Electronic Supplemental Information

Materials & Methods

Nanoparticle synthesis and characterization: The cerium oxide nanoparticles were synthesized by wet chemical process as previously described (3). Chemicals for CeO_2 nanoparticle synthesis, Ce $(NO_3)_3$, H_2O_2, were obtained from Sigma-Aldrich (St. Louis, MO). SiO_2 nanoparticles were purchased from Corpuscular Inc. (Cold Spring, NY). The surface chemistry of the cerium oxide nanoparticles was studied using a Physical Electronics (5400 PHI ESCA) spectrometer with a monochromatic Al Kα X-ray source operated at 300 W and base pressure of 1×10^{-9} Torr. The binding energy of the Au (4f7/2) at 84.0±0.1 eV was used to calibrate the binding energy scale of the spectrometer.

Assay for nitric oxide: A ferrous hemoglobin assay was adapted from Murphy & Noack (2) in which ferrous hemoglobin (Hb) (Sigma-Aldrich) and ·NO react to form oxidized ferric hemoglobin. S-nitroso-*N*-acetylpenicillamine (SNAP) (Molecular Probes), was used to generate ·NO. Briefly, 200 μM of SNAP was added to 25 mg/mL ferrous Hb in the presence or absence of nanoparticles or the spin-trap DEPMPO (Enzo Life Sciences) in 100 mM phosphate buffer (pH 7.0). The oxidation of Hb was monitored using a Hewlett-Packard 8453 diode array spectrophotometer. We followed changes to spectra at wavelengths of 411 nm (isosbestic point) and 421 nm. The change in absorbance per unit time was measured for 10 min at 30 s intervals. The concentration of ·NO reacting with Hb was obtained by the difference in absorbance between 401-421 nm using an extinction coefficient of 77 $mM^{-1}cm^{-1}$ (2).

Surface chemistry alteration by phosphate ions:

Phosphate buffer was prepared by dissolving monosodium phosphate (13.8 g/L) and its conjugate base, disodium phosphate (14.1 g/L), in 1 L of water to give a 0.1 M solution, and the

pH was adjusted by titration with 1 M HCl to reach a pH value of 7.4. Water dispersed CeO_2 NPs with higher levels of oxygen vacancies at their surface (200 μM) were suspended in equimolar phosphate buffer (pH 7.4) for 24 h at room temperature. The UV-visible spectra were recorded to determine surface chemistry of cerium using a UV-viable Hewlett-Packard 8453 diode array spectrophotometer in a 1.0 cm path length quartz cuvette.

·NO detection using copper-fluorescein method: To measure ·NO by an alternate method we followed ·NO levels using a copper-fluorescein (Cu-FL) probe as previously described (1). In these experiments, 100 μM of the ·NO generator, diethylamine NONOate diethylammonium salt (DEA/NO) (Sigma) was added to CuFL probe (1 μM) (Strum Chemicals, Newburyport, MA). Fluorescence was followed at an emission wavelength of 530 nm using an excitation wavelength of 503 nm in 50 mM sodium phosphate buffer, pH 7.0, containing 20 μM DPTA using a Varian Cary Eclipse fluorescence spectrophotometer (Palo Alto, CA) for 20 min at room temperature. Assays were carried out in the presence or absence of CeO_2 NP, SiO_2 NPs or glutathione (Fisher Scientific, Pittsburg, PA).

Transmission electron microscopy (TEM)

The CeO_2 nanoparticle morphology was characterized using high-resolution transmission electron microscopy (HRTEM). The CeO_2 NP preparations were deposited on carbon-coated copper grid (SPI supplies) for HRTEM analysis. HRTEM micrographs were obtained using FEI Tecnai F30 operated at 300 keV.

X-ray photoelectron spectroscopy (XPS)

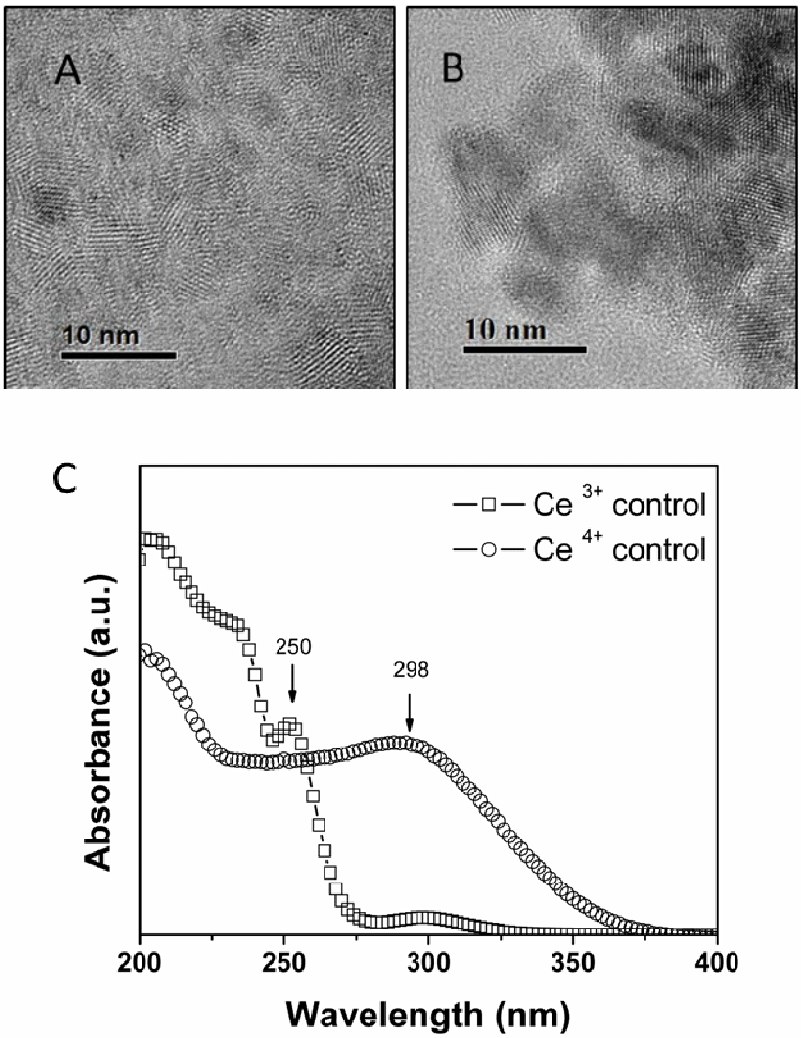
The CeO_2 nanoparticle were transferred onto silicon wafers (Kmbh Associates CZ Silicon, thickness of wafer: 350 μm) and air dried. The surface chemistry of the nanoparticles were studied using a Physical Electronics (5400 PHI ESCA) spectrometer with a monochromatic Al

Kα X-ray source operated at 300 W and base pressure of 1 $\times 10^{-9}$ Torr. The binding energy of the Au (4f7/2) at 84.0±0.1 eV was used to calibrate the binding energy scale of the spectrometer.

Zeta potential (ZP) and Particle Size Measurement

Water dispersed CeO_2 NPs with different 3+/4+ ratios were suspended in buffers according to the various conditions used in these studies and ZP and particle size measured. For surface chemistry alteration experiments, NPs were incubated for 24 h followed by ZP and particle size measurements using Zeta sizer (Nano-ZS) from Malvern Instruments.

Supplementary Figures



Supplementary Fig. S1: Comparison of CeO_2 NPs 3+ and 4+. A: HR-TEM image of CeO_2 NP higher 3+ B: HR-TEM image CeO_2 NPs higher 4+ C: UV-vis spectroscopy.

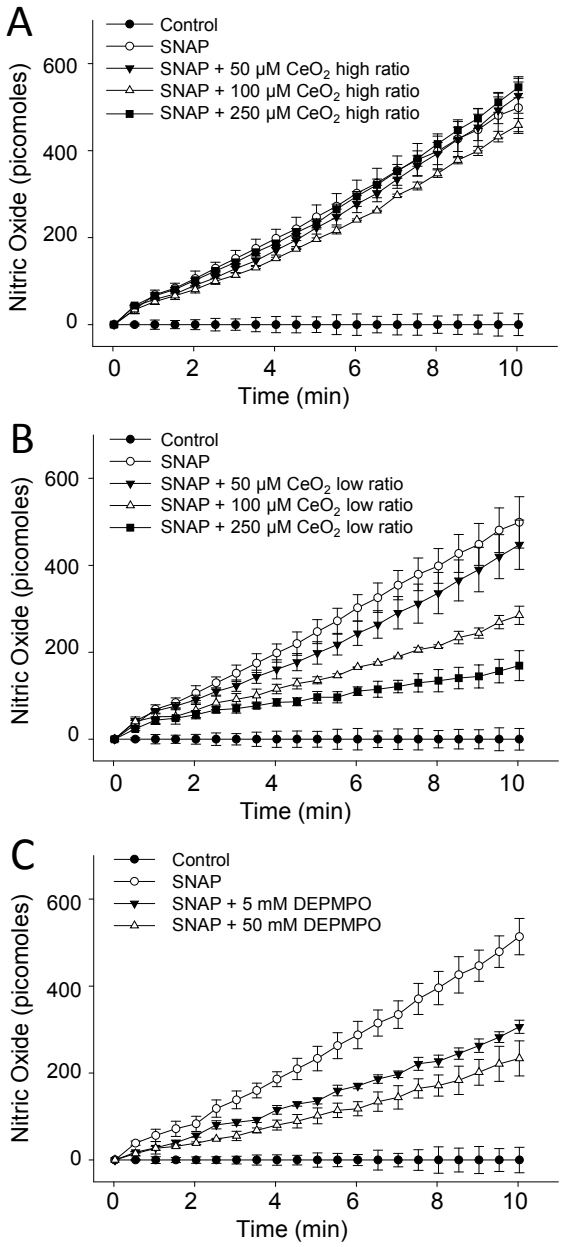
Reaction conditions	NP conc. (µM)	NO production rate[a] (pmol min^{-1} +/- SD)
SNAP (200 µM) control	0	51.6 ± 4.4
SNAP + CeO_2 (low 3+/4+)	50	42.1 ± 5.7
	100	25.8 ± 2.9
	250	14.3 ± 3.3
SNAP + CeO_2 (high 3+/4+)	250	52.5 ± 2.9
SNAP + CeO_2 + PO_4^{2-}	200	39.7 ± 5.9

[a]Rates are pmol min^{-1} and were calculated by determining the rate of change in absorbance per unit time, based on the molar extinction coefficient of conversion of HbO_2 to metHb in the presence of ·NO (401 nm-421 nm) ($\Delta\varepsilon$ = 77 $mM^{-1}cm^{-1}$). SD = standard deviation

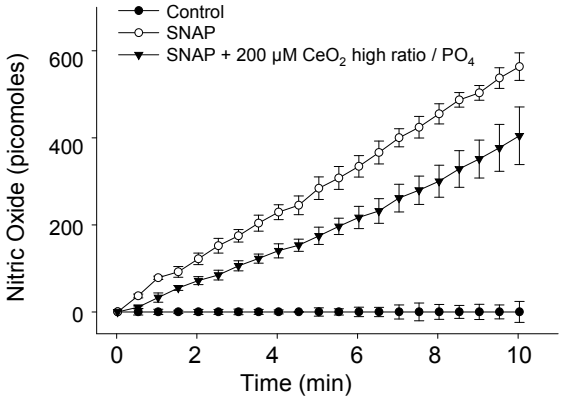
Supplementary Table S1: Changes in •NO levels in the presence of CeO2 nanoparticles

	3+	4+
Size (nm)	5-8	3-8
Zeta Potential – as synthesized (H_2O)(mV)	15.1	49.2
Zeta Potential – Hb assay buffer (mV)	-14.4	-21.7
Zeta Potential - CuFL assay buffer (mV)	-14.1	-20.5
XPS – Ce3+ (%)	75±3	20±5

Supplementary Table S2: Physiochemical properties of CeO_2 NPs



Supplementary Fig. S2: Effective scavenging of ·NO by CeO_2 NPs lacking surface oxygen vacancies. The concentration of ·NO in the presence or absence of CeO_2 NPs was quantified using the extinction coefficient for reaction with ferrous Hb (2). Data are derived from experimental data shown in Fig.1. Represented in all graphs: closed circles = 25 mg/mL Hb alone; open circles = 25 mg/mL Hb + 200 µM SNAP. **A**) CeO_2 NPs with high 3+/4+ ratio. **B**) CeO_2 NPs with low 3+/4+ ratio. **C**) DEPMPO addition. CeO_2 NPs or DEPMPO were added at the concentrations indicated. Graph is representative of 3 or more experiments.



Supplementary Fig.S3: Effective scavenging of ·NO by CeO_2 NPs with high level of surface oxygen vacancies upon incubation with phosphate. Concentration of ·NO in the presence or absence of CeO_2 NPs was quantified as described in Supplementary Fig S1. Closed circles = 25 mg/mL Hb alone; open circles = 25 mg/mL Hb + 200 µM SNAP; closed triangles = 25 mg/mL Hb + 200 µM SNAP + 200 µM CeO_2 high Ce^{3+}/PO_4. Graph is representative of 3 or more experiments.

Literature Cited in Supplement

1. **Lim, M. H., B. A. Wong, W. H. Pitcock, Jr., D. Mokshagundam, M. H. Baik, and S. J. Lippard.** 2006. Direct nitric oxide detection in aqueous solution by copper(II) fluorescein complexes. J Am Chem Soc **128:**14364-14373.
2. **Murphy, M. E., and E. Noack.** 1994. Nitric oxide assay using hemoglobin method. Methods Enzymol **233:**240-250.
3. **Patil, S., S. C. Kuiry, S. Seal, and R. Vanfleet.** 2002. Synthesis of nanocrystalline ceria particles for high temperature oxidation resistant coating. J Nanopart Res **4:**433-438.