Chapter 1

General introduction

1.1 Free radicals in cells

Free radicals are any atoms or molecules that have at least one unpaired electron. They fulfil various biological functions including signalling, aiding immune responses as well as cell maturation. They are also usually present whenever something is wrong with cells. Malnutrition, irradiation, or all kinds of diseases influence free radical generation. Free radicals are also built during the cell's natural metabolism, the aging process[1,2] as well as cell death. The radicals that are commonly present in biological systems are for instance superoxide radicals ($*O_2$), hydroxyl radical (*OH), and nitric oxide (*NO). These radicals are short lived and very reactive. Thus, we have limited information about which ones play a role, where they are created or under which conditions.

Free radicals can be found in several organelles. Mostly they are produced in mitochondria as by-product of normal cellular metabolism.[3] The main radical species that is formed in mitochondria is $*O_2$. The superoxide radicals will be disposed by superoxide dismutase that converts the radicals into less reactive molecules like oxygen and hydrogen peroxide (H_2O_2),[4] this result can also lead to the formation of *OH through the Fenton reaction.

Free radicals are also present during immune reactions to pathogens.[5] There $*O_2$ and *OH are formed by phagocytic cells to kill bacteria. When antibiotics are used, free radicals play a key role in the killing of bacteria as well. Interestingly, this is characteristic of killing and independent of the class of antibiotic that is used.

1.2 Detection methods for free radicals

There are several ways to detect free radicals in cells. The different methods can be divided in indirect and direct methods. Indirect methods measure the response of the cell towards a certain radical species. This is done via detecting the expression of certain genes, which encode enzymes involved in coping with stress (e.g superoxide dismutase or catalase)[6] that can be investigated using quantitative polymerase chain reactions (qPCR). The major advantage is that these enzymes are specific and we can differentiate between different species. However, we need to know in

advance which enzymes or which molecules are involved (which is often unknown). Furthermore, spatial and temporal information on location and time of free radicals generation are lost. Another way to detect radicals indirectly, is to measure the damage they do. Common ways to do this are for instance to detect DNA damage or lipid peroxidation.

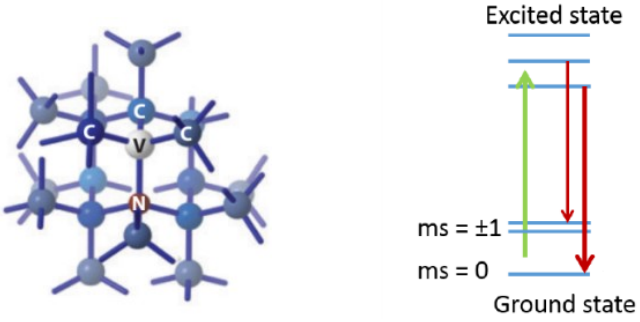
The most widely used method is based on 2',7'–dichlorofluorescin diacetate (DCFDA) for reactive oxygen species (ROS; $*O_2$ and *OH but also non-radical ROS as for instance H_2O_2). DCFDA fluoresces after reacting with ROS. However, this dye suffers from low specificity and can be toxic to cells at high concentration. It can also interact with components of cell culture media and does not provide sufficient spatial information due to the diffusion of the dye.

Among direct methods, magnetic resonance imaging (MRI) and electron spin resonance (ESR) spectroscopy are the golden standard for free radical detection. ESR has limitations when there's only small amount of radicals present. This is usually solved by using spin traps (compounds or nanoparticles that react with the radicals and form a relatively stable radical which can then be detected). This solution is similar to the methods of using dyes, but selective to radical molecules. Moreover, the sensitivity of the method is still low. MRI is routinely used to illuminate details from the inside of the human body. However, magnetic resonance signals are very small and thus difficult to detect. In conventional MRI, approximately 10^{12} - 10^{18} nuclei (or a factor of 1000 less electron spins) are needed to generate an observable signal. This limits the resolution to about 3 μm^2 at its best.[7,8]

1.3 Diamond magnetometry

Since magnetic resonance signals are very weak, these signals are difficult to detect. As a result, the spatial resolution is in the millimeter range or down to several micrometers at best. In contrast, nanoscale resolution can be achieved using diamond magnetometry,[9] which excels in higher spatial resolution and magnetic field sensitivity.

Diamond magnetometry makes use of the Nitrogen-Vacancy (NV) defect in diamond or in fluorescent nanodiamonds (FND).[10] As shown in Figure 1(a), the NV defect consists of a nitrogen atom (N) and a vacancy (V) couple as substitutes for two carbon atoms in the diamond crystal lattice. The NV center has a general tendency to trap an additional electron to form the negatively-charged state NV^-.[11] Only this negatively-charged state is interesting for magnetometry applications since it provides a spin triplet ground state level that can be initialized, manipulated, and readout by purely optical means.[9] The NV-center energy diagram is shown in Figure 1(b). The NV-center exhibits the red photoluminescence (PL) that can be detected from a single center and is photostable.[12]



(a) (b)

Figure 1. Schematic of (a) Nitrogen-vacancy (NV) center in the diamond crystal lattice.[10] b) NV-center energy level: The NV-center is excited by a green laser, then it emits a red photon. Its ground level is a spin triplet state that can be split into a singlet state $m_s = 0$ and a doublet $m_s = \pm 1$. This spin triplet ground level is the feature that allows magnetometry applications by purely optical means.

FND's excellent material properties allow optical polarization, manipulation and readout of its spin state thus promise various applications in nanotechnology and quantum information processing.[13] FNDs are suitable for applications in biology because of their biocompatibility. They show no toxicity and can work at room temperature. Since the readout can be done optically, the method is more sensitive and no conventional magnetic resonance imaging machines are required. This method also allows the implementation of different functions as particle tracking, optical imaging and different modalities to detect magnetic resonances.

The NV center can be used to detect fluctuation of magnetic fields by monitoring the decoherence (T_2) or relaxation (T_1) of its electron spin.[14] Magnetic field fluctuations caused by paramagnetic species, such as free radicals, is one type of magnetic signal in biological systems. T1 relaxation is a specific mode of diamond magnetometry. In a T1 measurement, the NV centers convert magnetic noise to an optical signal. Since light can be detected more sensitively than magnetic resonances themselves this method is so sensitive that the faint signal of a single electron spin is detectable.[15]

1.4 Thesis objectives

The aim of this thesis is to utilize relaxometry to measure free radicals in cells. Chapter 2 presents the first result of using relaxometry to detect free radicals in a biological environment. In chapter 3, we targeted nanodiamonds to mitochondria using antibodies. With this study, we demonstrated free radical detection in single organelles. Chapter 4 presents free radicals detection during oxidative stress response in young and aged cell. Additionally, we were able to differentiate between knock out yeast mutants with an impaired metabolism. In chapter 5, we investigated free radical production in human primary dendritic cell. Chapter 6 discussed free radical generation in single bacteria during UV irradiation and during antibiotic influence. Finally, in chapter 7, we discussed the importance of this thesis and future applications of this research.

References

[1] Harman, D. The Free Radical Theory of Aging. Antioxid. Redox Signal. 5, 557–561 2003.

[2] van der Laan, K. J., Morita, A., Perona-Martinez, F. P., and Schirhagl, R. Evaluation of the oxidative stress response of aging yeast cells in response to internalization of fluorescent nanodiamond biosensors. Nanomaterials, 10(2):372, 2020.

[3] Cadenas, E. Mitochondrial free radical production and cell signaling. Molecular aspects of medicine, 25(1-2), 17-26. 2004.

[4] Spinelli, J. B. & Haigis, M. C. The multifaceted contributions of mitochondria to cellular metabolism. Nat. Cell Biol. 20, 745–754, 2018.

[5] Steck, N. & Grassl, G.A. Free Radicals and Pathogens – Role for Reactive Intermediates in Innate Immunity. In Systems Biology of Free Radicals andAntioxidant (Laher I, ed). Springer. 2014.

[6] DiGuiseppi J, Fridovich I. CRC Crit. Rev. Toxicol. 12:315-42, 1984.

[7] Ciobanu, L., Seeber, D. A. & Pennington, C. H. 3D MR microscopy with resolution 3.7 μm by 3.3 μm by 3.3 μm. J. Magn. Reson. 158, 178–182, 2002.

[8] Glover, P. & Mansfield, P. Limits to magnetic resonance microscopy. Rep. Prog. Phys. 65, 1489–1511, 2002.

[9] Rondin, L., Tetienne, J.-P., Hingant, T., Roch, J.-F., Maletinsky, P., Jacques, V. Magnetometry with nitrogen-vacancy defects in diamond. Reports on Progress in Physics. 77: 5, 2014.

[10] Schirhagl, R., Chang, K., Loretz, M., Degen, C.L. Nitrogen-Vacancy Centers in Diamond: Nanoscale Sensors for Physics and Biology. Annu. Rev. Phys. Chem. 65:83–105, 2014.

[11] Haque, A. & Sumaiya, S. An Overview on the Formation and Processing of Nitrogen-Vacancy Photonic Centers in Diamond by Ion Implantation. J. Manuf. Mater. Process. 1, 6, 2017.

[12] Gruber, A., Dräbenstedt, A., Tietz, C., Fleury, L., Wrachtrup, J., and von Borczyskowski C. Scanning Confocal Optical Microscopy and Magnetic Resonance on Single Defect Centers. Science, 276(5321): 1997.

[13] Doherty, M.W., Manson, N.B., Delaney, P., Jelezko, F., Wrachtrup, J., Hollenberg, L.C.L. The nitrogen-vacancy colour centre in diamond. Phys. Rep. 528, 1–45, 2013.

[14] Hall, L.T., Cole, J.H., Hill, C.D., Holenberg, L.C.L. Sensing of fluctuating nanoscale magnetic fields using Nitrogen-Vacancy centers in diamond. Phys. Rev. Lett., 103, 220802, 2009.

[15] Grinolds, M. S., Hong, S., Maletinsky, P., Luan, L., Lukin, M.D., Walsworth, R.L., Yacoby, A. Nanoscale magnetic imaging of a single electron spin under ambient conditions. Nature Physics volume 9, pages 215–219, 2013.