Supporting Information

**pH Nanosensor Using Electronic Spins in Diamond**

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1. Surface chemical modification of fluorescent nanodiamonds to endow pH-sensing function

As mentioned in the main text, untreated fluorescent nanodiamonds (FNDs) have a surface graphite layer and a wide variety of surface functional groups (e.g., carboxy, amino, and hydroxyl groups). As shown in the FT-IR spectra (Fig. S1-1), the surface carboxy groups of FNDs without surface treatment (FNDΔ) were much fewer as compared with FNDs with uniform surface carboxylation (FND-COOH) (Fig. S1a). This was also supported by XPS measurements (Fig. S1-2). After air oxidation and mixed acid treatment, the C=C peak was decreased, whereas the C-C peak was increased drastically. Moreover, the C=O peak of FND-COOH was much higher than that of FNDΔ. Therefore, the graphite layer on the surface of FNDΔ was removed and carboxy groups were created instead by the surface carboxylation.

FNDs coated by poly-L-cysteine (FND-Cys) were prepared by polymerization of L-cysteine on the surface of FND particles (see Materials and Methods). As shown in Fig. 3, the C-H components was increased after polymerization. In addition, the XPS measurements in Fig. S1-2 shows that the C-S peak was also increased after polymerization, suggesting an increase in thiol groups. Therefore, we concluded that the surface of FND-COOH was successfully coated by poly-L-cysteine after polymerization of L-cysteine.

![Figure S1-1. Surface characterization of fluorescent nanodiamond (FND). FT-IR spectra of FND without treatment (FNDΔ; blue) and with carboxylation (FND-COOH; red). V(O-H) (O-H stretching at 3300 cm⁻¹) and V(C=O) (C=O stretching at 1790 cm⁻¹), which are characteristic of -COOH groups, were not clearly observed in FNDΔ.](image-url)
Figure S1-2. XPS measurements of FNDΔ (a), FND-COOH (b) and FND-Cys (c). The C1s peak (left) contains three peaks at 284, 285 and 286 eV of binding energy, which are attributed to C=C, C-C and C-O components, respectively. The O1s peak contains two peaks at 532 and 533 eV, which are attributed to C=O and C-O components, respectively. The S2p peaks at 163.5 and 168.5 eV are attributed to C-S components and oxidized reagents (e.g. SO₃⁻).

To confirm this prediction, we evaluated the pH dependence of $T_1$ of FNDΔ. As a result, $T_1$ of FNDΔ had no dependence on pH (Fig. S1-3a). As shown in the main text (Fig. 2), however, $T_1$ of FND-COOH obtained by surface carboxylation of FNDΔ (see Materials and Methods) had pH dependence around pH 5 (Fig. S1-3b). Additionally, $T_1$ of FND-Cys produced by polymerizing L-cysteine on the surface of FND-COOH showed pH dependence around pH 10 (Fig. S1-3c). The pH dependence of the zeta potential of FND-COOH was different from that of FND-Cys, as shown in Fig. 3(c). In both cases, however, the pH dependence of $T_1$ was correlated with the pH dependence of the zeta potential. In the five cases of FND-COOH shown in Fig. S1-3(b), the correlation coefficients were 0.89, 0.88, 0.87, 0.88 and 0.92, respectively. In the five cases of FND-Cys shown in Fig. S1-3(c), the correlation coefficients were 0.98, 0.90, 0.96, 0.93 and 0.89, respectively. Our above analyses strongly support our conclusion that the pH dependences of FND-COOH and FND-Cys were obviously acquired.
after the surface modification processes. Moreover, the pH dependence of FND-COOH and FND-Cys was retained after measurement, and they could be used repeatedly as pH sensors (Fig. S1-4a and b, respectively).

**Figure S1-3. Typical pH dependences of $T_1$ of FND.** Red, blue and black lines show the pH dependence of $T_1$ of untreated FNDs (FNDΔ, a), FND-COOHs (b) and FNDs with poly-cysteine coating (FND-Cys; c), respectively. Error bars are standard errors of the best fit of $T_1$ decay to a single exponential function.

**Figure S1-4. Typical results of $T_1$ reversibility of FNDs depending on pH changes.** Red and blue lines show the results of the $T_1$ reversibility of FND-COOH (a), FND-Cys (b), respectively. Error bars are standard errors of the best fit of $T_1$ decay to a single exponential function.

2. **Particle size characterization of FND-COOH**

The particle size distributions of the FNDs that we used were characterized by dynamic light scattering (DLS; Fig. S2a) and atomic force microscopy (AFM; Fig. S2b-d). First, we
measured DLS, which reported a size distribution of FND-COOH of ~50-200 nm in diameter (Fig. S2a). In addition, we applied monodispersed FND-COOH particles to a coverslip with grid lines and numbers. Via the grid number, we identified the positions of the FND-COOH particles located on the AFM images (Fig. S2b), and thus we could find the same particles on the fluorescence images (Fig. 2c). Next, as a proxy for particle diameter, we determined the individual heights of 43 particles from the AFM measurement data, and plotted the correlation between the particle diameters and the fluorescence intensities (d). As a result, the particle sizes of FND-COOH that we observed by fluorescence were mainly distributed within 50-100 nm.

**Figure S2. Size distributions of fluorescent nanodiamonds.** (a) DLS measurement of FND-COOH. (b, c) Typical AFM (b) and fluorescence (c) images of FND-COOH. The fluorescence image (d) was obtained from the same region of the cover slip. (d) Correlation plot between particle diameter measured by AFM (b) and fluorescence intensity (c). The correlation coefficient was 0.68 under the assumption that fluorescence intensity is proportional to the cube of the particle diameter. In total, 43 particles were used for the correlation analysis.

### 3. Characteristics of NV centers in FND-COOH

EPR measurements were performed to determine the concentration of negatively charged NV centers (NV\(^-\)) in FND-COOH. We obtained X-band EPR spectra (9.87 GHz) at half the magnetic field (double-quantum transition of NV\(^-\)) of FND-COOH and an equal amount of a 100-nm HPHT nanodiamond sample of known NV\(^-\) concentration (1.8 ppm) (Fig. S3a). By comparing these EPR spectra, we determined the concentration of NV\(^-\) in FND-COOH to be 0.5 ppm (= an average of 40 NV\(^-\) centers per particle). At this NV\(^-\) concentration, most NV\(^-\) centers cannot interact with each other.

Photoluminescence (PL) spectra were obtained from FND-COOH using 532-nm laser excitation. As a result, we observed zero-phonon lines of electroneutral (NV\(^0\)) and negatively charged (NV\(^-\)) centers (Fig. 3b). In general, the excitation efficiency of NV\(^0\) is lower than
that of NV\(^-\) using a 532-nm laser. Therefore, FND-COOH contains a number of NV\(^0\). Note that the ratio between NV\(^0\) and NV\(^-\) was independent of the surrounding pH (Fig. S3c).

We also measured ODMR frequency spectra of FNDs. Typical ODMR frequency spectra of FND-COOH are shown in Fig. S3d. Although FND-COOH contained a number of NV\(^0\) as shown above, the ODMR contrast around the resonant frequency (2870 MHz) reached more than 10\%. Zero-field peak splitting (the difference between two location parameters) and peak width (scale parameter) were about 7.3–11.3 MHz and 5.2–6.3 MHz, respectively, which are typical values for 50–100-nm-sized FNDs. Peak splitting was not correlated with peak width, implying that the splitting and peak broadening were not caused by spin dipole–dipole interactions. The results indicated that the NV\(^-\) centers in FND-COOH did not interact with each other, or that only a few NV\(^-\) centers interact with each other.

Overall, the above analyses suggest that the pH dependence of \(T_1\) is likely to depend neither on NV\(^-\)–NV\(^-\) nor on NV\(^-\)–NV\(^0\) interactions.

Figure S3. Characterization of NV centers in FND-COOH. (a) EPR spectra of NV\(^-\) in FND-COOH (blue). By comparing the EPR intensity with a 100-nm HPHT nanodiamond sample of known NV\(^-\) concentration (1.8 ppm), the NV\(^-\) concentration FND-COOH was determined to be 0.5 ppm. (b) Typical photoluminescence (PL) spectra of an FND-COOH particle. The two peaks at 575 and 637 nm are attributed to electroneutral (NV\(^0\)) and negatively charged (NV\(^-\)) centers. (c) pH independence of the ratio between NV\(^0\) and NV\(^-\) in FND-COOH. The ratio was obtained from the areas of zero-phonon peaks attributed to NV\(^0\) (572–582 nm) and NV\(^-\) (633–643 nm) after base line correction. We defined integrals of the best-fit Cauchy distribution as the areas of the peaks, and the error bars are the standard fitting errors of the best fit. The comparisons were performed independently for 15 samples (= 15 particles). As a result, the change of the ratio NV\(^0\)/NV\(^-\) from pH 11 to pH 3 was
-10±14%. (d) Typical ODMR spectra of FND-COOH. The spectra were fitted by using two Cauchy distributions with the same width, using the fitting location and scale parameters \( \{x_0, \gamma\} = \{2875.007 \text{ and } 2863.697 \text{ MHz, } 6.1242 \text{ MHz}\}, \{2874.898 \text{ and } 2865.194 \text{ MHz, } 5.1583 \text{ MHz}\}, \{2874.288 \text{ and } 2865.384 \text{ MHz, } 6.3353 \text{ MHz}\} \) and \( \{2874.039 \text{ and } 2866.671 \text{ MHz, } 5.3743 \text{ MHz}\}\), respectively.

4. Temporal resolution and measurement error

In this study, the fitting error of \( T_1 \) decay (see Fig. 2a) is the measurement error of the pH measurement, affecting the pH measurement precision directly. On the other hand, several biological measurements require a much higher time resolution than minutes. The large measurement error and the low time resolution limit the application of the method. Therefore, improvement of measurement accuracy and time resolution is a challenge for the future. In general, however, there is a trade-off between measurement error and time resolution. We performed \( T_1 \) measurement of the NV center in FND-COOH for various measurement times, and estimated measurement error for each measurement time (Fig. S4a). As a result, it was found that the measurement error decreased to 4% or less for a measurement time of about 80 min. Moreover, it was also found that the measurement error was inversely proportional to the square root of the measurement time (Fig. S4b). This means that the measurement error is not caused by problems of the measurement method itself but is governed by acquisition noise. Therefore, improvement of measurement accuracy and time resolution may be achieved in the future by improving measurement technology and appropriate data thinning.

![Figure S4](image)

**Figure S4.** Correlation of measurement error (standard fitting error) with measurement time (a) and the reciprocal of the square root of the measurement time (b). Error bars are standard deviations of three measurements.