Supplementary Material (ESI) for Chemical Communications

Supporting Information

Gold Nanodot-Based Luminescent Sensor for the Detection of Hydrogen Peroxide and Glucose

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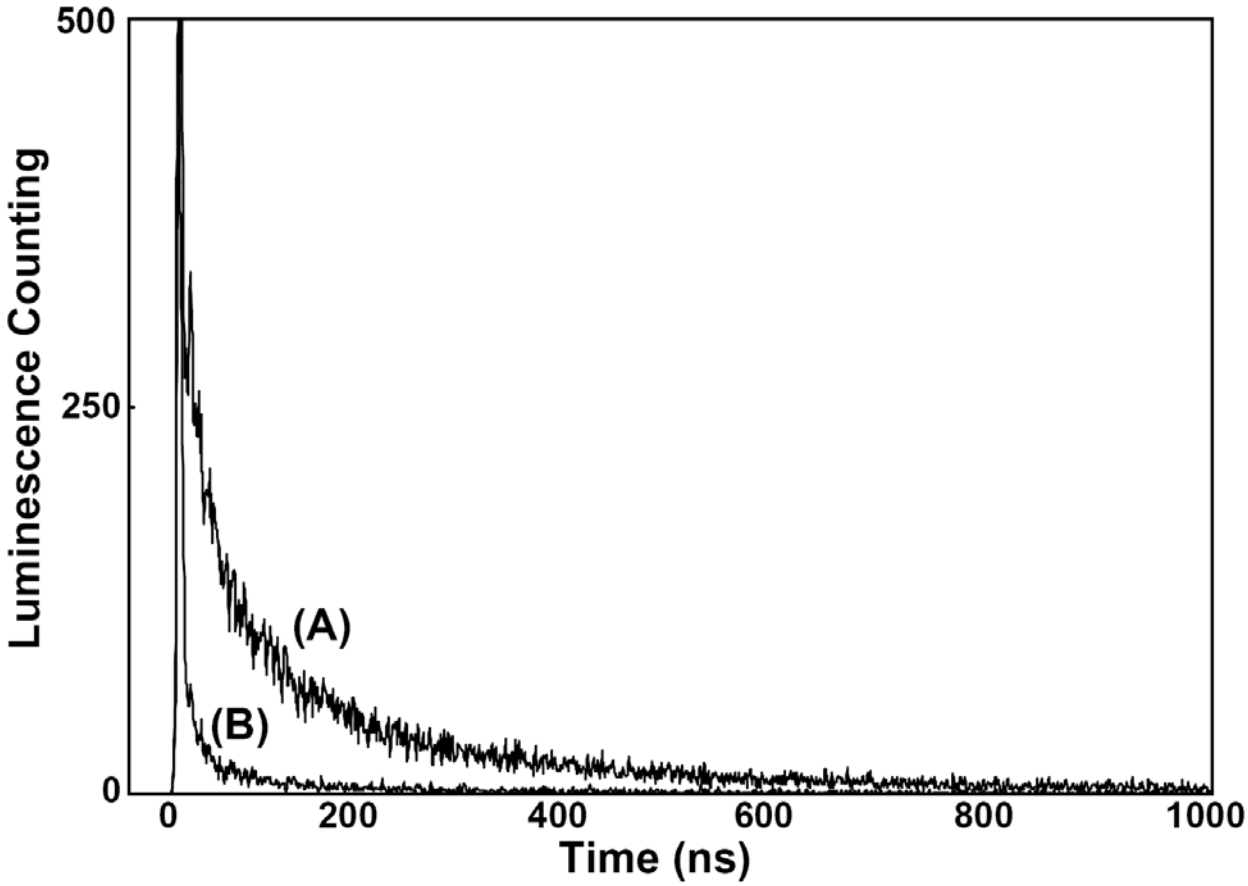

Figure S1. Measurement of the luminescence lifetime of the 11-MUA–Au NDs (100 nM) in the (A) absence and (B) presence of H_2O_2 (10 mM). The data were obtained after excitation at 375 nm. The luminescence decay was fitted to a biexponential decay. Other conditions were the same as those described in Figure 1.

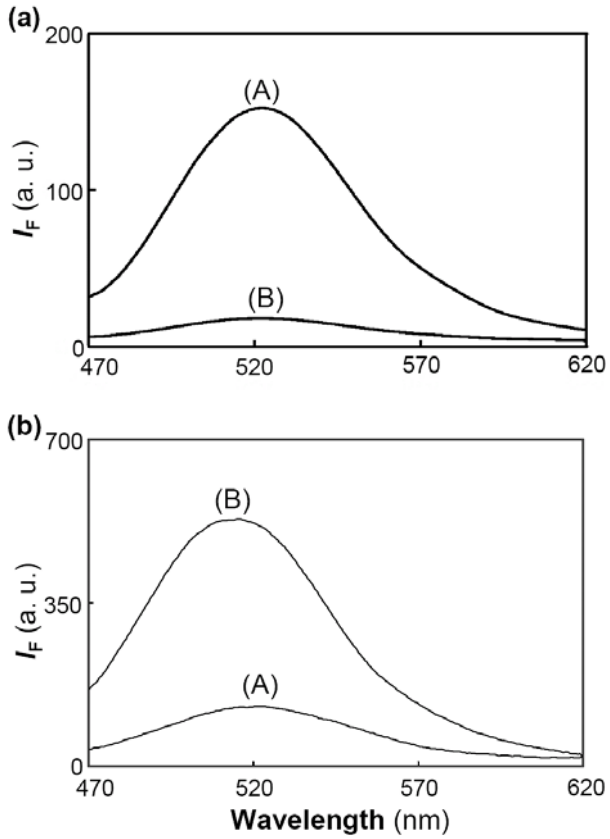

Figure S2. (a) Luminescence spectra of the 11-MUA–Au NDs (10 nM) in the (A) absence and (B) presence of $NaBH_4$ (10 mM). (b) Luminescence spectra of the 11-MUA–Au NDs (10 nM) at (A) 298 K and (B) 77 K. The luminescence intensities (I_F) are plotted in arbitrary units (a. u.); excitation wavelength: 375 nm.

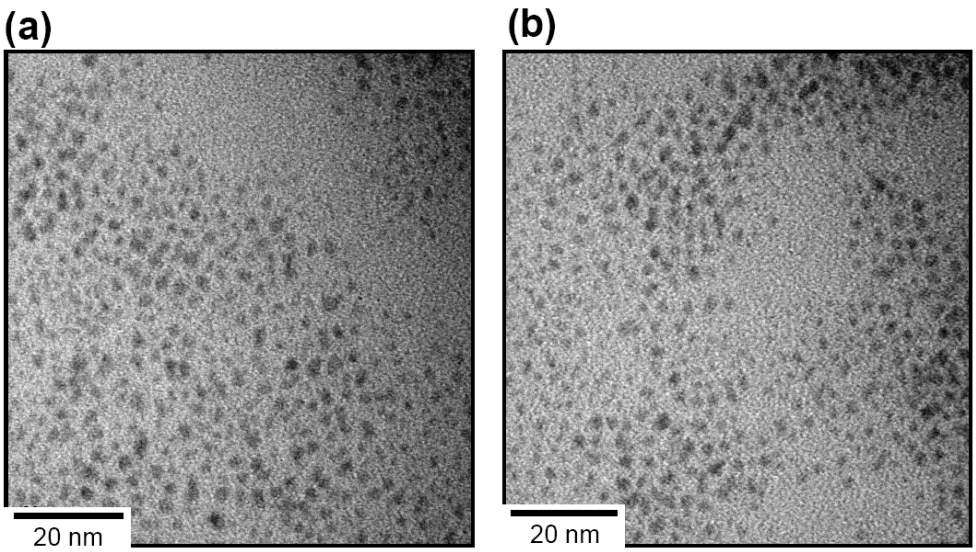

Figure S3. Transmission electron microscopy (TEM) images of 11-MUA–Au NDs (100 nM) in the (a) absence and (b) presence of H_2O_2 (10 mM). From analyses of these TEM images, we estimated that the 11-MUA–Au NDs (from counts of 100 particles) had average sizes of 2.0 (±0.3) and 2.0 (±0.4) nm in (a) and (b), respectively. Other conditions were the same as those described in Figure 1.

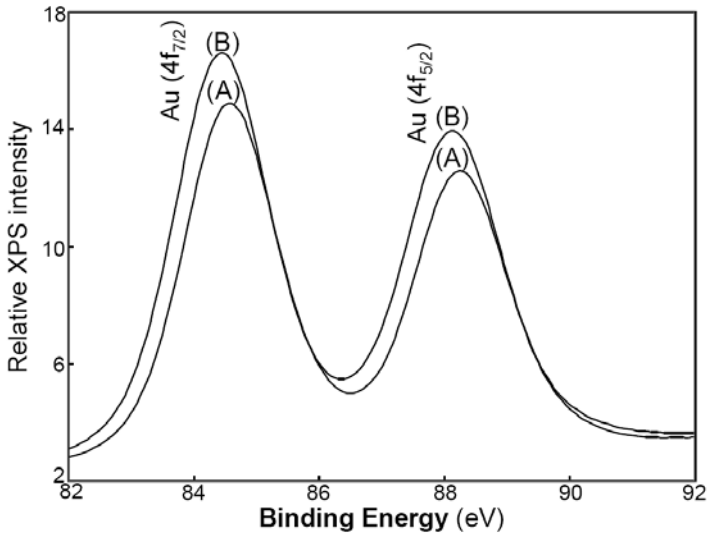

Figure S4. Au 4f core-level photoelectron spectra of the 11-MUA–Au NDs in the (A) absence and (B) presence of H_2O_2. Other conditions were the same as those described in Figure 1.

We used X-ray photoelectron spectroscopy (XPS) to investigate the oxidation states of the surfaces of the 11-MUA–Au NDs in the absence and presence of 10 mM H_2O_2 (Figure S4). The binding energy (BE) for the Au $4f_{7/2}$ electrons in the 11-MUA–Au NDs in the absence of H_2O_2 was 84.6 eV (Figure S4, curve A), i.e., falling within the range from 84.0 eV (Au) to 85.0 eV [polynuclear Au(I)–11-MUA complex].[1] The BE of the Au $4f_{7/2}$ electrons is a common signature for Au oxidation states when using the BE (285.3 eV) of the alkyl chain C 1s orbital as an internal reference.[2] On the other hand, the BE in the presence of H_2O_2 was 84.4 eV (Figure S4, curve B), suggesting a small shift for the Au $4f_{7/2}$ electrons to the BE of bulk Au crystallites. The zeta potentials of the 11-MUA–Au NDs in the absence and presence of 10 mM H_2O_2 were –27.8 and –25.2 mV, respectively. A decrease in negative zeta potential supports that some of the surface 11-MUA molecules were oxidized to form RS–SR. In addition, we found that the average particle size of the 11-MUA–Au NDs after their H_2O_2-mediated oxidation did not change (Figure S3), revealing that the changing density of 11-MUA units was a factor influencing the zeta potentials. Again, these results are consistent with that fewer 11-MUA units being bound to each Au ND in the presence of H_2O_2.

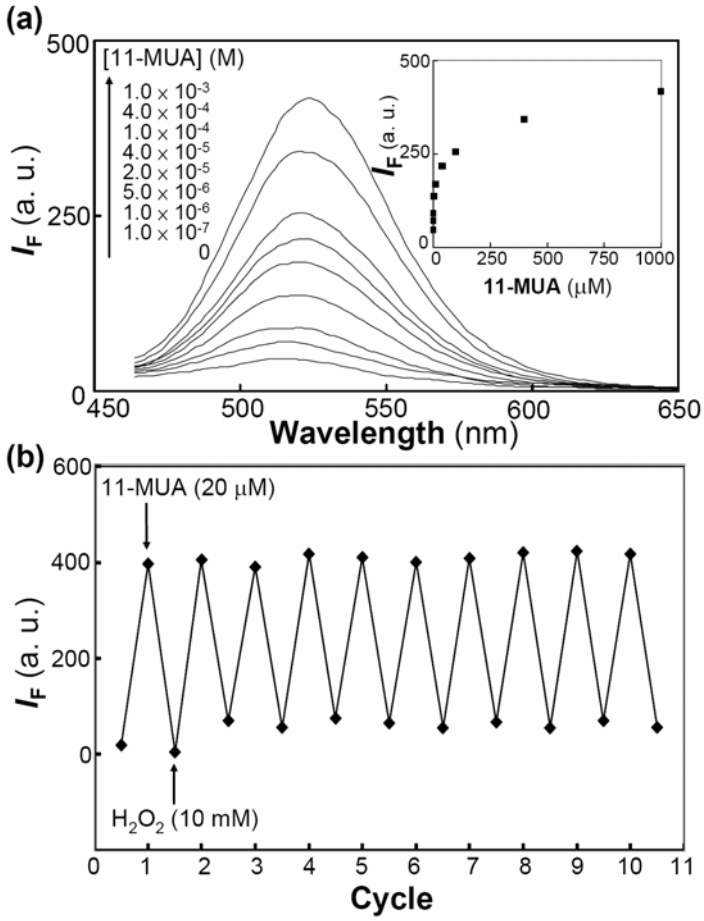

Figure S5. (a) Luminescence spectra of the 11-MUA–Au NDs (100 nM) that had been reacted with H_2O_2 (10 mM) after the addition of 11-MUA (0–1.0 mM). Inset: Plot of the luminescence intensity (520 nm) of the 11-MUA–Au NDs against the concentration of 11-MUA (0–1.0 mM). (b) Validation of the reuse of the 11-MUA–Au ND sensor for H_2O_2. During the sensing cycle, a luminescence spectrophotometer recorded the luminescence intensity of the 11-MUA–Au NDs at 520 nm. Other conditions were the same as those described in Figure 1. The relative standard deviation (RSD) of luminescence decrease caused by H_2O_2 and the luminescence restoration in the presence of 11-MUA was 1.4% and 2.0%, respectively.

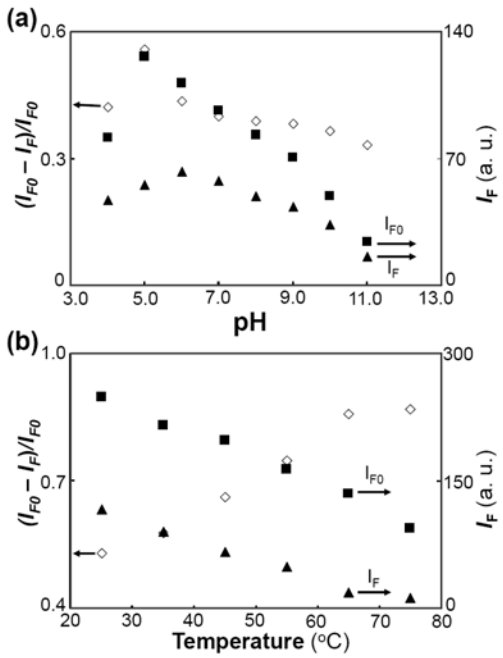

Figure S6. (a) pH- and (b) temperature-dependence response curves of the 11-MUA–Au ND (10 nM) nanosensor for H_2O_2 (1.0 mM). Temperatures: (a) 25 °C; (b) 25–75 °C. Other conditions were the same as those described in Figure 1. The I_{F0} and I_F is the luminescence intensities of the 11-MUA–Au NDs in the absence and presence of H_2O_2, respectively.

In Figure S6, the luminescence signals decreased upon increasing the reaction temperature, suggesting that increased collisions among the Au NDs caused the quenching. The luminescence of the 11-MUA–Au NDs in the presence of H_2O_2 almost disappeared completely at temperatures above 60 °C, mainly because of the rapid reaction between 11-MUA and H_2O_2. Aggregation of the Au NDs as a result of collisions is another reason for the low luminescence intensity of the solution at high temperature. Owing to the weak bonding between the possible products of 11-MUA and the Au NDs, the presence of fewer surface molecules at high temperature is another possible reason for the decreased luminescence.

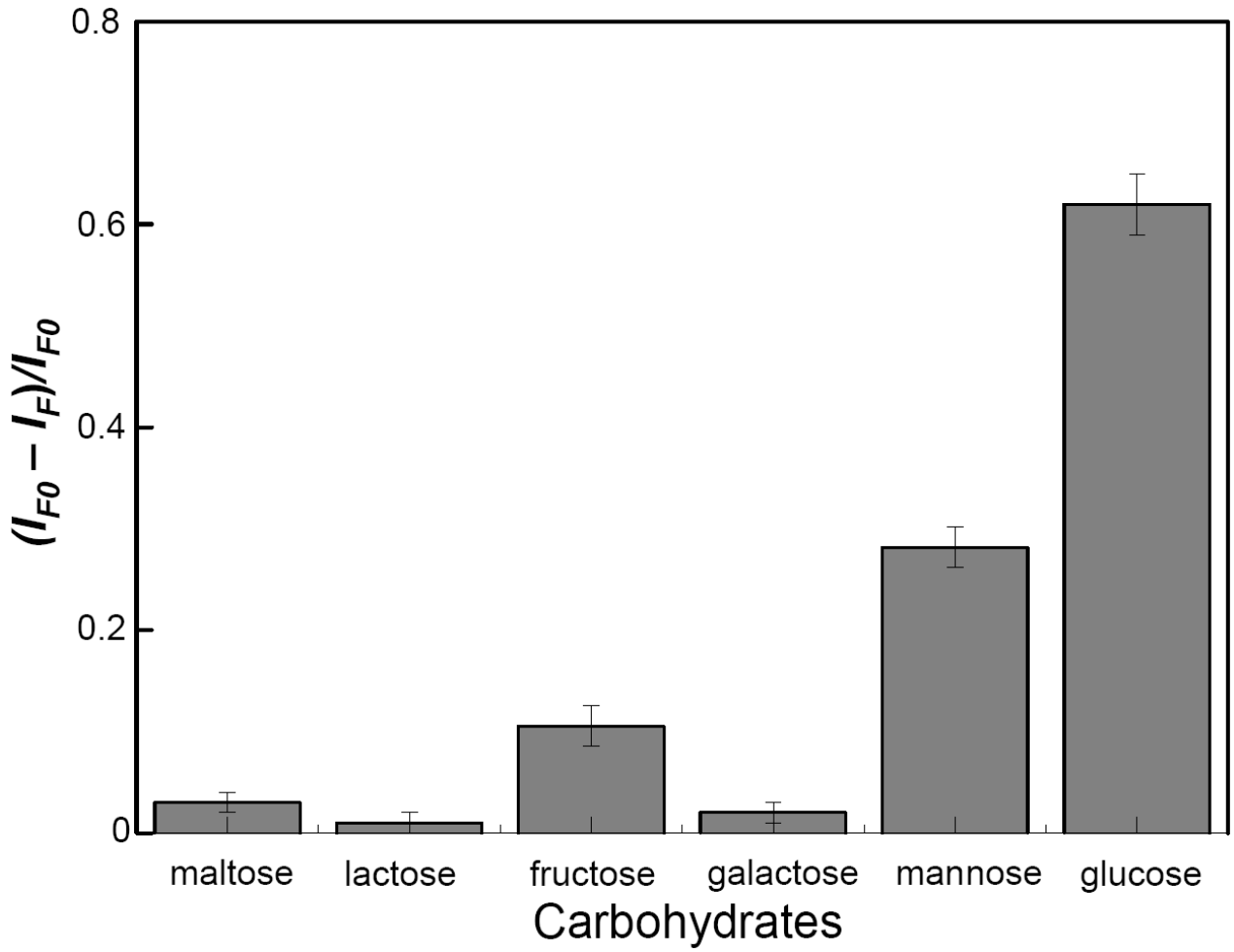

Figure S7. Specificity of the 11-MUA–Au ND sensor (10 nM) toward the detection of glucose over five different carbohydrates. The concentration of each carbohydrate was 10 mM. The error bars represent the standard deviation of three repeated measurements. Other conditions were the same as those described in Figure 3.

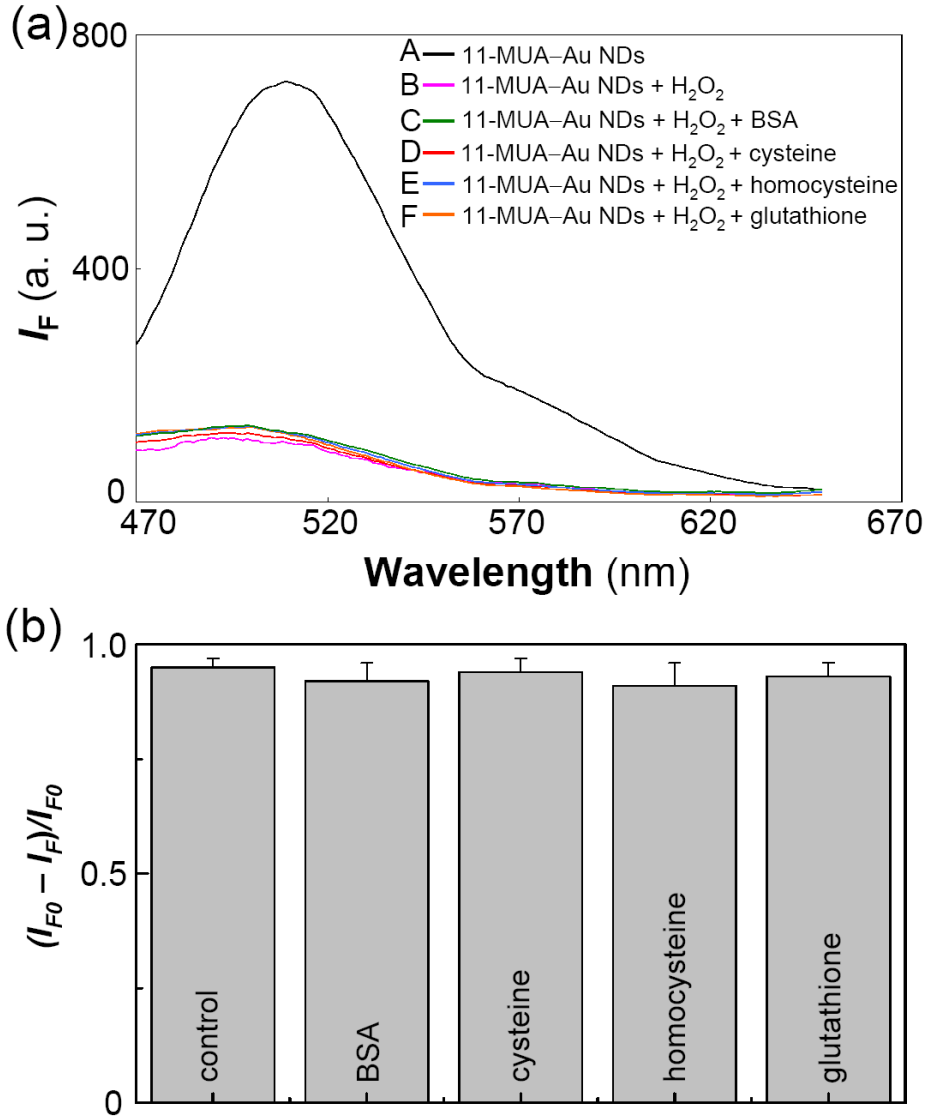

Figure S8. (a) Luminescence spectra of (A) 11-MUA–Au NDs (10 nM) in the presence of (B) H_2O_2, (C) H_2O_2 and BSA, (D) H_2O_2 and cysteine, (E) H_2O_2 and homocysteine, and (F) H_2O_2 and glutathione. (b) Plot of the luminescence decreased ratio $[(I_{Fo} - I_F)/I_{Fo}]$ of the 11-MUA–Au NDs in the presence of H_2O_2 and one of the interference molecules (cysteine, homocysteine, glutathione and BSA). The concentration of H_2O_2 and each interference molecule was 10 mM and 50 μM, respectively. The error bars represent the standard deviation of three repeated measurements. Other conditions were the same as those described in Figure 2.

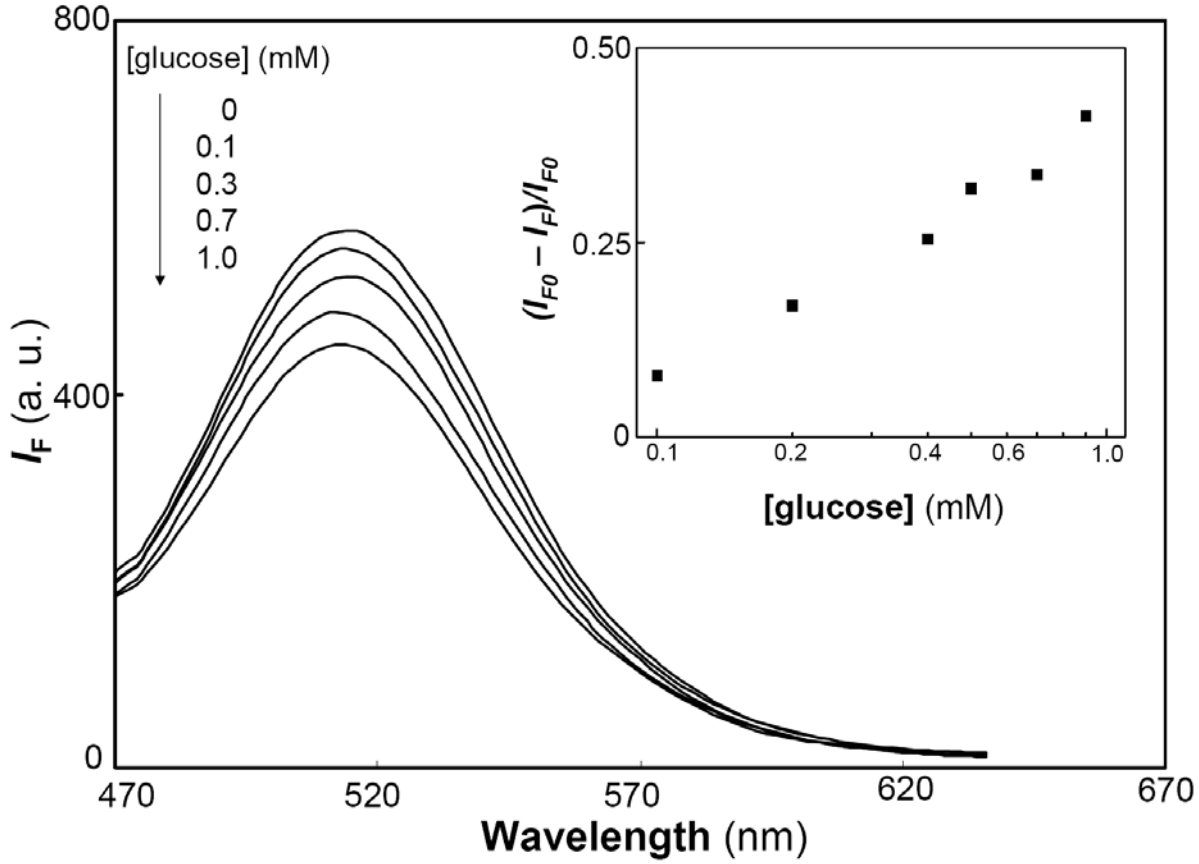

Figure S9. Validation of the use of the 11-MUA–Au ND sensor for the detection of glucose (0–1.0 mM) spiked in a serum sample. Inset: Luminescence ratio $[(I_{Fo} - I_F)/I_{Fo}]$ of the 11-MUA–Au NDs plotted with respect to the concentration of glucose. Other conditions were the same as those described in Figure 3.

EXPERIMENTAL SECTION

Materials. Fructose, glucose, lactose, maltose, manmose, galactose, glucose oxidase (GOx), H_2O_2, 11-MUA, and tetrakis(hydroxymethyl)phosphonium chloride (THPC) were obtained from Sigma (St. Louis, MO). Hydrogen tetrachloroaurate(III) trihydrate and all other reagents used in this study were purchased from Aldrich (Milwaukee, WI).

Synthesis of 11-MUA–Au NDs. The 11-MUA–Au NDs samples were synthesized through the THPC-mediated reduction of $HAuCl_4 \cdot 3H_2O$.[3] A THPC solution (1 mL), which had been prepared by adding 80% THPC solution (12 μL) to water (1 mL), was added to a solution of 1.0 N NaOH (0.5 mL) in water (45 mL). The mixture was stirred for 5 min, and then 1.0 wt% $HAuCl_4 \cdot 3H_2O$ (1.5 mL) was added rapidly. The color of the solution turned brown over the course of 1 min. Prior to use, the solution was stored at 4 °C. The average size of the as-prepared spherical Au NPs was determined to be 2.9 (± 0.5) nm using a transmission electron microscope (Tecnai 20 G2 S-Twin TEM, Philips/FEI, Hillsboro, OR). The particle concentration (ca. 0.94 μM) of the as-prepared Au NP solution was determined according to a procedure described previously.[4-6]

The Au NPs and 11-MUA were self-assembled by introducing 11-MUA stock solutions (100 mM) into the as-prepared Au NP solution at a concentration of 0.47 μM. The as-prepared Au NP solution (5.0 mL), DI water (3.0 mL), trisodium tetraborate (50 mM, pH 9.2, 1.0 mL), and 11-MUA (1.0 mL, 100 mM) were added, in that order, to a 10-mL volumetric flask. The mixture was left to react for 72 h in the dark at room temperature. The resulting Au NDs, which exhibited strong photoluminescence, are designated as "11-MUA–Au NDs." The as-prepared 11-MUA–Au NDs, which had a quantum yield of ca. 3.1%, exhibited absorption and photoluminescence bands centered at wavelengths of 375 and 520 nm, respectively. The luminescence intensity at 520 nm of 11-MUA–Au NDs was *ca*. 23,000-fold higher than that of the as-prepared Au NPs at 750 nm.[4] They were stable for at least 3 months at 4 °C in sodium phosphate solutions (10 mM; pH 4.00–11.00)

containing NaCl at concentrations of up to 250 mM.[6] Prior to use, the excess 11-MUA in the solution was removed through centrifugal filtration (13,500 g) for 40 min through a filter having a cutoff of 10 kDa (membrane nominal pore size ~ 1 nm). Most of the 11-MUA, precursors and possibly Au (I)-thiolate in the solution were removed. We then measured the luminescence of the removed solution, showing a very weak luminescence when excited at 375 nm. We prepared mixture of 11-MUA (10 mM) and $HAuCl_4$ (0.3 mM), and it luminesced at 620 nm when excited at 280 nm.[5] On the other hand, the suspended solution luminesced more strongly, which reveals that the luminescence signal is truly from the particles. The 11-MUA–Au ND solution was then resuspended in 5 mM sodium phosphate solution (pH 7.4). By comparing the absorbance of the original 11-MUA–Au ND solution at 375 nm to that of the resuspended solution, ca. 95% of the 11-MUA–Au ND was deemed to have been collected (data not shown). The corresponding particle concentration in the resuspended solution was estimated to be 1.05 μM, under the assumption that the 11-MUA–Au NDs had a uniform diameter of 2.0 (± 0.3) nm, which was estimated from a count of 100 11-MUA–Au ND particles in a TEM image. The HRTEM image suggests that the lattice fringes of the purified luminescent 11-MUA–Au NDs are consistent with metallic gold having a discerned lattice spacing of 2.4 Å, which corresponds to the d-spacing of the (111) crystal plane of fcc Au.[7] The TEM image and luminescence spectrun further revealed that the luminescence signal is truly from the purified 11-MUA–Au NDs (2.0 (± 0.3) nm; n = 100), not the aggregates of ultrasmall Au clusters.[4-6] The particle concentration of the as-prepared Au ND solution was determined to be 1.05 μM by using the equation ($n = 3m / 4\pi r^3 s$), assuming the presence of ideal spherical particles, in which n is the amount of Au ND per milliliter, m is the molar mass of Au in substance [g/mL], r is the particle radius [cm], and s is the specific gravity of colloidal gold [19.3 g/cm^3].[8] The m and r values were determined by conducting inductively coupled plasma mass spectroscopy (ICP-MS) and TEM measurements, respectively. This formula gives the number of Au ND per milliliter. This concentration was then

converted into number of Au ND per liter and divided by Avogadro's number (6.023×10^{23}) to get the final molar concentration of Au NDs.

We demonstrate the purified 11-MUA–Au NDs sample was stable (no precipitates formed) for at least 3 months when stored at 4 °C in the dark by light scattering experiments. The intensities of static light scattering of fresh- and stored- 11-MUA–Au NDs (100 nM) were determined to be close (320 ± 35 kcps, n = 5) by using a particles size analyzer (Zetasizer Nano, Malvern). In addition, the luminescence intensity of the stored 11-MUA–Au NDs was almost the same as that of the freshly prepared ones. The absorption and luminescence spectra of the as-prepared 11-MUA–Au ND solutions were measured using UV–Vis absorption (Cintra 10e, GBC, Victoria Australia) and fluorescence spectrophotometers (Cary Eclipse; Varian, CA, USA), respectively.

Luminescence Assays for H_2O_2 and Glucose. For H_2O_2 assays, aliquots (0.5 mL) of a solution of 10 mM sodium phosphate (pH 5.0) containing H_2O_2 (0–100 mM) and 11-MUA–Au NDs (10 nM) were maintained at 65 °C for 30 min. All solutions were transferred into a 1-mL quartz cuvette and then their luminescence spectra were measured using a fluorescence spectrophotometer operated with excitation at 375 nm. For glucose assays, aliquots (0.5 mL) of a solution of 10 mM sodium phosphate (pH 7.0) containing glucose (0–10 mM) and GOx (1 μM) were maintained at 37 °C for 5 min. The resulting solutions were diluted fivefold with a solution of 10 mM sodium phosphate (pH 5.0) containing 11-MUA–Au NDs (10 nM) and maintained at 65 °C for 30 min. All solutions were transferred into a 1-mL quartz cuvette and then their luminescence spectra were measured using a fluorescence spectrophotometer operated with excitation at 375 nm.

References

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