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Detecting free radicals in single cells using diamond relaxometry

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Chapter 7

General Discussion

7.1 T1 relaxometry in biological environment

The T1 relaxometry, which is a specific type of diamond magnetometry, converts magnetic resonance signals into optical signals based on fluorescent defects in diamonds. This has the advantage that optical signals are much easier to detect. While diamond magnetometry has already delivered groundbreaking results in physics, it is still new in the biomedical fields.

In **chapter 2**, we focused on applying the T1 relaxometry technique for the detection of free radicals in a biological environment. The T1 experiment allows sensing magnetic noise[1] by utilizing a train of laser pulses. This technique is relatively simple as it avoids the use of additional microwaves and magnets, which might disturb the biological samples and complicate the experiment. Since this technique was new in biological fields, there was no standard way to interpret the data yet. In chapter 2, we present the first results of using the T1 relaxation experiments for detecting a relevant biological free radical and known concentrations of gadolinium labels to develop a calibration for this system. We used nanodiamonds between 40 and 120 nm containing ensembles of specific defects called NV centers.

To perform a T1 relaxation measurement, we pumped the NV center in the ground state (with a laser at 532 nm) and observed how long the NV center can remain in this state. We used this method to provide real-time measurements of free radicals while they are generated in a chemical reaction. More specifically, we followed photolysis of H_2O_2 and the Haber-Weiss reaction. Both of these processes are important reactions in biological environments. The sensitivity of this technique allows measurement of small amounts of the *OH radicals (1 μM). This result can be achieved within a few minutes while the conventional probe hydroxyphenyl fluorescein (HPF) required 14 h to reveal the

concentration of *OH. Unlike with other fluorescent probes, T1 relaxometry is able to determine both increase and decrease of radical concentration in real time.

We investigated different diamond probes and their ability to sense gadolinium spin labels. We performed measurements between 0 and 10^8 nM of gadolinium and we are able to reach detection limits down to the nanomolar range. We also took into account the effect of salts and proteins (which are the components in the cell growth medium) on the performance of this T1 experiment. This is an important step towards quantifying signals in a biological environment.

7.2 Free radical detection in single cells using relaxometry

Free radicals play a crucial role in numerous diseases. Currently, very little is known about where and when radicals are build and how they work. Their short lifetime and reactivity make measuring them challenging. There are currently several methods available to detect free radicals inside cells; however none of them can determine the location and detect the radicals at high time resolution.

Nanodiamonds have superior physical and chemical properties and are excellently biocompatible.[2] The NV centers make it possible for nanodiamonds to function as biosensors to detect free radicals. NV centers can convert magnetic noise into an optical signal by changing their brightness according to the magnetic surroundings and allowing radical detection in real-time.

In **chapter 3**, we targeted nanodiamonds to mitochondria for various reasons. Mitochondria are the energy powerhouses in cells and thus play crucial roles in cell metabolism, the energy generation of cells and maintaining cell function. Free radicals are

produced by mitochondria as by-products during normal metabolism. In this chapter, we demonstrated diamond relaxometry to detect mitochondrial free radicals in single macrophage cells and isolated single mitochondria. To bring nanodiamonds to mitochondria, we coated nanodiamond with antiVDAC2 antibodies. We further compared free radical generation in macrophages between bare nanodiamonds and targeted nanodiamonds. Our results show significantly higher radical generation when the nanodiamonds were attached to mitochondria compared to somewhere else in the cell. We also inhibited and triggered free radical generation in mitochondria by adding stimuli or antioxidants. Our results show that T1 measurements are able to monitor radical changes in real time.

We also investigated oxidative stress responses and the aging process that produce free radicals. We chose yeast cells in chapter 4 as they are ideal to be used as aging model. They have a relatively short life cycle, are easy to maintain, well studied and it is possible to differentiate between young and old cell by size. We also observed the difference between wild type yeast and its mutant genes like superoxide dismutase 1 (SOD1), target of rapamycin 1 (TOR1), peroxin 19 (PEX19) regarding their stress responses and aging process with T1 relaxometry. With this powerful tool, we are able to follow free radical generation after chemically inducing stress. In addition, we can observe free radical reduction in presence of an antioxidant. It also allows us to follow the ageing process and differentiate between different strains and between young and old cells. We compared the result with fluorescence based probes such as H_2DCFDA and HPF. While they are able to measure the history of the samples, we are able to monitor fluctuations of free radicals concentration at the single cell level.

In **chapter 5**, we performed the first measurements in primary human cells. Dendritic cells are a specific type of immune cells. They are a type of phagocyte and antigen presenting cells. Free radicals play an important role in the immune response[3] which can be related to several diseases. Thus, mapping them in human primary dendritic cells can be a good indicator for early phase diagnosis. This study also takes our application one step closer to clinical research. In this chapter, we investigated NOX2 related production of free radicals and inhibited free radical generation using NOX inhibitors or antioxidants.

Free radical generation also plays a key role in the killing of bacteria by antibiotics. In **chapter 6,** we use relaxometry to detect radical generation on single bacteria. We demonstrate that the radical generation in *Staphylococcus aureus* increases in the presence of UV irradiation as well as vancomycin and is dependent on the dose of the antibiotic. We were able to reveal the dynamics of radical generation by following the radical generation for individual bacteria over the whole duration of the experiment. Diamond magnetometry is an efficient tool in providing invaluable information on free radical production and distribution in biological samples.

7.3 Further applications

While so far we are able to see the total radical formation, this technique will be improved if we can differentiate between radicals. There are several methods available to achieve this goal. Specific radicals/nuclei rotate at a characteristic frequency. Using specific pulse sequences, the diamond magnetometer can become sensitive to these specific frequencies. The simplest way to create spectral information is to do T1 measurement under the influence of magnetic fields. This method has been demonstrated in non-biological systems.[4] If that does not provide enough

spectral resolution to differentiate radicals, there are more complex pulsing modalities for example double electron electron resonance (DEER) protocol. This pulse sequence is regularly used in NMR or ESR spectroscopy and has also been implemented for diamond based magnetometry.[5]

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