Supporting information

Detection of Few Hydrogen Peroxide Molecules Using Self-Reporting Fluorescent Nanodiamond Quantum Sensors

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1. Experimental section

1.1 Materials

Nanodiamonds, ND-NV-10 and ND-NV-40 were purchased from Adámas Nanotechnologies; 3,3′,5,5′-tetramethylbenzidine solution (TMB, Liquid Substrate System for ELISA), hydrogen peroxide (H₂O₂) solution, sodium acetate and acetic acid were purchase from Sigma-Aldrich and were used without further purification, 3,3′,5,5′-tetramethylbenzidine powder was purchased from Thermofisher Scientific, H₂O was obtained from the Millipore purification system. Simulated Body Fluid was purchase from Biochemazone.

1.2 Experiments

Transmission Electron Microscopy (TEM)

One drop of a 0.1 mg/mL solution of ND-NV-10 or ND-NV-40 in MilliQ was placed onto an oxygen treated copper grid and dried at room temperature. A Jeol 1400 transmissions electron microscope was used to obtain bright field images

Dynamic Light Scattering (DLS)

500 µL of 0.1 mg/mL solutions of ND-NV-10 or ND-NV-40 in MilliQ were transferred into a borosilicate glass cuvette and measured at 25 °C with a 90° angle using a particle sizer (Malvern Zetasizer Nano-S90 (Nano series)). The hydrodynamic diameter distribution was presented as intensity. Zeta potential was measured at 25 °C with a Malvern Zetasizer Nano-S90 (Nano series).

UV-Vis Absorbance for catalytic activity analysis

50 µL of a 100mM H₂O₂ solution and 50 µL TMB solution were added in one of the wells of a 96-well microplate, and either100 µL H₂O, 100 µL 1 mg/mL ND-NV-10 or 100 µL 1mg/mL ND-NV-40 were added in. Absorbance scans from 300 to 800 nm were measured at the time point of 0 min, 10 min, 20 min, 30 min, 60 min, 90 min, and 120 min using a Tecan Spark 20M microplate reader.

X-Ray Photoelectron Spectroscopy
10 μL of a 1 mg/mL ND-NV-10 or ND-NV-40 aqueous solutions were deposited on a silicon substrate and dried at room temperature. XPS was conducted using a Kratos Axis UltraDLD spectrometer 3 (Kratos, Manchester, England) using an Al Kα excitation source with a photon energy of 1487 eV. The data was acquired in the hybrid mode using a 0° take-off angle, defined as the angle between the surface normal and the axis of the analyzer lens. Detailed region XP spectra were collected with setting analyzer pass energy at 80 eV, and a linear background was subtracted for all peak quantifications. The peak areas were normalized by the manufacturer supplied sensitivity factors and surface concentrations were calculated using CasaXPS software. C 1s high-resolution spectra were collected with analyzer pass energy of 20 eV. Neutralizer was always used during spectra collection.

1.3 Density functional theory (DFT) calculations
Density functional theory (DFT) calculations were performed using the Gaussian 09 software package\textsuperscript{1} and structural representations were generated with CYLview\textsuperscript{20}. All the geometry optimizations were carried out using the Minnesota hybrid meta-GGA functional M06-2X and valence double-zeta 6-31G(d) basis set. All of the optimized geometries were verified by frequency computations as minima (zero imaginary frequencies) or transition states (a single imaginary frequency corresponding to the desired reaction coordinate). The free energy values presented in Figure 3 in main text and Table S2 were derived from the electronic energy corrected by using the thermal and entropic corrections based on structural and vibration frequency data.

1.4 NV center spin relaxation time measurement
The spin relaxation time ($T_1$) of NV centers in nanodiamonds are performed in a customized confocal microscope. We use a 532nm laser to excite the NV center. The laser power (measured before the objective) is fixed at 50 μW for all the experiments. The laser is focussed onto the sample using an oil-immersion objective (Olympus UPlanSApo 60x oil, N.A. = 1.35). The fluorescence from the NV center is collected through the same objective and detected using an avalanche photodiode (APD). We use a 650nm longpass filter in front of the APD to eliminate the excitation laser and minimize the detection of NV\textsuperscript{0}.  

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The pulse sequence for the $T_1$ measurement is shown in the main text (Fig 4). It consists of a laser pulse (10μs long) to polarize the NVs in the $m_s = 0$ state. After a variable waiting time $\tau$, the subsequent laser pulse is used to read the spin state of the NV center. We use fluorescence photons detected in the first 500 ns of the laser pulse to constitute the signal. This all-optical $T_1$ measurement is prone to charge state fluctuation of the NV, which would manifest as a decrease in NV fluorescence as a function of time. To measure the actual $T_1$ time of the NV we use an additional control sequence with a linear chirp microwave pulse to invert the population from the $m_s = 0$ to $m_s = \pm 1$ state. The frequency range for the linear chirp microwave pulse is from 2850 MHz to 2950 MHz and is generated using an arbitrary waveform generator. The duration of the chirp and the Rabi frequency is adjusted to achieve the adiabatic passage condition. The $T_1$ measurements without inversion pulse is subtracted from the $T_1$ measurements with inversion pulse to obtain the spin relaxation time due to magnetic noise. The experiment is repeated several times with a total acquisition time of 10 minutes. The resulting $T_1$ data is fitted with a monoexponential decay function.

To measure the $T_1$ time statistics of the nanodiamonds, we used cleaned glass slide with a lithographically patterned MW structure. We placed a silicone gasket (cell well volume ~30μL) on top of the glass slide to confine the nanodiamonds and the analyte solution in the subsequent measurements. Next, we added ~10 μL of 10 μg/mL sample into the cell well and the sample was dried for several hours under ambient conditions. During the $T_1$ measurement, we added ~5 μL of buffer (acetate buffer for pH 4 and DPBS for pH 7) and covered the silicone well with a glass slide to avoid evaporation. The $T_1$ time is measured as described above on selected isolated fluorescence spots (with fluorescence counts between 400-600 kcts/s). Following the $T_1$ measurements in the buffer, we added ~5 μL of 100 mM H$_2$O$_2$ solution and repeated the $T_1$ measurement on the same fluorescent spots as before.

Furthermore, we also performed the similar relaxometry experiments with smaller H$_2$O$_2$ concentration and observe a change in $T_1$ time even for 100 pM concentration (Figure S11). In order to explore the catalytic activity of the ND-NV-10 sample in biological samples, we performed similar relaxometry experiments in simulated body fluid (SBF) to mimic the relevant biological environment (Figure S10). We observe a clear decrease in the $T_1$ time ($T_{1, SBF} \sim 31$ μs and $T_{1, H_2O_2} \sim 17$ μs) with the addition of H$_2$O$_2$ solution. Although the change in $T_1$ time is slightly
smaller than in the controlled environment (T1 change with SBF ~ 45% compared to ~56% or higher with acetate or DPBS buffer), we see a clear decreasing trend of T1 time with H2O2 solution. Similar experiments were performed with 14 ND-NV-40 nanoparticles under the same conditions (Figure S8 A and D). We observed only a small change in T1 time with the addition of the H2O2 solution. As discussed earlier, the small responsivity of ND-NV-40 to H2O2 molecules could be attributed to both the size of the NDs (relatively bigger than ND-NV-10, therefore the NVs are less sensitive to the surface noise) and the presence of fewer surface groups producing the radicals. Some of the nanodiamonds in both samples NV-NV-10 and NV-NV-40 showed an increase in T1 time with the addition of H2O2. The behaviour was prevalent in nanodiamonds with short NV T1 time (< 20 μs). Since the effects of optical artefacts are removed, we attribute the T1 increase to the removal of certain surface groups due to the oxidative activity of the H2O2. All the confocal and quantum sensing experiments are performed with Qudi software.3

1.5 Simulation of spin relaxation times

Without the OH or O2H radicals, the ms = 0 spin relaxation is determined by the intrinsic NV relaxation and the electron spin noise at the surface of the nanodiamond, with the relaxation rate given by

\[
\frac{1}{T_1^\text{buffer}} = \frac{1}{T_1^i} + \frac{1}{T_1^\text{noise}}.
\]

Here we take the intrinsic NV relaxation time \(T_1^i = 1\) ms, a value similar to the ones of NVs in bulk diamond. The relaxation rate \(1/T_1^\text{noise}\) due to the noisy electrons at diamond surface is calculated in a way similar to that of the OH or O2H radicals.

The correlation times \(\tau_c\) for the noisy electrons at diamond surface and for the OH or O2H radical spins are obtained using the results of Tetienne et al.4. We have

\[
\tau_c = \left(\frac{\mu_0 \gamma_e^2}{16\pi} \left(\frac{(8\pi \rho)^{1/2}}{\gamma_{\text{min}}^{3/2}} + R_{\text{vib}}\right)\right)^{-1},
\]
where $R_{\text{vib}} = 50$ GHz takes into account the intrinsic vibrational spin relaxation. The parameter related to the minimum allowed distance among the electron spins is 0.15 nm for the noisy electrons at diamond surface and is 0.2 nm for the OH or O$_2$H radicals. We assume a small thickness (0.1 nm, which is much smaller than the diamond sizes) for the surface electrons layer on top of the diamond surface. To obtain ~56% reduction of NV spin relaxation times observed in the experiments, the noisy electrons at the diamond has a density of 0.18/nm$^3$ while the OH or O$_2$H radicals has a density of 0.05/nm$^3$ for the simulation.

1.6 Sensitivity estimation

The smallest number of radicals that can be detected is given as $^4$,

$$N_{\text{min}} = \frac{1}{P} \sqrt{\Delta t} d_0^4 f(\sigma)$$

Where $P = \frac{c}{2\pi} \sqrt{\frac{AR_{\text{int}}}{2e}}$ and $f(\sigma) = \frac{\sigma^{1/4}(\sigma+B)^{3/2}}{\sigma+3B}$. In our experiment, $C \sim 0.2$, $R \sim 300$ kcts/s, $T_{\text{int}} \sim 600$ ns.

2. Tables and Figures
Figure S1. (A) TEM image ND-NV-10 (scale bar: 200 nm), (B) The size distribution histogram of ND-NV-10. (C) TEM image ND-NV-40 (scale bar: 200 nm), (D) The size distribution histogram of ND-NV-40.
Figure S2. Time-dependent absorbance changes at 652 nm in the present of ND-NV-10.
Figure S3. (A) TEM images of ND-NV-40 after 5 times fractionation by centrifugation at 12,000 rpm to remove the small sized NDs in the supernatant (scale bar = 50 nm); (B) Hydrodynamic diameter distribution of ND-NV-40 and ND-NV-40 after after 5 times fractionation by centrifugation at 12,000 rpm to remove the small sized NDs in the supernatant by DLS; (C) Time-dependent absorbance spectra of TMB in the reaction system of TMB + H₂O₂ + ND-NV-40 (after removing the small sized NDs).
Figure S4. Time-dependent absorbance spectra of TMB in the reaction system of TMB + H₂O₂ + ND-NV-10 in water ((pH = 7.3) (A), and DPBS buffer ((pH = 7.05))(B), (C) Absorbance spectra of TMB in different reaction systems after 5 min. Dark line: TMB + H₂O₂ + ND-NV-10 in water, red line TMB + H₂O₂+ ND-NV-10 in DPBS, blue line: TMB + H₂O₂+ ND-NV-10 in DPBS containing 10% FBS.
Figure S5. Zeta-potential of ND-NV-10 and ND-NV-40, data presented as mean ± SD, n = 3.

Figure S6. Confocal images of ND-NV-10 before (A) and after (B) adding H$_2$O$_2$.
Figure S7. Comparison of the $T_1$ relaxation time of ND-NV-10 nanoparticles at pH 4 (A-C) and at pH 7 (D-F). Blue dots represent the measured $T_1$ time with the buffer (acetate buffer for pH 4 and DPBS for pH 7) and the orange dots represent the $T_1$ time with the addition of H$_2$O$_2$ solution. The grey lines connect the individual ND measurements.

Figure S8. (A) Typical $T_1$ relaxation curve of NV in ND-NV-10 at pH 7 DPBS buffer (blue, dots) and with the addition of H$_2$O$_2$ (orange dots) solution. The solid lines are single exponential fit to
the measured data. (B) Comparison of the $T_1$ relaxation time of 15 ND-NV-10 nanoparticles. The grey lines connect the individual ND measurements. Inset: Box-and-whisker plot showing the distribution of $T_1$ time ($N = 45$).

Figure S9. Histogram of change in $T_1$ time ($\left( T_{1, \text{buffer}} - T_{1, H_2O_2} \right)/T_{1, \text{buffer}}$) for pH 4 (A) and pH 7 (B).

Figure S10. (A) Comparison of the $T_1$ relaxation times of ND-NV-10 nanoparticles in simulated body fluid (SBF) buffer. The grey lines connect the individual ND measurements. (B) Box-and-
whisker plot showing the distribution of $T_1$ time for 12 different nanodiamonds. Mean $T_1$ time is given in Table S3.

![Figure S11. Measured $T_1$ time of 5 different nanodiamonds of sample ND-NV-10 after the addition of 100 pM and 100 nM H$_2$O$_2$ concentration. The measurements were performed at pH 7.](image)

Table S1. XPS C1s core level fitting results of the presence of different functional groups on the surfaces.

|       | C-C      | C-O-C    | C=O      | O-C=O    |
|-------|----------|----------|----------|----------|
| ND-NV-10 | Binding Energy (eV) | 285.75   | 286.60   | 287.00   | 289.18   |
|       | Area (%) | 13.10    | 29.23    | 49.67    | 8.00     |
| ND-NV-40 | Binding Energy (eV) | 286.26   | 288.00   | 288.50   | 289.83   |
|       | Area (%) | 30.13    | 37.03    | 20.88    | 11.96    |
Table S2. Calculated energies and the geometry

| Promoter          | SCF energy (Hartree) | Free energy correction (Hartree) | Imaginary frequency (i cm\(^{-1}\)) |
|-------------------|----------------------|----------------------------------|------------------------------------|
| \(2 \text{ H}_2\text{O}\) |                      |                                  |                                    |
| I                 | -455.748810681       | 0.074552                         |                                    |
| TS\(_{\text{I}-\text{ii}}\) | -455.658725336       | 0.075844                         | 545.3                              |
| IIa               | -455.711574894       | 0.076579                         |                                    |
| IIb               | -455.711569278       | 0.076838                         |                                    |
| TS\(_{\text{II}-\text{iii}}\) | -455.673332106       | 0.070506                         | 1444.0                             |
| III               | -455.765981539       | 0.070684                         |                                    |
| \(\text{MeCOOH} + \text{H}_2\text{O}\) |                      |                                  |                                    |
| I                 | -608.353936339       | 0.109608                         |                                    |
| TS\(_{\text{I}-\text{ii}}\) | -608.284035824       | 0.105363                         | 509.9                              |
| IIa               | -608.322273983       | 0.106875                         |                                    |
| IIb               | -608.320264792       | 0.107186                         |                                    |
| TS\(_{\text{II}-\text{iii}}\) | -608.284008919       | 0.103070                         | 1462.6                             |
| III               | -608.368293236       | 0.102891                         |                                    |
| \(\text{ND(111)} + \text{H}_2\text{O}\) |                      |                                  |                                    |
| I                 | -2990.81199942       | 0.358543                         |                                    |
| TS\(_{\text{I}-\text{ii}}\) | -2990.74911635       | 0.358869                         | 444.2                              |
| IIa               | -2990.77505116       | 0.357670                         |                                    |
| IIb               | -2990.78186028       | 0.359426                         |                                    |
| TS\(_{\text{II}-\text{iii}}\) | -2990.73814749       | 0.353936                         | 1543.6                             |
| III               | -2990.82052324       | 0.353504                         |                                    |
Figure S12. Optimized structures (M06-2X/6-31G(d)) for the reaction of H₂O₂ decomposition.

Figure S13. (A) The gray dot-dashed lines are the spin relaxation times of NV centers, averaging over different elliptical shapes of NDs. The results are similar to the ones of spherical NDs (green, blue, red lines that are also shown in Fig. 5 of the main manuscript). Here, the averaging is performed by randomly chosen the length $L$ of a principal axis of the elliptical ND following a Weibull distribution, while the other two principal axes are fixed to the diameter $D$ of the ND, i.e., the x-axis of the plots in (A). The orientations of the elliptical NDs are also random with respect to the NV symmetry axis. (B) The Weibull distribution used in (A) has a mean value $D$ and has a standard deviation of $0.3D$. This distribution has a probability density function (PDF) similar to a normal distribution but the PDF is zero when the length $L$ is smaller than 0.
Table S3. Statistics of NV $T_1$ time.

| Sample            | Sample size (N) | Before $\text{H}_2\text{O}_2$ addition | After $\text{H}_2\text{O}_2$ addition |
|-------------------|-----------------|----------------------------------------|--------------------------------------|
| ND-NV-10 (pH = 4)| 44              | 62.887 Mean (µs) 43.516 SD (µs)         | 29.67 Mean (µs) 21.216 SD (µs)       |
| ND-NV-10 (pH = 7)| 45              | 27.156 Mean (µs) 25.216 SD (µs)         | 12.298 Mean (µs) 11.523 SD (µs)      |
| ND-NV-10 (SBF)   | 12              | 31.413 Mean (µs) 15.564 SD (µs)         | 16.896 Mean (µs) 12.976 SD (µs)      |
| ND-NV-40         |                 | 57.506 Mean (µs) 30.034 SD (µs)         | 48.553 Mean (µs) 23.042 SD (µs)      |

3. References

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