Single Nanoparticle-based Sensor for Hydrogen Peroxide ($\text{H}_2\text{O}_2$) via Cytochrome $c$-mediated Plasmon Resonance Energy Transfer

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Supporting Information

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EXPERIMENTAL METHODS

Materials. Sulfuric acid (H₂SO₄, 95%) and hydrogen peroxide (H₂O₂, 34.5%) were purchased from SAMCHUN. Ethanol (99.9%) was purchased from J.T.Baker. Chloroplatinic acid hexahydrate (H₂PtCl₆•6H₂O, 37.5%), 3-aminopropyltriethoxysilane (APTES, 99%), trimethoxyoctadecylsilane (TMOS, 90%), L-ascorbic acid (AA), cytochrome c (Cyt c) from bovine heart (≥95%), uric acid (UA, 99%), dopamine hydrochloride and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. Polydimethylsiloxane elastomer kits (Sylgard 184) was purchased from Dow Corning. 40, 50, and 100 nm GNPs were purchased from BBI solutions.

Au@Pt nanoparticle synthesis. Au@Pt core@shell nanoparticle was synthesized by decorating small Pt nanoparticles onto a 40 nm Au spherical nanoparticle. Briefly, 10 ml of the 40 nm Au nanoparticle was mixed with 10 ml of deionized water. Then this mixture solution was heated to 100 °C with vigorous stirring. As a reducing agent for Pt ion, AA was added into the above solution based on the desired Pt to Au molar ratio. The molar ratio of AA to H₂PtCl₆ was kept at 5:1 to make sure the complete reduction of Pt ions. Pt nanoshell on Au cores was then formed by adding Pt precursor solution to make the Au:Pt = 1:1 (molar ratio). The heating was kept for 1 h and cooled down at room temperature.

Sensor chip fabrication. The glass slides were cleaned in piranha solution (sulfuric acid:hydrogen peroxide = 7:3 v/v) for 60 min and then rinsed with ethanol. The cleaned glass slide was modified with 1 mM APTES and 1 mM TMOS by incubation in ethanol for 24 h. The glass was then rinsed with ethanol and followed by drying with N₂ gas. In order to immobilize nanoparticles on the glass, a small aliquot of nanoparticle solution (ca., 10 µl) was dropped onto the APTES/TMOS modified glass slide sensor chip. For confining a reaction solution on the sensor chip, PDMS well was fabricated. A 10:1 (by weight) mixture of PDMS base/curing agent was degassed under vacuum for 1 h and poured in square dish. After its curing at 70 °C for 1 h, the solidified PDMS film was cut into a rectangular well. The prepared PDMS well was placed on the nanoparticle immobilized glass slide before measurement of dark-field nanospectroscopy.
Single nanoparticle based detection of H$_2$O$_2$ via dark-field nanospectroscopy. In order to measure intrinsic spectra of single nanoparticles in solution, PBS solution was loaded in the PDMS well on the sensor chip. For measurement of single nanoparticle spectra, dark-field transmission optical microscope (Olympus BX43, Tokyo, Japan) equipped with hyperspectral imaging spectrophotometer (CytoViva Hyperspectral Imaging System (HSI), Auburn, AL, USA) was used. A 20× objective lens for imaging was used and integration time for collecting spectra was 0.25 second. For measuring initial spectra of nanoparticle probes before exposure to H$_2$O$_2$, PBS solution in the PDMS well was replaced with the reduced Cyt c solution dissolved in the PBS solution, which was prepared by 12 h incubation of native Cyt c with AA. And then H$_2$O$_2$ was injected to the PDMS well and the ratio between reduced Cyt c and H$_2$O$_2$ was set to 5:1 v/v. After 2 min reaction of them, changed scattering spectra of individual nanoparticles were collected as a consequence of H$_2$O$_2$ exposure. All collected spectra were normalized and then the change in spectral quenching dips was measured before and after H$_2$O$_2$ exposure. For all data, the reliability of the observed spectral shift and intensity ration from the single particle were examined by the standard deviation of the spectral shift from 5 different particles at the same condition.
Figure S1. Illustration of signaling pathways associated H$_2$O$_2$. High levels of H$_2$O$_2$ can induce apoptosis. On the other hand, low levels of H$_2$O$_2$ can regulate proliferation.
Figure S2. (A-B) Transmission electron microscope (TEM) images of the core-shell nanoparticle comprising a 40 nm Au core and 4 nm Pt satellites (40 nm Au@Pt). Scale bars are 50 nm (left) and 20 nm (right). (C) Image of high angle annular dark field scanning transmission electron microscopy (HAADF-STEM) analysis and elemental mapping obtained from energy-dispersive X-ray spectrometry (EDX) showing the presence of Au core-nanoparticle (pink) and corresponding Pt shell-layer (green). Scale bar is 20 nm.
Figure S3. Photograph of a PDMS well placed on the nanoparticle immobilized glass slide.
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Figure S6. Spectral changes measured via UV-Vis spectroscopy in accordance with the increase of \( \text{H}_2\text{O}_2 \) concentration.
Figure S7. Monitoring the enzymatic generation of superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) by xanthine oxidase (XOD) and superoxide dismutase (SOD), respectively. (A) Time-resolved spectra monitored during enzymatic generation of O$_2^-$ and H$_2$O$_2$ via the measurement of change in the quenching dip at 550 nm. (B) Plot for the time-resolved changes in the spectral quenching dip after injection of XOD and SOD enzyme.