of electroporation [212].

The work by Vahalová et al. [213] investigated the mechanisms by which PEF induces oxidative processes in biomolecules, in this case bovine serum albumin (BSA). The study developed a unique experimental platform for real-time, in-situ monitoring of protein oxidation using BAL. The results demonstrated that ROS generated at the anode during PEF applications lead to oxidative modifications of BSA, producing a measurable BAL signal. The BAL intensity increased in the presence of BSA, indicating that the protein molecules were involved in the chemiluminescent reactions. The authors proposed a reaction mechanism involving the formation of $^3(R{=}O)^*$ and $^1O_2$ as the primary BAL emitters. The study's findings suggest that PEF-induced ROS can cause structural and functional changes in proteins, which has implications for the use of PEF technology in biomedical and food industry applications. This work [213] was additionaly extended by the candidate and is discused in the chapter 6.

The application of BAL monitoring in studying PEF-treated biological samples provides a powerful tool for deciphering complex molecular dynamics that underlie the PEF effects. This approach not only bridges significant knowledge gaps regarding the interaction of electric fields with biological molecules but also enhances the capability to tailor PEF technology for specific industrial and therapeutic outcomes based on detailed molecular-level evidence.

2.6 Biological modulators

Pathogens such as viruses, bacteria, and fungi are known to infect plants and cause significant crop destruction worldwide. These infections can trigger defense responses in plants, including the production of ROS and

subsequent BAL. The study of BAL in plant-pathogen interactions is a valuable technique to investigate and understand the dynamics of these interactions and the underlying defense mechanisms.

2.6.1 Fungal pathogens

Fungal pathogens are among the primary culprits in plant infections, leading to severe crop losses. The interaction between plants and fungi can also lead to BAL, as shown in a study [214] on sweet potato *Ipomoea batatas* infected with the nonpathogenic fungus *Fusarium oxysporum*. The study revealed that living sweet potato tissues inoculated with viable fungal conidia emitted significant luminescence, whereas dead tissues or those treated with dead fungi did not. The intensity of BAL was directly correlated with the concentration of fungal conidia, and spectral analysis showed that the luminescence occurred in the 450-630 nm range. This finding highlights the importance of the fungal metabolic activity in inducing BAL in plant tissues, which could be used as an indicator of infection and plant response.

The study by Rastogi and Pospíšil [215] explored the production of H_2O_2 and $HO^{\bullet}$ in potato tubers during the necrotrophic phase of infection by the hemibiotrophic pathogen *Phytophthora infestans*. Using 3,3-diaminobenzidine tetrahydrochloride (DAB) imaging and electron paramagnetic resonance (EPR) spectroscopy, the researchers demonstrated significant formation of H_2O_2 and $HO^{\bullet}$ in infected tissues. This oxidative burst is associated with lipid peroxidation, indicated by enhanced BAL from the infected tubers. The BAL observed in such cases is a result of oxidative stress induced by the pathogen, which plays a role in the plant's defense strategy.

In his study, Allen [216] explored the phenomenon of BAL in both eukaryotic and prokaryotic cells, with a particular focus on polymorphonuclear leukocytes (PMNL). The research highlights that PMNLs, essential components of the acute inflammatory response, generate BAL during their microbicidal activity. This activity is characterized by increased glucose oxidation via the hexose monophosphate shunt and augmented non-mitochondrial oxygen consumption. These metabolic changes are linked to the activation of a membrane-associated NADPH oxidoreductase, which leads to the production of ROS. The experiments demonstrated the oxygen-dependence; PMNLs showed no BAL under anaerobic conditions until oxygen was introduced, triggering an immediate luminescent response. For the PMNL studies, human whole blood was used to isolate leukocytes, which were then subjected to phagocytosis using zymosan particles. Zymosan, polysaccharide extracted from *Saccharomyces cerevisiae*, isused as a model for microbial particles in studies on immune response and as a tool in the study of inflammatory mediators. The BAL was measured under varying oxygen conditions [216].

The topic of BAL modulation by fungal patogene was further explored and discussed by the candidate in the chapter 7.

2.6.2 Bacterial pathogen

In the study [217], *Arabidopsis thaliana*, a model plant species, was infected with the bacterial pathogen *Pseudomonas syringae* to investigate the role of BAL in plant resistance responses. This research highlights the use of BAL to report the activation of plant defense mechanisms, specifically hypersensitive cell death triggered by gene-for-gene interactions. BAL provides valuable temporal and spatial information on these resistance responses, which involve increases in intracellular calcium and reactive nitrogen species rather than ROS. The study emphasizes the non-invasive nature of BAL as a tool for monitoring plant immune responses in real-time .

2.6.3 Viruses

Viruses also trigger BAL in infected plants as part of their defense responses. The study by Kobayashi [28] investigated the response in cowpea (*Vigna unguiculata*) infected with cucumber mosaic virus through the detection and analysis of BAL. The response is a plant defense mechanism involving ROS generation, which leads to cell death at infection sites. The researchers used a photon counting imaging system to capture the spatiotemporal dynamics of BAL, associated with the oxidative burst. The study found that BAL in cowpea leaves infected with virus showed significant transient increases in BAL intensity, peaking between 10 and 16 hours post-inoculation. These emissions were linked to ROS production, as their intensity was markedly reduced when an antioxidant, Tiron, was applied. Spectral analysis indicated that the photon emissions were primarily due to chlorophyll molecules, suggesting that oxidative damage in chloroplasts plays a role in the emission mechanism [28].

2.7 Enviromental modulators

Various environmental stressors, such as pollutants, salinity, drought, flooding, nutrient insufficiency, heat, and chilling, can significantly alter BAL levels. These stress conditions disrupt cellular homeostasis, leading to increased production of ROS, which, in turn, elevate BAL as a byproduct of oxidative stress responses. Environmental factors represent a complex set of phenomena and circumstances that can influence BAL. These factors often overlap with other categories of stress conditions, such as chemical or physical modulators, yet they are grouped as environmental due to their broader impact on ecosystems and the organisms within them. For example, pollution could be considered a chemical modulator, while heat and chilling are often classified under physical factors like temperature. However, these are labeled as environmental factors because they arise from the organism's interaction with its external surroundings, encompassing a range of stress conditions that collectively shape biological responses.

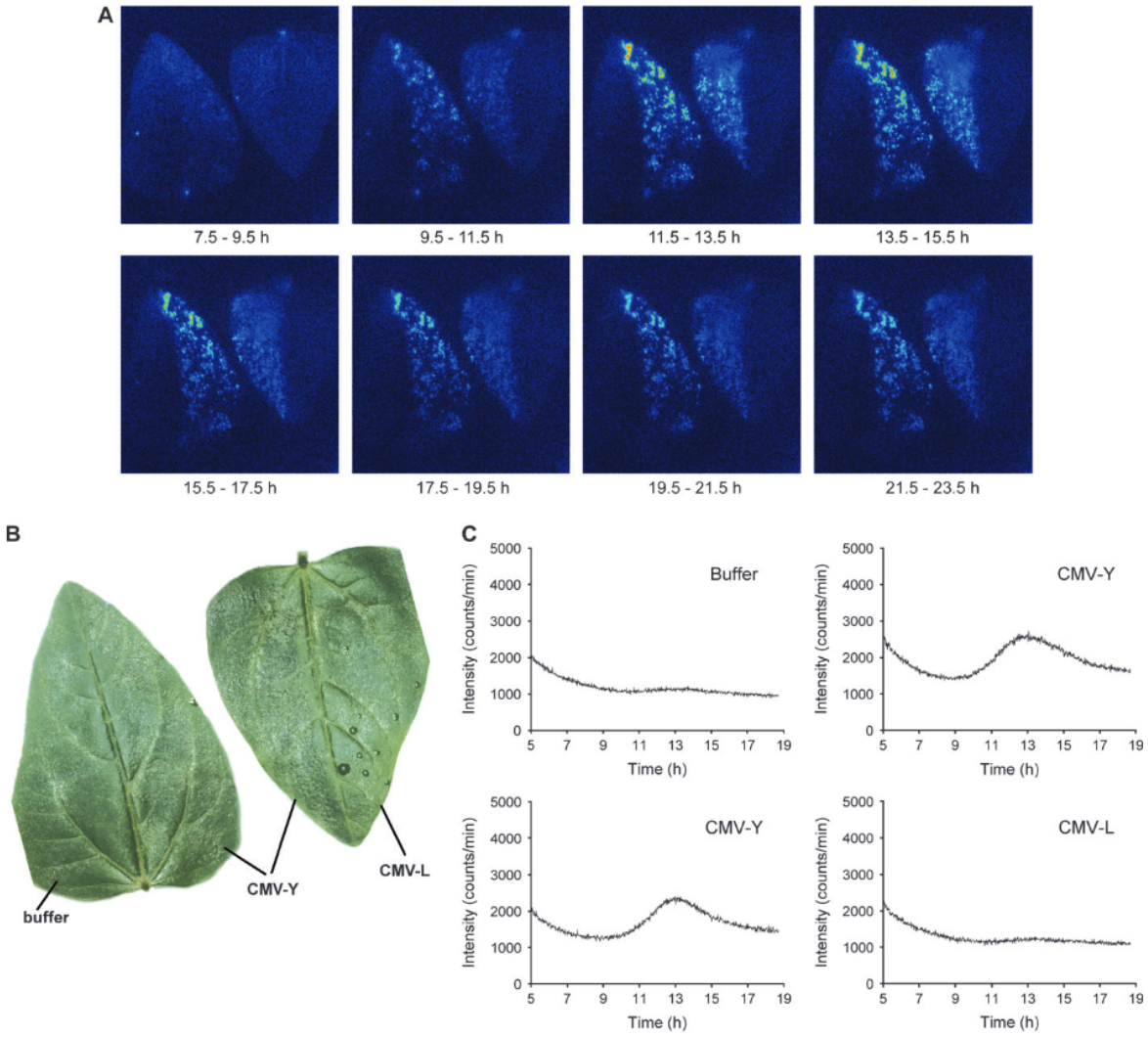

Figure 2.4: BAL of the hypersensitive response observed on cowpea leaves after inoculation with cucumber mosaic virus and its comparison among different strains of virus, CMV-Y (yellow), CMV-L (legume), and buffer treatment. (A) Time sequence of BAL images of two leaves after inoculation observed using the ×0.33 lens system. Images are constructed with time integration over the time range indicated below each image. (B) A sample photograph taken 24 h after inoculation. The condition of inoculation treatment is indicated. (C) Comparison of time-courses of BAL among different treatment regions for CMV-Y, CMV-L, and buffer. [28]

2.7.1 Heat and chilling

Temperature extremes, such as heat and chilling, are significant environmental factors that disrupt metabolic functions and induce stress responses, which manifest as changes in BAL. Both heat and cold stress can affect enzyme activity, protein stability, and membrane fluidity, leading to increased production of ROS and altered BAL levels. These temperature-related influences are particularly impactful because they directly affect the physical properties and stability of biological molecules and structures.

The study by Boveris et al. [218] examined the influence of temperature on BAL in both dry and soaked seeds. The researchers found that temperature plays a significant role in enhancing BAL, with both dry and soaked seeds exhibiting substantial increases in light emission at higher temperatures. For instance, dry seeds showed 10 times more emission at 52 °C and 40 times more at 110 °C compared to room temperature, while soaked seeds also displayed enhanced chemiluminescence as temperature rose. This temperature dependence is linked to two main processes: the enzymatic lipoxygenase reaction, which dominates at lower temperatures, and the non-enzymatic autooxidation of unsaturated fatty acids, which becomes more prominent at higher temperatures.

In the study by Prasad et al. [219] explored the biochemical responses of the unicellular green alga *Chlamydomonas reinhardtii* to heat stress, focusing on the production of $^1O_2$ and the associated lipid peroxidation mechanisms. The algae were subjected to a temperature of 40 °C and used various advanced techniques to monitor the formation of ROS, particularly $^1O_2$. The study found that heat stress induces significant lipid peroxidation in algae cells, marked by the generation of hydroperoxides and malondialdehyde (MDA). BAL measurements revealed the presence of $^3(R{=}O)^*$, which further suggestes the formation of electronically excited species during lipid peroxidation. Electron paramagnetic resonance (EPR) spin-trapping spectroscopy corroborated the presence of $^1O_2$, confirming that this ROS is produced during heat stress. The mechanism underlying $^1O_2$ formation was found to be primarily enzymatic, driven by lipoxygenase activity. This conclusion was supported by experiments showing that lipoxygenase inhibitors such as catechol and caffeic acid significantly suppressed the production of $^1O_2$.

The study by Hideg and Björn [220] investigated the impact of chilling stress on plants, particularly focusing on the increase in BAL as a response to this stress. Chilling stress, which occurs when plants are exposed to low but non-freezing temperatures, disrupts cellular homeostasis and triggers the production of ROS, initiating lipid peroxidation, leading to cellular damage.

2.7.2 Drought

Drought stress induces oxidative stress in plants by disrupting cellular water balance, leading to the generation of ROS. Specifically, during drought conditions, cells experience dehydration, which accelerates lipid peroxidation.

Experiments conducted on red bean seedlings (*Vigna angularis*) under drought conditions demonstrated a

marked increase in BAL, particularly from the root apex [221]. The photon emission intensity varied with the severity of drought, indicating that stronger drought stress caused higher BAL levels.

The temporal pattern of BAL in plants under drought conditions exhibits characteristic phases. Initially, a rapid increase in photon emission is observed as drought stress is applied, reflecting immediate oxidative stress and cellular responses. This is followed by a gradual decline as the plant adjusts to the stress or succumbs to damage [221]. In cases where the drought period is prolonged, BAL may decrease after an initial peak, corresponding to cellular damage that reduces the plant's overall metabolic activity. Moreover, rewatering after a period of drought can also trigger a resurgence in BAL, as damaged cells attempt to recover and resume normal metabolic processes. This rehydration-induced BAL surge is often stronger than the initial response to drought, potentially due to the activation of repair mechanisms and further ROS generation [221].

Drought stress has a profound impact on plant roots, significantly affecting their physiological and biochemical responses. Drought conditions trigger an increase in the production of ROS in plant roots, which can lead to oxidative stress and cellular damage. As part of the plant's defense mechanism, antioxidant enzymes such as ascorbate peroxidase (APX) become critical in mitigating the harmful effects of ROS. Research on soybean roots showed that under drought stress, there is a notable increase in APX activity, which helps to scavenge excess hydrogen peroxide produced during oxidative bursts [222]. This increase in APX activity was associated with a reduction in BAL, as the enzyme's action reduces the levels of ROS [223]. The enhanced activity of APX under drought conditions indicates that the plant is actively responding to stress by boosting its antioxidant defenses to protect root tissues from oxidative damage. These changes are crucial for maintaining root function and overall plant survival during prolonged periods of water scarcity.

The measurement of BAL offers a non-invasive method for detecting early drought stress in plants. Unlike conventional methods that rely on visible signs of damage, BAL monitoring can identify stress at the cellular level long before it manifests as wilting or tissue necrosis. This makes it a valuable tool for agricultural applications, where timely interventions can mitigate the effects of drought on crop yield and quality [221].

2.7.3 Nutrients

Nutrient sufficiency and insufficiency play critical roles in cellular metabolism, significantly impacting cell function, growth, and overall health. When nutrients are sufficient, cells experience optimal energy production through efficient ATP generation from glucose and fatty acids. This ensures that energy-dependent processes operate smoothly. Adequate amino acids are crucial for protein synthesis, supporting the production of enzymes, structural proteins, and signaling molecules. Sufficient nucleotides enable proper DNA and RNA synthesis, facilitating cell replication and repair. Vitamins and minerals, such as vitamin D and zinc, are vital for hormone production and signaling pathways, promoting cell growth and division. Additionally, antioxidants like vitamins C and E, along with selenium, maintain the antioxidant defense system, protecting cells from oxidative damage and ensuring metabolic flexibility [224, 225].

The study by Madl et al. [226] examined the effects of culture medium changes on BAL from cancer and non-cancer cells. The findings emphasize that changes in the cell culture medium alone caused a significant, cell-type-specific increase in BAL. This suggests that the medium, which provides nutrients and affects the cellular environment, plays a crucial role in modulating BAL. Specifically, the medium change induced a transient increase in BAL, with different cell types showing varying responses based on their susceptibility to external stressors. The increase in BAL following a culture medium exchange, can be attributed to the nutrient availability. The introduction of fresh medium alters the nutrient environment of the cells, leading to a sudden shift in cellular metabolism. Cells quickly adapt to the new conditions, which can temporarily increase ROS production.

2.7.4 Pollution

While pollution can be primarily chemical, it is classified as a complex circumstance when considering the broader context of environmental interactions. Pollutants can have combined physical (e.g., particulates causing mechanical irritation,), chemical (e.g., toxic chemical reactions), and biological (e.g., microorganisms, metabolites) effects on biological systems. The multifaceted nature of pollution justifies its classification as a complex modulator.

Studies have demonstrated that strong environmental stressors, such as toxic chemicals, can suppress seedling development, leading to a decrease in BAL. Conversely, mild stress conditions may actually enhance BAL as seedlings activate their defense mechanisms. For instance, experiments with wastewater sediment solutions revealed that while strong stress suppressed both germination and BAL, mild stress conditions could increase BAL without significantly hindering growth [227].

The study by Ohya et al. [228] explored the effect of sodium chloride (NaCl) stress on BAL in red bean *Vigna angularis* seedlings. The key findings highlight that NaCl-induced stress significantly affects BAL, with varying results depending on the concentration of NaCl in the solution. At lower concentrations, such as 0.01 M NaCl, there was minimal impact on BAL, suggesting that the plants could acclimate to mild salt stress. However, at higher concentrations, such as 0.1 M and 1 M NaCl, photon emission decreased, indicating physiological damage and growth inhibition due to NaCl stress. This reduction in BAL intensity was linked to the decreased production of ROS. Interestingly, at a very high concentration of NaCl (4.5 M), there was a dramatic increase of BAL intensity, which was associated with severe cellular damage, including the destruction of cell membranes. This intense BAL was attributed to the excessive generation of ROS due to the breakdown of cellular structures, including organelles [228].

The study by Hossain et al. [229] investigated the effects of cadmium (Cd) stress on soybean plants (*Glycine max*). Cd^{2+} disturbs normal cellular functions by replacing chemicaly similar metals (Zn^{2+}, Ca^{2+}, and Fe^{2+}) from proteins [230]. In addition, Cd^{2+} has high affinity for protein sulfhydryl groups and thus can cause enzyme inactivation [231]. Under cadmium stress, the study observed increased BAL, indicating heightened oxidative

stress and cellular damage [229].

2.7.5 Chronobiological rhythms

Chronobiological rhythms are the natural cycles that govern the physiological and behavioral functions of living organisms. These rhythms help organisms adapt to the regular changes in their environment, such as the cycle of day and night, seasonal variations, and other periodic phenomena. Chronobiology, the study of these rhythms, categorizes them into several types based on their duration: circadian (roughly 24-hour cycles), ultradian (periods ranging from minutes to hours), and infradian (days, weeks, months, or years) rhythms [232].

Circadian rhythms help organisms anticipate and respond to daily environmental changes. These rhythms are controlled by a central genetic oscillator that involves transcriptional and translational feedback loops. Genes form a complex network of feedback loops that regulate various physiological processes [233, 234]. The relationship between circadian rhythms and ROS homeostasis is intricate and involves multiple layers of regulation. The circadian clock regulates the expression of genes involved in both the production and scavenging of ROS [235]. For instance, genes encoding ROS-generating enzymes such as NADPH oxidases [236–238] and superoxide dismutases [239] exhibit circadian patterns of expression. This regulation ensures that ROS levels fluctuate in a controlled manner throughout the day, peaking at times that align with the metabolic activities and environmental stresses. The circadian clock influences the expression and activity of these antioxidant enzymes, aligning their peak activity with times of high ROS production. [239]. Studies have shown that plants with a well-synchronized circadian clock are better equipped to handle oxidative stress, as they can more effectively manage ROS levels through timely activation of antioxidant defenses [240, 241].

Sensing BAL from the human body has been explored for its correlation with circadian rhythms by several studies [29, 242–245], as described in more detail below.

Kobayashi's et al. [242] study utilized a highly sensitive imaging system to document BAL from the human body, uncovering a distinct diurnal rhythm in their intensity. These emissions peaked in the late afternoon, correlating with changes in metabolic rates, which are known to follow circadian patterns. This research underscores the rhythmic nature of BAL and suggests that they are linked to the body's metabolic processes, which are governed by the circadian clock.

Scholkmann et al. [29] focused on the spatio-temporal dynamics of BAL from human hands, demonstrating variability based on time of day and individual differences. Their findings indicate that photon emission intensities were not only lower around noon but also exhibited patterns that varied day-to-day. These fluctuations in BAL from the hands are indicative of underlying rhythmic biological processes, potentially offering insights into personal circadian rhythm profiles.

Van Wijk et al. [243] explored photon emissions from 29 different body sites, emphasizing the rhythmic variations in BAL intensity across different times of the day and sites. Their findings indicate that emission intensity was generally lowest in the morning and highest in the evening.

Study by Cifra et al. [244] provided detailed observations of BAL from human hands, emphasizing their time-dependent nature. The research highlighted a significant rhythmic fluctuation over 24 hours, with emissions decreasing around midday and increasing in the afternoon. The study suggests that these emissions are closely associated with the circadian rhythms of the human body, possibly linked to the daily cycles of metabolic and oxidative activities.

Gallep et al. [245] has shown that BAL in seedlings exhibits pronounced circadian cycles, with BAL patterns aligning closely with local gravitational oscillations. These rhythms suggest a biological synchronization with environmental cycles, highlighting the complexity between internal biological clocks and external environment.

This topic was further explored and discused by the candidate in the chapter 8.

3 Aims of the doctoral thesis

The goal of this thesis is to employ both theoretical and experimental approaches to comprehensively study biological autoluminescence (BAL). This involves developing a deeper understanding of the mechanisms behind BAL generation, identifying the factors that influence its modulation, and refining the methods used for its detection and analysis. Through this dual approach, the thesis aims to bridge the gap between theoretical models and practical applications, enhancing our ability to utilize BAL as a tool for scientific research and medical diagnostics.

The specific aims of the doctoral thesis are as follows:

1) Develop methodology to measure biological autoluminescence from various biological systems.
2) Conduct experiments on various living organisms and biological materials to investigate the role of different modulators in influencing biological autoluminescence.
3) Perform experiments involving biological matter and deliver a spectral analysis.
4) Investigate the factors influencing the kinetics of biological autoluminescence.
5) Discuss the potential applications of detecting biological autoluminescence in living systems.

4 | KINETICS OF BIOLOGICAL AUTOLUMINESCENCE

The following chapter discusses an ongoing study on the kinetics of BAL, aiming to develop a mathematical model that captures the underlying mechanisms of this complex biochemical phenomenon. The work presented here is in progress and, as such, the findings and models should be considered provisional and subject to further validation and refinement. The theoretical foundations laid out in this chapter are derived from existing literature and are intended to provide a conceptual framework for understanding the dynamics of BAL. These theoretical insights are crucial for building the future mathematical model.

4.1 INTRODUCTION

While there is a growing qualitative understanding of the biological and chemical processes behind the generation of BAL, a unified quantitative framework is still missing. Currently, the knowledge base is fragmented and scattered across a vast literature. In our study, we consolidate this dispersed information and apply the fundamental laws of chemical kinetics to establish a comprehensive framework. This framework not only identifies but also quantifies the key steps in the production of BAL at the cellular level. It provides estimates of the concentration of endogenously generated electronically excited species and the amount of associated photon emission. Our model aims to quantify the complex biochemical processes leading to the BAL. This involves analyzing the production rates of ROS, their interactions with biological molecules, their detoxification by cellular antioxidants, and modulation by prooxidants.

The study of the kinetics involved in the buildup of biological antioxidative levels (BAL) focuses on the rates at which reactive oxygen species (ROS) are produced, interact with biological molecules, and are detoxified by cellular antioxidant mechanisms. Several factors influence the kinetics of ROS generation, including the availability of essential substrates like oxygen and NADPH, the activity of enzymes that produce ROS, and the presence of metal ions that catalyze the formation of highly reactive species. Conversely, the elimination of ROS is managed by antioxidant defenses, such as enzymatic systems including superoxide dismutase (SOD), catalase, and glutathione peroxidase, along with non-enzymatic antioxidants like ascorbate. Additionally, the process includes the rates at which oxidative damage propagates, the formation of high-energy intermediates, the subsequent creation of electronically excited species, and ultimately, photon emission. This process is simplified in the Fig. 4.1.

The study of kinetics in complex process in living systems, biological autoluminescence, utilizes systems of differential equations to describe and predict the behavior of reactants and products over time. The reaction rate constant, denoted usually as k, is a fundamental parameter in the kinetics of a chemical reaction. It quantifies

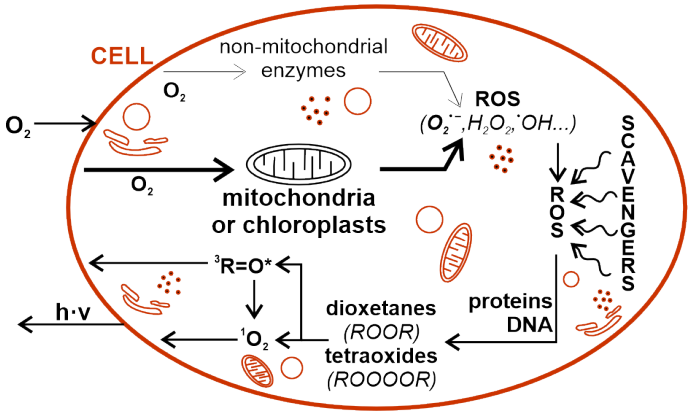

Figure 4.1: A simplified linearized framework of the processes starting from consumption of oxygen and leading to photon emission, based on [46]

the speed at which a reaction proceeds. Generally, for a bimolecular reaction 4.1, the rate might be given by rate = k[A][B], where [A] and [B] are the concentrations of reactants A and B, respectively. The rate constant k here determines how rapidly product C is formed from reactants.

$$A + B \rightarrow C \tag{4.1}$$

4.2 Kinetics of the process leading to BAL

To accurately study these complex biochemical events, it is essential to clearly define the initial conditions of the system. This model focuses on proteins, which are the most abundant biomolecules by dry weight in cells and thus serve as primary targets for ROS. Proteins are targeted not only because of their abundance but also due to their involvement in high-rate biochemical reactions, which are critical for maintaining cellular structure and function [246]. The initial conditions include the concentration of the oxidizable substrate, which is approximated based on the effective concentration of amino acid residues in cells, considering an average of 600 amino acids per protein [246]. This approximation provides a realistic basis for the substrate levels available for reactions. Additionally, the model represents spontaneous BAL occurring in complete darkness, without the presence of any chromophores that might influence the luminescence. Oxygen, ferrous ions, and antioxidants are assumed to be at a steady state at the onset of the reaction (Tab. 4.1). Enzymes necessary for catalyzing these reactions are always available and are not a limiting factor in the model, ensuring continuous and effective reaction progression. These reactions typically occur in water, reflecting the intracellular environment of the organisms, and under physiological temperatures and pH levels that align with those found in living systems.

Table 4.1: Initial concentrations of molecules and ferrous ions involved in the model

	Concentration [M]	**Ref.**
$[^3O_2]$	$3\times10^{-6} - 30\times10^{-6}$	[247]
$[Fe^{2+}]$	10^{-6}	[126]
$[AscH^-]$	$0.1\times10^{-3} - 5\times10^{-3}$	[248]
[GSH]	$0.5\times10^{-3} - 10\times10^{-3}$	[249]
[RH]	$15\times10^{-3} - 3$	[246]

Oxygen consumption by cells

Oxygen is fundamental to life, crucial for function of all aerobic organisms, influencing various cellular and metabolic processes. Oxygen consumption in cells is a well-studied area, providing insights into cellular metabolism and the redox balance within organisms. The rate at which different cells utilize oxygen varies significantly, influenced by factors such as cell type, size, and metabolic activity. Larger cells, especially those with many mitochondria, smaller cells like bacteria or red blood cells, which lack mitochondria, consume much less oxygen. There appears to be a correlation between the size of a cell, its protein content, and rate of oxygen consumption rate by mitochontria. Oxygen concentration within mitochondria is usually in the range 3 to 30 μM [247].

ROS production

Mitochondria are the primary producers of ROS, when no specialized enzymes like those from the NOX family are present in the cell. They play a crucial role because oxygen entering the mitochondrial matrix is essential for the last step of the electron-transport chain. Almost all of the molecular oxygen an aerobic organism consumes is used in ATP generation through oxidative phosphorylation, primarily facilitated by the enzyme cytochrome c oxidase within the inner mitochondrial membrane. During this process, molecular oxygen undergoes one-electron reduction by the electron transport chain, ultimately being reduced to water. Not all oxygen molecules consumed by mitochondria are fully reduced; a portion leads to the formation of the superoxide anion radical, a precursor to other ROS. This occurs due to electron leakage from the mitochondrial electron transport chain, which combines with molecular oxygen in its ground state. It is estimated that about 0.1 to 4% of the oxygen a cell uses contributes to ROS production [218]. This rate might be overestimated, as many experiments expose mitochondria directly to atmospheric oxygen levels of 21%, a hyperoxic condition for these cell components. Under physiological conditions, the actual rate of ROS production is likely significantly lower, possibly one to two orders of magnitude less. In different tissues, the percentage of oxygen contributing to ROS can range from about 0.12 to 0.8% or even lower [51, 250].

As outlined in the chapter 2, the reaction detailed in equation 2.1 emphasizes the pivotal role of superoxide

anion radical ($O_2^{\bullet -}$) in initiating oxidative processes within cells. A specific rate of production for $O_2^{\bullet -}$ by mitochondria has been assumed to be $d[O_2^{\bullet -}]/dt = 0.57 \times 10^{-6}$ [251]. The $O_2^{\bullet -}$ is not a very strong oxidant but plays a crucial role in the propagation of oxidative chain reactions as it is a precursor to hydrogen peroxide H_2O_2 (reaction 1). H_2O_2 is further detoxified either enzymatically (as seen in reactions 2 and 3) or through decomposition by iron ions. The reaction 5, also known as the Fenton reaction, is particularly significant as it facilitates the formation of $HO^{\bullet}$ from hydrogen peroxide. Reactions in the Tab. 4.3 present the rate constants of possible inorganic reactions involving ROS.

Reactions of ROS with biomolecules

In the context of the protein-centered model, amino acids are of particular interest due to their role as the building blocks of proteins—the most abundant biomolecules by dry weight in cells and primary targets for reactive oxygen species [246]. This focus is crucial as it helps to elucidate the interactions between ROS and proteins at the molecular level. Amino acids, integral to protein structures, are thus critical subjects of study. Tab. 4.4 presents the rate constants for the reactions of various amino acids with three different reactive species: the $HO^{\bullet}$, $^1O_2$, and ozone (O_3). $HO^{\bullet}$ is known for its high reactivity, which is reflected in the comparatively large rate constants observed for its reactions with amino acids. This reactivity can be attributed to the non-selective and aggressive nature of the $HO^{\bullet}$, which reacts rapidly with most organic molecules, including amino acids. In contrast, the rate constants for the reactions of amino acids with $^1O_2$ and O_3 are several orders of magnitude lower than those with $HO^{\bullet}$. This suggests that $^1O_2$ and O_3 are significantly less reactive towards amino acids. $^1O_2$, while still a potent oxidant, tends to react more selectively with specific molecular targets, such as double bonds in unsaturated fatty acids or certain aromatic amino acids. The reaction of $^1O_2$ with biomolecules (reaction 15) can lead to the formation of various products, one of which is dioxetane.

Propagation and termination

Table 4.5 summarizes the rate constants for various propagation and termination reactions involving organic radicals. The reactions of alkyl radicals ($R^{\bullet}$) and peroxyl radicals ($ROO^{\bullet}$) with antioxidants like glutathione (GSH) and ascorbate ($AscH^-$), illustrate the crucial role of antioxidants in mitigating radical damage. These antioxidants donate hydrogen atoms to the radicals, effectively preventing further propagation of the radical chain, underscoring the protective function of antioxidants in biological systems. The reaction (reaction 19) of $R^{\bullet}$ with molecular oxygen, producing $ROO^{\bullet}$, occurs with rate constants in the range of 10^8 - 10^{10} $M^{-1}s^{-1}$. This rapid reaction highlights the strong affinity of oxygen for organic radicals, a key step in protein oxidation.

Formation of high-energy intermediates and excited molecules

Table 4.6 provides a comprehensive overview of the rate constants associated with various reactions that lead to the formation of high-energy intermediates and excited molecules. Reaction 31 represents a simplified

summary of multiple complex reactions that occur when two $ROO^{\bullet}$ interact and lead to the formation of electronically excited species. The listed rate constant, ranging from 10^2 to 10^8 $M^{-1}s^{-1}$, encompass the overall process, which actually involves several intermediate steps and pathways. The reactions involved in individual steps typically occur at a wide range of rate constants, reflecting the diversity of the processes involved. For instance, the recombination of $ROO^{\bullet}$ to form tetraoxides (ROOOOR) (reaction 32) can exhibit rate constants spanning across five orders of magnitude. The recombination of $ROO^{\bullet}$ and cyclization to form dioxetanes are two competing processes with distinct likelihoods depending on the local concentration of $ROO^{\bullet}$. Recombination to form ROOOOR generally occurs with higher rate constants, especially at elevated concentrations of $ROO^{\bullet}$, making it the more probable reaction under such conditions. In contrast, peroxycyclization (reaction 33), which leads to the formation of dioxetanes ($ROOR_{dioxetane}$), tends to have lower rate constants and is more likely to occur when the concentration of $ROO^{\bullet}$ is lower, reducing the probability of intermolecular recombination.

Reactions 34 to 37 include reactions in solvent cage complex, this refers to a transient structure formed during certain chemical reactions, particularly in radical processes. It occurs when two reactive species, are produced in close proximity within a solvent. The cage effect keeps these species close together, limiting their ability to diffuse apart. As a result, the species may react with each other or undergo further transformations within the cage before escaping into the bulk solvent. Cage complexes are important in determining the fate of reactive intermediates. For instance, reactions 36 and 37 both involve the decomposition of reactive intermediates, but they differ significantly in their outcomes and rate constants. Reaction 37, that produces $^1O_2$, is more likely to occur due to its higher rate constant. Reaction 36, on the other hand, occurs relatively slowly, with a rate constant of around 10 s^{-1} and is therefore less likely to dominate under the same conditions. Reaction 38 involves the decomposition of a ROOR generating $^3(R{=}O)^*$. The rate constant for this reaction is extremely high, indicating that this process occurs very quickly once initiated. Reaction 39 describes the energy transfer from $^3(R{=}O)^*$ to $^1O_2$. The rate constant for this reaction is quite high, indicating that this reaction is relatively fast and efficient, and also implying that $^3(R{=}O)^*$ does not participate in BAL in high amounts.

Transition of electronically excited molecules to the ground state

Table 4.7 details a series of reactions where electronically excited molecules transition to their ground state. Reaction 40 outlines the radiationless transition of $^1O_2$ to $^3O_2$. This process occurs without photon emission ($h\nu$). Reaction 41 involves the transition from $^1O_2$ to $^3O_2$ accompanied by the emission of a photon. Reaction 42 describes a process that can involve the simultaneous transition of both $^1O_2$ molecules from the singlet state to the triplet state, during which a photon is emitted. The rate constants listed suggest that this photon-emitting process by $^1O_2$ is relatively slow compared to radiationless transitions, indicating that most of the energy from $^1O_2$ is dissipated in the solvent and does not produce light at all. Reaction 43 features a reaction involving an $^3O_2$ transitioning to its ground state, emitting a photon.

4.3 Rate constants of chemical reactions involved in BAL generation

Table 4.2: Rate constants for enzyme-catalyzed reactions

ID	Reaction	Rate constant	Ref.
1	$2O_2^{\bullet -} + 2H_2O + \xrightarrow{SOD} H_2O_2 + {}^3O_2 + HO^-$	2.3×10^9 $M^{-1}s^{-1}$	[251]
2	$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$	5×10^7 $M^{-1}s^{-1}$	[252]
3	$2H_2O_2 \xrightarrow{CAT} 2H_2O + {}^3O_2$	7.6×10^6 –7.9×10^6 $M^{-1}s^{-1}$	[253]

Table 4.3: Rate constants of inorganic reactions involving ROS

ID	Reaction	Rate constant	Ref.
4	$2HO^\bullet \rightarrow \frac{1}{4}({}^1O_2 + {}^3O_2) + H_2O$	5.5×10^9 $M^{-1}s^{-1}$ 5×10^9 $M^{-1}s^{-1}$	[154] [254]
5	$H_2O_2 + Fe^{2+} \rightarrow HO^\bullet + HO^- + Fe^{3+}$	76-100 $M^{-1}s^{-1}$ 56 $M^{-1}s^{-1}$	[126] [154]
6	$H_2O_2 + Fe^{3+} \rightarrow HO_2^\bullet + H^+ + Fe^{2+}$	0.01 $M^{-1}s^{-1}$ 0.01 - 0.02 $M^{-1}s^{-1}$	[255] [256]
7	$2HO^\bullet \rightarrow \frac{1}{4}({}^1O_2 + {}^3O_2) + H_2O$	5.5×10^9 $M^{-1}s^{-1}$ 5×10^9 $M^{-1}s^{-1}$	[154] [254]
8	$2HO^\bullet \rightarrow H_2O_2$	4.7×10^9 $M^{-1}s^{-1}$ $(4 \pm 1) \times 10^9$ $M^{-1}s^{-1}$ 5.5×10^9 $M^{-1}s^{-1}$	[257] [258] [259]
9	$HO^\bullet + HO_2^\bullet \rightarrow \frac{1}{2}({}^1O_2 + {}^3O_2) + H_2O$	7.1×10^9 $M^{-1}s^{-1}$ 6×10^9 $M^{-1}s^{-1}$ 6×10^9 $M^{-1}s^{-1}$	[154] [258] [259]
10	$2HO_2^\bullet \rightarrow \frac{1}{2}({}^1O_2 + {}^3O_2) + H_2O_2$	8.3×10^5 $M^{-1}s^{-1}$ $(3.4 \pm 2.5) \times 10^6$ $M^{-1}s^{-1}$ $(8.6 \pm 0.62) \times 10^5$ $M^{-1}s^{-1}$ $(8.3 \pm 0.7) \times 10^5$ $M^{-1}s^{-1}$	[154] [258] [260] [261]
11	$HO_2^\bullet \rightarrow H^+ + O_2^{\bullet -}$	7.5×10^6 s^{-1} 1.6×10^5 s^{-1} 7.9×10^5 s^{-1}	[154] [255] [256]
12	$H_2O_2 + O_2^{\bullet -} \rightarrow HO^\bullet + OH^- + {}^3O_2$	16 $M^{-1}s^{-1}$ (0.13 ± 0.07) $M^{-1}s^{-1}$ 0.005 - 2.25 $M^{-1}s^{-1}$	[154] [262] [263]
13	$HO^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O$	3×10^7 $M^{-1}s^{-1}$	[154]

Continued on next page

Table 4.3 – continued from previous page

ID	Reaction	Rate constant	Ref.
		$(2.6 \pm 0.8) \times 10^7$ $M^{-1}s^{-1}$	[258]
		2.7×10^7 $M^{-1}s^{-1}$	[259]

Table 4.4: Rate constants for reactions between amino acid residues in proteins and ROS

ID	Reaction	Rate constant	Ref.
14	$HO^\bullet + RH \rightarrow R^\bullet + H_2O$	reactions of $HO^\bullet$ with amino acids:	
		His: 4.8×10^9 $M^{-1}s^{-1}$	[264]
		4.3 - 5.0×10^9 $M^{-1}s^{-1}$	[265]
		1.9 - 5.0×10^9 $M^{-1}s^{-1}$	[258]
		Trp: 13×10^9 $M^{-1}s^{-1}$	[264]
		7.1 - 14×10^9 $M^{-1}s^{-1}$	[265]
		Tyr: 9.4 - 10.5×10^9 $M^{-1}s^{-1}$	[265]
		Phe: 5.8 - 7.2×10^9 $M^{-1}s^{-1}$	[265]
		5.8 - 7.2×10^9 $M^{-1}s^{-1}$	[258]
		Arg: 0.73 - 5.7×10^9 $M^{-1}s^{-1}$	[265]
		0.57 - 3.5×10^9 $M^{-1}s^{-1}$	[258]
		Cys: 19×10^9 $M^{-1}s^{-1}$	[264]
		5.9 - 40×10^9 $M^{-1}s^{-1}$	[258]
		Met: 7.4×10^9 $M^{-1}s^{-1}$	[264]
		6.0 - 8.2×10^9 $M^{-1}s^{-1}$	[265]
		6.5 - 8.5×10^9 $M^{-1}s^{-1}$	[258]
		Val: 0.66 - 0.67×10^9 $M^{-1}s^{-1}$	[265]
		0.66 - 0.72×10^9 $M^{-1}s^{-1}$	[258]
		Leu: 1.6 - 1.9×10^9 $M^{-1}s^{-1}$	[265]
		1.6 - 1.8×10^9 $M^{-1}s^{-1}$	[258]
		Ile: 1.7×10^9 $M^{-1}s^{-1}$	[265]
		1.7 - 1.8×10^9 $M^{-1}s^{-1}$	[258]
		Gly: 0.0073 - 0.017×10^9 $M^{-1}s^{-1}$	[265]
		0.0046 - 0.26×10^9 $M^{-1}s^{-1}$	[258]
		Ala: 0.074 - 0.079×10^9 $M^{-1}s^{-1}$	[265]
		0.046×10^9 $M^{-1}s^{-1}$	[258]
		Ser: 0.23 - 0.32×10^9 $M^{-1}s^{-1}$	[265]
		0.25 - 0.32×10^9 $M^{-1}s^{-1}$	[258]
		Thr: 0.36 - 0.51×10^9 $M^{-1}s^{-1}$	[265]

Continued on next page

Table 4.4 – continued from previous page

ID	Reaction	Rate constant	Ref.
		0.39 - 0.51 × 10^9 $M^{-1}s^{-1}$	[258]
		Lys: 0.35 - 0.60 × 10^9 $M^{-1}s^{-1}$	[265]
		0.35 - 0.65 × 10^9 $M^{-1}s^{-1}$	[258]
		Pro: 0.28 - 0.65 × 10^9 $M^{-1}s^{-1}$	[265]
		0.31 - 0.65 × 10^9 $M^{-1}s^{-1}$	[258]
		Asp: 0.031 - 0.075 × 10^9 $M^{-1}s^{-1}$	[265]
		0.033 - 0.075 × 10^9 $M^{-1}s^{-1}$	[258]
		Asn: 0.049 × 10^9 $M^{-1}s^{-1}$	[265]
		0.032 - 0.049 × 10^9 $M^{-1}s^{-1}$	[258]
		Glu: 0.127 - 0.23 × 10^9 $M^{-1}s^{-1}$	[265]
		0.14 - 0.23 × 10^9 $M^{-1}s^{-1}$	[258]
		Gln: 0.54 × 10^9 $M^{-1}s^{-1}$	[265]
		0.16 - 0.54 × 10^9 $M^{-1}s^{-1}$	[258]
15	RH + $^1O_2$ → ROOH	reactions of aromatic aminoacids with $^1O_2$	
		His: 3.4 × 10^7 $M^{-1}s^{-1}$	[266]
		Trp: 1.3 × 10^7 $M^{-1}s^{-1}$	[266]
		Tyr: 0.2 - 0.5 × 10^7 $M^{-1}s^{-1}$	[266]
		Phe: 0.07 × 10^7 $M^{-1}s^{-1}$	[266]
16	RH + O_3 → product	reactions of aromatic aminoacids with O_3	
		His: 1.7 × 10^3 $M^{-1}s^{-1}$	[267]
		Trp: 5.4 × 10^5 $M^{-1}s^{-1}$	[267]
		Phe: 2.1 × 10^3 $M^{-1}s^{-1}$	[267]

Table 4.5: Rate constants for organic radical reactions: propagation and termination

ID	Reaction	Rate constant	Ref.
17	$R^\bullet$ + GSH → RH + $GS^\bullet$	(1.05 ± 0.05) × 10^5 $M^{-1}s^{-1}$	[268]
		2 × 10^6	[269]
18	$R^\bullet$ + $AscH^-$ → RH + $Asc^{\bullet-}$	10^7 – 10^8 $M^{-1}s^{-1}$	[270]
		2.9 × 10^7	[271]
19	$R^\bullet$ + $^3O_2$ → $ROO^\bullet$	reactions of organic radicals $R^\bullet$ with $^3O_2$ to produce $ROO^\bullet$ have rate constants in the range:	
		10^8 - 10^{10} $M^{-1}s^{-1}$	[272]
20	$ROO^\bullet$ + GSH → ROOH + $GS^\bullet$	8 × 10^3 – 5 × 10^4 $M^{-1}s^{-1}$	[272]

Continued on next page

Table 4.5 – continued from previous page

ID	Reaction	Rate constant	Ref.
21	$ROO^{\bullet} + AscH^{-} \rightarrow ROOH + Asc^{\bullet -}$	10^6 – 10^8 $M^{-1}s^{-1}$	[273]
		2×10^8 $M^{-1}s^{-1}$	[274]
22	$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$	100 $M^{-1}s^{-1}$ (lipids)	[275]
		9×10^{-3} – 90 $M^{-1}s^{-1}$ (lipids)	[276]
		10^3–10^4 $M^{-1}s^{-1}$	[277]
23	$ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O$	10^3–10^7 $M^{-1}s^{-1}$	[278]
24	$ROOH + Fe^{2+} \rightarrow Fe^{3+} + RO^{\bullet} + HO^{-}$	1.5×10^3 $M^{-1}s^{-1}$ (PUFA)	[47]
25	$ROOH + Fe^{3+} \rightarrow Fe^{2+} + ROO^{\bullet} + H^{+}$	0.02 $M^{-1}s^{-1}$	[279]
26	$RO^{\bullet} \rightarrow R^{\bullet} + R{=}O$	β-fragmentation:	
		10^6 s^{-1}	[246]
		$>10^6$ s^{-1}	[280]
		$>10^7$ s^{-1}	[281]
27	$RO^{\bullet} + RH \rightarrow R^{\bullet} + ROH$	10^6 $M^{-1}s^{-1}$	[246]
		10^7–10^9	[282]
28	$RO^{\bullet} + ROOH \rightarrow ROO^{\bullet} + ROH$	$\geq 2\times10^5$ $M^{-1}s^{-1}$	[283]
29	$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR_{linear}$	5×10^7 $M^{-1}s^{-1}$ (lipids)	[284]
30	$R^{\bullet} + R^{\bullet} \rightarrow RR$	7.3×10^8	[285]
		5×10^8 $M^{-1}s^{-1}$	[286]
		1×10^6	[154]

Table 4.6: Rate constants of generation of high-energy intermediates and electronically excited molecules

ID	Reaction	Rate constant	Ref.
31	$ROO^{\bullet} + ROO^{\bullet} \rightarrow\rightarrow {}^3O_2 + {}^3(R{=}O)^* + {}^1O_2$	peroxyradicals react with organic matter with rate constants in the range:	
		10^2 to 10^8 $M^{-1}s^{-1}$	[272]
32	$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOOOR$	2×10^4 - 2×10^7 $M^{-1}s^{-1}$	[280]
		2×10^7 $M^{-1}s^{-1}$ (lipid)	[287]
		10^3 – 10^8 $M^{-1}s^{-1}$	[283]
33	$ROO^{\bullet} \rightarrow ROOR_{dioxetane}$	peroxycyclization:	
		10–10^3 s^{-1} (lipid)	[288]
34	$ROOOOR \rightarrow \{RO^{\bullet} + O_2 + {}^{\bullet}OR\}_{cage}$	10^3–10^5 s^{-1}	[289]
35	$\{RO^{\bullet} + O_2 + {}^{\bullet}OR\}_{cage} \rightarrow 2RO^{\bullet} + {}^3O_2$	10^3–10^5 s^{-1}	[290]

Continued on next page

Table 4.6 – continued from previous page

ID	Reaction	Rate constant	Ref.
36	$\{RO^{\bullet} + O_2 + {}^{\bullet}OR\}_{cage} \rightarrow {}^3(R{=}O)^* + ROH + {}^3O_2$	$10\ s^{-1}$	[139]
37	$\{RO^{\bullet} + O_2 + {}^{\bullet}OR\}_{cage} \rightarrow R{=}O + ROH + {}^1O_2$	$30 - 1.4\times10^4\ s^{-1}$	[139]
38	$ROOR \rightarrow {}^3(R{=}O)^* + R{=}O$	10^7 - $10^{10}\ s^{-1}$	[139]
39	${}^3(R{=}O)^* + {}^3O_2 \rightarrow {}^1(R{=}O) + {}^1O_2$	$10^8\ M^{-1}s^{-1}$ $1.3\times10^9 - 2.55\times10^9$	[291] [292]

Table 4.7: Rate constants for transition of electronically excited molecules to the ground state

ID	Reaction	Rate constant	Ref.
40	${}^1O_2 \rightarrow {}^3O_2$	radiationless transition: $2.9\times10^5\ s^{-1}$ $2.8\times10^5\ s^{-1}$ $4.4\times10^5\ s^{-1}$	 [293] [294] [295]
41	${}^1O_2 \rightarrow {}^3O_2 + h\nu$	$0.11\ s^{-1}$ $1\ s^{-1}$ $0.1\ s^{-1}$	[296] [297] [298]
42	${}^1O_2 + {}^1O_2 \rightarrow {}^3O_2 + {}^3O_2 + h\nu$	$(3.3 \pm 0.6)\times10^{-2}\ M^{-1}s^{-1}$ $(2.65 \pm 0.8)\times10^{-2}\ M^{-1}s^{-1}$ $0.1\ M^{-1}s^{-1}$	[299] [142] [154]
43	${}^3(R{=}O)^* \rightarrow {}^1(R{=}O) + h\nu$	$4.6\times10^4\ s^{-1}$	[300]

5 | Chemical modulators: Pro- and Anti- oxidants

Following sections are based on following conference proceedings:

Červinková, K., Nerudová, M., Hašek, J., and Cifra, M.

Chemical modulation of the ultra-weak photon emission from Saccharomyces cerevisiae and differentiated HL-60 cells

In Photonics, Devices, and Systems VI, 9450, 169-175, 2015.

DOI: 10.1117/12.2070424

5.1 Antioxidants and prooxidants modulation of BAL

As it was described previously in 2.4, BAL is closely associated with the activity of ROS, that can cause significant cellular damage, including lipid peroxidation and protein oxidation. Organisms naturally counteract this damage through antioxidant defense systems, which can reduce ROS levels and thus modulate BAL intensity. In our studies, we investigated how both antioxidants and prooxidants influence BAL signals. Antioxidants, which scavenge ROS, are expected to decrease BAL by reducing oxidative stress. Conversely, prooxidants increase ROS levels, potentially enhancing BAL. By studying these effects in yeast (*Saccharomyces cerevisiae*) and human leukemia cells (HL-60), we aim to gain a deeper understanding of how BAL is modulated, providing insights into the oxidative state of organisms and their ability to respond to external changes.

5.2 Introduction

5.2.1 Examined biological models

We studied two biological models to investigate BAL: yeast cells (*S. cerevisiae*) and differentiated human promyelocytic leukemia cells (HL-60). *S. cerevisiae* emits photons spontaneously due to high metabolic activity during yeast culture growth, while BAL in HL-60 cells is chemically induced by phorbol 12-myristate 13-acetate (PMA). Both models' signals can be modulated by antioxidants. This study specifically focused on the chemical modulation of both spontaneous and induced BAL, using (*S. cerevisiae*) for spontaneous emission and HL-60 cells for chemically induced emission.

Yeast cells (*S. cerevisiae*)

S. cerevisiae is a simple eukaryotic yeast system extensively used in research due to its well-characterized metabolism and genetics. In this study, we measured BAL from yeast cells cultivated in a batch culture. The growth cycle begins with a lag phase immediately after inoculation into a nutrient-rich medium containing glucose, a fermentable carbon source. During the lag phase, cells adapt to their environment.

As the cells begin to proliferate and consume the medium's nutrients, they enter the log phase, where glucose is metabolized through glycolysis to generate energy. Once glucose is depleted, a diauxic shift occurs, transitioning the culture from glycolysis to respiration. This shift results in a decrease in the growth rate. Eventually, when the medium is exhausted, the culture enters the stationary phase, where cell division ceases and growth stabilizes.

For this study, we used *S. cerevisiae* cells from the Euroscarf collection, with the genetic background BY4741, MATa.

Differentiated human promyelocytic leukemia cells (HL-60)

The human promyelocytic leukemia cell line (HL-60) is widely used in blood research due to its ability to differentiate into various types of blood cells. When exposed to specific chemicals, such as all-trans retinoic acid (ATRA) or dimethylsulfoxide (DMSO), HL-60 cells can mature into cells with a neutrophil-like morphology. This makes HL-60 an effective in vitro model for studying neutrophils.

Similar to isolated neutrophils, differentiated HL-60 cells can produce extracellular reactive oxygen species (ROS) rapidly, known as a respiratory burst, when stimulated by specific biological or chemical agents. In differentiated HL-60 cells, superoxide anion radicals, primarily produced by the NADPH oxidase enzymatic complex, are the main ROS. These ROS play a crucial role in the immune response by attacking and killing pathogens.

5.3 Experimental design

5.3.1 Preparation of the samples

Yeast cells (*S. cerevisiae*)

Before cultivation in liquid medium, yeast cultures were stored on agar plates (1% yeast extract, 2% peptone, 2% agar, 2% D-glucose in distilled water) at 4 °C in a refrigerator and transferred to new plates monthly. For cultivation, 10 mL of autoclaved YPD medium (1% yeast extract (Chemos CZ, s.r.o., CZ), 2% peptone (Chemos CZ, s.r.o., CZ), 2% D-glucose (Ing. Petr Švec – Penta s.r.o., CZ) in ultra-pure water) was added to a 100 mL glass Erlenmeyer flask. The glucose was filtration-sterilized (200 nm pore filter) and added after medium sterilization. The medium was inoculated with yeast cells and cultivated at 30 °C on an orbital shaker (Yihder

Technology Co., CN) at 180 rpm for 16 hours. Afterward, a portion of the cell suspension was transferred to a larger 250 mL Erlenmeyer flask containing 200 mL of fresh YPD medium. The cell concentration was adjusted to 5×10^6 cells/mL to ensure consistency at the start of each experiment.

The flask was fitted with a stopper with a central glass tube, through which air was pumped using a diaphragm air pump (SCHEGO, DE) to aerate the medium and prevent cell sedimentation, which could affect the BAL due to setup geometry. Air was filtered through a HEPA 12 filter (Philips, CZ) to avoid contamination.

During the first 5-7 hours of measurement, spontaneous photon emission was recorded. Following this, an antioxidant solution was injected, and signal recording continued. The effects of three different antioxidants—ascorbic acid, mannitol, and glutathione (all Sigma Aldrich, CZ)—were compared, all at a final concentration of 1 mM. Additionally, changes in the BAL with three different final concentrations (1 mM, 5 mM, and 10 mM) of ascorbic acid were evaluated. In all cases, 800 µL of antioxidant solution was used for injection.

Differentiated human promyelocytic leukemia cells (HL-60)

HL-60 cells were maintained in 89% RPMI 1640 medium (Biotech, CZ) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biotech, CZ) and 1% L-glutamine-penicillin-streptomycin solution (Sigma Aldrich, CZ) at 37 °C in a 5% CO_2 atmosphere. To differentiate HL-60 cells into a neutrophil-like model, they were exposed to all-trans-retinoic acid (Sigma Aldrich, CZ) at a final concentration of 1 µM and incubated for 6 days. For BAL measurement, 3 mL of cell suspension with a concentration of 2.7×10^6 cells/mL was transferred to a Petri dish. Respiratory burst was induced by adding phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich, CZ), dissolved in 96% ethanol, to achieve a final concentration of 54 nM in the cell suspension.

BAL was measured for 45 minutes, after which the measurement was paused to introduce an antioxidant solution. The measurement then resumed for an additional 35 minutes. We evaluated the effects of three different antioxidants—ascorbic acid, mannitol, and glutathione—at three different concentrations each. The final concentrations were 1 mM, 5 mM, and 1 µM for ascorbic acid and glutathione, and 1 mM, 5 mM, and 10 mM for mannitol. Stock solutions of each antioxidant were prepared to ensure that 20 µL of solution was used for each measurement.

5.3.2 BAL measurement equipment and set-up

The BAL from both cell types were detected using a sensitive low-noise PMT, model H7360-01 (Hamamatsu Photonics Deutschland, DE). The PMT system has an average dark count was less than 13 counts per second (cps). To prevent interference from ambient light, the PMT was housed in a light-tight chamber specifically designed for BAL measurements from biological samples. The chamber's temperature is controlled by a thermo-control unit (UWE Electronic, DE) and was adjusted according to the physiological needs of the biological model: 30 °C for yeast cells and 37 °C for HL-60 cells.

Two slightly modified measurement setups were used, depending on the type of cell sample. For HL-60

cells, the PMT was positioned above the uncovered Petri dish (Fig. 5.1A), while for yeast cells, it was placed at the side, through the glass wall of an Erlenmeyer flask (Fig. 5.1B). In both cases, the PMT was positioned as close as possible to the sample. This close proximity is crucial for detecting low-intensity signals, as it maximizes the likelihood of photon detection from the sample.

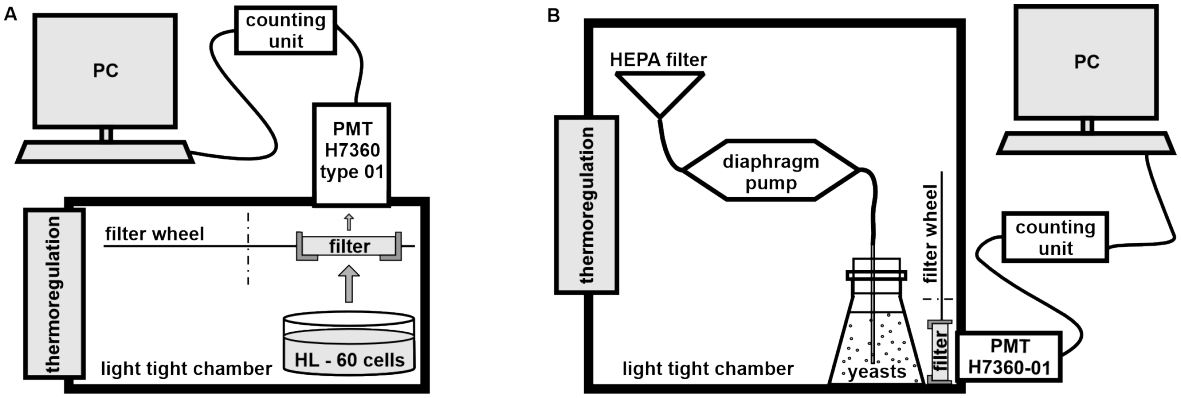

Figure 5.1: Measurement set-ups: (A) HL-60 cells; (B) *Saccharomyces cerevisiae*

5.4 Results

Yeast cells (*S. cerevisiae*)

Sample preparation and experiments with *S. cerevisiae* were considerably time-consuming, with each BAL measurement, including waiting periods, taking over 30 hours. The graphs in Figure 5.2 illustrate the temporal developments of BAL from *S. cerevisiae*. Fig. 5.2A shows the unmodulated BAL from yeast cultures during the lag, log, and early stationary phases of growth. Graph 5.2B details the changes in BAL intensity with varying concentrations of ascorbic acid (1 mM, 5 mM, and 10 mM), all of which resulted in a decrease in BAL intensity. Graph 5.2C compares the effects of three antioxidants—ascorbic acid, mannitol, and glutathione—at a concentration of 1 mM. Both ascorbic acid and glutathione modulated the BAL signal as expected, demonstrating antioxidant effects. In contrast, mannitol did not affect BAL intensity.

In Figure 5.2A, the BAL at t=0 hours is between 100 and 150 counts. In contrast, Figures 5.2B and 5.2C show BAL values below 50 counts at t=0 hours. The intensity of the measured signal is influenced by background noise, which is particularly noticeable at the start of measurements when cell concentration and activity are low. This discrepancy is attributed to the cultivation medium, which itself exhibits photon emission that varies depending on its maturity. Through extensive BAL measurements of yeast cultures, we have observed that the longer the time since the medium was autoclaved, the lower the BAL signal. The medium used for the sample in Figure 5.2A was fresher compared to the media used for the samples in Figures 5.2B and 5.2C. Thus, Figure 5.2A illustrates a typical BAL curve for a yeast culture, highlighting the influence of medium maturity on BAL measurements.

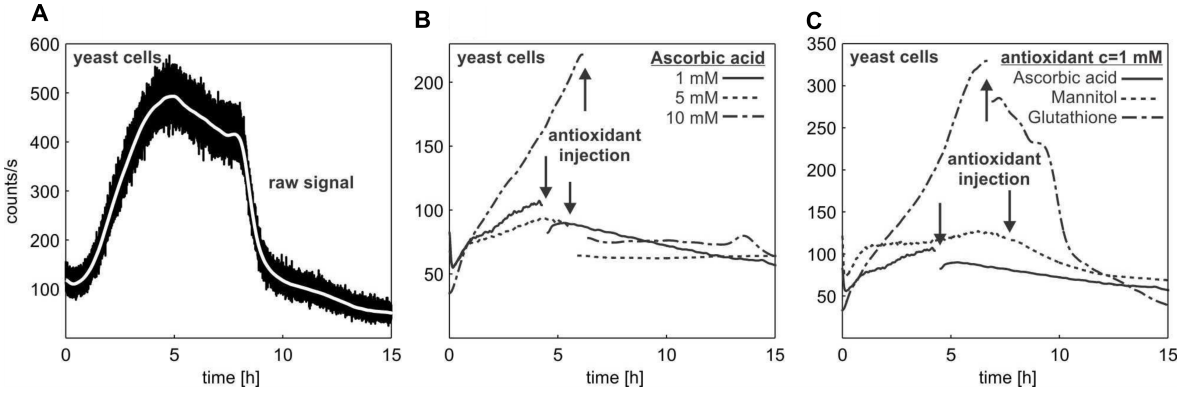

Figure 5.2: BAL signal from Saccharomyces cerevisiae: (A) raw signal, typical temporal development without any antioxidant injection; (B) ascorbic acid in three different concentrations; (C) 1 mM of ascorbic acid, mannitol and glutathione.

Differentiated human promyelocytic leukemia cells (HL-60)

The curves shown in Figure 5.3 represent the BAL signal measured from differentiated HL-60 cells. All data (10 measurements) were collected within a single day from the same cell culture. Fig. 5.3A displays a typical BAL signal from HL-60 cells induced by PMA, with maximum intensity reached approximately 45 minutes after induction. This timing is ideal for BAL modulation by antioxidants, as the difference between unmodulated and modulated signals is most pronounced. The shape of this curve is similar to the signal from induced neutrophils isolated from blood (data not shown), validating the use of differentiated HL-60 cells as an in vitro model for BAL research.

Fig. 5.3B, 5.3C, and 5.3D illustrate the effects of ascorbic acid, mannitol, and glutathione on BAL intensity. Ascorbic acid induces the most significant reduction in BAL intensity, with each of the three concentrations tested reducing it by up to 90% (eg. from 500 to 50 counts/s in Fig. 5.3B). Fig. 5.3D shows that the effect of glutathione on BAL modulation depends on its concentration. A 1 mM concentration of glutathione suppresses BAL by 55%, while higher concentrations seem to have a lasting effect. A 0.5 mM concentration also reduces BAL intensity quickly (by 40%), but the intensity begins to rise almost immediately after application. Conversely, all concentration of mannitol increases BAL intensity(Fig 5.3C).

5.5 Conclusion

The consistent reduction in BAL by ascorbic acid and glutathione across yeast and HL-60 cells underscores the efficacy of these antioxidants in mitigating oxidative stress by scavenging reactive oxygen species. This reduction in oxidative stress is critical for maintaining cellular health and can prevent oxidative damage. The unique response of mannitol in HL-60 cells, exhibiting prooxidant properties, starkly contrasts with its neutral effect in yeast. This observation prompts a deeper inquiry into the environmental and cellular conditions that influence mannitol's role. It suggests that mannitol's chemical behavior may depend on specific cellular contexts, such

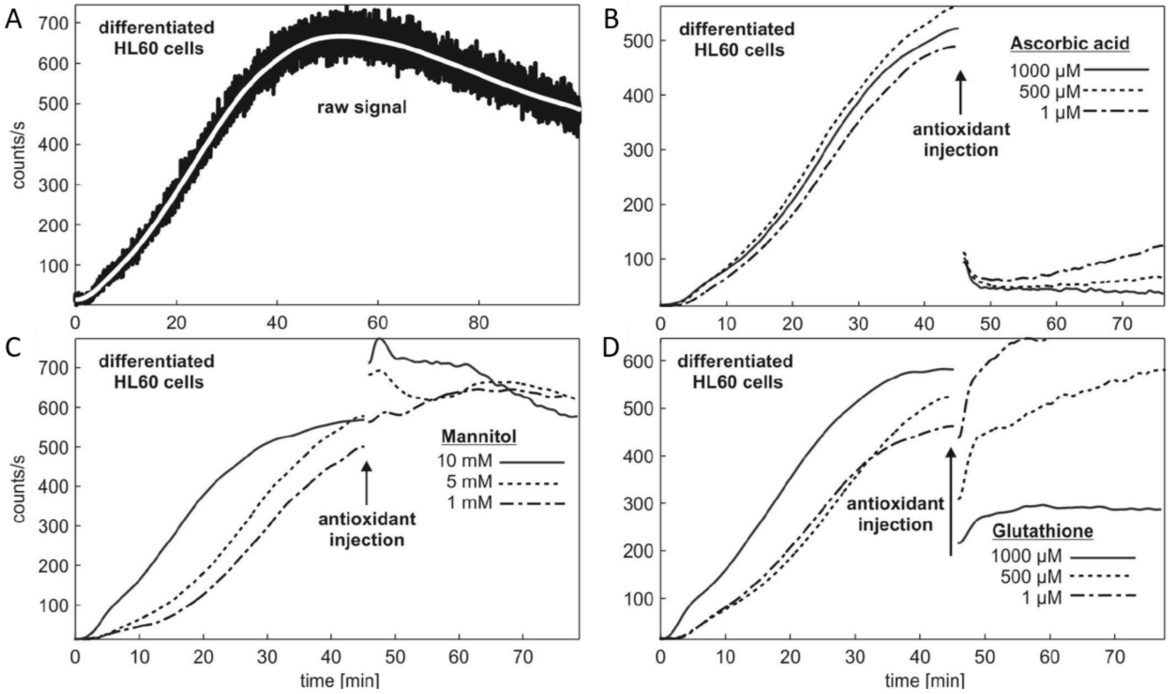

Figure 5.3: BAL signal from differentiated HL-60 cells: (A) raw signal, temporal development without any antioxidant injection; (B) ascorbic acid in three different concentrations; (C) mannitol in three different concentrations; (D) glutathione in three different concentrations.

as the presence of certain cellular enzymes, pH levels, or the concentration of transition metals.

5.6 Additional contributions of the candidate to the research topic

The candidate's expertise and specialized knowledge in handling and experimenting with *S. cerevisiae* yeast cells, HL-60 human promyelocytic leukemia cells, and various chemical modulators have been pivotal in subsequent research projects. Her skill in developing and refining experimental methods, along with her technical know-how, has been helpful for the success of these studies. These contributions have led to the publication of following peer-reviewed papers, in which the candidate is credited as a co-author:

5.6.1 BAL combined with metabolomics in monitoring of oxidative stress

The studies examined the use of BAL as a tool for monitoring oxidative stress, particularly in connection with metabolomic analyses. The research utilized HL-60 cells, a model for neutrophil-like cells, to study the dynamic processes of oxidative metabolism. By inducing respiratory bursts by a chemical modulator PMA, and analyzing related metabolites, the studies revealed strong correlations between BAL signals and specific biochemical changes. This approach highlights the potential of combining BAL measurements with metabolomics to gain deeper insights into oxidative stress and related physiological processes.

The candidate contributed to these studies by providing expertise in the protocols for HL-60 cell handling and BAL measurements and participated in the preparation of the manuscript.

Burgos, R. C. R., Červinková, K., van der Laan, T., Ramautar, R., van Wijk, E. P., Cifra, M., Koval, S., Berger, R., Hankemeier, T., and van der Greef, J.

Tracking Biochemical Changes Correlated with Ultra-Weak Photon Emission Using Metabolomics

Journal of Photochemistry & Photobiology, B: Biology, 163, 237-245, 2016.

DOI: 10.1016/j.jphotobiol.2016.08.030

and

Burgos, R. C. R., Schoeman, J. C., van Winden, L. J., Červinková, K., Ramautar, R., van Wijk, E. P., Cifra, M., Berger, R., Hankemeier, T., and van der Greef, J.

Ultra-Weak Photon Emission as a Dynamic Tool for Monitoring Oxidative Stress Metabolism

Scientific reports, 7(1), 1229, 2017.

DOI: 10.1038/s41598-017-01229-x

5.6.2 BAL modulation by ascorbic acid in yeast

Vahalová, P., Červinková, K., & Cifra, M.

Biological Autoluminescence for Assessing Oxidative Processes in Yeast Cell Cultures

Scientific Reports, 11(1), 10852, 2021.

DOI: 10.1038/s41598-021-89753-9

The study discusses the application of BAL to monitor oxidative processes in yeast cell cultures. The research highlights how increased ROS levels influence BAL. Antioxidant ascorbic acid's role is examined in the context of its ability to modulate ROS levels within these systems.The study measures how different concentrations of ascorbic acid affect spontaneous BAL.

The candidate's contributions to the project included aiding in the development of the methodology, assisting with formal analysis, and preparatory examination of the antioxidant effects on BAL.

5.6.3 Effect of antioxidants and prooxidants on BAL during PEF treatment

This study examined the combined effects of physical and chemical modulators on BAL measurements. It focused on the impact of PEF treatment as a physical modulator on protein oxidation, while also introducing antioxidant and prooxidant agents to explore their synergistic effects with pulsed electric field (PEF). By analyzing these interactions, we aim to enhance our understanding of how physical and chemical factors together influence BAL. Detailed findings and methodologies are discussed in the following chapter 6.

6 | Physical modulators: Pulsed electric field

This chapter is based on this accepted paper:

Červinková, K.., Vahalová, P., Poplová, M., Zakar, T., Havelka, D., Paidar, M., Kolivoška, V., and Cifra, M.

Modulation of Pulsed Electric Field Induced Oxidative Processes in Protein Solutions by Pro- and Antioxidants Sensed by Biochemiluminescence

Scientific Reports, accepted.

The candidate played a part in planning the experiments and helped improve the methodology. She was responsible for conducting BAL experiments and some biochemical analysis, organizing the resulting data, and worked on visualizing the data. She also contributed to the formal analysis and investigation. The candidate supported the initial drafting of the manuscript and was actively involved in reviewing and editing it to produce a final version of the paper.

6.1 Monitoring BAL in proteins subjected to PEF treatment

This study extends the previous work by Vahalová et al. [213] by adding a deeper biochemical and biophysical analysis of the oxidative effects induced by ROS generated through Pulsed electric field (PEF) treatment. The study broades this investigation by employing additional techniques to analyze the structural and chemical changes in proteins. The candidate also explores the role of enzymatic antioxidants in modulating the effects of ROS on proteins. This expanded analysis not only confirms the previous findings [213], but also offers new insights into how oxidative damage can be influenced by the presence of prooxidants and antioxidants, thereby contributing valuable knowledge to the field of protein chemistry and PEF applications.

6.2 Introduction

PEF treatment is known to induce the formation of ROS within cells and biological samples (See 2.5.6). While ROS contribute significantly to the structural and functional modifications of proteins, it is important to note that they may not be the only factors involved. Conventional assays often lack the sensitivity to detect minor changes in protein oxidation or small-scale generation of ROS. This enhanced sensitivity is crucial for mapping immediate and transient phenomena that conventional techniques might overlook. Using BAL to monitor

ROS generation in real-time can overcome these limitations, enabling the detection of low levels of BAL that correspond with early and potentially reversible changes in protein structure and function.

This study explored the effects of PEF treatment on proteins, using bovine serum albumin (BSA) as a model. PEF treatment was applied to induce the generation of ROS, and real-time monitoring of intrinsic BAL was conducted to assess the oxidative processes occurring within the samples. Recognizing that biological systems inherently contain both prooxidants and antioxidants, which play crucial roles in modulating oxidative processes, the study examined various conditions to explore the synergistic effects between PEF, prooxidants, and antioxidants. This approach allowed for a comprehensive investigation into how PEF interacts with these elements to influence protein oxidation. To provide a deeper understanding of the molecular changes, complementary biochemical analyses were performed, including carbonyl detection, sulfhydryl group analysis, fluorescence of aromatic amino acids, hydrophobic residue exposure assessment, and dynamic light scattering (DLS) for protein size measurements. Additionally, inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to evaluate potential metal released from electrodes into the solution. The Fig. 6.1 represents a comprehensive experimental setup and workflow for of the research study.

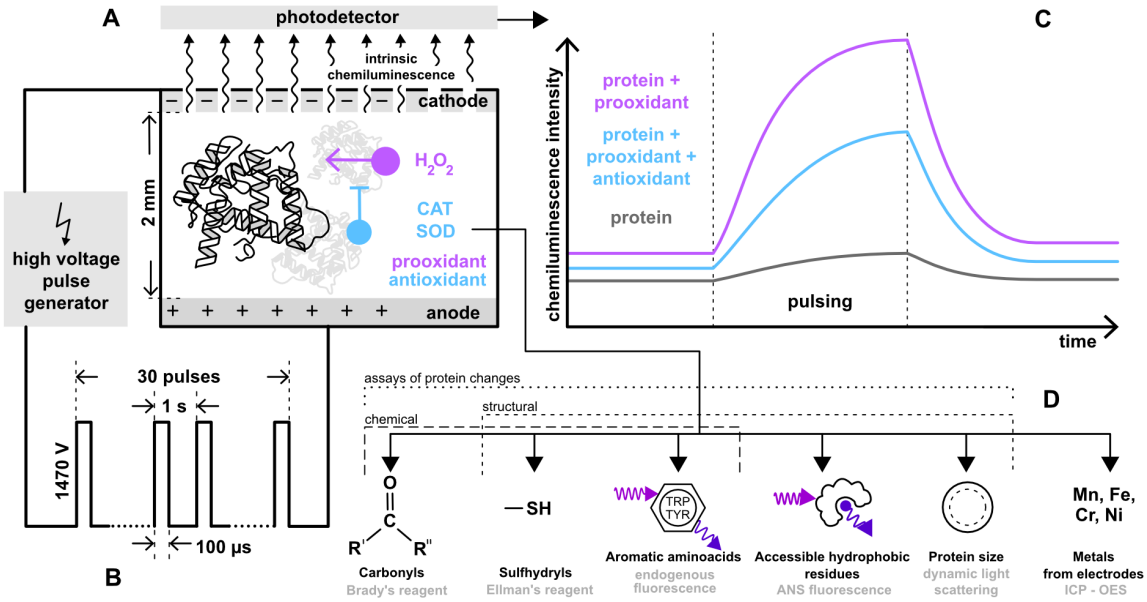

Figure 6.1: Overview of the main concepts and messages of the paper. The BAL signal (C) is generated from the solution of BSA (A) when exposed to a sequence of intense electric pulses (B). No external BAL labels are required; the signal arises due to components endogenous to the sample. The BAL reports on oxidative processes in the BSA solution. We analyzed the behavior of BAL in the presence or absence of prooxidant as well as enzymatic antioxidants and respective combinations. Furthermore, we used various biochemical and biophysical techniques (D) to analyze the effects of the treatment on BSA.

6.3 Experimental design

6.3.1 Pulsed electric field equipment and treatment

A chamber for conducting the PEF treatment on samples employed in this study was designed and crafted at the Institute of Photonics and Electronics at the Czech Academy of Sciences in Prague, Czechia. This chamber was machined from a block of the dielectric polymethylmethacrylate (PLEXIGLAS GS, Zenit); it featured an anode at the bottom and a cathode at the top arranged in a thin-layer parallel-plate configuration (gap between electrodes of 2.00 mm). The anode was solid and manufactured from a plate of stainless steel (type 1.4571, thickness 6 mm, AKROS). The cathode was manufactured from a plate of stainless steel (type 1.4304, thickness 130 µm, PragoBoard) and, to enable sensing of BAL, it contained hexagonally arranged drilled holes, resulting in 50 % void area. Both cathode and anode had 26 mm diameter, defining the cross-sectional area of the liquid sample within the PEF chamber (5.31 cm^2). The PEF chamber volume was 1.06 mL. The model of the chamber used is illustrated in Fig. 6.2 [213].

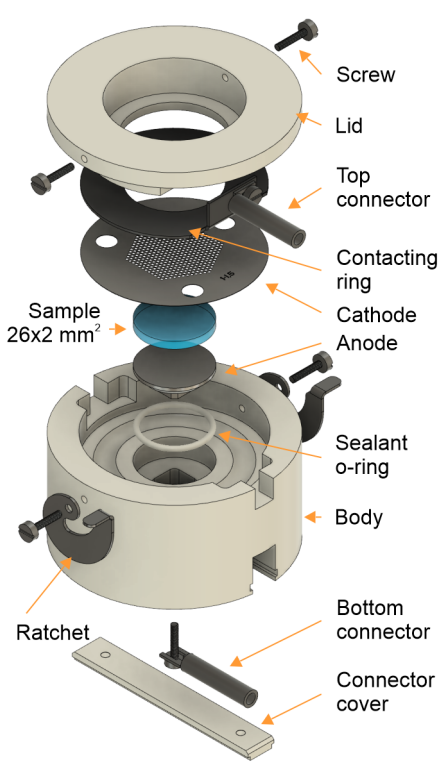

Figure 6.2: 3D model of the PEF chamber and its compartments, adopted from [213]

The PEF was formed by the pulse generator (ELECTROcell B15, Leroy-Biotech), which also continuously recorded applied voltage and resulting current flowing between the anode and cathode. The characteristics of the initial pulse in each PEF sequence were independently recorded using high voltage (P5100, Durlclth) and

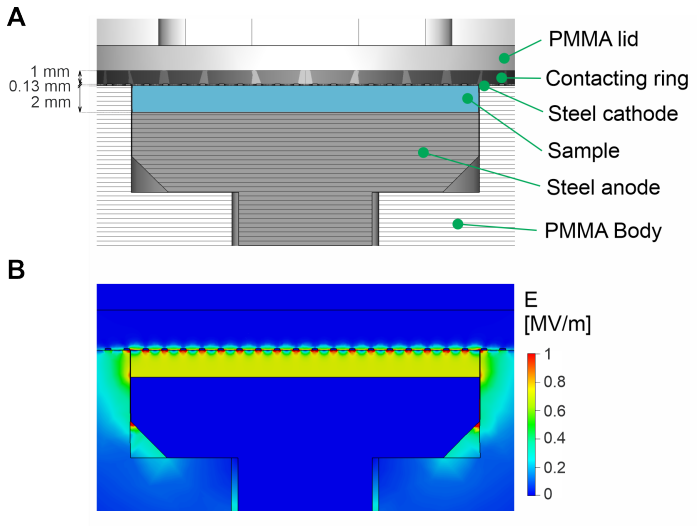

Figure 6.3: (A) Cross-sectional image of the PEF chamber employed in this study. (B) Distribution of electric field strength in the PEF chamber for applied voltage of 1470 V.

current (ICP5150, Instrance) probes connected to the oscilloscope (GDS-2202E, GW Instek), see Fig. 6.6 for the measurement setup. PEF consisted of 30 unipolar pulses, each with voltage amplitude of 1470 V and a width of 100 µs, fired at a frequency of 1 Hz. For the employed PEF chamber, the applied voltage of 1470 V corresponds to the electric field strength of 0.735 MV·m^{-1} Fig. 6.3 shows the distribution of electric field strength in the PEF chamber calculated in the CST Microwave Studio for applied 1470 V. Fig. 6.4 shows the PEF profile (A) and representative current transients obtained in the first pulse for PB and BSA (B), Fig. 6.5 depicts the voltage transient applied in each pulse.

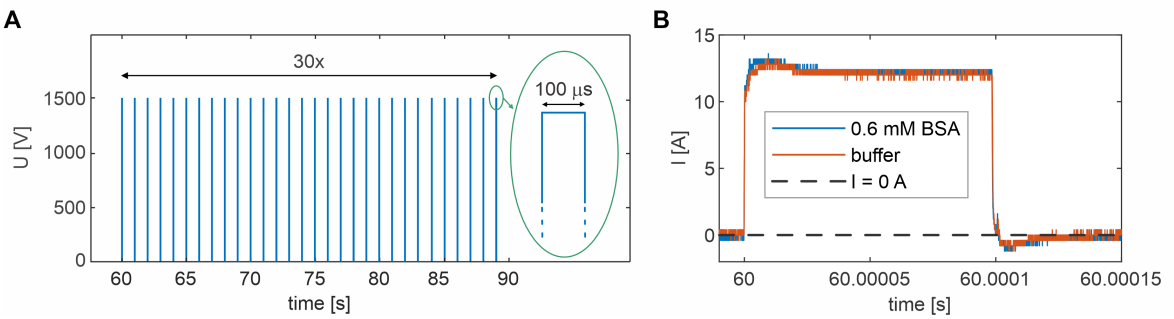

Figure 6.4: (A) PEF profile applied in this study. (B) Representative current transients obtained in the first pulse of PEF for PB and BSA sample.

6.3.2 BAL measurement equipment

The PEF chamber filled with the sample was situated within a light-tight black box designed and constructed in the Institute of Photonics and Electronics at the Czech Academy of Sciences in Prague, Czechia. This box incorporated a PMT module H7360-01, Hamamatsu Photonics K.K. mounted to the ceiling of the box. This PMT

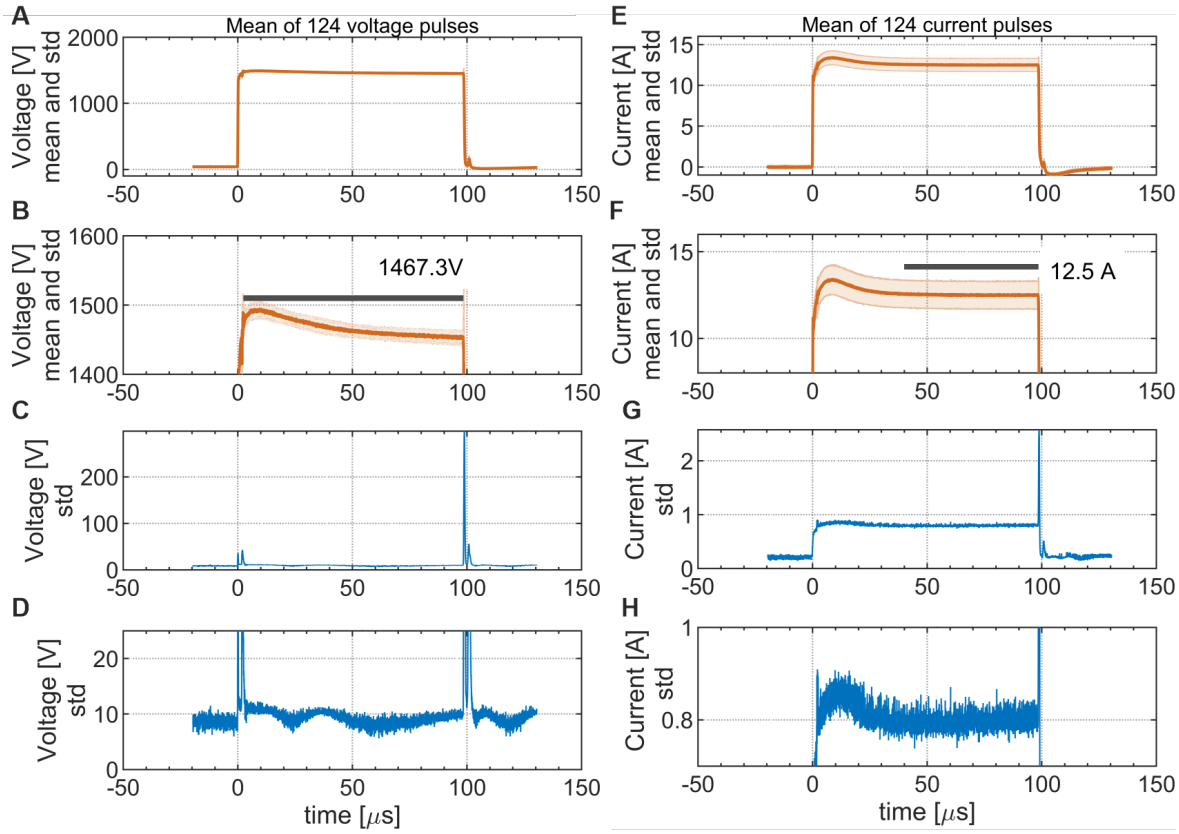

Figure 6.5: (A-D) Voltage profile applied in each pulse of PEF - mean value (A, close-up B) and standard deviation (C, close-up D). (E-H) Resulting current transients - mean value (E, close-up F) and standard deviation (G, close-up H). This dataset was compiled from representative curves obtained for all samples inspected in this study.

senses photons within a spectral range from 300 to 650 nm, i.e. covering the emission wavelength of species relevant for this study. The PMT was connected to a counting unit (C8855, Hamamatsu Photonics K.K.), offering an interface for computer connection. The PEF chamber was positioned close to the PMT, maintaining a fixed distance of 8 mm between the PMT module and the cathode. Before actual experiments, background measurements of photon counts were performed, encompassing both empty box without the PEF chamber ("dark counts") and the dry, empty PEF chamber installed in the box, with values stabilizing at 21 ± 6 counts·s^{-1}. To distinguish the relative contributions of BAL e emitters, we employed a shortpass filter (84-708, Edmund Optics) and a longpass filter (62-984, Edmund Optics), with a cut-off wavelength of 550 nm and a diameter of 25 mm. The filter holder dimensions were mechanically designed to pass the luminescence from the sample to the detector only through the filter, if present, to avoid any unfiltered light leakage from the sides. To quantitatively assess BAL contributions (Fig. 6.12), we considered the quantum efficiency (QE) of the chosen PMT (Fig. 6.7) in our wavelength-resolved measurements. QE, representing the percentage of incident photons effectively converted into photoelectrons emitted from the photocathode of the PMT, varies with the wavelength of the incident light. This characteristic is necessary for converting measured photon counts to correct BAL magnitude. The average QE for the wavelength range of 300 to 550 nm was 12.2%, while for the range of 550 to 650 nm, it was 0.55%, with these values being used in the conversion. See Fig. 6.7 for the quantum efficiency (QE) profile of the PMT used in this study.

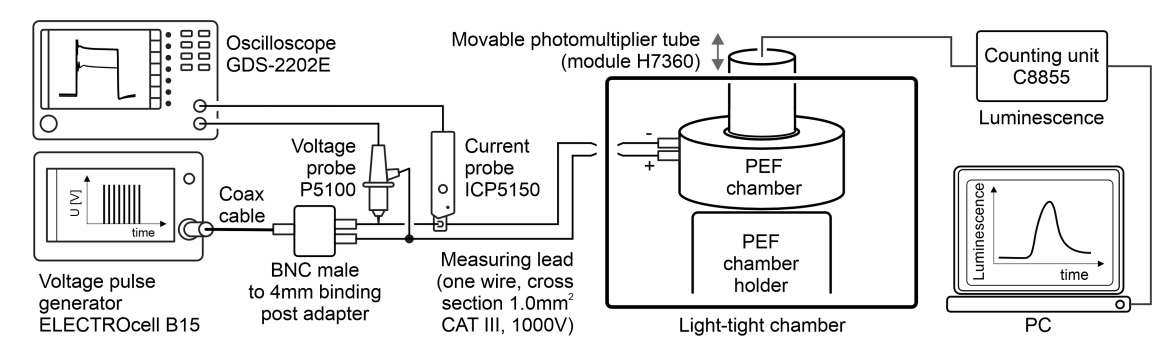

Figure 6.6: Scheme of the experimental platform employed in this study to generate PEF, measure applied voltage and resulting current and luminescence signal.

6.3.3 Examined samples

The experiments were conducted using bovine serum albumin (BSA), a versatile, cost-effective, and widely studied protein that serves as a standard model in many biochemical applications. BSA was selected due to its ease of handling, availability, and well-documented properties. This water-soluble protein, derived from bovine blood, is composed of 583 amino acids with a molecular weight of approximately 66.4 kDa. Structurally, it is a large globular protein consisting of a single polypeptide chain organized into three homologous domains [302]. These characteristics make BSA an excellent model system for studying protein behavior under various

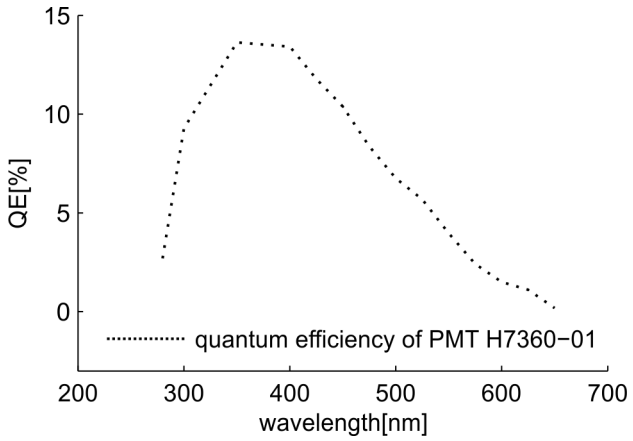

Figure 6.7: Quantum efficiency of the PMT used in this study, adopted from [301].

conditions. BSA is able to bind and transport a wide range of molecules within biological systems, including fatty acids, hormones, bilirubin, and metal ions such as Zn^{2+}, Cu^{2+}, Ca^{2+}, Ni^{2+}, and Mg^{2+}, as well as metallic complexes [303–306]. Although BSA can also bind Fe^{2+}, this interaction is relatively weak compared to specialized iron-binding proteins like transferrin [307]. The protein's binding versatility not only facilitates the distribution and protection of these molecules but also plays a critical role in maintaining osmotic pressure, buffering pH, and serving as a reservoir for essential nutrients and ions.

The isoelectric point (pI) of BSA falls within the range of 4.7 to 5.0 [308], meaning that at physiological pH (7.4), the protein carries a net negative charge. Its diffusion coefficient has been measured at 6.3×10^{-11} $m^2 \cdot s^{-1}$ (the value for 23 °C) [309], with an electrophoretic mobility of 1.4×10^{-8} $m^2 \cdot s^{-1} \cdot V^{-1}$ [310]. These properties were considered when selecting BSA as the model protein for our experiments, as they are crucial for understanding how the protein interacts with other molecules and responds to external stimuli.

To ensure a controlled comparison, a parallel experiment was conducted using an inorganic phosphate buffer (PB) with identical pH and conductivity as the BSA solution. This allowed us to isolate the effects of the protein from the buffer environment.

Additionally, H_2O_2 was used as a pro-oxidant agent to simulate oxidative stress, while superoxide dismutase (SOD) and catalase (CAT) were employed as antioxidant agents. These antioxidants were selected for their well-established roles in mitigating oxidative damage. Specifically, CAT and SOD were chosen for their distinct capabilities in detoxifying different ROS. CAT is highly efficient in breaking down H_2O_2 into water and oxygen, while SOD targets $O_2^{\bullet -}$. Their complementary actions allowed us to investigate the complex relations between pro-oxidants, antioxidants, and the effects of external physical stimuli, PEF.

6.3.4 Preparation of the samples

Milli-Q water (Millipore, resistivity 18.2 MΩ·cm) was used to prepare all solutions and perform all cleaning procedures. BSA (heat shock fraction, lyophilized powder, Sigma - Aldrich, A3803-50G) was dissolved at a

concentration of 40 mg·mL^{-1} (0.6 mM). The pH was 7.2 ± 0.2, its conductivity was 0.033 ± 0.003 S·m^{-1}. We chose phosphate buffer (PB) as a control, purely inorganic sample, with identical pH and conductivity as the BSA solution, comprising 0.612 mM NaH_2PO_4 and 1.411 mM Na_2HPO_4. Solid chemicals used in the preparation of PB were $NaH_2PO_4 \cdot 2\ H_2O$ (P-Lab, D 03102) and $Na_2HPO_4 \cdot 12\ H_2O$ (P-Lab, H 08102). For selected experiments, hydrogen peroxide (H_2O_2, a non-stabilized 30% aqueous solution, p.a., Penta, 23980-11000), was introduced to the sample (either PB or BSA) in final concentrations (0.1 mM, 1 mM, and 10 mM). For selected experiments, CAT from bovine liver (lyophilized powder, 2000-5000 units·mg^{-1} protein, Sigma - Aldrich, C9322-1G) was introduced to the sample in a final concentration of 3.2×10^{-5} mM. For selected experiments, SOD from bovine erythrocytes (lyophilized powder, ≥3000 units·mg^{-1} protein, Sigma - Aldrich, S7571-30KU) was introduced to the sample in a final SOD concentration of 9.9×10^{-5} mM. These concentrations of CAT and SOD were selected empirically to ensure a measurable decrease in BAL of the BSA/H_2O_2 sample within the experimental time window. Our objective was to showcase the impact of CAT and SOD on BAL rather than quantifying the observed changes or relating them to the catalytic activity of CAT and SOD.

All prepared solutions were stored under ambient pressure and temperature in contact with lab air during experimentation and hence additionally contained approximately 0.3 mM of dissolved oxygen.

6.3.5 Timing of the experimental procedures

Maintaining precise timing in the preparation of samples (namely adding H_2O_2, CAT, and SOD to BSA and PB) and in transporting such prepared samples to the PEF chamber was found to be crucial for the reproducibility of realized experiments, as it influenced the immediate BAL response of samples due to fast kinetics of involved chemical reactions. Due to the time required for the PEF chamber assembly and carrying out IS measurements, it was not technically possible to monitor BAL signals immediately after the sample preparation.

In the following description, time t = 0 s refers to the point at which luminescence monitoring was commenced (see Fig. 6.8). For samples containing either CAT or SOD, the respective enzyme was added to the sample in an Eppendorf tube at -180 s, and the resulting mixture was briefly vortexed. For samples containing H_2O_2, it was added to the sample in an Eppendorf tube at -170 s, and the resulting mixture was briefly vortexed. At t = -150 s, a respective sample was introduced to the open PEF chamber (cathode demounted). In particular, the volume of 1.24 mL was introduced, which is slightly more than the volume of the PEF chamber (1.06 mL). Upon mounting the cathode to the PEF chamber, an excess portion of the sample was forced to move through the cathode perforation, ensuring the absence of air bubbles in the interior of the PEF chamber. The chamber was subsequently placed in the light-tight box, connected to the LCR meter, and the impedance spectra (magnitude Z and phase shift Θ) were measured around -90 s and -60 s, respectively. Luminescence was monitored from 0 s to 500 s. In the period between 0 s and 60 s, no PEF was applied to electrodes, allowing background signal to be collected (pre-pulsing period). Between t = 60 s and 90 s, PEF was applied (pulsing period) with the profile as described above (and also shown in Fig. 6.1), with an electric current being recorded. For t = 90 s

to t = 500 s, no PEF was applied (post-pulsing period), while luminescence was still recorded to evaluate the remnant signal. Significant changes in the BAL were observed only up to 150 s and, therefore, only this time period is presented in figures and discussed in the manuscript. The impedance spectra (magnitude and phase shift) were measured again around t = 530 s and t = 560 s, respectively.

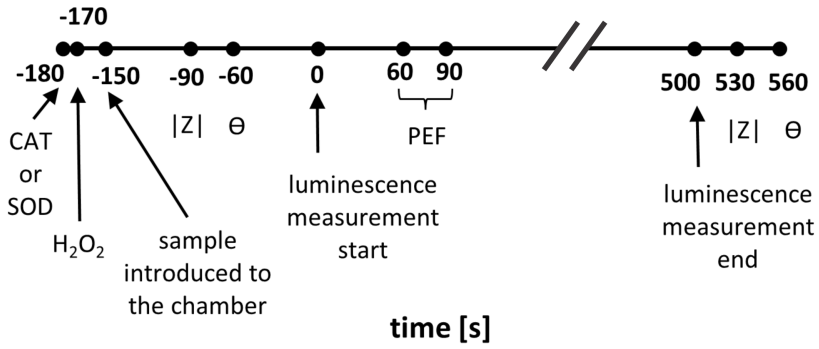

Figure 6.8: Timing scheme followed in the preparation of samples and subsequent PEF experiments. Z and Θ stand for the impedance magnitude and impedance phase measurement by LCR meter, respectively.

Monitoring temperature in the PEF experiment

PEF experiments were performed at ambient pressure and initial temperature of 22 °C. The temperature of the PEF chamber was monitored in selected experiments using a thermal IR camera (Therm-App, Opgal Optronic Industries) in conjunction with a smartphone running the Therm-App Plus application. The camera sensor was directed to a black opaque adhesive tape in direct contact with the cathode. Calibration was conducted using a thermometer (SuperFast ThermoJack PRO, Dostmann, 5020-0552). The tape was affixed to a glass beaker filled with water (23 °C and 42 °C). The emissivity of the tape was adjusted so that the temperatures displayed by the IR camera corresponded accurately with the actual temperatures. The emissivity was set

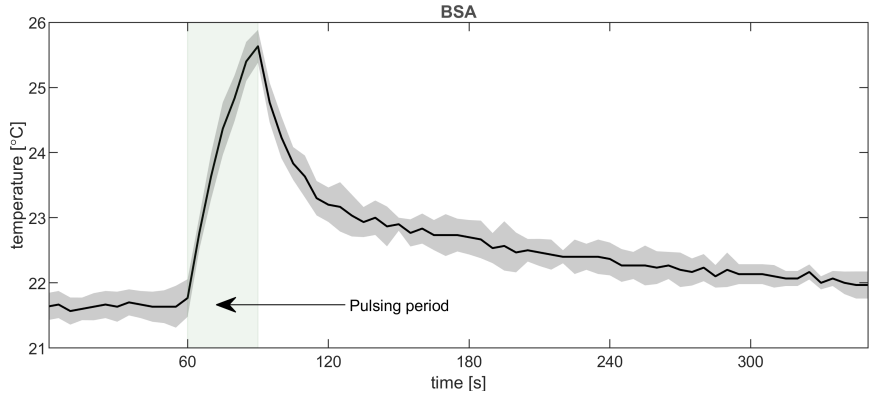

Figure 6.9: Evolution of temperature in the PEF experiment with BSA sample as measured by a thermal IR camera directed to a black opaque adhesive tape being in direct contact with the cathode.

to 0.6. A representative temperature profile is shown in Fig. 6.9.

During the pulsing period, the temperature transiently increased, with the maximum of 26 °C (i.e. by 4 °C) at the end of the period. This increase is attributed to the Joule heating due to ionic migration in the PEF. Such subtle temperature increase is believed to have no substantial effect on the conformation of BSA. The experimentally observed temperature increase of 4 °C is much less compared to the theoretically predicted value for the applied PEF profile and resulting current (circa 12 °C), which we attribute to an efficient heat flow from the solution via electrodes.

6.4 Results

6.4.1 ROS generation and BAL

During PEF treatment, the intense electric field induces the migration of ions in the solution, particularly towards the electrodes, where electrochemical reactions occur. At the anode, water oxidation generates hydroxyl radicals ($HO^{\bullet}$) and hydroperoxyl radical ($HO_2^{\bullet}$). These ROS are highly reactive and can initiate oxidation of the BSA molecules near the anode. The oxidative processes involve the abstraction of hydrogen atoms from amino acid residues in BSA, leading to the formation of BSA radicals ($BSA^{\bullet}$). These radicals can further react with molecular oxygen (O_2), forming peroxyl radicals ($BSAOO^{\bullet}$), see scheme in Fig. 6.10C.

6.4.2 Role of H_2O_2

Hydrogen peroxide (H_2O_2) plays a critical role in amplifying the oxidative processes. When introduced into the system, H_2O_2 undergoes electrooxidation at the anode, generating additional ROS, particularly $HO^{\bullet}$ and $HO_2^{\bullet}$ radicals. These radicals further enhance the oxidative damage to BSA by attacking amino acid residues, leading to increased formation of $BSA^{\bullet}$ and $BSAOO^{\bullet}$, thereby intensifying the BAL (Fig. 6.10A). The study proposes that the presence of metal cations in BSA, such as iron, catalyzes the Fenton-like decomposition of H_2O_2, further accelerating the production of highly reactive hydroxyl radicals. This catalytic activity significantly boosts the BAL signal, indicating a more extensive oxidative modification of the protein.

6.4.3 Effect of antioxidants

The study also explores the effects of antioxidant enzymes, specifically catalase (CAT) and superoxide dismutase (SOD), on the BAL signal (Fig. 6.11 C) and the underlying oxidative processes. Catalase reduces the availability of H_2O_2 by decomposing it into water and oxygen, thereby limiting the electrooxidation of H_2O_2 and subsequent ROS generation. This leads to a noticeable decrease in the BAL signal, indicating reduced oxidative stress on the BSA molecules.

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet -}$) into hydrogen peroxide and molecular oxygen. While this process generates additional H_2O_2, the study finds that SOD still

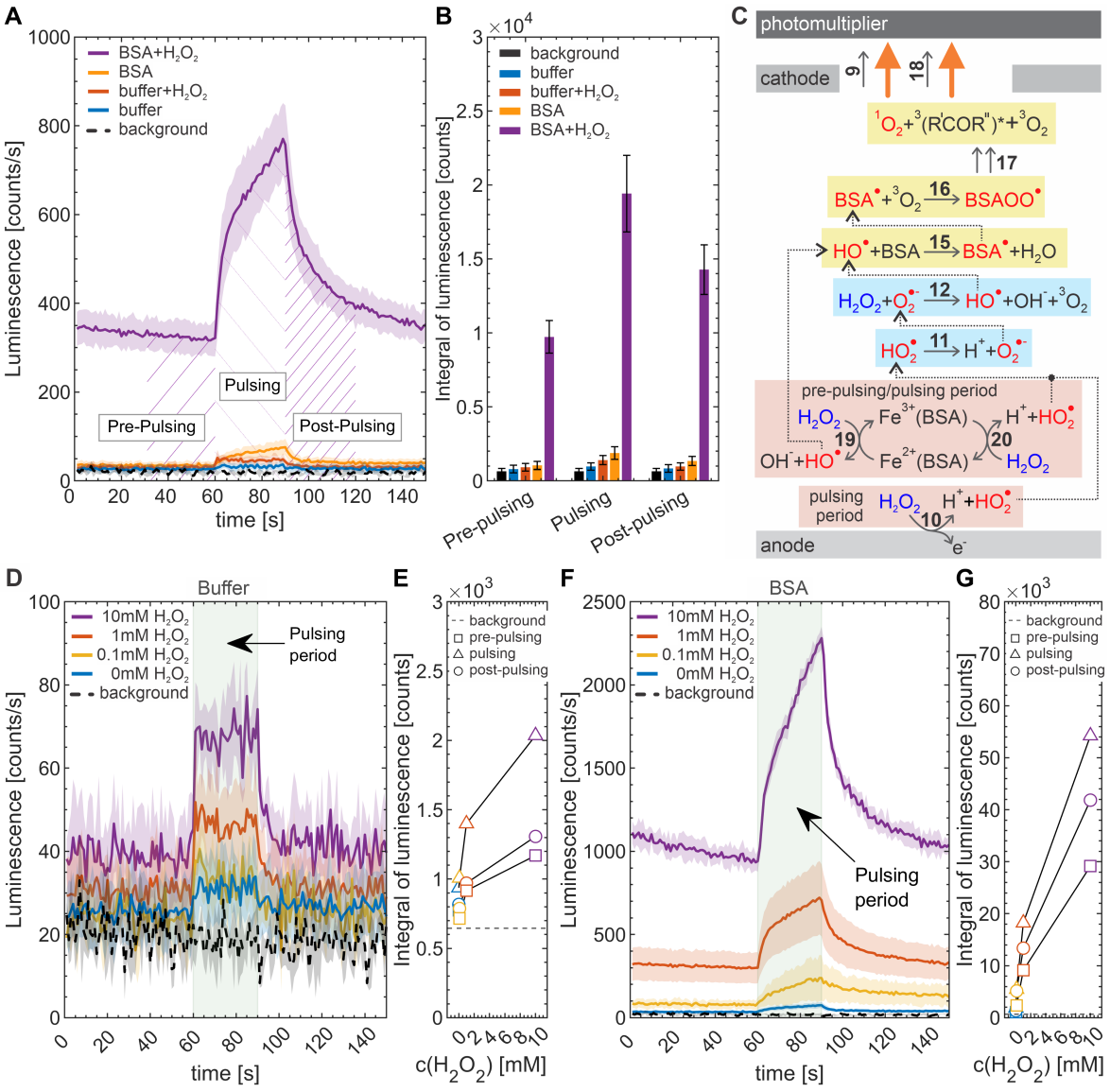

Figure 6.10: (A) Averaged luminescence transients obtained for PEF performed with PB and BSA solution, both in the absence and presence of H_2O_2 (1 mM). Hatched areas denote time intervals in which luminescence signal was integrated, with results shown in (B). (C) Summary of important processes leading to intensified BAL in the BSA/H_2O_2 system. Averaged luminescence transients obtained in PEF experiments with (D) PB and (F) BSA solution with varied H_2O_2 concentration. Individual luminescence signals were integrated in the pre-pulsing, pulsing, and post-pulsing period, with averaged results shown in (E) and (G). In (D), (F) the standard deviation is in shaded error bars.

reduces the overall BAL signal. This suggests that SOD effectively mitigates the formation of superoxide-related ROS, thereby slowing down the oxidative processes initiated by PEF. The study notes that the reduction in BAL in the presence of SOD is more pronounced during and after the pulsing period, implying that SOD not only counters the effects of ROS generated by the Fenton reaction but also those formed directly by the electrooxidation of H_2O_2.

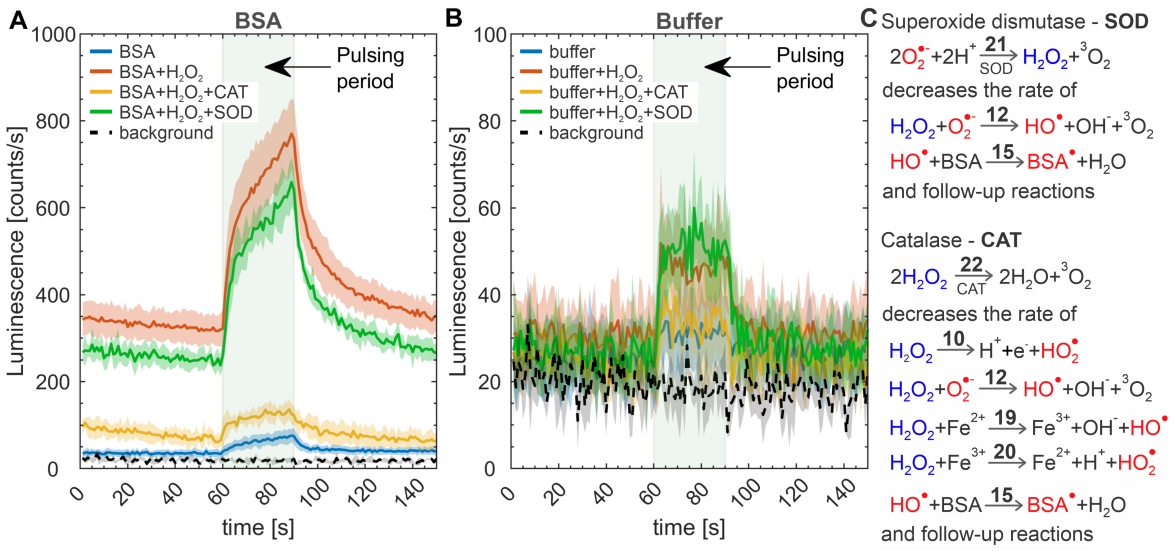

Figure 6.11: Averaged BAL transients obtained in PEF experiments performed with (A) BSA and (B) PB in the absence and presence of H_2O_2 and with additionally introduced antioxidant enzymes (CAT and SOD). (C) A summary of important reactions modulating the BAL response of the BSA/H_2O_2 system by SOD and CAT

6.4.4 Optical spectral analysis

The spectral analysis conducted using optical filters provided critical insights into the specific BAL emissions generated during PEF treatment of BSA solutions in the presence of 1 mM H_2O_2. By employing short-pass and long-pass filters with a 550 nm cut-off, the study was able to discern the different contributors to the BAL (Fig. 6.12). The spectral analysis indicated that $^1O_2$ contributed more significantly to the BAL than $^3(R{=}O)^*$. When the long-pass filter was used to capture emissions above 550 nm, where $^1O_2$ primarily emits, a stronger BAL signal was observed compared to the signal detected with the short-pass filter, which captured the emission from $^3(R{=}O)^*$. This result suggests that the generation of $^1O_2$ played a more dominant role in the BAL reactions occurring under the given experimental conditions. It is important to note that this emission likely stems from a dimolar emission of $^1O_2$, as monomolar emission at 1270 nm is not observed, given that the quantum efficiency of the protomultiplier at this wavelength is effectively zero (Fig 6.7).

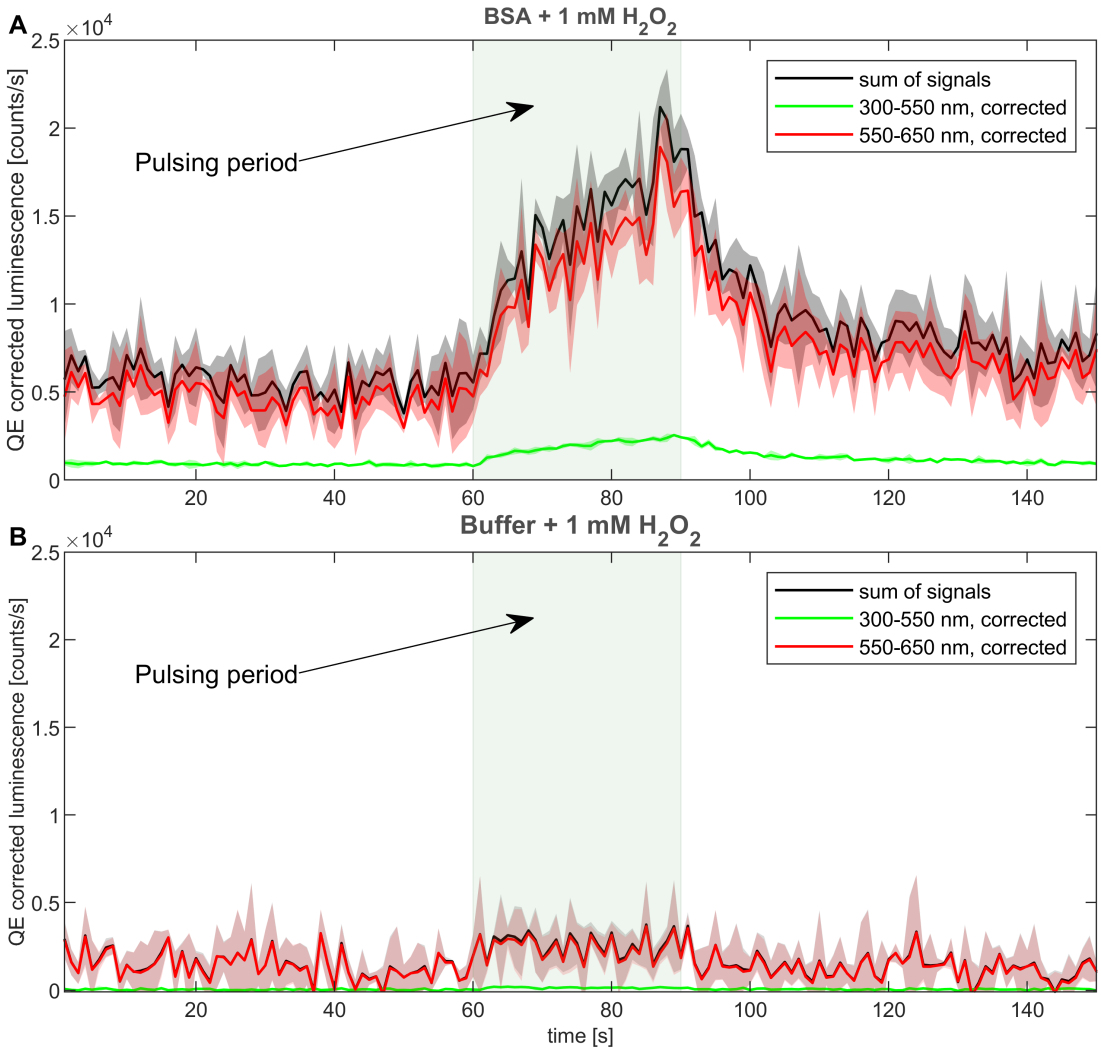

Figure 6.12: Averaged luminescence transients obtained in PEF experiments performed with (A) BSA and (B) PB solution, both additionally containing 1 mM H_2O_2, with luminescence being measured separately in the wavelength range of 300 nm to 550 nm and 550 nm to 650 nm. Shown data are corrected for the averaged QE of the employed PMT in the respective wavelength range. The mathematical sum of corrected signals obtained in both wavelength ranges is additionally plotted.

6.4.5 Biochemical and biophysical analysis

To localize structural changes in the BSA molecule in samples treated by PEF either in the absence or presence of H_2O_2 as described above, we further performed a series of their biochemical and biophysical analyses, see Fig. 6.13. To evaluate the net effect of PEF, we additionally inspected respective samples not introduced to the PEF chamber (control) and samples introduced to the chamber, but with no PEF applied (sham).

Dynamic light scattering measurements were performed to evaluate relative changes of the size of the BSA molecule. Without H_2O_2, PEF was found to have no profound effect. Introducing H_2O_2 led to a slight, but not significant, increase in the average effective hydrodynamic radius (by ca. 15%) compared to case in the absence of H_2O_2. We attribute the small observed shift to a partial unfolding or contribution of a small number of aggregates upon mild oxidation of BSA molecules. Fig. 6.13A,B.

Ellman's reagent was applied to determine the amount of free sulfhydryl (-SH) groups. For BSA without H_2O_2, no statistically relevant differences between PEF treated and control/sham samples were found. The introduction of H_2O_2 to BSA led to approximately 24 to 29% decrease of -SH concentration for PEF treated as well as for control/sham samples, implying that H_2O_2 and further ROS generated from H_2O_2 cause extensive oxidation of -SH groups. Fig. 6.13C.

Brady's reagent was employed to determine the amount of carbonyl groups as parts of newly formed ketone and aldehyde structures as another marker of oxidative damage. PEF was found not to affect the carbonyl content, while the introduction of H_2O_2 led to significantly elevated amounts for all three inspected samples. Fig. 6.13D.

We have further measured spectral characteristics of **fluorescence** excited at 295 nm to determine tryptophan residues and at 280 nm to determine the sum of tryptophan and tyrosine residues. Fluorescence intensity and emission wavelength maxima were virtually the same for all inspected samples. This implies that the introduction of H_2O_2 to the bulk of the sample causes no observable oxidation of aromatic amino acid residues in the BSA molecule. While $HO^{\bullet}$ electrogenerated in PEF at the anode is highly likely to react with aromatic amino acid residues in BSA molecules, these reactions are spatially confined to the diffusion layer of the anode, which has thickness of only tens to hundreds of nm. The fluorescence measurements, which reflect the averaged response of the entire sample volume, therefore cannot reveal changes in such narrow reaction zone. Fig. 6.13E,F.

ANS (8-anilino-1-naphthalenesulfonic acid) was further introduced to samples as a fluorescence probe to sense structural changes in surface hydrophobic parts of the BSA molecule. Performing PEF in the absence of H_2O_2 increased signal by 6% and in the presence of H_2O_2 by circa 10% (the addition of H_2O_2 to BSA by itself had no effect), implying that these two factors synergically lead to slight exposure of hydrophobic moieties to the surrounding environment. Fig. 6.13G,H.

Overall, the introduction of H_2O_2 to BSA caused a concentration decrease of free -SH groups and elevated content of carbonyl moieties, both confirming oxidative damage. The effects of the PEF treatment on overall

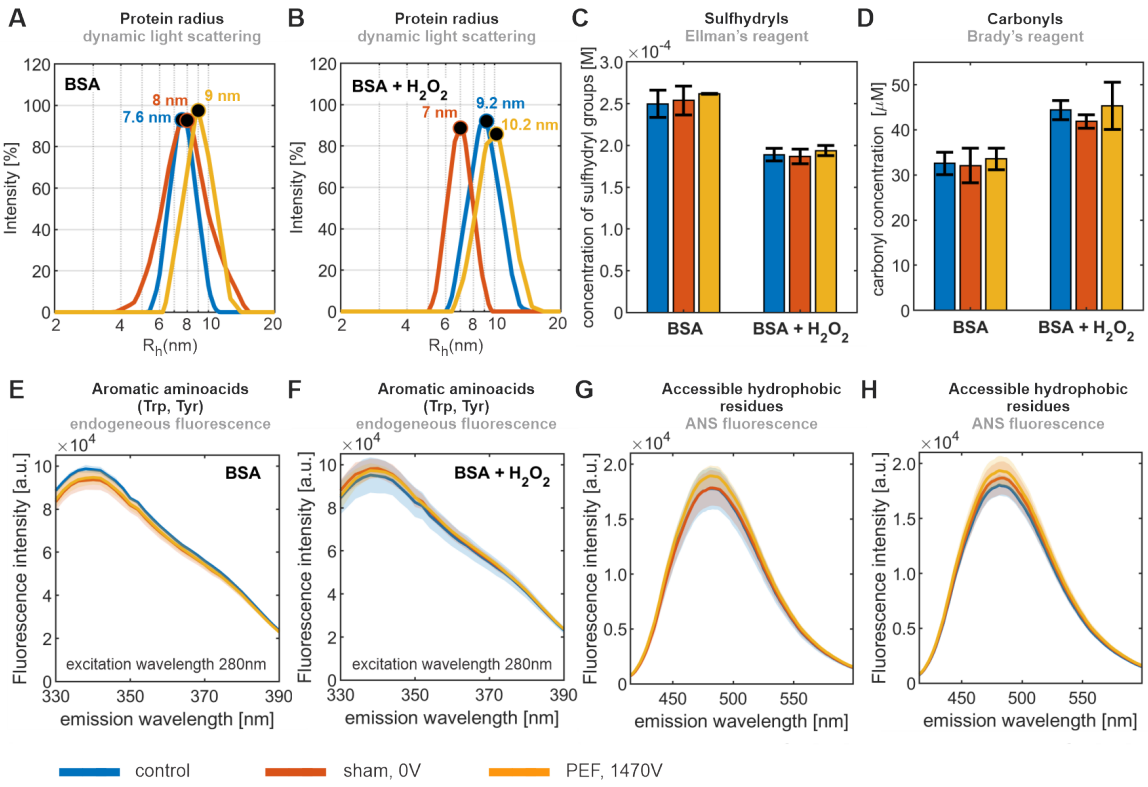

Figure 6.13: Results of biophysical and biochemical analyses performed for BSA and BSA/H_2O_2 samples (subjected to PEF, sham and control): averaged distribution of effective R_h values (A), (B), content of free sulfhydryl groups (C), content of carbonyl (aldehyde and ketone) groups (D), fluorescence spectra excited at 280 nm (E), (F) and fluorescence spectra of ANS introduced to samples excited at 370 nm (G), (H).

sample characteristics were minor. This observation is ascribed to the confinement of electrogenerated highly reactive ROS ($HO^{\bullet}$) at the anode surface limiting its impact on the sample bulk (BAL observed after PEF treatment was, as discussed above, ascribed to reactions involving significantly less reactive $O_2^{\bullet-}$ and various BSA radical species). Fluorescence analysis demonstrated that aromatic amino acid residues (tyrosine and tryptophan) and generally hydrophobic parts of the BSA molecule remained largely intact in all samples, suggesting that oxidative damage by H_2O_2 was located in hydrophilic (non-aromatic) regions of the BSA molecule. While this observation can seemingly be in a contradiction with the general notion that aromatic amino acid residues are more prone to the oxidation by ROS compared to their aliphatic counterparts [265], one has to also consider varied accessibility of individual residues in the protein structure to short-living $HO^{\bullet}$. The combination of ANS fluorescence and dynamic light scattering measurements results suggested that oxidative damage by H_2O_2 led either to partial unfolding or slight aggregation of BSA molecules.

Aromatic residues (*tyrosine*, *phenylalanine*, *tryptophan*, *histidine*) are highly susceptible to the radical attack due to their conjugated structure, which can undergo hydroxylation, ring-opening or form cross-links, such as dityrosine in tyrosine. *Arginine* is an aliphatic amino acid, which has reactivity comparable to that of aromatic amino acids, ascribed to its delocalized guanidino tail that forms a hydroxyl or carbonyl derivative upon oxidation. Sulfur-containing aliphatic residues (*cysteine* and *methionine*) are also prone to radical attack at rates comparable to aromatic residues. Cysteine's thiol group can be oxidized to sulfinic/sulfonic acids or to form disulfides, crucial for protein folding and stability, while methionine oxidation leads to sulfoxide, sulfon or aldehyde, which can affect protein function. Purely aliphatic alkyl residues are less reactive than sulfur or aromatic residues, but can still be oxidized to hydroxyl or carbonyl derivatives. Branched chains (*leucine*, *isoleucine* and *valine*) are more reactive than linear ones (*alanine*, *glycine*). *Threonine* and *serine* are moderately reactive aliphatic amino acids, containing oxidizable hydroxyl groups, leading to side chain cleavage or carbonyl group formation. *Lysine* with comparable reactivity has a terminal amino group, which is converted to an aldehyde. *Proline*, as a special cyclic aliphatic amino acid, also has comparable reactivity and forms a wide variety of products including carbonyls. *Aspartate*, *glutamate* and their amide derivatives *asparagine* and *glutamine* belong to less reactive residues. Their radical attack is targeted to carboxylate/amide groups, leading to decarboxylation or other oxidative changes that affect protein charge and interactions [246, 311–313].

6.5 Conclusion

The study offers a comprehensive exploration of the oxidative processes induced by PEF treatment in protein solutions, specifically BSA. By employing BAL as a sensitive detection method, the research elucidates the complex network of oxidative reactions occurring at the molecular level. These reactions are driven by electrogenerated ROS and are intricately modulated by the presence of prooxidants, such as H_2O_2, and antioxidants like CAT and SOD. The novelty of this study lies in its innovative combination of prooxidant and antioxidant

agents with PEF treatment, allowing for an in-depth investigation into the synergistic effects between physical and chemical modulators of oxidative processes. This approach not only accelerates the understanding of how PEF amplifies the damaging effects of prooxidants but also reveals the protective mechanisms conferred by antioxidants. Ultimately, the study provides a detailed understanding of the molecular dynamics involved in PEF-induced protein modifications, highlighting the significant role of prooxidants and antioxidants in modulating oxidative damage and protein stability under PEF conditions. These insights have broad implications for the application of PEF technology in fields such as bioelectrochemistry, food processing, and medical treatments, where controlling oxidative processes is critical.

7 Biological modulators

7.1 Respiratory burst in differentiated HL-60 Cells induced by yeast

In the experiment, differentiated neutrophil-like HL-60 cells were used. These cells are a well-established model for studying white blood cells and are particularly valuable for examining their role in the respiratory burst—a critical defense mechanism in which ROS are produced to combat invading microorganisms.

Following the same cultivation and differentiation protocol as previously described in 5.3.1, yeast cells were added to the HL-60 cell suspension to simulate an immune response. The respiratory burst was then monitored using biological autoluminescence (BAL), which provided insights into the kinetics of ROS production. Through this analysis, observations were made regarding how quickly the cells responded to the yeast stimulus, the timing and magnitude of peak ROS production, and the duration and decay of the burst.

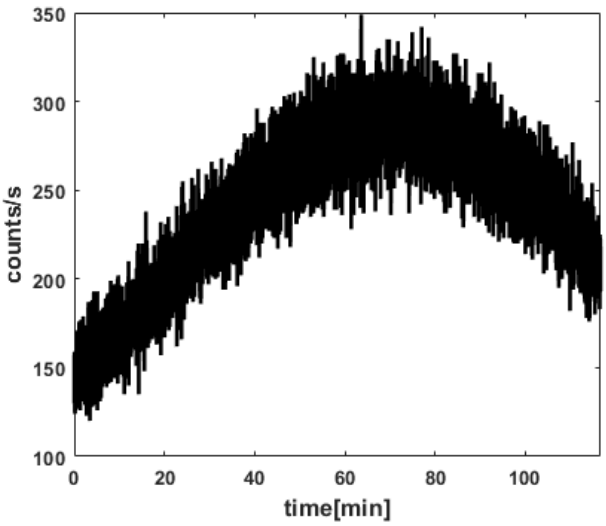

Figure 7.1: BAL in HL-60 Cells modulated by *S. cerevisiae*

This experiment was conducted as a one-time investigation, primarily driven by curiosity, and was not developed into a further study. The goal was to gain an initial understanding of the respiratory burst dynamics in differentiated neutrophil-like HL-60 cells in response to yeast stimulation. While the findings offer a snapshot of ROS kinetics, the data are not polished or extensively analyzed. Nevertheless, the use of BAL suggests it could be a valuable complementary tool in immunological studies, potentially providing additional insights into ROS production and cellular responses.

8 Environmental modulators

This chapter is based on following published paper:

Gallep, C. M., Moraes, T.A, Červinková, K., Cifra, M., Katsumata, M., Barlow, P.W.
Lunisolar tidal synchronism with biophoton emission during intercontinental wheat-seedling germination tests
Plant signaling & behavior, 9(5), e28671, 2014.
DOI: 10.4161/psb.28671

The candidate was responsible for handling and managing biological materials, conducting BAL experiments, and curating the resulting data.

8.1 Lunisolar tidal synchronism with biological autoluminescence in wheat seedlings

The synchronization of biological processes with the external environment is a well-documented phenomenon (see Section 2.7.5). This chapter focuses on the lunisolar tidal synchronism observed by monitoring BAL during the germination of wheat (*Triticum aestivum*) seedlings. It explores how local gravimetric tidal accelerations, driven by the positions of the moon and sun, correlate with BAL in these seedlings. This correlation provides valuable insights into the external regulation of plant metabolic rhythms.

8.2 Introduction

It has been observed that plants exhibit circadian-like rhythms that synchronize with the temporal variations in local gravimetric tidal acceleration, which reflects the lunisolar gravitational influence on Earth [314]. Similar synchronization has been identified in the spontaneous BAL of developing wheat seedlings [315]. BAL correlates with metabolic activity during germination and early seedling growth. Previous studies [316] demonstrated lag-phase and acclimatization effects in BAL rhythms when seedlings from Brazil were transported to Germany, suggesting that these rhythms may depend on location. To further explore this relationship, additional transcontinental tests were conducted across Brazil, Japan, and Czechia.

8.3 Experimental design

8.3.1 Locations

To investigate the influence of lunisolar tides on BAL in wheat seedlings, a transcontinental experiment was conducted across three geographically distinct locations: Limeira (Brazil), Hamamatsu (Japan), and Prague (Czechia). In Brazil, the primary tests involved local Brazilian wheat seeds. The Japanese site was chosen to test the effects of geographic displacement by using wheat seeds transported from Brazil. Meanwhile, in Prague, local wheat seeds were used in simultaneous germination tests, allowing for a comparative analysis between the native and transported seeds across these transcontinental locations. This diverse selection of locations was critical to examining the influence of gravimetric tides on BAL in different global contexts. All experiments ran in parallel starting the same date, under the same internal laboratory conditions

8.3.2 Equipment

In Brazil, and Czechia, Hamamatsu H7630 PMT units were employed, to measure BAL from 25 wheat seeds placed in Petri dishes with distilled water soaked in filter paper, all prepared in dark conditions to minimize external light interference. In Japan, a miniaturized device, the Hamamatsu Type 6100 Biophoton Assay System, was used, to measured photon BAL from 6 seeds in a smaller Petri dishes. Across all locations, the samples and equipment were placed in a way that ensured low and stable background noise levels.

8.4 Results

The results from the experiments provided evidence of the relationship between BAL from germinating wheat seedlings and local gravimetric tides across the three test locations. In Brazil and Czechia, seedlings displayed BAL rhythms that were synchronized with the local gravimetric tidal variations. In Japan, the Brazilian wheat seeds transported for testing exhibited biophoton emission (BAL) patterns that, despite the geographical displacement, remained largely synchronized with the local gravimetric tidal variations. Interestingly, the results from Japan showed minimal disturbances in the periodicity of the BAL rhythms, even after the seeds were transported from Brazil. The gravimetric tides in Japan, which are nearly 180° longitudinally opposite to those in Brazil, shared similar profiles, potentially explaining why the transported seeds required little to no acclimatization to the new environment.

8.5 Conclusion

This multinational study revealed that BAL of germinating wheat seeds are synchronized with local gravitational tides, regardless of the seedlings' geographic location. Even when seedlings were transported across continents, there were no notable phase lags or disruptions in this synchronization, highlighting a strong link

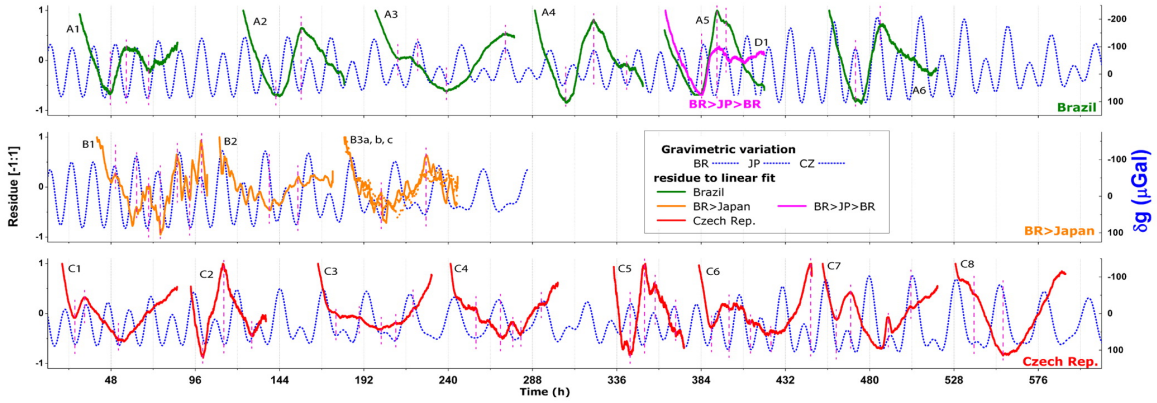

Figure 8.1: Normalized residue of BAL data to a linear growth fit: Brazilian samples/BR (A series, top), traveled to Japan/JP (B, middle) and back to Brazil (D1, top on A5), and Czech samples/CZ (C, bottom), plotted against the contemporaneous local gravimetric acceleration oscillation (δ).

between the seedlings' biological rhythms and lunisolar gravitational forces. This study adds to the growing evidence that the biological rhythms of plants may be intimately linked with and regulated by environmental lunar-solar gravitational forces.

9 Results

The thesis presents a thorough investigation into the phenomenon of biological autoluminescence (BAL), a spontaneous photon emission from biological cells and tissues, and its potential applications. The research explores the complicated interrelation between BAL and various chemical, physical, biological, and environmental modulators, providing significant insights into the underlying mechanisms and practical implications of BAL in scientific and industrial fields.

Chapter 4 establishes a foundational understanding of the kinetics behind BAL primarily through an extensive review of existing literature. This chapter gathers and reviews information from various studies to describe the dynamics of reactive oxygen species (ROS) generation and decay, and their impact on BAL generation. Importantly, it compiles the rate constants connected to the fundamental mechanisms of BAL generation for the first time in such an extent. The development of a quantitative model to predict the contributions of different biochemical processes to BAL is identified as forthcoming work, aimed at further advancing the understanding of this complex phenomenon.

The experiments detailed in chapter 5 provide insight into how antioxidants and prooxidants influence BAL, enhancing our understanding of cellular oxidative mechanisms modulated by external chemical agents. This investigation uses two specific biological models: yeast *(Saccharomyces cerevisiae)* and differentiated human promyelocytic leukemia cells (HL-60). The study demonstrated that antioxidants typically reduce BAL by lowering ROS levels. However, in HL-60 cells, mannitol increased BAL, indicating prooxidant activity. These findings emphasize that chemical agents can have dual roles in biological systems, depending on the specific context.

Chapter 6 explores the connection between pulsed electric field (PEF) treatment and BAL monitoring, highlighting BAL as an additional technique to understand the effects of physical stressors on protein structures. This chapter examines how PEF influences the structural integrity of proteins and their oxidative states through electrically generated ROS. PEF treatment impacts autoluminescence, making BAL a valuable indicator of molecular responses to PEF. Additionally, this chapter investigates the synergy between PEF treatments and chemical modulators, specifically peroxide and enzymes such as catalase and superoxide dismutase. It examines how the combination of PEF and these chemical agents affects BAL, suggesting that PEF may enhance the effects of prooxidants and that certain enzymes can protect biological matter against damage by ROS during PEF treatment. This synergistic effect could lead to new applications of BAL monitoring in cases where combined physical and chemical treatments are applied, as it is real-time and non-invasive. Lastly, in the context of optical spectral analysis, the chapter reveals that the main contribution to BAL under PEF with externally

added hydrogen peroxide (H_2O_2) is from singlet oxygen ($^1O_2$). This finding is significant as it ties the observed BAL enhanced by PEF directly to specific oxidative pathways.

Focusing on the biological factors that influence BAL, chapter 7 discusses how interactions between cells and microbial agents impact oxidative stress and subsequent BAL. This chapter explores the respiratory burst in neutrophil-like cells induced by yeast, illustrating how these cells increase ROS production in response to microbial invasion. It also shows how monitoring BAL can offer insights into the cellular defense mechanisms and their kinetic properties.

Chapter 8 provides a comprehensive analysis of how lunar and solar tidal forces correlate with the patterns of BAL in plant systems. By linking environmental cycles with biological rhythms, it explores the broader implications of BAL as a marker for understanding and predicting biological responses to natural environmental changes.

10 Conclusions

10.1 Contribution of the dissertation

This thesis offers a comprehensive exploration of biological autoluminescence (BAL), examining its mechanisms of generation, detection methods, and the diverse modulators influencing its activity. By providing an extensive overview of the current state of the art, the thesis systematically categorizes the chemical, physical, biological, and environmental factors that affect BAL.

A significant theoretical contribution of this thesis is the collection of rate constants for building a kinetic model of BAL, as detailed in Chapter 4. This work lays the groundwork for future quantitative modeling that will enhance our understanding of the dynamic processes governing BAL. The compilation of these constants marks a critical step toward developing a comprehensive kinetic model that could predict the behavior of BAL across various biological contexts.

The experimental work conducted by the candidate, encompassed in Chapters 5, 6, 7, and 8, showcases the practical application of theoretical concepts. These chapters detail experiments on the effects of different modulators on BAL using model organisms such as yeast and human promyelocytic leukemia cells (HL-60), proteins, and germinating seeds. Through these studies, the candidate has not only provided empirical evidence supporting theoretical predictions but has also developed and refined experimental protocols and methods, enhancing the precision and reliability of BAL measurements.

Chapter 6 focused on the impact of physical modulators like pulsed electric fields, incorporating optical spectral analysis to elucidate how specific wavelengths of light contribute to the overall BAL, indicating the primary role of singlet oxygen.

In conclusion, this thesis not only advances our theoretical understanding of BAL but also enhances practical approaches to its measurement and manipulation. The incorporation of spectral analysis into the study of BAL adds an additional layer of depth to our understanding.

10.2 Future applications

10.2.1 Neurology

As dramatic advances in contemporary medicine extend life expectancy, the cognitive decline associated with aging remains a significant challenge. Neurological diseases like Alzheimer's and Parkinson's typically manifest around 75 years of age, underscoring the need for strategies to delay or prevent the deterioration of mental functions to ensure a dignified, productive, and active end of life.

As highlighted in chapter 4, approximately 0.1 to 4% of the total molecular oxygen consumed by the body leaks into superoxide anion radicals, precursors for the generation of reactive oxygen species (ROS). The brain, consuming about 20% of the body's oxygen intake [317], is especially susceptible to the effects of these ROS due to its high metabolic demand. This susceptibility makes the brain particularly vulnerable to oxidative stress, which can significantly impair neural functions. Monitoring BAL offers a promising avenue for advancing our understanding of oxidative stress in neurological contexts. BAL, as a non-invasive technique, can be used to detect and quantify the presence of ROS in real-time. This capability could be pivotal for early detection of oxidative stress in neural tissues, a known contributor to neurodegenerative diseases. By identifying elevated ROS levels early, interventions could be implemented sooner, potentially slowing or reversing the oxidative damage that leads to cognitive decline.

10.2.2 Biopharmaceuticals production

The manufacturing of biopharmaceuticals, particularly biologics like monoclonal antibodies, recombinant proteins, and vaccines, is a highly complex process that involves cultivating living cells to produce therapeutic compounds. These cell lines, often derived from mammalian, bacterial, or yeast cells, must be maintained in precise conditions that optimize their productivity and longevity. The process begins with the selection of a suitable cell line, followed by cell culture, where cells are grown in bioreactors under carefully controlled conditions of temperature, pH, oxygen, and nutrient levels. Throughout the process, ensuring that cells are thriving is essential, as their health directly impacts the quality and quantity of the biologic product.

BAL monitoring could be implemented as a sophisticated tool for overseeing this delicate manufacturing process, since it is sensitive to oxidative stress and metabolic changes within cells. In biopharmaceutical manufacturing, BAL can be used to continuously monitor the metabolic state of cell cultures in real-time, offering early warnings of cellular stress or suboptimal conditions. This allows for timely adjustments to environmental factors within bioreactors, such as nutrient supply, oxygenation, or waste removal, ensuring that cells continue to thrive and produce at optimal levels. The benefits of BAL monitoring extend beyond just maintaining product quality. By providing real-time insights into cellular health, BAL could enable more efficient production processes. This efficiency translates into reduced energy and resource consumption, such as lower water usage, and optimized energy use in maintaining bioreactor conditions. Furthermore, minimizing cellular stress and metabolic waste could reduce downstream purification processes, which often involve extensive filtration, chromatography, and sterilization to remove impurities and ensure the safety of the final product.

In the highly regulated biopharmaceutical industry, where safety, quality, and compliance with good manufacturing practices (GMP) are paramount, BAL could offer a non-invasive, real-time monitoring solution that complements traditional analytical methods. This would contribute to the overall robustness of the production process, helping manufacturers consistently meet stringent quality standards while also improving sustainability. By integrating BAL into the manufacturing workflow, biopharmaceutical companies can enhance their

ability to produce biologics, complex medicines that have revolutionized the treatment of diseases such as cancer, autoimmune disorders, and rare genetic conditions—more efficiently and with a reduced environmental footprint.

10.2.3 Biofuel production

BAL monitoring can play a pivotal role in optimizing biofuel production from waste materials, especially when using bacteria and yeast to convert organic waste into fuels like ethanol and biodiesel. As this technology provides real-time, non-invasive insights into the metabolic activity and oxidative stress of these microorganisms, which are crucial for maximizing biofuel production efficiency. By detecting subtle metabolic shifts early, BAL enables operators to adjust environmental conditions, such as nutrient levels and pH, to keep microbial populations healthy and productive.

In addition to process optimization, BAL monitoring can aid in selecting the most efficient microbial strains or mutants for biofuel production. During strain development, various bacterial or yeast mutants are tested for their ability to convert waste materials into biofuels. BAL can help identify mutants with the lowest oxidative stress and highest metabolic efficiency under production conditions, indicating their potential for high biofuel yield. By selecting strains that demonstrate superior performance through BAL signals, researchers and producers can ensure that the most efficient and robust microorganisms are used in the biofuel production process, leading to more sustainable and higher-yielding operations.

10.2.4 Monitoring of waste water treatment

Wastewater treatment is a complex and essential process aimed at removing contaminants from water before it is released back into the environment or reused. The treatment process typically involves multiple stages, each designed to progressively remove solids, organic matter, and chemical pollutants. In primary treatment, large particles and sediments are mechanically removed through screening and settling. Secondary treatment, often biological in nature, is where microorganisms play a vital role in breaking down organic pollutants. This stage usually takes place in aeration tanks or biofilters, where bacteria and other microbes consume organic matter and convert it into carbon dioxide, water, and biomass. Finally, tertiary treatment further purifies the water, often through chemical, filtration, or disinfection processes, to remove any remaining contaminants such as nutrients, heavy metals, or pathogens.

BAL can be a powerful tool in optimizing the biological stage of wastewater treatment, where microbial activity is critical. This can be particularly valuable in the case when the performance of microbes is directly linked to the efficiency of pollutant breakdown. For example, if the BAL signal indicates elevated oxidative stress, it may suggest that microorganisms are struggling to process the organic load, signaling the need for adjustments in aeration, pH, or nutrient levels.

By continuously monitoring BAL, plant operators can make informed decisions to optimize the conditions for microbial growth and activity, preventing the onset of issues such as process upsets, incomplete treatment, or toxic shocks to the microbial community. Moreover, BAL can help identify the early stages of biofilm formation or other inefficiencies that might compromise the treatment process. This real-time monitoring allows for proactive management, reducing the need for reactive interventions that can be more resource-intensive and costly.

In addition to improving process efficiency, BAL monitoring can enhance the sustainability of wastewater treatment operations. By optimizing microbial performance, plants can reduce energy consumption associated with aeration and mixing, which are often the most energy-intensive aspects of the treatment process. Furthermore, by ensuring that biological treatment is operating at peak efficiency, BAL can help minimize the need for chemical additives, reducing both costs and the environmental impact of treatment plants. Overall, the application of BAL in wastewater treatment can lead to cleaner effluent, more stable operations, and a reduced environmental footprint, aligning with the growing demand for sustainable water management solutions.

BIBLIOGRAPHY

[1] O. Kučera, K. Červinková, M. Nerudova, and M. Cifra, "Spectral perspective on the electromagnetic activity of cells," *Current topics in medicinal chemistry*, vol. 15, no. 6, pp. 513–522, 2015.

[2] D. Johnston, J. C. Magee, C. M. Colbert, and B. R. Christie, "Active Properties of Neuronal Dendrites," *Annual Review of Neuroscience*, vol. 19, no. 1, pp. 165–186, Mar. 1996.

[3] W. Balcavage, T. Alvager, J. Swez, C. Goff, M. Fox, S. Abdullyava, and M. King, "A Mechanism for Action of Extremely Low Frequency Electromagnetic Fields on Biological Systems," *Biochemical and Biophysical Research Communications*, vol. 222, no. 2, pp. 374–378, May 1996.

[4] D. C. Gadsby, "Ion channels versus ion pumps: the principal difference, in principle," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 5, pp. 344–352, May 2009.

[5] R. H. Funk, T. Monsees, and N. Özkucur, "Electromagnetic effects – From cell biology to medicine," *Progress in Histochemistry and Cytochemistry*, vol. 43, no. 4, pp. 177–264, Feb. 2009.

[6] L. V. Titova, A. K. Ayesheshim, A. Golubov, R. Rodriguez-Juarez, R. Woycicki, F. A. Hegmann, and O. Kovalchuk, "Intense THz pulses down-regulate genes associated with skin cancer and psoriasis: a new therapeutic avenue?" *Scientific Reports*, vol. 3, no. 1, p. 2363, Aug. 2013.

[7] Y. Wang, Y. Xiong, M. Chen, F. Liu, H. He, Q. Ma, P. Gao, G. Xiang, and L. Zhang, "The biological effects of terahertz wave radiation-induced injury on neural stem cells," *iScience*, vol. 26, no. 10, p. 107418, Oct. 2023.

[8] I. V. Lundholm, H. Rodilla, W. Y. Wahlgren, A. Duelli, G. Bourenkov, J. Vukusic, R. Friedman, J. Stake, T. Schneider, and G. Katona, "Terahertz radiation induces non-thermal structural changes associated with Fröhlich condensation in a protein crystal," *Structural Dynamics*, vol. 2, no. 5, p. 054702, Sep. 2015.

[9] D. Havelka, M. Cifra, O. Kučera, J. Pokorný, and J. Vrba, "High-frequency electric field and radiation characteristics of cellular microtubule network," *Journal of theoretical biology*, vol. 286, pp. 31–40, 2011.

[10] M. Cifra, J. Pokorný, D. Havelka, and O. Kučera, "Electric field generated by axial longitudinal vibration modes of microtubule," *Biosystems*, vol. 100, no. 2, pp. 122–131, May 2010.

[11] A. Merla and G. L. Romani, "Biomedical Applications of Functional Infrared Imaging."

[12] M. Etehadtavakol and E. Y. K. Ng, "BREAST THERMOGRAPHY AS A POTENTIAL NON-CONTACT METHOD IN THE EARLY DETECTION OF CANCER: A REVIEW," *Journal of Mechanics in Medicine and Biology*, vol. 13, no. 02, p. 1330001, Apr. 2013.

[13] H. Journeedekorver, E. Midena, and A. Singh, "Infrared Thermotherapy: From Laboratory to Clinic," *Ophthalmology Clinics of North America*, vol. 18, no. 1, pp. 99–110, Mar. 2005.

[14] S. Bayata and A. Türel Ermertcan, "Thermotherapy in dermatology," *Cutaneous and Ocular Toxicology*, vol. 31, no. 3, pp. 235–240, Sep. 2012.

[15] J. E. Lloyd, "Fireflies of Melanesia: bioluminescence, mating behavior, and synchronous flashing (Coleoptera: Lampyridae)," *Environmental Entomology*, vol. 2, no. 6, pp. 991–1008, 1973, publisher: Oxford University Press Oxford, UK.

[16] P. J. Herring, "Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea," *Journal of the Marine Biological Association of the United Kingdom*, vol. 87, no. 4, pp. 829–842, 2007, publisher: Cambridge University Press.

[17] M. S. Grober, "Brittle-star bioluminescence functions as an aposematic signal to deter crustacean predators," *Animal Behaviour*, vol. 36, no. 2, pp. 493–501, Apr. 1988.

[18] E. Widder, "Bioluminescence," in *Adaptive mechanisms in the ecology of vision*. Springer, 1999, pp. 555–581.

[19] M. Cifra and P. Pospíšil, "Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications," *Journal of Photochemistry and Photobiology B: Biology*, vol. 139, pp. 2–10, Oct. 2014.

[20] O. Kučera and M. Cifra, "Cell-to-cell signaling through light: just a ghost of chance?" *Cell Communication and Signaling*, vol. 11, no. 1, p. 1, 2013.

[21] P. Pospíšil, "Ultra-weak photon emission from living systems – from mechanism to application," *Journal of Photochemistry and Photobiology B: Biology*, vol. 139, p. 1, Oct. 2014.

[22] M. Rác, M. Sedlářová, and P. Pospíšil, "The formation of electronically excited species in the human multiple myeloma cell suspension," *Scientific Reports*, vol. 5, p. 8882, Mar. 2015.

[23] A. Prasad, P. Gouripeddi, H. R. N. Devireddy, A. Ovsii, D. P. Rachakonda, R. V. Wijk, and P. Pospíšil, "Spectral Distribution of Ultra-Weak Photon Emission as A Response to Wounding in Plants: An In Vivo Study," *Biology*, vol. 9, no. 6, p. 139, Jun. 2020.

[24] A. Rastogi and P. Pospíšil, "Ultra-weak photon emission as a non-invasive tool for the measurement of oxidative stress induced by UVA radiation in Arabidopsis thaliana," *Journal of Photochemistry and Photobiology B: Biology*, vol. 123, pp. 59–64, Jun. 2013.

[25] R. Van Wijk, E. P. Van Wijk, H. A. van Wietmarschen, and J. v. d. Greef, "Towards whole-body ultra-weak photon counting and imaging with a focus on human beings: A review," *Journal of Photochemistry and Photobiology B: Biology*, vol. 139, pp. 39–46, Oct. 2014.

[26] R. C. R. Burgos, J. C. Schoeman, L. J. v. Winden, K. Červinková, R. Ramautar, E. P. A. Van Wijk, M. Cifra, R. Berger, T. Hankemeier, and J. v. d. Greef, "Ultra-weak photon emission as a dynamic tool for monitoring oxidative stress metabolism," *Scientific Reports*, vol. 7, no. 1, p. 1229, Dec. 2017.

[27] C. d. M. Gallep and D. Robert, "Time-resolved ultra-weak photon emission as germination performance indicator in single seedlings," *Journal of Photochemistry and Photobiology*, vol. 1, p. 100001, Mar. 2020.

[28] M. Kobayashi, K. Sasaki, M. Enomoto, and Y. Ehara, "Highly sensitive determination of transient generation of biophotons during hypersensitive response to cucumber mosaic virus in cowpea," *Journal of Experimental Botany*, vol. 58, no. 3, pp. 465–472, Dec. 2006.

[29] F. Scholkmann, E. van de Kraats, R. van Wijk, E. van Wijk, and J. van der Greef, "Spatio-temporal dynamics of spontaneous ultra-weak photon emission (autoluminescence) from human hands measured with an EMCCD camera: Dependence on time of day, date and individual subject," *Matters*, Mar. 2018.

[30] H. Saeidfirozeh, A. Shafiekhani, M. Cifra, and A. A. Masoudi, "Endogenous Chemiluminescence from Germinating Arabidopsis Thaliana Seeds," *Scientific Reports*, vol. 8, no. 1, p. 16231, Dec. 2018.

[31] E. Cadenas, "Biochemistry of oxygen toxicity," *Annual review of biochemistry*, vol. 58, no. 1, pp. 79–110, 1989.

[32] E. Cadenas, A. Boveris, and B. Chance, "Low-level chemiluminescence of bovine heart submitochondrial particles," *Biochemical Journal*, vol. 186, no. 3, pp. 659–667, 1980.

[33] E. Cadenas and H. Sies, "Low Level Chemiluminescence of Liver Microsomal Fractions Initiated by tert-Butyl Hydroperoxide," *European Journal of Biochemistry*, vol. 124, no. 2, pp. 349–356, 1982, _eprint: https://febs.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1432-1033.1982.tb06599.x.

[34] M. E. Murphy and H. Sies, "[63] Visible-range low-level chemiluminescence in biological systems," in *Methods in Enzymology*, ser. Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants. Academic Press, Jan. 1990, vol. 186, pp. 595–610.

[35] A. Boveris, R. A. Sanchez, A. I. Varsavsky, and E. Cadenas, "Spontaneous chemiluminescence of soybean seeds," *FEBS Letters*, vol. 113, no. 1, pp. 29–32, 1980, _eprint: https://febs.onlinelibrary.wiley.com/doi/pdf/10.1016/0014-5793%2880%2980487-X.

[36] C. D. M. Gallep, "Auto-luminescence in seedlings: possible indicators for the gravimetric tide?" *Frontiers in Photonics*, vol. 5, p. 1421686, Aug. 2024.

[37] M. Havaux, C. Triantaphylides, and B. Genty, "Autoluminescence imaging: a non-invasive tool for mapping oxidative stress," *Trends in plant science*, vol. 11, no. 10, pp. 480–484, 2006.

[38] S. Cohen and F. Popp, "Biophoton emission of the human body," *Journal of Photochemistry and Photobiology B: Biology*, vol. 40, no. 2, pp. 187–189, 1997.

[39] C. Choi, W. M. Woo, M. B. Lee, J. S. Yang, K. S. Soh, J. S. Yang, G. Yoon, M. Kim, C. Zaslawsky, and J. J. Chang, "Biophoton emission from the hands," *JOURNAL-KOREAN PHYSICAL SOCIETY*, vol. 41, pp. 275–278, 2002.

[40] R. Bajpai, S. Kumar, and V. Sivadasan, "Biophoton emission in the evolution of a squeezed state of frequency stable damped oscillator," *Applied Mathematics and Computation*, vol. 93, no. 2, pp. 277–288, 1998.

[41] K. Kobayashi, H. Okabe, S. Kawano, Y. Hidaka, and K. Hara, "Biophoton emission induced by heat shock," *PloS one*, vol. 9, no. 8, p. e105700, 2014.

[42] F. A. Popp, W. Nagl, K. Li, W. Scholz, O. Weingärtner, and R. Wolf, "Biophoton emission: New evidence for coherence and DNA as source," *Cell Biochemistry and Biophysics*, vol. 6, no. 1, pp. 33–52, 1984.

[43] F.-A. Popp, Q. Gu, and K.-H. Li, "Biophoton emission: Experimental background and theoretical approaches," *Modern Physics Letters B*, vol. 8, no. 21n22, pp. 1269–1296, 1994.

[44] R. Van Wijk and E. P. Wijk, "An Introduction to Human Biophoton Emission," *Forschende Komplementärmedizin / Research in Complementary Medicine*, vol. 12, no. 2, pp. 77–83, Apr. 2005.

[45] D. Xing, S. Tan, Y. Tang, Y. He, and D. Li, "Observation of biophoton emission from plants in the process of defense response," *Chinese Science Bulletin*, vol. 44, no. 23, pp. 2159–2162, 1999, publisher: Springer.

[46] P. Pospíšil, A. Prasad, and M. Rác, "Mechanism of the Formation of Electronically Excited Species by Oxidative Metabolic Processes: Role of Reactive Oxygen Species," *Biomolecules*, vol. 9, no. 7, p. 258, Jul. 2019.

[47] B. Halliwell and J. M. Gutteridge, *Free radicals in biology and medicine*. Oxford University Press, USA, 2015.

[48] R. B. Hamanaka and N. S. Chandel, "Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes," *Trends in Biochemical Sciences*, vol. 35, no. 9, pp. 505–513, Sep. 2010.

[49] R.-Z. Zhao, S. Jiang, L. Zhang, and Z.-B. Yu, "Mitochondrial electron transport chain, ROS generation and uncoupling (Review)," *International Journal of Molecular Medicine*, May 2019.

[50] S. Guan, L. Zhao, and R. Peng, "Mitochondrial Respiratory Chain Supercomplexes: From Structure to Function," *International Journal of Molecular Sciences*, vol. 23, no. 22, p. 13880, Nov. 2022.

[51] M. P. Murphy, "How mitochondria produce reactive oxygen species," *Biochemical Journal*, vol. 417, no. 1, pp. 1–13, Jan. 2009.

[52] P. Hernansanz-Agustín and J. A. Enríquez, "Generation of Reactive Oxygen Species by Mitochondria," *Antioxidants*, vol. 10, no. 3, p. 415, Mar. 2021.

[53] K. Bedard and K.-H. Krause, "The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology," *Physiological Reviews*, vol. 87, no. 1, pp. 245–313, Jan. 2007, publisher: American Physiological Society.

[54] F. Augsburger, A. Filippova, D. Rasti, T. Seredenina, M. Lam, G. Maghzal, Z. Mahiout, P. Jansen-Dürr, U. G. Knaus, J. Doroshow, R. Stocker, K.-H. Krause, and V. Jaquet, "Pharmacological characterization of the seven human NOX isoforms and their inhibitors," *Redox Biology*, vol. 26, p. 101272, Sep. 2019.

[55] C. C. Winterbourn, A. J. Kettle, and M. B. Hampton, "Reactive Oxygen Species and Neutrophil Function," *Annual Review of Biochemistry*, vol. 85, no. 1, pp. 765–792, Jun. 2016.

[56] M. Canton, R. Sánchez-Rodríguez, I. Spera, F. C. Venegas, M. Favia, A. Viola, and A. Castegna, "Reactive Oxygen Species in Macrophages: Sources and Targets," *Frontiers in Immunology*, vol. 12, p. 734229, Sep. 2021.

[57] M. Vulcano, S. Dusi, D. Lissandrini, R. Badolato, P. Mazzi, E. Riboldi, E. Borroni, A. Calleri, M. Donini, A. Plebani, L. Notarangelo, T. Musso, and S. Sozzani, "Toll Receptor-Mediated Regulation of NADPH Oxidase in Human Dendritic Cells," *The Journal of Immunology*, vol. 173, no. 9, pp. 5749–5756, Nov. 2004.

[58] H. Buvelot, K. M. Posfay-Barbe, P. Linder, J. Schrenzel, and K.-H. Krause, "*Staphylococcus aureus* , phagocyte NADPH oxidase and chronic granulomatous disease," *FEMS Microbiology Reviews*, p. fuw042, Dec. 2016.

[59] R. Holmdahl, O. Sareila, L. M. Olsson, L. Bäckdahl, and K. Wing, "Ncf1 polymorphism reveals oxidative regulation of autoimmune chronic inflammation," *Immunological Reviews*, vol. 269, no. 1, pp. 228–247, Jan. 2016.

[60] N. Browne, M. Heelan, and K. Kavanagh, "An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes," *Virulence*, vol. 4, no. 7, pp. 597–603, Oct. 2013.

[61] I. Szanto, L. Rubbia-Brandt, P. Kiss, K. Steger, B. Banfi, E. Kovari, F. Herrmann, A. Hadengue, and K.-H. Krause, "Expression of *NOX1* , a superoxide-generating NADPH oxidase, in colon cancer and inflammatory bowel disease," *The Journal of Pathology*, vol. 207, no. 2, pp. 164–176, Oct. 2005.

[62] I. R. Hanna, L. L. Hilenski, A. Dikalova, Y. Taniyama, S. Dikalov, A. Lyle, M. T. Quinn, B. Lassègue, and K. K. Griendling, "Functional association of nox1 with p22phox in vascular smooth muscle cells," *Free Radical Biology and Medicine*, vol. 37, no. 10, pp. 1542–1549, Nov. 2004.

[63] A. M. Briones, F. Tabet, G. E. Callera, A. C. Montezano, A. Yogi, Y. He, M. T. Quinn, M. Salaices, and R. M. Touyz, "Differential regulation of Nox1, Nox2 and Nox4 in vascular smooth muscle cells from WKY and SHR," *Journal of the American Society of Hypertension*, vol. 5, no. 3, pp. 137–153, May 2011.

[64] B. Bánfi, A. Maturana, S. Jaconi, S. Arnaudeau, T. Laforge, B. Sinha, E. Ligeti, N. Demaurex, and K.-H. Krause, "A Mammalian H $^+$ Channel Generated Through Alternative Splicing of the NADPH Oxidase Homolog *NOH-1*," *Science*, vol. 287, no. 5450, pp. 138–142, Jan. 2000.

[65] X.-L. Cui, D. Brockman, B. Campos, and L. Myatt, "Expression of NADPH Oxidase Isoform 1 (Nox1) in Human Placenta: Involvement in Preeclampsia," *Placenta*, vol. 27, no. 4-5, pp. 422–431, Apr. 2006.

[66] T. Ago, T. Kitazono, J. Kuroda, Y. Kumai, M. Kamouchi, H. Ooboshi, M. Wakisaka, T. Kawahara, K. Rokutan, S. Ibayashi, and M. Iida, "NAD(P)H Oxidases in Rat Basilar Arterial Endothelial Cells," *Stroke*, vol. 36, no. 5, pp. 1040–1046, May 2005.

[67] S. Kobayashi, Y. Nojima, M. Shibuya, and Y. Maru, "Nox1 regulates apoptosis and potentially stimulates branching morphogenesis in sinusoidal endothelial cells," *Experimental Cell Research*, vol. 300, no. 2, pp. 455–462, Nov. 2004.

[68] Y.-A. Suh, R. S. Arnold, B. Lassegue, J. Shi, X. Xu, D. Sorescu, A. B. Chung, K. K. Griendling, and J. D. Lambeth, "Cell transformation by the superoxide-generating oxidase Mox1," *Nature*, vol. 401, no. 6748, pp. 79–82, Sep. 1999.

[69] G. Gavazzi, B. Banfi, C. Deffert, L. Fiette, M. Schappi, F. Herrmann, and K.-H. Krause, "Decreased blood pressure in NOX1-deficient mice," *FEBS Letters*, vol. 580, no. 2, pp. 497–504, Jan. 2006.

[70] J. L. Arbiser, J. Petros, R. Klafter, B. Govindajaran, E. R. McLaughlin, L. F. Brown, C. Cohen, M. Moses, S. Kilroy, R. S. Arnold, and J. D. Lambeth, "Reactive oxygen generated by Nox1 triggers the angiogenic switch," *Proceedings of the National Academy of Sciences*, vol. 99, no. 2, pp. 715–720, Jan. 2002.

[71] H. Sauer, M. M. Bekhite, J. Hescheler, and M. Wartenberg, "Redox control of angiogenic factors and CD31-positive vessel-like structures in mouse embryonic stem cells after direct current electrical field stimulation," *Experimental Cell Research*, vol. 304, no. 2, pp. 380–390, Apr. 2005.

[72] F. J. Miller, X. Chu, B. Stanic, X. Tian, R. V. Sharma, R. L. Davisson, and F. S. Lamb, "A Differential Role for Endocytosis in Receptor-Mediated Activation of Nox1," *Antioxidants & Redox Signaling*, vol. 12, no. 5, pp. 583–593, Mar. 2010.

[73] S. Jiang, J. Streeter, B. M. Schickling, K. Zimmerman, R. M. Weiss, and F. J. Miller, "Nox1 NADPH oxidase is necessary for late but not early myocardial ischaemic preconditioning," *Cardiovascular Research*, vol. 102, no. 1, pp. 79–87, Apr. 2014.

[74] U. Maitra, N. Singh, L. Gan, L. Ringwood, and L. Li, "IRAK-1 Contributes to Lipopolysaccharide-induced Reactive Oxygen Species Generation in Macrophages by Inducing NOX-1 Transcription and Rac1 Activation and Suppressing the Expression of Antioxidative Enzymes," *Journal of Biological Chemistry*, vol. 284, no. 51, pp. 35 403–35 411, Dec. 2009.

[75] G. Cheng, Z. Cao, X. Xu, E. G. Meir, and J. Lambeth, "Homologs of gp91 phox : cloning and tissue expression of Nox3, Nox4, and Nox5," *Gene*, vol. 269, no. 1-2, pp. 131–140, May 2001.

[76] M. Geiszt, J. B. Kopp, P. Várnai, and T. L. Leto, "Identification of Renox, an NAD(P)H oxidase in kidney," *Proceedings of the National Academy of Sciences*, vol. 97, no. 14, pp. 8010–8014, Jul. 2000.

[77] T. Ago, T. Kitazono, H. Ooboshi, T. Iyama, Y. H. Han, J. Takada, M. Wakisaka, S. Ibayashi, H. Utsumi, and M. Iida, "Nox4 as the Major Catalytic Component of an Endothelial NAD(P)H Oxidase," *Circulation*, vol. 109, no. 2, pp. 227–233, Jan. 2004.

[78] J. T. Colston, S. D. De La Rosa, J. R. Strader, M. A. Anderson, and G. L. Freeman, "H $_2$ O $_2$ activates Nox4 through PLA $_2$ -dependent arachidonic acid production in adult cardiac fibroblasts," *FEBS Letters*, vol. 579, no. 11, pp. 2533–2540, Apr. 2005.

[79] F. Hahner, F. Moll, T. Warwick, D. Hebchen, G. Buchmann, J. Epah, W. Abplanalp, T. Schader, S. Günther, R. Gilsbach, R. Brandes, and K. Schröder, "Nox4 promotes endothelial differentiation through chromatin remodeling," *Redox Biology*, vol. 55, p. 102381, Sep. 2022.

[80] J. Li, M. Stouffs, L. Serrander, B. Banfi, E. Bettiol, Y. Charnay, K. Steger, K.-H. Krause, and M. E. Jaconi, "The NADPH Oxidase NOX4 Drives Cardiac Differentiation: Role in Regulating Cardiac Transcription Factors and MAP Kinase Activation," *Molecular Biology of the Cell*, vol. 17, no. 9, pp. 3978–3988, Sep. 2006.

[81] Y. Yoshikawa, T. Ago, J. Kuroda, Y. Wakisaka, M. Tachibana, M. Komori, T. Shibahara, H. Nakashima, K. Nakashima, and T. Kitazono, "Nox4 Promotes Neural Stem/Precursor Cell Proliferation and Neurogenesis in the Hippocampus and Restores Memory Function Following Trimethyltin-Induced Injury," *Neuroscience*, vol. 398, pp. 193–205, Feb. 2019.

[82] Y.-M. Lee, B.-J. Kim, Y.-S. Chun, I. So, H. Choi, M.-S. Kim, and J.-W. Park, "NOX4 as an oxygen sensor to regulate TASK-1 activity," *Cellular Signalling*, vol. 18, no. 4, pp. 499–507, Apr. 2006.

[83] J. H. Kim, J. Lee, S.-J. Bae, Y. Kim, B.-J. Park, J.-W. Choi, J. Kwon, G.-H. Cha, H. J. Yoo, E.-K. Jo, Y. S. Bae, Y.-H. Lee, and J.-M. Yuk, "NADPH oxidase 4 is required for the generation of macrophage migration inhibitory factor and host defense against Toxoplasma gondii infection," *Scientific Reports*, vol. 7, no. 1, p. 6361, Jul. 2017.

[84] B. Bánfi, G. Molnár, A. Maturana, K. Steger, B. Hegedûs, N. Demaurex, and K.-H. Krause, "A Ca2+-activated NADPH Oxidase in Testis, Spleen, and Lymph Nodes," *Journal of Biological Chemistry*, vol. 276, no. 40, pp. 37 594–37 601, Oct. 2001.

[85] H. K. A. Gole, D. L. Tharp, and D. K. Bowles, "Upregulation of Intermediate-Conductance Ca2+-Activated K+ Channels (KCNN4) in Porcine Coronary Smooth Muscle Requires NADPH Oxidase 5 (NOX5)," *PLoS ONE*, vol. 9, no. 8, p. e105337, Aug. 2014.

[86] C. Dupuy, R. Ohayon, A. Valent, M.-S. Noël-Hudson, D. Dème, and A. Virion, "Purification of a Novel Flavoprotein Involved in the Thyroid NADPH Oxidase," *Journal of Biological Chemistry*, vol. 274, no. 52, pp. 37 265–37 269, Dec. 1999.

[87] P. Manikandan and S. Nagini, "Cytochrome P450 Structure, Function and Clinical Significance: A Review," *Current Drug Targets*, vol. 19, no. 1, Jan. 2018.

[88] R. Rapoport, D. Sklan, and I. Hanukoglu, "Electron leakage from the adrenal-cortex mitochondrial P450Scc and P450C11 systems: NADPH and steroid dependence," *Archives of biochemistry and biophysics*, vol. 317, no. 2, pp. 412–416, 1995, publisher: Elsevier.

[89] E. Kellogg and I. Fridovich, "Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system." *Journal of Biological Chemistry*, vol. 250, no. 22, pp. 8812–8817, Nov. 1975.

[90] Y. Yamaguchi, T. Matsumura, K. Ichida, K. Okamoto, and T. Nishino, "Human Xanthine Oxidase Changes its Substrate Specificity to Aldehyde Oxidase Type upon Mutation of Amino Acid Residues in the Active Site: Roles of Active Site Residues in Binding and Activation of Purine Substrate," *Journal of Biochemistry*, vol. 141, no. 4, pp. 513–524, Jan. 2007.

[91] C. J. Lowenstein, J. L. Dinerman, and S. H. Snyder, "Nitric Oxide: A Physiologic Messenger," vol. 120, no. 3, 1994.

[92] I. Fleming, "Biology of Nitric Oxide Synthases."

[93] K. Ikeda, O. G. G. Jr, and Y. Yamori, "DIETARY N ^{g} -NITR0- l -ARGININE INDUCES SUSTAINED HYPERTENSION IN NORMOTENSIVE WISTAR-KYOTO RATS," *Clinical and Experimental Pharmacology and Physiology*, vol. 19, no. 8, pp. 583–586, Aug. 1992.

[94] H.-Y. Yun, V. Dawson, and T. Dawson, "Nitric oxide in health and disease of the nervous system," *Nitric oxide in the nervous system*, vol. 2, pp. 300–310, 1997.

[95] L. E. Gustafsson, C. U. Wiklund, N. P. Wikhund, M. G. Persson, and S. Moncada, "Modulation of autonomic neuroeffector transmission by nitric oxide in guinea pig ileum," *Biochemical and Biophysical Research Communications*, vol. 173, no. 1, pp. 106–110, Nov. 1990.

[96] D. B. Stolz, R. Zamora, Y. Vodovotz, P. A. Loughran, T. R. Billiar, Y.-M. Kim, R. L. Simmons, and S. C. Watkins, "Peroxisomal localization of inducible nitric oxide synthase in hepatocytes: Peroxisomal localization of inducible nitric oxide synthase in hepatocytes," *Hepatology*, vol. 36, no. 1, pp. 81–93, Jul. 2002.

[97] A. Cyr, L. Chambers, P. K. Waltz, S. P. Whelan, L. Kohut, E. Carchman, M. Dyer, J. Luciano, B. Kautza, H. D. Gomez, L. E. Otterbein, M. R. Rosengart, S. Shiva, and B. S. Zuckerbraun, "Endotoxin Engages Mitochondrial Quality Control *via* an iNOS-Reactive Oxygen Species Signaling Pathway in Hepatocytes," *Oxidative Medicine and Cellular Longevity*, vol. 2019, pp. 1–9, Oct. 2019.

[98] D. Stuehr, S. Pou, and G. M. Rosen, "Oxygen Reduction by Nitric-oxide Synthases," *Journal of Biological Chemistry*, vol. 276, no. 18, pp. 14 533–14 536, Jan. 2001.

[99] H. Sato, I. Sagami, S. Daff, and T. Shimizu, "Autoxidation Rates of Neuronal Nitric Oxide Synthase: Effects of the Substrates, Inhibitors, and Modulators," *Biochemical and Biophysical Research Communications*, vol. 253, no. 3, pp. 845–849, Dec. 1998.

[100] J. Weaver, S. Porasuphatana, P. Tsai, S. Pou, L. J. Roman, and G. M. Rosen, "A comparative study of neuronal and inducible nitric oxide synthases: Generation of nitric oxide, superoxide, and hydrogen peroxide," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1726, no. 3, pp. 302–308, Nov. 2005.

[101] L. Piacenza, A. Zeida, M. Trujillo, and R. Radi, "The superoxide radical switch in the biology of nitric oxide and peroxynitrite," *Physiological Reviews*, vol. 102, no. 4, pp. 1881–1906, Oct. 2022.

[102] J. Zeng, S. Deng, Y. Wang, P. Li, L. Tang, and Y. Pang, "Specific Inhibition of Acyl-CoA Oxidase-1 by an Acetylenic Acid Improves Hepatic Lipid and Reactive Oxygen Species (ROS) Metabolism in Rats Fed a High Fat Diet," *Journal of Biological Chemistry*, vol. 292, no. 9, pp. 3800–3809, Mar. 2017.

[103] G. M. Small, K. Burdett, and M. J. Connock, "A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase," *Biochemical Journal*, vol. 227, no. 1, pp. 205–210, Apr. 1985.

[104] S. Takahashi, K. Abe, and Y. Kera, "Bacterial d-amino acid oxidases: Recent findings and future perspectives," *Bioengineered*, vol. 6, no. 4, pp. 237–241, Jul. 2015.

[105] K. Ryuichi and Y. Yosihiro, "D-Amino-acid oxidase and its physiological function," *International journal of biochemistry*, vol. 24, no. 4, pp. 519–524, 1992, publisher: Elsevier.

[106] C. Kiryu, M. Makiuchi, J. Miyazaki, T. Fujinaga, and K. Kakinuma, "Physiological production of singlet molecular oxygen in the myeloperoxidase-HPOP-chloride system," *FEBS Letters*, 1999.

[107] M. Tarr and D. P. Valenzeno, "Singlet oxygen: the relevance of extracellular production mechanisms to oxidative stress in vivo," *Photochemical & Photobiological Sciences*, vol. 2, no. 4, pp. 355–361, Apr. 2003.

[108] S. I. Ahmad, Ed., *Ultraviolet Light in Human Health, Diseases and Environment*, ser. Advances in Experimental Medicine and Biology. Cham: Springer International Publishing, 2017, vol. 996. [Online]. Available: http://link.springer.com/10.1007/978-3-319-56017-5

[109] D. Livingstone, "Contaminant-stimulated Reactive Oxygen Species Production and Oxidative Damage in Aquatic Organisms," *Marine Pollution Bulletin*, vol. 42, no. 8, pp. 656–666, Aug. 2001.

[110] P. S. J. Lakey, T. Berkemeier, H. Tong, A. M. Arangio, K. Lucas, U. Pöschl, and M. Shiraiwa, "Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract," *Scientific Reports*, vol. 6, no. 1, p. 32916, Sep. 2016.

[111] S. Ahmad, "Oxidative stress from environmental pollutants," *Archives of Insect Biochemistry and Physiology*, vol. 29, no. 2, pp. 135–157, Jan. 1995.

[112] Kiran, R. Bharti, and R. Sharma, "Effect of heavy metals: An overview," *Materials Today: Proceedings*, vol. 51, pp. 880–885, 2022.

[113] B. Poljšak and R. Fink, "The Protective Role of Antioxidants in the Defence against ROS/RNS-Mediated Environmental Pollution," *Oxidative Medicine and Cellular Longevity*, vol. 2014, pp. 1–22, 2014.

[114] T. V. Sirota, "A Chain Reaction of Adrenaline Autoxidation is a Model of Quinoid Oxidation of Catecholamines," *Biophysics*, vol. 65, no. 4, pp. 548–556, Jul. 2020.

[115] G. S. Behonick, M. J. Novak, E. W. Nealley, and S. I. Baskin, "Toxicology update: the cardiotoxicity of the oxidative stress metabolites of catecholamines (aminochromes)," *Journal of Applied Toxicology*, vol. 21, no. S1, pp. S15–S22, Dec. 2001.

[116] S. Toyokuni, "Iron and carcinogenesis: from Fenton reaction to target genes," *Redox Report*, vol. 7, no. 4, pp. 189–197, Aug. 2002.

[117] A. Maetzke and S. J. Knak Jensen, "Reaction paths for production of singlet oxygen from hydrogen peroxide and hypochlorite," *Chemical Physics Letters*, vol. 425, no. 1-3, pp. 40–43, Jul. 2006.

[118] C. A. Juan, J. M. Pérez De La Lastra, F. J. Plou, and E. Pérez-Lebeña, "The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies," *International Journal of Molecular Sciences*, vol. 22, no. 9, p. 4642, Apr. 2021.

[119] B. Halliwell and S. Chirico, "Lipid peroxidation: its mechanism, measurement, and significance," *The American journal of clinical nutrition*, vol. 57, no. 5, pp. 715S–725S, 1993, publisher: Elsevier.

[120] E. Niki, Y. Yoshida, Y. Saito, and N. Noguchi, "Lipid peroxidation: Mechanisms, inhibition, and biological effects," *Biochemical and Biophysical Research Communications*, vol. 338, no. 1, pp. 668–676, Dec. 2005.

[121] L. J. Marnett, "Lipid peroxidation—DNA damage by malondialdehyde," *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 424, no. 1-2, pp. 83–95, Mar. 1999.

[122] M. Csala, T. Kardon, B. Legeza, B. Lizák, J. Mandl, É. Margittai, F. Puskás, P. Száraz, P. Szelényi, and G. Bánhegyi, "On the role of 4-hydroxynonenal in health and disease," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1852, no. 5, pp. 826–838, May 2015.

[123] M. J. Davies, "Protein and Peptide Alkoxyl Radicals Can Give Rise to C-Terminal Decarboxylation and Backbone Cleavage," *Archives of Biochemistry and Biophysics*, vol. 336, no. 1, pp. 163–172, Dec. 1996.

[124] C. L. Hawkins and M. J. Davies, "Detection, identification, and quantification of oxidative protein modifications," *The Journal of Biological Chemistry*, vol. 294, no. 51, pp. 19 683–19 708, Dec. 2019.

[125] F. Khabiri, R. Hagens, C. Smuda, A. Soltau, V. Schreiner, H. Wenck, K.-P. Wittern, H.-J. Duchstein, and W. Mei, "Non-invasive monitoring of oxidative skin stress by ultraweak photon emission (UPE)-measurement. I: mechanisms of UPE of biological materials," *Skin Research and Technology*, vol. 14, no. 1, pp. 103–111, 2008.

[126] B. Halliwell and J. M. Gutteridge, "[1] Role of free radicals and catalytic metal ions in human disease: an overview," vol. 186, pp. 1–85, 1990.

[127] C. Von Sonntag, *Free-Radical-Induced DNA Damage and Its Repair: A Chemical Perspective*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2006. [Online]. Available: https://link.springer.com/10.1007/3-540-30592-0

[128] W. Chamulitrat, N. Takahashi, and R. P. Mason, "Peroxyl, alkoxyl, and carbon-centered radical formation from organic hydroperoxides by chloroperoxidase," *Journal of Biological Chemistry*, vol. 264, no. 14, pp. 7889–7899, May 1989.

[129] J. A. Howard, "Measurement of Absolute Propagation and Termination Rate Constants for Alkylperoxyls in Solution by the Hydroperoxide Method," *Israel Journal of Chemistry*, vol. 24, no. 1, pp. 33–37, 1984.

[130] C. von Sonntag, P. Dowideit, X. Fang, R. Mertens, X. Pan, M. N. Schuchmann, and H.-P. Schuchmann, "The fate of peroxyl radicals in aqueous solution," *Water Science and Technology*, vol. 35, no. 4, pp. 9–15, 1997, publisher: Elsevier.

[131] G. Cilento, "Dioxetanes as intermediates in biological processes," *Journal of theoretical biology*, vol. 55, no. 2, pp. 471–479, 1975.

[132] W. Adam and A. V. Trofimov, "Contemporary trends in dioxetane chemistry," p. 40, 2009.

[133] W. Adam and G. Cilento, *Chemical and biological generation of excited states*. Elsevier, 1982.

[134] R. Lee, G. Gryn'ova, K. U. Ingold, and M. L. Coote, "Why are sec-alkylperoxyl bimolecular self-reactions orders of magnitude faster than the analogous reactions of tert-alkylperoxyls? The unanticipated role of CH hydrogen bond donation," *Physical Chemistry Chemical Physics*, vol. 18, no. 34, pp. 23 673–23 679, 2016.

[135] G. D. Mendenhall, X. C. Sheng, and T. Wilson, "Yields of excited carbonyl species from alkoxyl and from alkylperoxyl radical dismutations," *Journal of the American Chemical Society*, vol. 113, no. 23, pp. 8976–8977, 1991.

[136] G. S. Timmins, R. E. dos Santos, A. C. Whitwood, L. H. Catalani, P. Di Mascio, B. C. Gilbert, and E. J. H. Bechara, "Lipid Peroxidation-Dependent Chemiluminescence from the Cyclization of Alkylperoxyl Radicals to Dioxetane Radical Intermediates," *Chemical Research in Toxicology*, vol. 10, no. 10, pp. 1090–1096, Oct. 1997.

[137] G. F. Fedorova, A. V. Trofimov, R. F. Vasil'ev, and T. L. Veprintsev, "Peroxy-radical-mediated chemiluminescence: mechanistic diversity and fundamentals for antioxidant assay," *Arkivoc*, vol. 8, pp. 163–215, 2007.

[138] D. V. Kazakov, V. P. Kazakov, G. Y. Maistrenko, D. V. Mal'zev, and R. Schmidt, "On the Effect of 1, 4-Diazabicyclo [2.2. 2] octane on the Singlet-Oxygen Dimol Emission: Chemical Generation of (1O2) 2 in Peroxide Reactions," vol. 111, no. 20, pp. 4267–4273, 2007.

[139] A. C. Velosa, W. J. Baader, C. V. Stevani, C. M. Mano, and E. J. H. Bechara, "1,3-Diene Probes for Detection of Triplet Carbonyls in Biological Systems," *Chemical Research in Toxicology*, vol. 20, no. 8, pp. 1162–1169, Aug. 2007.

[140] Q. Niu and G. D. Mendenhall, "Structural effects on the yields of singlet molecular oxygen (1. DELTA. gO2) from alkylperoxyl radical recombination," *Journal of the American Chemical Society*, vol. 112, no. 4, pp. 1656–1657, 1990.

[141] C. M. Mano, F. M. Prado, J. Massari, G. E. Ronsein, G. R. Martinez, S. Miyamoto, J. Cadet, H. Sies, M. H. G. Medeiros, E. J. H. Bechara, and P. Di Mascio, "Excited singlet molecular O2 ($1\Delta g$) is generated enzymatically from excited carbonyls in the dark," *Scientific Reports*, vol. 4, Aug. 2014.

[142] G. A. Fisk and G. N. Hays, "A study of the 0.634 pm dimol emission from excited molecular oxygen," *Chemical Physics Letters*, vol. 79, no. 2, pp. 331–333, 1981.

[143] M. Poplová, "Photocount statistics and spectral analysis of ultraweak photon emission from biological systems," January 2024.

[144] H. P. K.K., "Photomultiplier tubes, basics and applications, Fourth edition," 2017.

[145] Oxford Instruments Andor, "Electron Multiplying CCD Cameras."

[146] O. I. Andor, "What is an EMCCD Camera?" 2021.

[147] Hamamatsu Photonics K.K., "Electron Multiplying Charge-Coupled Devices (EMCCDs)."

[148] J. Sławiński, "Biophotons from stressed and dying organisms: toxicological aspects," *Indian Journal of Experimental Biology*, vol. 41, no. 5, pp. 483–493, 2003, publisher: NISCAIR-CSIR, India.

[149] A. Rastogi and P. Pospíšil, "Spontaneous ultraweak photon emission imaging of oxidative metabolic processes in human skin: effect of molecular oxygen and antioxidant defense system," *Journal of Biomedical Optics*, vol. 16, no. 9, p. 096005, 2011.

[150] C. L. Oros and F. Alves, "Leaf wound induced ultraweak photon emission is suppressed under anoxic stress: Observations of Spathiphyllum under aerobic and anaerobic conditions using novel in vivo methodology," *PLOS ONE*, vol. 13, no. 6, p. e0198962, Jun. 2018.

[151] Z. Rajfur, "Photon emission from chemically perturbed yeast cells," *Journal of bioluminescence and chemiluminescence*, vol. 9, no. 2, pp. 59–63, 1994, publisher: Wiley Online Library.

[152] A. Boveris, E. Cadenas, R. Reiter, M. Filipowski, Nasake, and B. Chance, "Organ chemiluminescence: Noninvasive assay for oxidative radical reaction," *Proceedings of the National Academy of Sciences*, vol. 77, pp. 347–351, 1980.

[153] M. Kobayashi, M. Takeda, T. Sato, Y. Yamazaki, K. Kaneko, K.-I. Ito, H. Kato, and H. Inaba, "In vivo imaging of spontaneous ultraweak photon emission from a rat's brain correlated with cerebral energy metabolism and oxidative stress," *Neuroscience research*, vol. 34, no. 2, pp. 103–113, 1999.

[154] I. P. Ivanova, S. V. Trofimova, I. M. Piskarev, N. A. Aristova, O. E. Burhina, and O. O. Soshnikova, "Mechanism of chemiluminescence in Fenton reaction," *Journal of Biophysical Chemistry*, vol. 03, no. 01, pp. 88–100, 2012.

[155] A. Prasad and P. Pospišil, "Two-dimensional imaging of spontaneous ultra-weak photon emission from the human skin: role of reactive oxygen species," *Journal of Biophotonics*, vol. 4, no. 11-12, pp. 840–849, Nov. 2011.

[156] M. Poplová, A. Prasad, E. Van Wijk, P. Pospíšil, and M. Cifra, "Biological Auto(chemi)luminescence Imaging of Oxidative Processes in Human Skin," *Analytical Chemistry*, vol. 95, no. 40, pp. 14 853–14 860, 2023, _eprint: https://doi.org/10.1021/acs.analchem.3c01566.

[157] P. Vahalová, K. Červinková, and M. Cifra, "Biological autoluminescence for assessing oxidative processes in yeast cell cultures," *Scientific Reports*, vol. 11, no. 1, p. 10852, May 2021.

[158] M. Bereta, M. Teplan, D. E. Chafai, R. Radil, and M. Cifra, "Biological autoluminescence as a noninvasive monitoring tool for chemical and physical modulation of oxidation in yeast cell culture," *Scientific Reports*, vol. 11, p. 328, 2021.

[159] A. Rastogi and P. Pospíšil, "Effect of exogenous hydrogen peroxide on biophoton emission from radish root cells," *Plant Physiology and Biochemistry*, vol. 48, no. 2-3, pp. 117–123, Feb. 2010.

[160] B. Halliwell, "Commentary: Vitamin C: Antioxidant or Pro-Oxidant In Vivo?" *Free Radical Research*, vol. 25, no. 5, pp. 439–454, Jan. 1996.

[161] N. Inoue, K. Akasaka, H. Arimoto, and H. Ohrui, "Effect of ascorbic acid on the chemiluminescence of polyphenols," *Bioscience, biotechnology, and biochemistry*, vol. 70, no. 6, pp. 1517–1520, 2006.

[162] E. A. Lissi, J. Escobar, C. Pascual, M. d. Castillo, T. H. Schmitt, and P. D. Mascio, "Visible chemiluminescence associated with the reaction between methemoglobin or oxyhemoglobin with hydrogen peroxide," *Photochemistry and photobiology*, vol. 60, no. 5, pp. 405–411, 1994.

[163] B. Gonzalez Flecha, S. Llesuy, and A. Boveris, "Hydroperoxide-initiated chemiluminescence: An assay for oxidative stress in biopsies of heart, liver, and muscle," *Free Radical Biology and Medicine*, vol. 10, no. 2, pp. 93–100, Jan. 1991.

[164] A. Jaśkowska, R. Borc, I. Milczarek, A. Dudziak, and E. Śpiewla, "Kinetics studies of ultraweak luminescence induced by ascorbic acid in Characeae cells and their structures," *Luminescence: The journal of biological and chemical luminescence*, vol. 16, no. 1, pp. 51–56, 2001, publisher: Wiley Online Library.

[165] H. Wefers and H. Sies, "Oxidation of glutathione by the superoxide radical to the disulfide and the sulfonate yielding singlet oxygen," *European Journal of Biochemistry*, vol. 137, no. 1-2, pp. 29–36, 1983.

[166] M. L. Salin and S. M. Bridges, "Chemiluminescence in Wounded Root Tissue: Evidence for Peroxidase Involvement," *Plant Physiology*, vol. 67, no. 1, pp. 43–46, Jan. 1981.

[167] Y. Wenli and Z. Yaping, "Chemiluminescence evaluation of oxidative damage to biomolecules induced by singlet oxygen and the protective effects of antioxidants," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1725, no. 1, pp. 30–34, Aug. 2005.

[168] K. Kakinuma, E. Cadenas, A. Boveris, and B. Chance, "Low level chemiluminescence of intact polymorphonuclear leukocytes," *FEBS Letters*, vol. 102, no. 1, pp. 38–42, Jun. 1979.

[169] D. T. Sawyer and J. S. Valentine, "How super is superoxide?" *Accounts of Chemical Research*, vol. 14, no. 12, pp. 393–400, Dec. 1981.

[170] R. A. Gus'kova, I. I. Ivanov, V. K. Kol'tover, V. V. Akhobadze, and A. B. Rubin, "Permeability of bilayer lipid membranes for superoxide (O2-) radicals," *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 778, no. 3, pp. 579–585, Dec. 1984.

[171] C. L. Soares and E. H. Bechara, "Enzymatic Generation of Triplet Biacetyl," *Photochemistry and Photobiology*, vol. 36, no. 1, pp. 117–119, 1982, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1751-1097.1982.tb04351.x.

[172] F. S. Knudsen, C. A. A. Penatti, L. O. Royer, K. A. Bidart, M. Christoff, D. Ouchi, and E. J. H. Bechara, "Chemiluminescent Aldehyde and β-Diketone Reactions Promoted by Peroxynitrite," *Chemical Research in Toxicology*, vol. 13, no. 5, pp. 317–326, May 2000.

[173] F. B. Jensen, "Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O2 and CO2 transport," *Acta Physiologica Scandinavica*, vol. 182, no. 3, pp. 215–227, 2004, publisher: Wiley Online Library.

[174] J. J. Pignatello, E. Oliveros, and A. MacKay, "Advanced Oxidation Processes for Organic Contaminant Destruction Based on the Fenton Reaction and Related Chemistry," *Critical Reviews in Environmental Science and Technology*, vol. 36, no. 1, pp. 1–84, Jan. 2006.

[175] J.-W. Chen, C.-M. Chen, and C.-C. Chang, "A fluorescent pH probe for acidic organelles in living cells," *Organic & Biomolecular Chemistry*, vol. 15, no. 37, pp. 7936–7943, 2017.

[176] H. Rottensteiner and F. L. Theodoulou, "The ins and outs of peroxisomes: Co-ordination of membrane transport and peroxisomal metabolism," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1763, no. 12, pp. 1527–1540, Dec. 2006.

[177] S. Birtic, B. Ksas, B. Genty, M. J. Mueller, C. Triantaphylidès, and M. Havaux, "Using spontaneous photon emission to image lipid oxidation patterns in plant tissues," *The Plant Journal*, vol. 67, no. 6, pp. 1103–1115, 2011.

[178] S. Miyamoto, G. E. Ronsein, T. C. Corrêa, G. R. Martinez, M. H. G. Medeiros, and P. Di Mascio, "Direct evidence of singlet molecular oxygen generation from peroxynitrate, a decomposition product of peroxynitrite," *Dalton Transactions*, no. 29, p. 5720, 2009.

[179] D. Slawinska, K. Polewski, and J. Slawinski, "The stress-induced electromagnetic emission from biosystems: chemiluminescence response of plants to mechanical and chemical damage," *Journal of Electroanalytical Chemistry*, vol. 343, no. 3, pp. 483–488, 1992.

[180] A. Prasad, M. Sedlářová, A. Balukova, M. Rác, and P. Pospíšil, "Reactive Oxygen Species as a Response to Wounding: In Vivo Imaging in Arabidopsis thaliana," *Frontiers in Plant Science*, vol. 10, p. 1660, Jan. 2020.

[181] A. Prasad, M. Sedlářová, R. S. Kale, and P. Pospíšil, "Lipoxygenase in singlet oxygen generation as a response to wounding: in vivo imaging in Arabidopsis thaliana," *Scientific Reports*, vol. 7, no. 1, p. 9831, Dec. 2017.

[182] T. Roach, L. Colville, R. P. Beckett, F. V. Minibayeva, M. Havaux, and I. Kranner, "A proposed interplay between peroxidase, amine oxidase and lipoxygenase in the wounding-induced oxidative burst in Pisum sativum seedlings," *Phytochemistry*, vol. 112, pp. 130–138, Apr. 2015.

[183] A. Görlach, K. Bertram, S. Hudecova, and O. Krizanova, "Calcium and ROS: A mutual interplay," *Redox Biology*, vol. 6, pp. 260–271, Dec. 2015.

[184] K. Nakamura and M. Hiramatsu, "Ultra-weak photon emission from human hand: Influence of temperature and oxygen concentration on emission," *Journal of Photochemistry and Photobiology B: Biology*, vol. 80, no. 2, pp. 156–160, Aug. 2005.

[185] P. Roschger, R. Scott, B. Devaraj, and H. Inaba, "Observation of phase transitions in intact leaves by intrinsic low-level chemiluminescence," *Photochemistry and photobiology*, vol. 57, no. 3, pp. 580–583, 1993, publisher: Wiley Online Library.

[186] A. Prasad and P. Pospísil, "Ultraweak photon emission induced by visible light and ultraviolet A radiation via photoactivated skin chromophores: in vivo charge coupled device imaging," *Journal of biomedical optics*, vol. 17, no. 8, p. 085004, 2012.

[187] P. Evelson, C. P. Ordóñez, S. Llesuy, and A. Boveris, "Oxidative stress and in vivo chemiluminescence in mouse skin exposed to UVA radiation," *Journal of Photochemistry and Photobiology B: Biology*, vol. 38, no. 2-3, pp. 215–219, Apr. 1997.

[188] E. V. Naumova, Y. A. Vladimirov, L. V. Beloussov, V. V. Tuchin, and I. V. Volodyaev, "Methods of Studying Ultraweak Photon Emission from Biological Objects: I. History, Types and Properties, Fundamental and Application Significance," *Biophysics*, vol. 66, no. 5, pp. 764–778, Sep. 2021.

[189] B. Tarusov, A. Polivoda, and A. Zhuravlev, "Detection of chemiluminescence in the liver of irradiated mice," *Radiobiologiya*, vol. 1, no. 1, pp. 150–151, 1961.

[190] A. E. Zaqaryan and H. G. Badalyan, "Chemiluminescence of Rats' Whole Blood after X-ray and γ-irradiation," vol. 9, no. 1, p. 4, 2016.

[191] P. J. Hore and H. Mouritsen, "The Radical-Pair Mechanism of Magnetoreception," *Annual Review of Biophysics*, vol. 45, no. 1, pp. 299–344, Jul. 2016.

[192] N. Ikeya and J. R. Woodward, "Cellular autofluorescence is magnetic field sensitive," *Proceedings of the National Academy of Sciences*, vol. 118, no. 3, p. e2018043118, Jan. 2021.

[193] H. Wang and X. Zhang, "Magnetic Fields and Reactive Oxygen Species," *International Journal of Molecular Sciences*, vol. 18, no. 10, p. 2175, Oct. 2017.

[194] P. Politański, E. Rajkowska, M. Pawlaczyk-Łuszczyńska, A. Dudarewicz, A. Wiktorek-Smagur, M. Śliwińska-Kowalska, and M. Zmyślony, "Static magnetic field affects oxidative stress in mouse cochlea," *International Journal of Occupational Medicine and Environmental Health*, vol. 23, no. 4, Jan. 2010.

[195] B. Feng, C. Ye, L. Qiu, L. Chen, Y. Fu, and W. Sun, "Mitochondrial ROS Release and Subsequent Akt Activation Potentially Mediated the Anti-Apoptotic Effect of a 50-Hz Magnetic Field on FL Cells," *Cellular Physiology and Biochemistry*, vol. 38, no. 6, pp. 2489–2499, 2016.

[196] J. W. Mundell, M. I. Brier, E. Orloff, S. A. Stanley, and J. S. Dordick, "Alternating Magnetic Fields Drive Stimulation of Gene Expression via Generation of Reactive Oxygen Species."

[197] L. Potenza, L. Ubaldi, R. De Sanctis, R. De Bellis, L. Cucchiarini, and M. Dachà, "Effects of a static magnetic field on cell growth and gene expression in Escherichia coli," *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, vol. 561, no. 1-2, pp. 53–62, Jul. 2004.

[198] M. Buemi, D. Marino, G. Di Pasquale, F. Floccari, M. Senatore, C. Aloisi, F. Grasso, G. Mondio, P. Perillo, N. Frisina, and others, "Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field," *Nephron*, vol. 87, no. 3, pp. 269–273, 2001, publisher: S. Karger AG.

[199] S. H. Meglič and T. Kotnik, "Electroporation-based applications in biotechnology," in *Handbook of Electroporation*, 2017, pp. 307–321.

[200] S. A. Shirley, "Delivery of Cytokines Using Gene Electrotransfer," in *Handbook of Electroporation*, D. Miklavčič, Ed. Cham: Springer International Publishing, 2017, pp. 1755–1768.

[201] S. Bhonsle, R. E. Neal, and R. V. Davalos, "Preclinical Studies on Irreversible Electroporation," in *Handbook of Electroporation*, 2016, pp. 1527–1542.

[202] V. Todorovic and M. Cemazar, "Combined Treatment of Electrochemotherapy with Immunomodulators," in *Handbook of Electroporation*, 2016, pp. 1717–1731.

[203] J. Gehl and G. Serša, "Electrochemotherapy and Its Clinical Applications," in *Handbook of Electroporation*, D. Miklavčič, Ed. Cham: Springer International Publishing, 2017, pp. 1771–1786.

[204] W. Zhao and R. Yang, "Pulsed Electric Fields for Inactivation of Endogenous Enzymes in Foods," in *Handbook of Electroporation*, D. Miklavčič, Ed. Cham: Springer International Publishing, 2017, pp. 2239–2251.

[205] F. Schottroff, A. Krottenthaler, and H. Jäger, "Stress Induction and Response, Inactivation, and Recovery of Vegetative Microorganisms by Pulsed Electric Fields," in *Handbook of Electroporation*, D. Miklavčič, Ed. Cham: Springer International Publishing, 2017, pp. 2539–2557.

[206] Z. S. Salameh, K. N. Aycock, N. Alinezhadbalalami, K. M. Imran, I. H. McKillop, I. C. Allen, and R. V. Davalos, "Harnessing the Electrochemical Effects of Electroporation-Based Therapies to Enhance Anti-tumor Immune Responses," *Annals of Biomedical Engineering*, vol. 52, pp. 48–56, Nov. 2023.

[207] G. Pataro, G. Donsì, and G. Ferrari, "Modeling of Electrochemical Reactions During Pulsed Electric Field Treatment," in *Handbook of Electroporation*, D. Miklavčič, Ed. Cham: Springer International Publishing, 2017, pp. 1059–1088.

[208] S. Zhang, R. Yang, W. Zhao, Q. Liang, and Z. Zhang, "The first ESR observation of radical species generated under pulsed electric fields processing," *LWT - Food Science and Technology*, vol. 44, no. 4, pp. 1233–1235, May 2011.

[209] M. A. Malik, M. A. Sheikh, and N. A. Mir, "A review on pulsed electric field modification of proteins: Effect on the functional and structural properties," *Food Bioscience*, vol. 61, p. 104636, Oct. 2024.

[210] W. Zhao, R. Yang, Y. Tang, W. Zhang, and X. Hua, "Investigation of the Protein-Protein Aggregation of Egg White Proteins under Pulsed Electric Fields," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 9, pp. 3571–3577, May 2009.

[211] R. Axelrod, M. Beyrer, and A. Mathys, "Impact of the electric field intensity and treatment time on whey protein aggregate formation," *Journal of Dairy Science*, vol. 105, no. 8, pp. 6589–6600, Aug. 2022.

[212] M. Bereta, M. Teplan, T. Zakar, H. Vuviet, M. Cifra, and D. E. Chafai, "Biological autoluminescence enables effective monitoring of yeast cell electroporation," *Biotechnology Journal*, vol. 19, no. 4, p. 2300475, 2024, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/biot.202300475.

[213] P. Vahalová, D. Havelka, E. Vaněčková, T. Zakar, V. Kolivoška, and M. Cifra, "Biochemiluminescence Sensing of Protein Oxidation by Reactive Oxygen Species Generated by Pulsed Electric Field," *Sensors and Actuators B: Chemical*, vol. 385, p. 133676, 2023.

[214] T. Makino, K. Kato, H. Lyozumi, H. Honzawa, Y. Tachiiri, and M. Hiramatsu, "Ultraweak luminescence generated by sweet potato and Fusarium oxysporum interactions associated with a defense response," *Photochemistry and photobiology*, vol. 64, no. 6, pp. 953–956, 1996.

[215] A. Rastogi and P. Pospíšil, "Production of hydrogen peroxide and hydroxyl radical in potato tuber during the necrotrophic phase of hemibiotrophic pathogen Phytophthora infestans infection," *Journal of Photochemistry and Photobiology B: Biology*, vol. 117, pp. 202–206, Dec. 2012.

[216] R. C. Allen, "Chemiluminescence from Eukaryotic and Prokaryotic Cells: Reducing Potential and Oxygen Requirements," *Photochemistry and Photobiology*, vol. 30, no. 1, pp. 157–163, 1979.

[217] M. Bennett, M. Mehta, and M. Grant, "Biophoton Imaging: A Nondestructive Method for Assaying R Gene Responses," *Molecular Plant-Microbe Interactions*, vol. 18, no. 2, pp. 95–112, 2005.

[218] A. Boveris, A. I. Varsavsky, S. G. D. Silva, and R. A. Sánchez, "CHEMILUMINESCENCE OF SOYBEAN SEEDS: SPECTRAL ANALYSIS, TEMPERATURE DEPENDENCE AND EFFECT OF INHIBITORS," *Photochemistry and Photobiology*, vol. 38, no. 1, pp. 99–104, Jul. 1983.

[219] A. Prasad, U. Ferretti, M. Sedlářová, and P. Pospíšil, "Singlet oxygen production in Chlamydomonas reinhardtii under heat stress," *Scientific Reports*, vol. 6, p. 20094, Feb. 2016.

[220] É. Hideg and L. Olof Björn, "Ultraweak light emission, free radicals, chilling and light sensitivity," *Physiologia Plantarum*, vol. 98, no. 2, pp. 223–228, Oct. 1996.

[221] T. Ohya, S. Yoshida, R. Kawabata, H. Okabe, and S. Kai, "Biophoton Emission Due to Drought Injury in Red Beans: Possibility of Early Detection of Drought Injury," *Japanese Journal of Applied Physics*, vol. 41, no. Part 1, No. 7A, pp. 4766–4771, Jul. 2002.

[222] R. Kausar, Z. Hossain, T. Makino, and S. Komatsu, "Characterization of ascorbate peroxidase in soybean under flooding and drought stresses," *Molecular Biology Reports*, vol. 39, no. 12, pp. 10 573–10 579, Dec. 2012.

[223] S. Komatsu, A. H. M. Kamal, T. Makino, and Z. Hossain, "Ultraweak photon emission and proteomics analyses in soybean under abiotic stress," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1844, no. 7, pp. 1208–1218, Jul. 2014.

[224] K. E. Wellen and C. B. Thompson, "Cellular Metabolic Stress: Considering How Cells Respond to Nutrient Excess," *Molecular Cell*, vol. 40, no. 2, pp. 323–332, Oct. 2010.

[225] Z. Liu, Z. Ren, J. Zhang, C.-C. Chuang, E. Kandaswamy, T. Zhou, and L. Zuo, "Role of ROS and Nutritional Antioxidants in Human Diseases," *Frontiers in Physiology*, vol. 9, p. 477, May 2018.

[226] P. Madl, T. Verwanger, M. Geppert, and F. Scholkmann, "Oscillations of ultra-weak photon emission from cancer and non-cancer cells stressed by culture medium change and TNF-α," *Scientific Reports*, vol. 7, no. 1, p. 11249, Sep. 2017.

[227] C. M. Gallep and S. R. Dos Santos, "Photon-counts during germination of wheat (Triticum aestivum) in wastewater sediment solutions correlated with seedling growth," *Seed Science and Technology*, vol. 35, no. 3, pp. 607–614, 2007.

[228] T. Ohya, H. Kurashige, H. Okabe, and S. Kai, "Early Detection of Salt Stress Damage by Biophotons in Red Bean Seedling," *Japanese Journal of Applied Physics*, vol. 39, no. Part 1, No. 6A, pp. 3696–3700, Jun. 2000.

[229] Z. Hossain, T. Makino, and S. Komatsu, "Proteomic study of β-aminobutyric acid-mediated cadmium stress alleviation in soybean," *Journal of Proteomics*, vol. 75, no. 13, pp. 4151–4164, Jul. 2012.

[230] S. A. Hasan, Q. Fariduddin, B. Ali, S. Hayat, and A. Ahmad, "Cadmium: toxicity and tolerance in plants," *J Environ Biol*, vol. 30, no. 2, pp. 165–174, 2009.

[231] F. F. Nocito, C. Lancilli, B. Giacomini, and G. A. Sacchi, "Sulfur Metabolism and Cadmium Stress in Higher Plants."

[232] F. Scholkmann, "Chronobiological Aspects of Spontaneous Ultra-Weak Photon Emission in Humans: Ultradian, Circadian and Infradian Rhythms," in *Ultra-Weak Photon Emission from Biological Systems: Endogenous Biophotonics and Intrinsic Bioluminescence*. Springer, 2023, pp. 289–304.

[233] M. Rosbash, "Circadian Rhythms and the Transcriptional Feedback Loop (Nobel Lecture)," *Angew. Chem.*, 2021.

[234] R. Zhang, N. F. Lahens, H. I. Ballance, M. E. Hughes, and J. B. Hogenesch, "A circadian gene expression atlas in mammals: Implications for biology and medicine," *Proceedings of the National Academy of Sciences*, vol. 111, no. 45, pp. 16 219–16 224, Nov. 2014.

[235] M. L. Fanjul-Moles and G. O. López-Riquelme, "Relationship between Oxidative Stress, Circadian Rhythms, and AMD," *Oxidative Medicine and Cellular Longevity*, vol. 2016, no. 1, p. 7420637, Jan. 2016.

[236] M. Zhou, W. Wang, S. Karapetyan, M. Mwimba, J. Marqués, N. E. Buchler, and X. Dong, "Redox rhythm reinforces the circadian clock to gate immune response," *Nature*, vol. 523, no. 7561, pp. 472–476, Jul. 2015.

[237] J. S. O'Neill and A. B. Reddy, "Circadian clocks in human red blood cells," *Nature*, vol. 469, no. 7331, pp. 498–503, Jan. 2011.

[238] M. Putker and J. S. O'Neill, "Reciprocal Control of the Circadian Clock and Cellular Redox State - a Critical Appraisal," *Molecules and Cells*, vol. 39, no. 1, pp. 6–19, Jan. 2016.

[239] A. Jiménez, F. Sevilla, and M. C. Martí, "Reactive oxygen species homeostasis and circadian rhythms in plants," *Journal of Experimental Botany*, vol. 72, no. 16, pp. 5825–5840, Aug. 2021.

[240] A. N. Dodd, N. Salathia, A. Hall, E. Kévei, R. Tóth, F. Nagy, J. M. Hibberd, A. J. Millar, and A. A. R. Webb, "Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage," *Science*, vol. 309, no. 5734, pp. 630–633, Jul. 2005.

[241] T. P. Michael, P. A. Salomé, H. J. Yu, T. R. Spencer, E. L. Sharp, M. A. McPeek, J. M. Alonso, J. R. Ecker, and C. R. McClung, "Enhanced Fitness Conferred by Naturally Occurring Variation in the Circadian Clock," *Science*, vol. 302, no. 5647, pp. 1049–1053, Nov. 2003.

[242] M. Kobayashi, D. Kikuchi, and H. Okamura, "Imaging of Ultraweak Spontaneous Photon Emission from Human Body Displaying Diurnal Rhythm," *PLoS ONE*, vol. 4, no. 7, p. e6256, Jul. 2009.

[243] R. Van Wijk, E. P. Van Wijk, and R. P. Bajpai, "Photocount distribution of photons emitted from three sites of a human body," *Journal of Photochemistry and Photobiology B: Biology*, vol. 84, no. 1, pp. 46–55, Jul. 2006.

[244] M. Cifra, E. Van Wijk, H. Koch, S. Bosman, and R. Van Wijk, "Spontaneous ultra-weak photon emission from human hands is time dependent," *Radioengineering*, vol. 16, no. 2, p. 15, 2007.

[245] C. de Mello Gallep, "Ultraweak, spontaneous photon emission in seedlings: toxicological and chronobiological applications: UPE in seedlings - applications," *Luminescence*, vol. 29, no. 8, pp. 963–968, Dec. 2014.

[246] M. J. Davies, "Protein oxidation and peroxidation," *Biochemical Journal*, vol. 473, no. 7, pp. 805–825, Apr. 2016.

[247] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *The Journal of Physiology*, vol. 552, no. 2, pp. 335–344, Oct. 2003.

[248] N. Smirnoff, "Ascorbic acid metabolism and functions: A comparison of plants and mammals," *Free Radical Biology and Medicine*, vol. 122, pp. 116–129, Jul. 2018.

[249] G. Wu, Y.-Z. Fang, S. Yang, J. R. Lupton, and N. D. Turner, "Glutathione metabolism and its implications for health," vol. 134, no. 3, pp. 489–492, 2004.

[250] J. St-Pierre, J. A. Buckingham, S. J. Roebuck, and M. D. Brand, "Topology of superoxide production from different sites in the mitochondrial electron transport chain," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 44 784–44 790, 2002.

[251] E. Cadenas and K. J. Davies, "Mitochondrial free radical generation, oxidative stress, and aging," *Free Radical Biology and Medicine*, vol. 29, no. 3-4, pp. 222–230, 2000.

[252] B. Chance, H. Sies, and A. Boveris, "Hydroperoxide metabolism in mammalian organs." vol. 59, no. 3, pp. 527–605, 1979.

[253] Y. Ogura and I. Yamazaki, "Steady-State Kinetics of the Catalase Reaction in the Presence of Cyanide," *The Journal of Biochemistry*, vol. 94, no. 2, pp. 403–408, Jul. 1983.

[254] F. Collin, "Chemical Basis of Reactive Oxygen Species Reactivity and Involvement in Neurodegenerative Diseases," *International Journal of Molecular Sciences*, vol. 20, no. 10, p. 2407, Jan. 2019, number: 10 Publisher: Multidisciplinary Digital Publishing Institute.

[255] R. F. Pontes, J. E. Moraes, A. Machulek, and J. M. Pinto, "A mechanistic kinetic model for phenol degradation by the Fenton process," *Journal of Hazardous Materials*, vol. 176, no. 1-3, pp. 402–413, Apr. 2010.

[256] A. A. Burbano, D. D. Dionysiou, and M. T. Suidan, "Effect of oxidant-to-substrate ratios on the degradation of MTBE with Fenton reagent," *Water Research*, vol. 42, no. 12, pp. 3225–3239, Jun. 2008.

[257] R. A. Crowell, R. Lian, M. C. Sauer, D. A. Oulianov, and I. A. Shkrob, "Geminate recombination of hydroxyl radicals generated in 200 nm photodissociation of aqueous hydrogen peroxide," *Chemical Physics Letters*, vol. 383, no. 5-6, pp. 481–485, Jan. 2004.

[258] F. Ross and A. B. Ross, "Selected specific rates of reactions of transients from water in aqueous solution. III. Hydroxyl radical and perhydroxyl radical and their radical ions," Notre Dame Univ., IN (USA). Radiation Lab., Tech. Rep. NSRDS-NBS-59, Jan. 1977.

[259] G. V. Buxton, C. L. Greenstock, W. P. Helman, and A. B. Ross, "Critical Review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (.OH/.O $^-$ in Aqueous Solution," *Journal of Physical and Chemical Reference Data*, vol. 17, no. 2, pp. 513–886, Apr. 1988.

[260] B. H. J. Bielski, "Reevaluation of the spectral and kinetic properties of HO_2 and O_2^- free radicals," *Photochemistry and Photobiology*, vol. 28, no. 4-5, pp. 645–649, Oct. 1978.

[261] B. H. J. Bielski, D. E. Cabelli, R. L. Arudi, and A. B. Ross, "Reactivity of HO $_2$ /O $^-$ $_2$ Radicals in Aqueous Solution," *Journal of Physical and Chemical Reference Data*, vol. 14, no. 4, pp. 1041–1100, Oct. 1985.

[262] J. Weinstein and B. H. Bielski, "Kinetics of the interaction of perhydroxyl and superoxide radicals with hydrogen peroxide. The Haber-Weiss reaction," vol. 101, no. 1, pp. 58–62, 1979.

[263] W. Koppenol, "The Haber-Weiss cycle – 70 years later," *Redox Report*, vol. 6, no. 4, pp. 229–234, Aug. 2001.

[264] E. Choe and D. B. Min, "Chemistry and Reactions of Reactive Oxygen Species in Foods," *Journal of Food Science*, vol. 70, no. 9, pp. R142–R159, Nov. 2005.

[265] T. Masuda, S. Nakano, and M. Kondo, "Rate constants for the reactions of OH radicals with the enzyme proteins as determined by the p-nitrosodimethylaniline method," *Journal of radiation research*, vol. 14, no. 4, pp. 339–345, 1973, publisher: Journal of Radiation Research Editorial Committee.

[266] A. Michaeli and J. Feitelson, "REACTIVITY OF SINGLET OXYGEN TOWARD AMINO ACIDS AND PEPTIDES," *Photochemistry and Photobiology*, vol. 59, no. 3, pp. 284–289, Mar. 1994.

[267] V. K. Sharma and N. J. Graham, "Oxidation of Amino Acids, Peptides and Proteins by Ozone: A Review," *Ozone: Science & Engineering*, vol. 32, no. 2, pp. 81–90, Apr. 2010, publisher: Taylor & Francis _eprint: https://doi.org/10.1080/01919510903510507.

[268] T. Nauser, W. H. Koppenol, and J. M. Gebicki, "The kinetics of oxidation of GSH by protein radicals," *Biochemical Journal*, vol. 392, no. 3, pp. 693–701, Dec. 2005.

[269] L. K. Folkes, M. Trujillo, S. Bartesaghi, R. Radi, and P. Wardman, "Kinetics of reduction of tyrosine phenoxyl radicals by glutathione," *Archives of Biochemistry and Biophysics*, vol. 506, no. 2, pp. 242–249, Feb. 2011.

[270] A. S. Domazou, W. H. Koppenol, and J. M. Gebicki, "Efficient repair of protein radicals by ascorbate," *Free Radical Biology and Medicine*, vol. 46, no. 8, pp. 1049–1057, Apr. 2009.

[271] J. M. Gebicki, "Oxidative stress, free radicals and protein peroxides," *Archives of Biochemistry and Biophysics*, vol. 595, pp. 33–39, Apr. 2016.

[272] P. Neta, R. E. Huie, and A. B. Ross, "Rate Constants for Reactions of Peroxyl Radicals in Fluid Solutions," *Journal of Physical and Chemical Reference Data*, vol. 19, no. 2, pp. 413–513, Mar. 1990.

[273] T. Miyazaki, T. Yoshimura, K. Mita, K. Suzuki', and M. Watanabe, "RATE CONSTANT FOR REACTION OF VITAMIN C WITH PROTEIN RADICALS IN y-IRRADIATED AQUEOUS ALBUMIN SOLUTION AT 295 K," *Radiat. Phys. Chem.*, vol. 45, pp. 199–202, 1994.

[274] H. Sies, W. Stahl, and A. R. Sundquist, "Antioxidant Functions of Vitamins," *Annals of the New York Academy of Sciences*, vol. 669, no. 1, pp. 7–20, Sep. 1992.

[275] E. Niki, N. Noguchi, H. Tsuchihashi, and N. Gotoh, "Interaction among vitamin C, vitamin E, and beta-carotene." vol. 62, no. 6, pp. 1322S–1326S, 1995.

[276] E. Cadenas and H. Sies, "The lag phase," *Free Radical Research*, vol. 28, no. 6, pp. 601–609, 1998.

[277] J. G. Nathanael, B. Yuan, C. R. Hall, T. A. Smith, and U. Wille, "Damage of amino acids by aliphatic peroxyl radicals: a kinetic and computational study," *Organic & Biomolecular Chemistry*, vol. 21, no. 11, pp. 2390–2397, Mar. 2023, publisher: The Royal Society of Chemistry.

[278] S. Toppo, L. Flohé, F. Ursini, S. Vanin, and M. Maiorino, "Catalytic mechanisms and specificities of glutathione peroxidases: Variations of a basic scheme," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1790, no. 11, pp. 1486–1500, Nov. 2009.

[279] T. Fang, P. S. J. Lakey, J. C. Rivera-Rios, F. N. Keutsch, and M. Shiraiwa, "Aqueous-Phase Decomposition of Isoprene Hydroxy Hydroperoxide and Hydroxyl Radical Formation by Fenton-like Reactions with Iron Ions," *The Journal of Physical Chemistry A*, vol. 124, no. 25, pp. 5230–5236, Jun. 2020.

[280] C. von Sonntag and H.-P. Schuchmann, "The Elucidation of Peroxyl Radical Reactions in Aqueous Solution with the Help of Radiation-Chemical Methods," *Angewandte Chemie International Edition in English*, vol. 30, no. 10, pp. 1229–1253, Oct. 1991.

[281] H. A. Headlam, A. Mortimer, C. J. Easton, and M. J. Davies, "β-Scission of C-3 (β-Carbon) Alkoxyl Radicals on Peptides and Proteins: A Novel Pathway Which Results in the Formation of α-Carbon Radicals and the Loss of Amino Acid Side Chains," *Chemical Research in Toxicology*, vol. 13, no. 11, pp. 1087–1095, Nov. 2000.

[282] B. Mihaljević, "Kinetics of oxidation of iron(II) ions with lipid hydroperoxides," *International Journal of Chemical Kinetics*, vol. 51, no. 6, pp. 422–434, Jun. 2019.

[283] K. U. Ingold, "Peroxy radicals," *Accounts of Chemical Research*, vol. 2, no. 1, pp. 1–9, 1969, publisher: ACS Publications.

[284] C. F. Babbs and M. G. Steiner, "Simulation of free radical reactions in biology and medicine: a new two-compartment kinetic model of intracellular lipid peroxidation," *Free Radical Biology and Medicine*, vol. 8, no. 5, pp. 471–485, 1990.

[285] X. Fang, F. Jin, H. Jin, and C. v. Sonntag, "Reaction of the superoxide radical with the N-centered radical derived from N-acetyltryptophan methyl ester," *Journal of the Chemical Society, Perkin Transactions 2*, no. 2, pp. 259–264, 1998.

[286] C. L. Hawkins and M. J. Davies, "Generation and propagation of radical reactions on proteins," *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, vol. 1504, no. 2-3, pp. 196–219, Apr. 2001.

[287] K. Hasegawa and L. Patterson, "Pulse radiolysis studies in model lipid systems: formation and behavior of peroxy radicals in fatty acids," vol. 28, no. 4, pp. 817–823, 1978.

[288] K. M. Schaich, "Lipid Oxidation: Theoretical Aspects," in *Bailey's Industrial Oil and Fat Products*, 1st ed., F. Shahidi, Ed. Wiley, Jul. 2005.

[289] K. Adamic, J. A. Howard, and K. U. Ingold, "Absolute rate constants for hydrocarbon autoxidation. XVI. Reactions of peroxy radicals at low temperatures," *Canadian Journal of Chemistry*, vol. 47, no. 20, pp. 3803–3808, Oct. 1969.

[290] S. L. Khursan, "Organic tetroxides and mechanism of peroxy radical recombination," in *PATAI'S Chemistry of Functional Groups*, Z. Rappoport, Ed. Chichester, UK: John Wiley & Sons, Ltd, Jun. 2014, pp. 1–34.

[291] E. J. Bechara, W. J. Baader, and C. V. Stevani, "2 Hydrogen Peroxide," *Hydrogen Peroxide Metabolism in Health and Disease*, p. 17, 2017, publisher: CRC Press.

[292] A. P. Darmanyan and C. S. Foote', "Solvent Effects on Singlet Oxygen Yield from n,pi* and pi,pi* Triplet Carbonyl Compounds," *J. Phys. Chem.*, vol. 97, no. 19, pp. 5032–5, 1993.

[293] M. Bregnhøj, M. Westberg, F. Jensen, and P. R. Ogilby, "Solvent-dependent singlet oxygen lifetimes: temperature effects implicate tunneling and charge-transfer interactions," *Physical Chemistry Chemical Physics*, vol. 18, no. 33, pp. 22 946–22 961, Aug. 2016, publisher: The Royal Society of Chemistry.

[294] C. A. Davis, K. McNeill, and E. M.-L. Janssen, "Non-Singlet Oxygen Kinetic Solvent Isotope Effects in Aquatic Photochemistry," *Environmental Science & Technology*, vol. 52, no. 17, pp. 9908–9916, Sep. 2018, publisher: American Chemical Society.

[295] F. Wilkinson, W. P. Helman, and A. B. Ross, "Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Compilation," *Journal of Physical and Chemical Reference Data*, vol. 24, no. 2, pp. 663–677, Mar. 1995.

[296] T. D. Poulsen, P. R. Ogilby, and K. V. Mikkelsen, "Solvent Effects on the O_2 ($a\Delta_g$)-O_2 ($X\,^3\sigma_g^-$) Radiative Transition: Comments Regarding Charge-Transfer Interactions," *The Journal of Physical Chemistry A*, vol. 102, no. 48, pp. 9829–9832, Nov. 1998.

[297] R. L. Jensen, L. Holmegaard, and P. R. Ogilby, "Temperature Effect on Radiative Lifetimes: The Case of Singlet Oxygen in Liquid Solvents," *The Journal of Physical Chemistry B*, vol. 117, no. 50, pp. 16 227–16 235, Dec. 2013.

[298] P. Bilski, R. N. Holt, and C. F. Chignell, "Properties of singlet molecular oxygen O2(1Dg) in binary solvent mixtures of different polarity and proticity," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 109, pp. 243–249, 1997.

[299] P. Borrell and N. H. Rich, "The rate constant for the "dimol" transition of singlet oxygen, O 2 (a 1 δ g), and the likely symmetry of the emitting species," vol. 99, no. 2, pp. 144–147, 1983.

[300] G. Porter, R. W. Yip, J. M. Dunston, A. J. Cessna, and S. E. Sugamori, "Detection and lifetime of the triplet state of acetone in solution," *Transactions of the Faraday Society*, vol. 67, pp. 3149–3154, 1971.

[301] N. Rafieiolhosseini, M. Poplová, P. Sasanpour, H. Rafii-Tabar, M. R. Alhossaini, and M. Cifra, "Photocount statistics of ultra-weak photon emission from germinating mung bean," *Journal of Photochemistry and Photobiology B: Biology*, vol. 162, pp. 50–55, Sep. 2016.

[302] T. Peters, *All about albumin*, 1996, publisher: Elsevier.

[303] J. Masuoka and P. Saltman, "Zinc(II) and copper(II) binding to serum albumin. A comparative study of dog, bovine, and human albumin." *Journal of Biological Chemistry*, vol. 269, no. 41, pp. 25 557–25 561, Oct. 1994.

[304] Y. Zhang and D. E. Wilcox, "Thermodynamic and spectroscopic study of Cu(II) and Ni(II) binding to bovine serum albumin," *JBIC Journal of Biological Inorganic Chemistry*, vol. 7, no. 3, pp. 327–337, Mar. 2002.

[305] K. A. Majorek, P. J. Porebski, A. Dayal, M. D. Zimmerman, K. Jablonska, A. J. Stewart, M. Chruszcz, and W. Minor, "Structural and immunologic characterization of bovine, horse, and rabbit serum albumins," *Molecular Immunology*, vol. 52, no. 3-4, pp. 174–182, Oct. 2012.

[306] T. Topală, A. Bodoki, L. Oprean, and R. Oprean, "Bovine serum albumin interactions with metal complexes," *Medicine and Pharmacy Reports*, vol. 87, no. 4, pp. 215–219, Nov. 2014.

[307] X. Xu, L. Zhang, D. Shen, H. Wu, and Q. Liu, "Oxygen-dependent Oxidation of Fe(II) to Fe(III) and Interaction of Fe(III) with Bovine Serum Albumin, Leading to a Hysteretic Effect on the Fluorescence of Bovine Serum Albumin," *Journal of Fluorescence*, vol. 18, no. 1, pp. 193–201, Jan. 2008.

[308] O. Regev, R. Khalfin, E. Zussman, and Y. Cohen, "About the albumin structure in solution and related electro-spinnability issues," *International Journal of Biological Macromolecules*, vol. 47, no. 2, pp. 261–265, Aug. 2010.

[309] A. K. Gaigalas, J. B. Hubbard, M. McCurley, and S. Woo, "Diffuslon of Bovine Serum Albumin in Aqueous Solutions," 1991.

[310] J. F. Miller, "Determination of Protein Charge in Aqueous Solution Using Electrophoretic Light Scattering: A Critical Investigation of the Theoretical Fundamentals and Experimental Methodologies," *Langmuir*, vol. 36, no. 29, pp. 8641–8654, Jul. 2020, publisher: American Chemical Society.

[311] C. Giulivi, N. J. Traaseth, and K. J. A. Davies, "Tyrosine oxidation products: analysis and biological relevance," *Amino Acids*, vol. 25, no. 3-4, pp. 227–232, Dec. 2003.

[312] G. Xu and M. R. Chance, "Hydroxyl Radical-Mediated Modification of Proteins as Probes for Structural Proteomics," *Chemical Reviews*, vol. 107, no. 8, pp. 3514–3543, Aug. 2007.

[313] M. Hellwig, "The Chemistry of Protein Oxidation in Food," *Angewandte Chemie International Edition*, vol. 58, no. 47, pp. 16 742–16 763, Nov. 2019.

[314] P. W. Barlow and J. Fisahn, "Lunisolar tidal force and the growth of plant roots, and some other of its effects on plant movements," *Annals of Botany*, vol. 110, no. 2, pp. 301–318, Jul. 2012.

[315] T. A. Moraes, P. W. Barlow, E. Klingelé, and C. M. Gallep, "Spontaneous ultra-weak light emissions from wheat seedlings are rhythmic and synchronized with the time profile of the local gravimetric tide," *Naturwissenschaften*, vol. 99, no. 6, pp. 465–472, Jun. 2012.

[316] C. M. Gallep, T. A. Moraes, S. R. Dos Santos, and P. W. Barlow, "Coincidence of biophoton emission by wheat seedlings during simultaneous, transcontinental germination tests," *Protoplasma*, vol. 250, no. 3, pp. 793–796, Jun. 2013.

[317] M. Vaz, C. Rajkumar, J. Wong, R. S. Mazzeo, A. G. Turner, H. S. Cox, G. L. Jennings, and M. D. Esler, "Oxygen consumption in the heart, hepatomesenteric bed, and brain in young and elderly human subjects, and accompanying sympathetic nervous activity," *Metabolism*, vol. 45, no. 12, pp. 1487–1492, Dec. 1996.

List of publications related to the doctoral thesis

Authors approved the quantitative contributions.

Papers in Peer-Reviewed Journals with Impact Factor

[A1] Červinková, K., Vahalová, P., Poplová, M., Zakar, T., Havelka, D., Paidar, M., Kolivoška, V., & Cifra, M. (2024). Modulation of Pulsed Electric Field Induced Oxidative Processes in Protein Solutions by Pro- and Antioxidants Sensed by Biochemiluminescence. Scientific Reports, accepted
| Impact factor / Quartile (2023): 3.8 / Q1
| Candidate's contribution: 50%
| Data curation (lead), Formal analysis, Investigation (lead), Methodology, Software, Visualization, Writing – original draft (supporting), Writing – review & editing

[A2] Vahalová, P., Červinková, K., & Cifra, M. (2021). Biological autoluminescence for assessing oxidative processes in yeast cell cultures. Scientific Reports, 11(1), 10852.
| Impact factor / Quartile (2021): 5.0 / Q1
| Candidate's contribution: 5%
| Methodology (supporting)

[A3] Burgos, R. C. R., Schoeman, J. C., Winden, L. J. V., Červinková, K., Ramautar, R., Van Wijk, E. P., Cifra, M., Berger, R., Hankemeier, T., & Greef, J. V. D. (2017). Ultra-weak photon emission as a dynamic tool for monitoring oxidative stress metabolism. Scientific reports, 7(1), 1229.
| Impact factor / Quartile (2017): 4.1 / Q1
| Candidate's contribution: 5%
| Investigation (supporting), Methodology (supporting), Writing – original draft (supporting)

[A4] Burgos, R. C. R., Červinková, K., van der Laan, T., Ramautar, R., van Wijk, E. P., Cifra, M., Koval, S., Berger, R., & van der Greef, J. (2016). Tracking biochemical changes correlated with ultra-weak photon emission using metabolomics. Journal of Photochemistry and Photobiology B: Biology, 163, 237-245.
| Impact factor / Quartile (2016): 2.7 / Q2
| Candidate's contribution: 10%
| Investigation (supporting), Methodology (supporting), Writing – original draft (supporting)

[A5] Kučera, O., Červinková, K., Nerudová, M., & Cifra, M. (2015). Spectral perspective on the electromagnetic activity of cells. Current topics in medicinal chemistry, 15(6), 513-522.
| Impact factor / Quartile (2015): 2.9 / Q2
| Candidate's contribution: 15%
| Writing – original draft (supporting)

[A6] Gallep, C. M., Moraes, T. A., Červinková, K., Cifra, M., Katsumata, M., & Barlow, P. W. (2014). Lunisolar tidal synchronism with biophoton emission during intercontinental wheat-seedling germination tests. Plant signaling & behavior, 9(5), e28671.
| Impact factor / Quartile (2014): 1.7 / Q2
| Candidate's contribution: 10%
| Data curation (supporting), Investigation (supporting)

Papers and Abstracts in Conference Proceedings Listed in the Web of Knowledge

[C1] Bereta, M., Janoušek, L., Cifra, M., & Červinková, K. (2016, May). Low frequency electromagnetic field effects on ultra-weak photon emission from yeast cells. In 2016 ELEKTRO (pp. 478-481). IEEE.
| Candidate's contribution: 5 %
| Methodology (supporting)

[C2] Červinková, K., Nerudová, M., Hašek, J., & Cifra, M. (2015, January). Chemical modulation of the ultra-weak photon emission from Saccharomyces cerevisiae and differentiated HL-60 cells. In Photonics, Devices, and Systems VI (Vol. 9450, pp. 169-175). SPIE.
| Impact factor (2015): 0.533
| Candidate's contribution: 60%
| Data curation (lead), Formal analysis, Investigation (lead), Methodology, Visualization, Writing – original draft (lead)

[C3] Nerudová, M., Červinková, K., Hašek, J., & Cifra, M. (2015, January). Optical spectral analysis of ultra-weak photon emission from tissue culture and yeast cells. In Photonics, Devices, and Systems VI (Vol. 9450, pp. 162-168). SPIE.
| Candidate's contribution: 15 %
| Impact factor (2015): 0.533
| Investigation (supporting), Methodology, Writing – original draft (supporting)

[C4] Červinková, K., Nerudová, M., Cifra, M., Hašek, J., & Vrba, J. (2014, April). Two-channel measurement of the ultra-weak photon emission from a yeast culture during its growth. In 2014 24th International Conference Radioelektronika (pp. 1-3). IEEE.
| Candidate's contribution: 60 %
| Data curation (lead), Formal analysis, Investigation (lead), Methodology, Visualization, Writing – original draft (lead)

[C5] Červinková, K., Nerudová, M. (2014, May). Effects of diverse experimental conditions on the ultra-weak photon emission from yeast cells Saccharomyces cerevisiae, In POSTER 2014 - 18th International Student Conference on Electrical Engineering. Prague: Czech Technical University.
| Candidate's contribution: 60 %
| Data curation (lead), Formal analysis, Investigation (lead), Methodology, Visualization, Writing – original draft (lead)

[C6] Nerudová, M.; Červinková, K. (2014, May). Optical spectral analysis of ultra-weak photon emission from differentiated Human promyelocytic leukemia cells, In POSTER 2014 - 18th International Student Conference on Electrical Engineering. Prague: Czech Technical University.
| Candidate's contribution: 15 % | Investigation (supporting), Methodology, Writing – original draft (supporting)

[C7] Červinková, K., Nerudová, M., Cifra, M., Hašek, J., & Vrba, J. (2013, August). Ultra Weak Photon Emission from Saccharomyces Cerevisiae. In PIERS Proceedings.
| Candidate's contribution: 60 %
| Data curation (lead), Formal analysis, Investigation (lead), Methodology, Visualization, Writing – original draft (lead)

Citations in Web of Science

H-index = 5

Times cites (without self-citations) = 66

Research Projects and Contracts

[P1] SubTHz on-chip devices for controlling protein nanomachines, GX20-06873X, Czech Science Foundation GA ČR.
Member of the team

[P2] Zkoumání fyzikálních základů interakce elektromagnetického pole s biomolekulami, buňkami a tkání, No. SAV-15-22, Bilateral project between Czech and Slovak Academy of Sciences.
Member of the team

[P3] Research and Measurement of Signals Generated by Nanostructures, No. P102/11/0649, Czech Science Foundation GA ČR.
Member of the team

[P4] Photonic biosignals: measurement and characterization, No. GP13-29294S, Czech Science Foundation GA ČR.
Member of the team

[P5] Interactions of EM field with biological systems and theirs applications in medicine, SGS14/189/OHK3/3T/13, Grant agency of the Czech Technical University in Prague.
Member of the team

[P6] Study of Electromagnetic Processes in biomolecules, cells and tissue, SGS13/077/OHK3/1T/13, Grant agency of the Czech Technical University in Prague.
Member of the team

Kateřina Červinková

EDUCATION

2013 – present	**PhD candidate, Radioelectronics**
	Czech Technical University in Prague (CTU in Prague), Czechia
2010 – 2013	**MSc, Biomedical Engineering**
	CTU in Prague, Czechia
2007 – 2010	**BSc, Biomedical Technician**
	CTU in Prague, Czechia

WORK EXPERIENCE

2013 – present	**Junior Researcher**
	Institute of Photonics and Electronics of the Czech Academy of Sciences, Czechia
2018 – 2021	parental leave

RESEARCH MOBILITY

2016	**Technion - Israel Institute of Technology**, Israel
	Protein engineering of lipase for improved stability in methanol
2014	**Leiden University**, The Netherlands
	Ultra-weak photon emission from cells: UPE detection and metabolomics

TRAINING

2017	**Center for Economic Research and Graduate Education - Economics Institute (CERGE-EI)**
	Intermediate R Programming
	Data Visualization using Tableau
2016	**Institute of Molecular Genetics of the Czech Academy of Sciences**, Czechia
	Microscopy methods in biomedicine
2011	**Politecnico di Milano**, Italy
	Discrete and Geometric Tomography
2011	**University Catholic of Louvain**, Belgium
	Bionic man : not so unrealistic!

FELLOWSHIPS & AWARDS

2022 – present	URSI WIRS Czechia, secretary
2024	Doreen J. Putrah cancer research foundation
2014	Mobilita-Akce 200 scholarship