Supplementary Information

Self Color-Changing Ordered Mesoporous Ceria for Reagent-Free Colorimetric Biosensing

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Experimental

Materials

Cerium(IV) oxide nanoparticles with ~20 nm in diameter (Free CeO_2 NPs), Sodium acetate, phosphate buffered saline (PBS), glucose oxidase from *Aspergillus niger* (GOx), cholesterol oxidase from *Streptomyces sp.* (ChOx), β-D-glucose, cholesterol, galactose, maltose, arabinose, fructose, lactose, fructose, glycerol, sodium phosphate (monobasic and dibasic), and human serum were purchased from Sigma-Aldrich (Milwaukee, WI). Hydrogen peroxide (35%, H_2O_2) was purchased from Junsei Chemical Co. (Japan). All the chemicals were of analytical grade or higher, and all solutions were prepared with DI water purified by a Milli-Q Purification System (Millipore, USA).

Synthesis and characterization of m-CeO_2

Mesostructured silica (KIT-6) was prepared following a previous method.(Kleitz et al. 2003) $Ce(NO_3)_3{\cdot}6H_2O$ (2 g) was dissolved in ethanol (15 mL), and KIT-6 (1 g) was added to the solution. Thereafter, ethanol was evaporated at 50 °C with mild stirring and the $Ce(NO_3)_3{\cdot}6H_2O$@KIT-6 composite was further dried in a vacuum oven for 6 hours. The composite was placed in an open crucible and heated to 350 °C for 6 hours to remove nitrate functional groups, and maintained at 550 °C for 6 hours. Prepared CeO_2@KIT-6 was stirred for six hours with added 2M NaOH two times to ensure removal of the KIT-6 silica. The products were recovered by centrifuging and washing with water and ethanol several times. Finally, the m-CeO_2 was dried at vacuum oven overnight. The m-CeO_2-s was synthesized in the same procedure except that it was heated in a quasi-closed crucible.

Characterization of m-CeO_2

Nitrogen physisorption was carried out at 77 K using a Tristar II 3020 (Micromeritics Instrument Co.). The structure of the synthetic material were examined using SEM with an S-

4200 field-emission SEM (Hitachi), and TEM with a JEOL EM-2010 microscope (JEOL Co.), and using an HR-TEM with an JEOL JEM-2010 (JEOL Co.). Powder XRD patterns were obtained using the PANalytical X'Pert diffractometer with Cu Kα radiation (λ=1.5406 Å). XPS was performed on a VG SCIENTIFIC ESCALAB 250 (KRATOS NOVA).

Preparation of m-CeO_2 entrapping crosslinked enzyme, m-CeO_2 with adsorbed enzyme, and free CeO_2 NPs with adsorbed enzyme

The m-CeO_2 entrapping crosslinked enzyme was prepared as follows. m-CeO_2 (10 mg) was mixed with free GOx (1 mL, 10 mg/mL) or ChOx (1 mL, 6 mg/mL) in a buffer solution (100 mM sodium acetate buffer, pH 5.3), vortexed for 30 s, sonicated for 10 s, and incubated at room temperature with shaking (250 rpm) for 30 minutes. Then, the samples were briefly washed with aqueous buffer (100 mM sodium phosphate, pH 8.0), and incubated in the same buffer containing glutaraldehyde (0.1%, w/w) at 200 rpm for 30 minutes. After two washings with phosphate buffer (100 mM, pH 8.0), Tris-HCl buffer (100 mM, pH 8.0) was added and incubated at 200 rpm for 30 minutes to block unreacted aldehyde groups. After Tris-capping, the samples were washed once with phosphate buffer (100 mM, pH 8.0), twice with sodium acetate buffer (100 mM, pH 5.3), and stored at 4°C until use. The m-CeO_2 with adsorbed enzyme was prepared following the same procedures but fresh phosphate buffer (100 mM, pH 8.0) was used instead of the glutaraldehyde solution. Free CeO_2 NPs with adsorbed enzyme were also prepared with the same procedures but CeO_2 NPs and fresh phosphate buffer (100 mM, pH 8.0) were used instead of m-CeO_2 and glutaraldehyde solution, respectively.

Detection of H_2O_2 using m-CeO_2

The m-CeO_2 entrapping crosslinked enzyme was prepared as follows. m-CeO_2 (10 mg) was mixed with free GOx (1 mL, 10 mg/mL) or ChOx (1 mL, 6 mg/mL) in H_2O_2 detection

was performed in a transparent 96-well plate by employing the following procedures: A mixture containing m-CeO_2 or free CeO_2 NPs (0.2 mg each) and H_2O_2 (20 μL, 100 mM) in 180 μL sodium acetate buffer (100 mM, pH 5.3) was incubated at room temperature for 3 minutes. The reacted well-plate was directly used to obtain images representing the progress of the reaction. For the quantification of color intensity, the well-plate was scanned with a conventional office scanner, followed by analyzing with 'ImageJ' software with the optional plugin 'RGB to CMYK'.

Detection of glucose and cholesterol using m-CeO_2 entrapping enzymes

Glucose detection was performed in a 96-well plate as follows. A solution containing m-CeO_2 entrapping crosslinked GOx (20 μL, 10 mg/mL) and carbohydrates including glucose (20 μL at diverse concentrations) in 160 μL sodium acetate buffer (100 mM, pH 5.3) was incubated at 37°C for 10 minutes. After the reaction, the reacted well-plate was directly used with the same procedures as those used for H_2O_2 detection. For cholesterol detection, cholesterol was first dissolved in Triton X-100 at 10% (w/v), and diluted with water to the predetermined concentrations. The other procedures were the same as those used for glucose detection.

The operational stability for glucose sensing was checked in aqueous buffer (100 mM sodium acetate, pH 5.3) at room temperature under non-shaking conditions. The initial and residual activities at each time point were determined by measuring the color intensity, as described above. The relative activity (%) was calculated using the ratio of the residual activity to the initial activity of each sample. Reversibility was measured after cycles involving the typical reaction with glucose (10 mM) and brief washing with aqueous buffer (100 mM sodium acetate, pH 5.3) to remove unreacted glucose in the sample. After regaining the initial white color of m-CeO_2 entrapping GOx, the sample was reused for the

measurement of residual activity.

Glucose and cholesterol detection in human serum

The initial glucose and cholesterol concentration of human serum were first determined with a glucose assay kit and cholesterol assay kit, respectively (Sigma-Aldrich). Predetermined amounts of glucose or cholesterol were then added into the human serum to make spiked samples, which represent normal, boundary, and high levels of blood glucose or cholesterol. Finally, the glucose or cholesterol level of each spiked sample (20 μL, 10-fold dilution) was determined by the same procedure described above. The recovery rate [recovery (%) = measured value/actual value × 100] and the coefficient of variation [CV (%) = SD / average × 100] were determined to evaluate the precision and reproducibility of the assays.

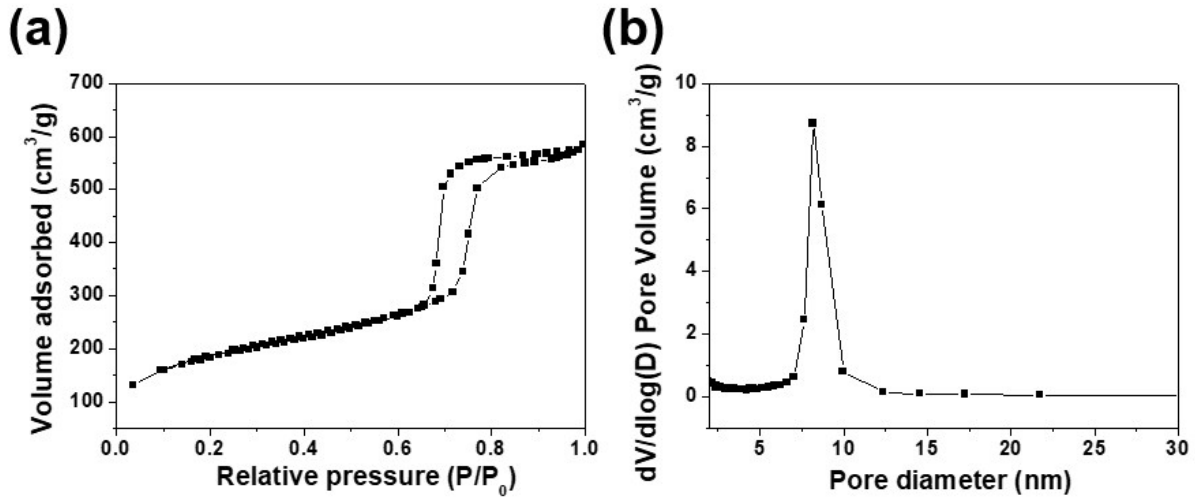

Figure S1. (a) Nitrogen adsorption–desorption isotherms of KIT-6 and (b) its corresponding pore size distribution calculated from adsorption branches of isotherms using a BJH method.

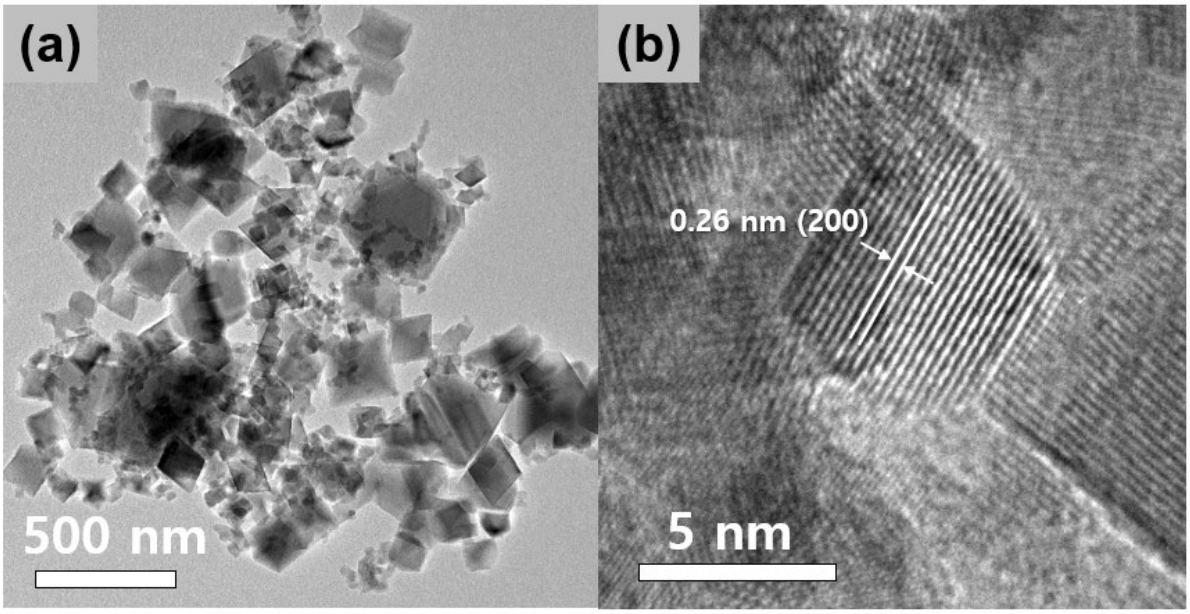

Figure S2. (a) TEM and (b) HR-TEM image of CeO_2 NPs.

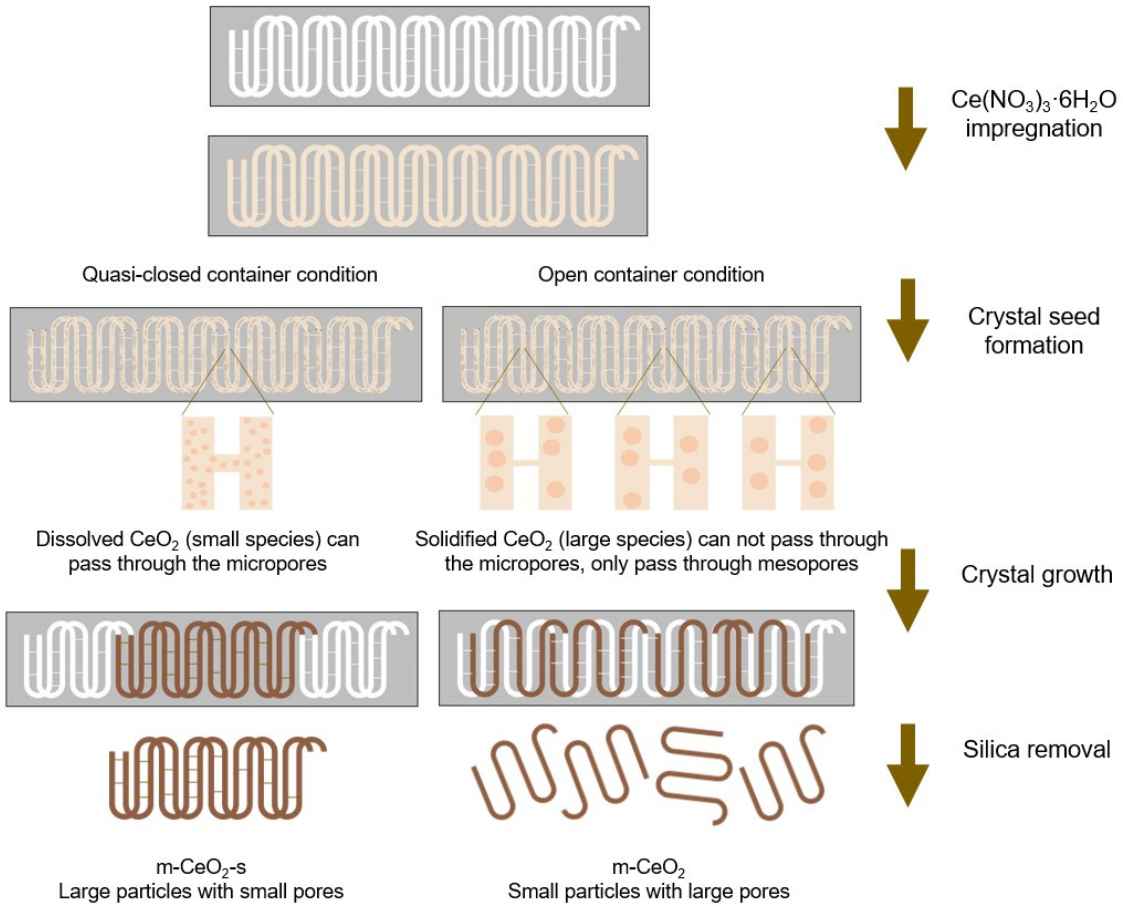

Figure S3. Schematic illustration of showing the process of formation m-CeO_2-s and m-CeO_2.

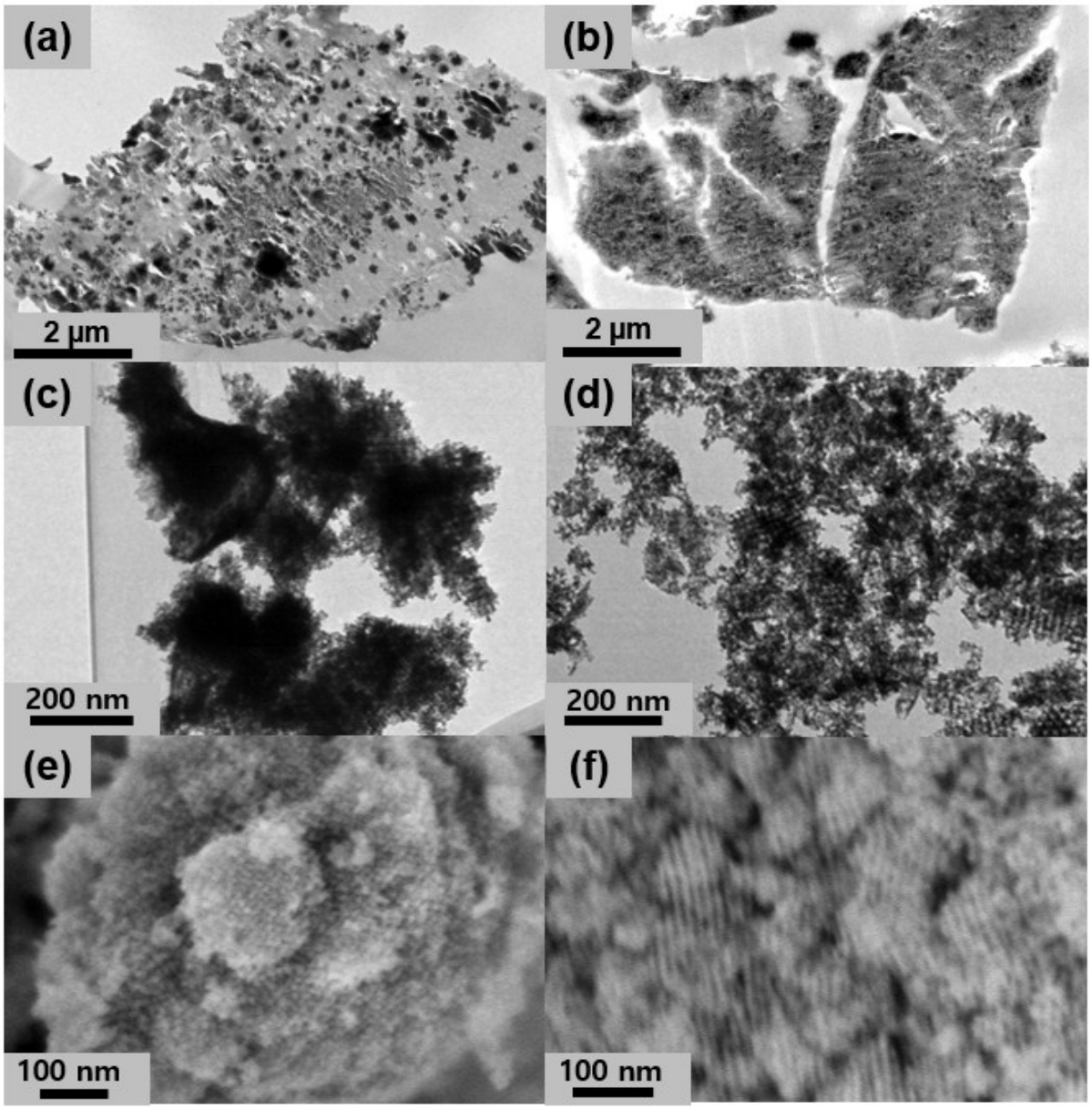

Figure S4. (a) Microtomed TEM image of CeO_2@KIT-6 synthesized in quasi-closed crucible and (b) in open crucible. (c) TEM image of m-CeO_2-s and (d) m-CeO_2 (e) SEM image of m-CeO_2-s and (f) m-CeO_2.

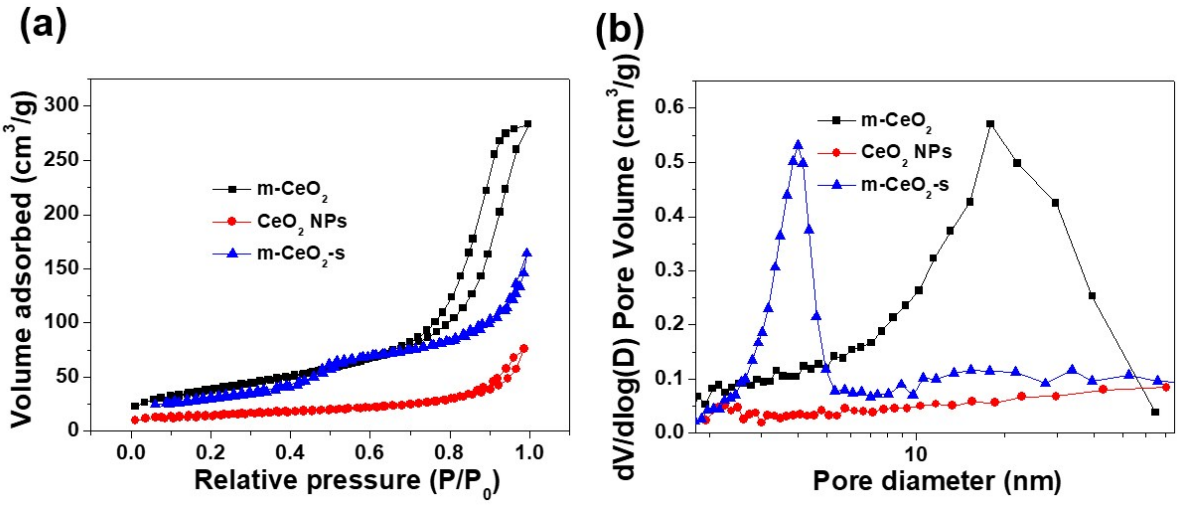

Figure S5. (a) Nitrogen adsorption–desorption isotherms of m-CeO_2, CeO_2 NPs and m-CeO_2-s, and (b) their corresponding pore size distribution calculated from adsorption branches of isotherms using a BJH method.

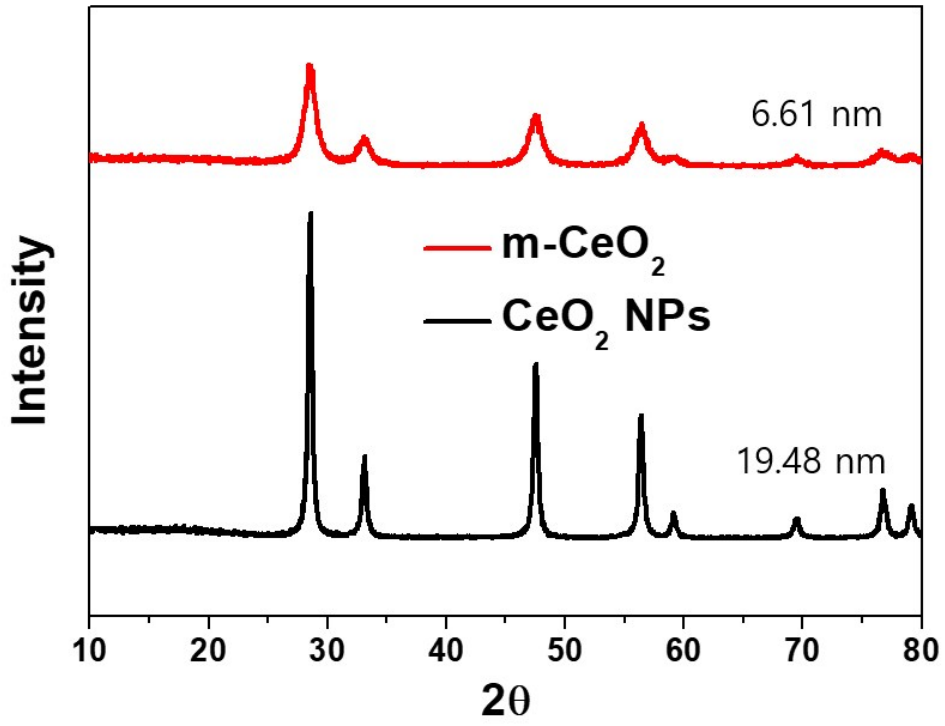

Figure S6. XRD partterns of m-CeO_2 and CeO_2 NPs. Crystallite sizes calculated by the Scherrer equation were 6.61 nm for m-CeO_2 and 19.48 nm for CeO_2 NPs.

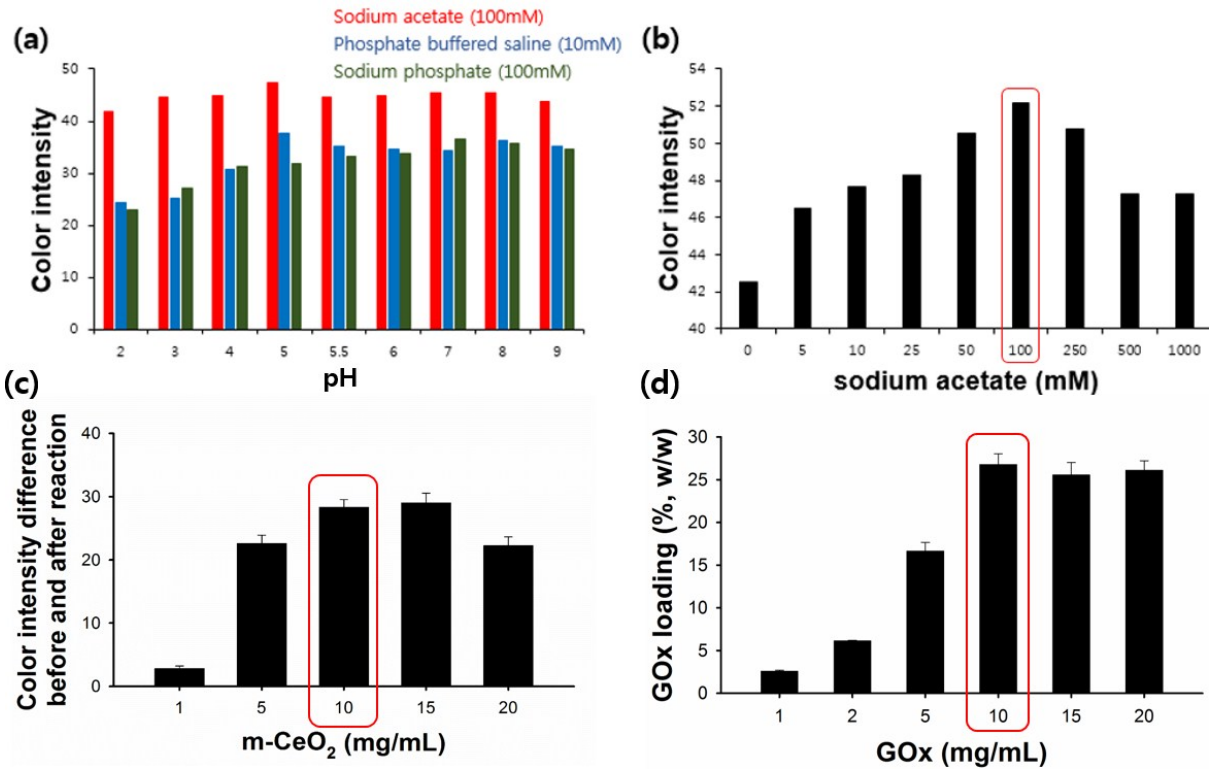

Figure S7. Optimization of buffer system (a & b) and initial concentrations of m-CeO_2 and GOx for the detection of target glucose

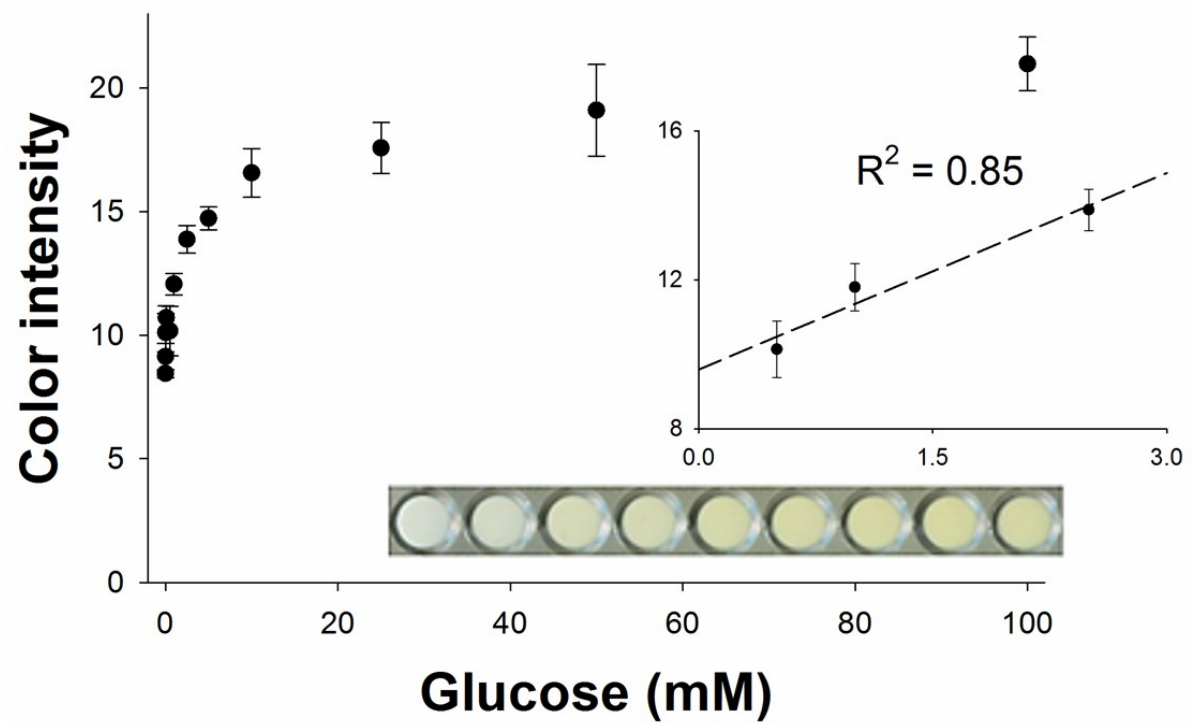

Figure S8. A dose-response curve with its real image and the corresponding linear calibration plot for glucose detection using free CeO_2 NPs with physically-adsorbed GOx on their surface.

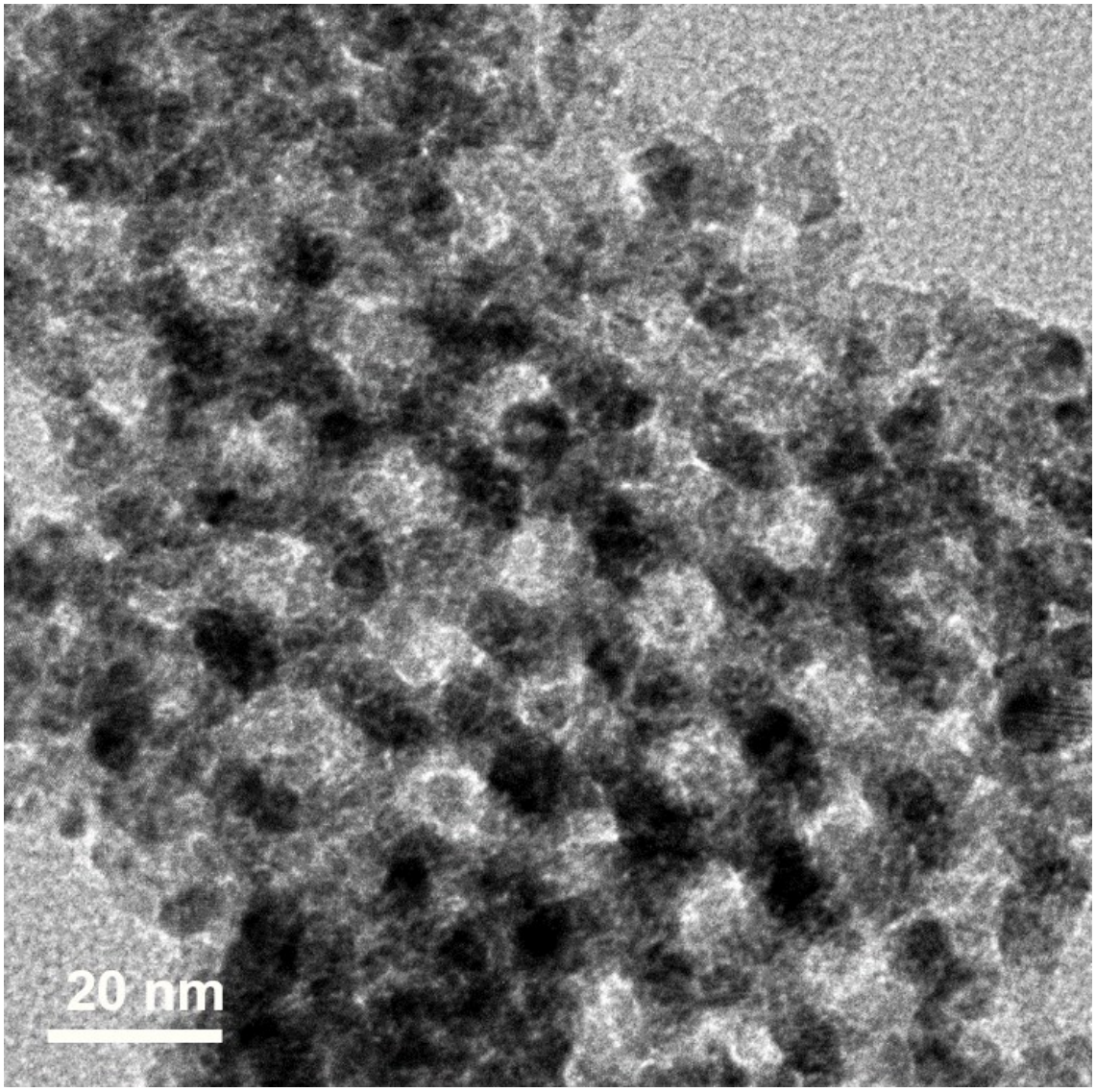

Figure S9. TEM image of m-CeO_2 after 10 cycles with hydrogen peroxide.