Nanodiamond relaxometry-based detection of free radical species when produced in chemical reactions in biologically relevant conditions.

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**Secondary time constant**

From the analysis of the relaxation curves, we have recognized the effect of two components in the relaxation of the ensemble of NVs. Accordingly, the data were fitted using the two-exponential function,

\[
PL(\tau) = I_{\text{inf}} + C_a e^{-\tau/T_a} + C_b e^{-\tau/T_b} \quad (S1)
\]

\[T_1 = \max(T_a, T_b)\]

\[T_f = \min(T_a, T_b)\]

To determine which time constant is the one sensitive to the external magnetic noise, we have processed the data from Fig 4 and plot the slow and fast time constants in figures S1 a) and b). Tf has been defined as the fast constant.

Figure S1: Comparison of time constants a) Tf and b) T1 calculated from the “Nanodiamonds covered with a protein corona” data.

Although the change on both constants shows a correlation with the change in the spin-label concentration, the range of that change is one order of magnitude wider for the slow time constant. We understand this wider range as an increased sensitivity of the slow time constant over Tf. Then, we chose this constant (T1) to represent the effect of the extrinsic magnetic noise in the ensemble’s relaxation.
Dependence of the size of the nanodiamonds on the relaxation constant T1

The size of the nanodiamonds used in the experiment is an important parameter to set in relaxometry experiments. At first, particles of different sizes host a different amount of defects. Larger particles contain more NV centers and thus are brighter and easier to detect. On the other hand, when using the NV centers for sensing, having more NVs in the particles can deteriorate the signal if some of them are not exposed to the external magnetic noise. If the NV centers are too close to one another they will also start to sense each other rather than the external environment. This process decreases sensitivity. In this case, deep defects in the particle (too far away to sense the external spins) would only add noise to the signal.

Fig. S2 Spin-Lattice (T1) relaxation constants of three sizes of nanodiamonds in water (a) and gadolinium (b). Under the same conditions, the T1 constant changes depending on the size of the nanodiamond. The sensitivity to Gd3+ (100 nM) also depends on the size of the nanoparticles. The error bars show the standard error of the mean from 5 particles (6 for the samples with size 70 nm).
Fig. S3 Values of the T1 constant for the data presented in Fig. S2 for water (a) and gadolinium (b)

To investigate which size is suited best, nanodiamonds of different sizes were allowed to adhere to the surface of a bottom glass culture dish. After removing the medium, 100 nM of gadolinium were added. The results of these measurements are shown in Figure S2. This experiment reveals two main conclusions. The value of the relaxation constant depends on the size of the nanodiamond hosting the defects. The smaller the particle the longer is the relaxation time. This is unexpected from particles with a single defect where the size dependency is reversed. Also in bulk diamonds T1 times decrease with the distance to the surface. In our case, we can explain this behaviour considering that larger particles contain not just more NVs but also a higher density. The interaction between many NV centers promotes the relaxation of the ensemble, decreasing the T1 value. A second observation over this result is made by considering the change of the T1 constant after adding gadolinium to the sample. The standard deviation for the different particle size is 114.2 µs (53%) for the 40 nm, 81.5 µs (44%) for the 70 nm and 19.6 µs (24%) for the 120 nm. However, as the error bars (standard error) show, the 40 nm particles show highest dispersion of values compared with the particles of 70 and 120 nm. The reason is that smaller particle contains fewer defects. Thus, a lower number of defects leads to a greater spread. On the contrary, on big particles, having a high amount of NVs, the differences are compensated in the average photoluminescent signal. Another possible contributor might be charge tunnelling which has been reported for diamonds with high nitrogen concentrations. Such charge tunnelling can also show a decay curve but wouldn’t be sensitive to spin noise. To calculate the sensitivity we calculated the signal to noise ratio. As signal we defined here the average T1 values while the noise was given by the standard deviation of the different T1 measurements. We obtain a signal to noise ratio of about 6 for 70 nm FNDs while for 120 nm and 40 nm it is around 3.

Taking this result into account, we suggest using bigger particles in applications where reproducibility is crucial (this is the case if it is needed to compare measurements with different particles) and smaller particles when it is important to have highest possible signals.

To reduce the variability of values of T1 while retaining long T1, the following experiments were performed using the 70 nm nanodiamonds.
Figure S4 shows the effect of the size on the relaxation curve. The figure shows the relaxation curves of the nanodiamonds immersed in 100 nM Gd$^{3+}$ (same dataset used in figure S2-b). Curves recorded from 40 nm nanodiamonds are shown in red, from 70 nm in green and 120 nm in blue. Diamonds with the same size form groups of curves with similar decay time.

![Figure S4](image)

Fig. S4 Relaxation curves grouped by nanoparticle size: red 40 nm, green, 70 nm and blue 120 nm.
Measuring free radicals in-situ:

*Photolysis of H₂O₂:*

Fig. S5. Spin-lattice relaxation time of a 70 nm particle immersed in water. The T₁ constant for the particle in absence of UV light was 976.5 µs, while the value of T₁ after turning the UV light on was 1055 µs. The relaxation constant increases 8% after turning the UV lamp on. This shifting is considered negligible and part of the experimental error.

Fig. S6 Raw dataset used in figure 5 in the article.
Measuring the concentration of hydroxyl radical by HTA:

Fig. S7: The formation of HTA by Fenton reaction of Fe(ClO$_4$)$_2$ (10 µM) with H$_2$O$_2$ (1000 µM) and Na$_2$TH (200 µM) over 20 min.

Fig. S8: The spectra measured after the formation of HTA by photolysis of H$_2$O$_2$ (9.7 M) with Na2TH (200 µM) using UV lamp ($\lambda$=275 nm) for 20 min.
Fig. S9: Calibration of HTA with intensities plotted against concentrations.
Calibration curve

To determine the concentration of free radicals corresponding to a certain value of $T_1$, we have calculated the inverse function of the fitting function presented in equation 3 of the main article.

$$C_{\cdot OH}(T_1) = -0.12 \ln \left( \frac{T_1 - 116.6}{170.4} \right)$$

Figure S10: Inverse of the calibration curve presented in figure 6.
References

1 Manson, N.B., Hedges, M., Barson, M.S., Ahlefeldt, R., Doherty, M.W., Abe, H., Ohshima, T. and Sellars, M.J., 2018. NV−–N+ pair centre in 1b diamond. *New Journal of Physics*, 20(11), p.113037.