1 # Supporting Information

2

3 **Experimental Section**

4 **Hydrothermal synthesis of cerium oxide nanorods.** An aqueous solution of NaOH

5 (14 mL, 1M, 5M or 9 M) was directly poured into a rapidly stirred solution of cerium

6 (III) nitrate hexahydrate (2 mL, 0.52 g, 0.4 M) ($Ce(NO_3)_3 \cdot 6H_2O$, 99.95% metals basis,

7 Sigma-Aldrich) in MilliQ-water. After being heavily stirred for 30 min, the lilac

8 suspension was transferred into a 25 mL Teflon-lined stainless-steel autoclave and

9 heated to 100 °C for 24 hours. The lilac product was separated from the turbid

10 suspension by centrifugation (10000 rpm, 10 min), washed five times with MilliQ-

11 water (50 mL each) until the pH reached 7. After drying at 60 °C overnight, the resulting

12 yellow product was carefully hand ground. The obtained CeO_{2-x} nanorods were

13 suspended in MilliQ-water (1 mg/mL) by sonication (a few seconds) and stirred directly

14 at room temperature before use.

15

16 **Transmission electron microscopy (TEM) analysis.** The TEM samples were

17 prepared by dispersing the nanoparticle powders in water, placing a droplet onto a

18 carbon coated copper grid and drying at ambient temperature. The FEI Tecnai 12

19 Biotwin TEM (U.S.A) was used to observe the morphology of the CeO_{2-x} nanorods.

20 The length and width of the CeO_{2-x} nanorods were measured using Image J. For each

21 sample, the total number of nanoparticles counted was 150.

22

23 **Dynamic Light Scattering (DLS).** The surface charge (zeta-potential, mV) of CeO_{2-x}

24 nanorods were determined using a ZetaSizer Nano series Nano-ZS (Zetasizer Nano

25 ZS90, Malvern, UK), performed at 25 °C for samples appropriately diluted in PBS.

26

27 **Powder X-ray diffraction patterns (XRD).** X-ray diffractograms of the samples were

28 obtained with a step size of 0.0025° on a Bruker D8 Advance ECO powder

29 diffractometer (Germany) diffractometer using Cu K_α radiation. The data were

30 collected with a scan rate of 0.02°/s and a 2θ ranging from 20° to 80° at ambient

31 temperature. All peaks of XRD patterns were indexed to a fluorite-type CeO_2 (space

32 group: Fm3m, JCPDS 34-394).

33

34 **X-ray photoelectron spectroscopy (XPS).** Samples were analyzed using a Scienta

35 Omicron ESCA-2SR with operating pressure ca. 1×10^{-9} Torr. Monochromatic Al Kα

36 x-rays (1486.6 eV) were generated at 250W (15 kV; 20mA) with photoelectrons

37 collected from a 2 mm diameter analysis spot. Photoelectrons were collected at a 0°

38 emission angle with source to analyzer angle of 54.7°. A hemispherical analyzer

39 determined electron kinetic energy, using a pass energy of 200 eV for wide/survey

40 scans, and 50 eV for high resolution scans. A flood gun was used for charge

41 neutralization of non-conductive samples. All binding energies were referenced to the

42 binding energy of C (1s, 284.8 eV). Deconvolution of the XPS spectra was carried out
43 using XPSPEAK (version 4.1) software. The complex signals were deconvoluted into
44 10 peaks with respect to physical constrains arising from the final-state occupation of
45 the Ce 4f level, namely $3d_{3/2}$ (910-895eV) and $3d_{5/2}$ (895-875 eV) spin-orbit coupling (3d
46 multiplet splitting assigned to 18.5 ± 0.5 eV), ratio of intensities (3:2), iterative Shirley
47 (background) and a Gauss/Lorenz peak type. The concentrations of Ce^{3+} and Ce^{4+} were
48 calculated by the following equations:

49 $$Concentration\ (Ce^{3+}) = \frac{ACe^{3+}}{ACe^{3+} + ACe^{4+}} \times 100$$

50 $$Concentration\ (Ce^{4+}) = \frac{ACe^{4+}}{ACe^{3+} + ACe^{4+}} \times 100$$

51 *A* is the integrated area of peak according to Ce^{3+} and Ce^{4+}.

52

53 **Phenol red assay.** The catalytic activity of the CeO_{2-x} nanorods was determined by the
54 bromination of phenol red described by Tremel *et al*[1]. For each assay, it contains phenol
55 red (PR, 25 µM), potassium bromide (KBr, 10 mM), Hydrogen peroxide (H_2O_2, 10
56 µM). The assay was performed for 10 min at 37°C in 5 mM MES buffer (pH 6.0) by
57 varying the types of nanorods (R=1, R=4.5, R=9). For each measurement, blank tests
58 and controls were performed, each measurement was carried out 3 times.
59 To get the kinetic parameters, two independent sets of experiments were performed of
60 cerium nanorods (R=1, R=4.5, R=9) in MES buffer (5 mM, pH 6.0 with 25 µM phenol
61 red). (1) H_2O_2 concentration was varied (0-1000 µM) while keeping constant the
62 concentrations of Br^- (10 mM) and CeO_{2-x} (0.04 mg/mL). (2) Br^- concentration was
63 varied (0-80 mM) while keeping constant the concentrations of H_2O_2 (250 µM) and
64 CeO_{2-x} (0.04 mg/mL). To evaluate the kinetic parameters with respect to the Br_4PR
65 concentration, the dA_{592nm}/dt values were converted to the equivalent $d[Br4PR]/dt$
66 values using the Lambert-Beer law with an extinction coefficient of Br_4PR (εBr_4PR)
67 determined to be 72200 $M^{-1}cm^{-1}$. The values were fit to the Michaelis-Menten model
68 and kinetic parameters (K_m, V_{max}) were determined by Lineweaver-Burk linearization.
69

70 **Human cell line and virus culture**. HCT-8 colon epithelial cells (ATCC®CCL-
71 244™) and H-CoV-OC43 (ATCC®VR1558™) were purchased from American Type
72 Culture Collection (ATCC). HCT-8 cells were maintained in Dulbecco's Modified
73 Eagle Medium (DMEM) (Corning, USA) with 10% fetal bovine serum (FBS) (Gibco,
74 USA) and 1% penicillin/streptomycin (Gibco, USA). The cells were grown as
75 monolayers at 37 °C with 5% CO_2. H-CoV-OC43 was grown and propagated in HCT-
76 8 cells at 33 °C with 5% CO_2 in DMEM with 2% FBS and 1% penicillin/streptomycin.
77 The incubation temperature, i.e., 33°C, was chosen as it reflects the physiological
78 condition of the nasopharynx, where the virus OC43 was discovered[2]. This incubation
79 condition has been validated by the American Type Culture Collection (ATCC, see
80 https://www.atcc.org/products/vr-1558), which considered 33°C to be ideal for the
81 replication of OC43 and its infectivity assays. Infected cells were lysed 8 days post-

82 infection by two freeze-thaw cycles. The virus-containing fluid was cleared by
83 centrifugation, aliquoted and stored at -80 °C.

84

85 **50% tissue culture infection dose (TCID$_{50}$).** Virus titers were tested by TCID$_{50}$ assay.
86 HCT-8 cells (2 x10^4 cells/well) were cultured in 96-well plates. Suspensions of OC43
87 (10$^{6.5}$TCID50/mL) were incubated with different formulations for 15 minutes. The
88 CeO$_{2-x}$ nanorods were removed by centrifuge at 5000 g for 10 minutes. The incubated
89 virus suspensions were subsequently collected and then were serially diluted (in 10-
90 fold steps) from 10^{-2} to 10^{-10} and inoculated into confluent HCT-8 cell monolayers and
91 incubated under 33 °C for 2 hours. After 2 hours, the monolayer was rinsed with PBS,
92 overlaid with medium (2% FBS in DMEM) and incubated at 33 °C. Viral cytopathic
93 effects were examined at day 6 using an inverted microscope. The final titer was
94 calculated as described previously.[3]

95

96 **Immunofluorescence Assay (IFA).** For the IFA assay, HCT-8 cells (5 x10^4 cells/well)
97 were cultured into a 3.5-mm glass bottom dish (Cellvis). Suspensions of OC43
98 (10$^{6.5}$TCID50/mL) were incubated with different formulations for 15 minutes. The
99 CeO$_{2-x}$ nanorods were removed by centrifuge at 5000 g for 10 minutes. The incubated
100 virus suspensions were subsequently collected and then infected with HCoV-OC43 at
101 a multiplicity of infection (MOI) of 0.5 and incubated at 33°C for 48 hours. After 48
102 hours, HCT-8 cells were fixed with 4% paraformaldehyde (PFA). The fixed cells were
103 washed twice with PBS and permeabilized with 0.5% Triton X-100 for 10 minutes at
104 room temperature. Afterwards, samples were blocked for 45 mins at room temperature
105 in 5% goat serum in PBS and incubated for overnight at 4 °C with primary anti-HCoV-
106 OC43 S antibodies (Cusabio, diluted 1:500 into PBS containing 3% goat serum).
107 Subsequently, samples were incubated for 1 hour with Alexa Fluor 568-labeled goat
108 anti-rabbit IgG (Thermo Fisher Scientific, diluted 1:1000 in PBS). Nuclei were stained
109 with Hoechst 33258 (Thermo Fisher Scientific) for minutes. After immunostaining, in
110 all the cases, cells were washed with 0.5% TWEEN-20 in PBS. Finally, stained cultures
111 were mounted on glass slides in ProLong Diamond antifade medium (Thermo Fisher
112 Scientific) and stored at 4 °C. The infection rate (%) was calculated by the following
113 equation. For each sample, cells were counted in five different fields.

114 % of infected cells $= \dfrac{OC\ 43\ positive\ cell\ number}{Total cell number (Hoechst)} \times 100$

115

116 **Cell Counting Kit-8 for Cytotoxicity studies.** HCT-8 cells were seeded in 96-well
117 plates (Corning Costar, USA) at a density of 1×10^4 cells/well and overnight at 33 °C
118 in a humidified 5% CO$_2$-containing atmosphere. Cells were then exposed to the CeO$_{2-}$
119 $_x$ nanorods or formulations containing CeO$_{2-x}$ nanorods (of various concentrations in
120 the range of 0.005 to 0.32 mg/mL), 1 mM Br$^-$, and 10 mM H$_2$O$_2$ for 72 hours. Viability
121 of the treated or untreated cells were quantified using a cell counting kit-8 (CCK-8 kit;
122 Dojindo Molecular Technologies, Japan). After incubating cells with the CCK-8 kit

123 reagents for 1-2 hours, the absorbance of each well at 450 nm was measured. Empty
124 wells with CCK-8 assay reagents only were used as blanks and were subtracted from
125 the final reading. Relative cell viability was calculated by normalizing the absorbance
126 readings using that of untreated cells. All assays were carried out in quintuplicate.
127
128 **LIVE/DEAD staining for Cytotoxicity studies.** Once seeded HCT-8 cells in 96 well
129 plates (Corning Costar, USA) at a density of 1×10^4 cells/well, the CeO_{2-x} nanorods or
130 formulations containing CeO_{2-x} nanorods (of various concentrations in the range of
131 0.005 to 0.32 mg/mL), 1 mM Br⁻, and 10 mM H_2O_2 were introduced into the cells and
132 then incubated for 24 hours. A LIVE/DEAD Viability/Cytotoxicity Kit (Molecular
133 Probes, Invitrogen, USA) was used to test the cell survival rate. Cells were then
134 incubated with calcein-AM (acetoxymethyl) (1 mM) and ethidium homodimer-1 (2
135 mM) for 30 min at 37°C to label live and dead cells, respectively. Images of cells were
136 taken by using confocal microscopy and the numbers of live and dead cells were
137 counted by using ImageJ. Cell survival rate (%) was then calculated as Live Cells/(Live
138 Cells + Dead Cells) × 100.
139
140 **Lactate dehydrogenase (LDH) assay for Cytotoxicity studies.** HCT-8 cells were
141 seeded in 96 well plates (Corning Costar, USA) at a density of 1×10^4 cells/well and
142 cultured overnight. The LDH toxicity assay kit (Cayman Chemical, USA) was used as
143 an indicator of cell membrane integrity and thus a measurement of cytotoxicity. In
144 short, Triton X-100 and Assay Buffer were added into wells in triplicate as a group of
145 maximum release and a group of spontaneous release according to the manufacture's
146 specifications. In addition, the CeO_{2-x} nanorods or formulations containing CeO_{2-x}
147 nanorods (of various concentrations in the range of 0.005 to 0.32 mg/mL), 1 mM Br⁻,
148 and 10 mM H_2O_2 were introduced into the cells as a testing group. The plate was
149 incubated at 37°C for 12 hours. After that, 100 µL of cell supernatant in each well was
150 transferred to a new 96-well plate containing 100 µL of LDH Reaction Solution in each
151 well. The new plate was then incubated with gentle shaking for 30mins at 37°C. The
152 final absorbance was measured at 490 nm. All experiments were performed in
153 quintuplicate. The % cytotoxicity was calculated as [(Experimental Value A490 -
154 Spontaneous Release A490)/(Maximum Release A490 - Spontaneous Release
155 A490)]*100.
156
157 **Statistical Analysis.** Data were analyzed using GraphPad Prism 7.0 software and
158 presented as mean ± SD. Student's t test or one-way ANOVA followed by Tukey's
159 HSD test was applied for comparisons between two groups or among multiple groups.
160
161

References

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2. T. Keck, R. Leiacker, H. Riechelmann and G. Rettinger, *Laryngoscope*, 2000, **110**, 651-654.

3. M. Lock, M. Korn, J. Wilson, M. Sena-Esteves and G. Gao, *Cold Spring Harb. Protoc.*, 2019, **2019**.
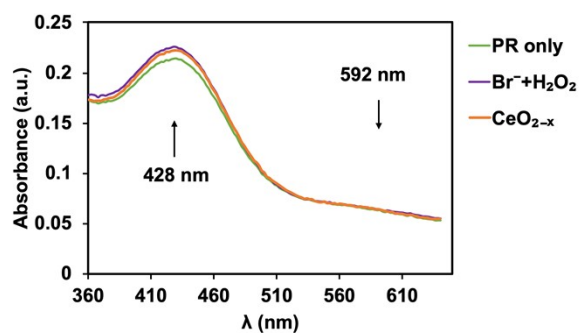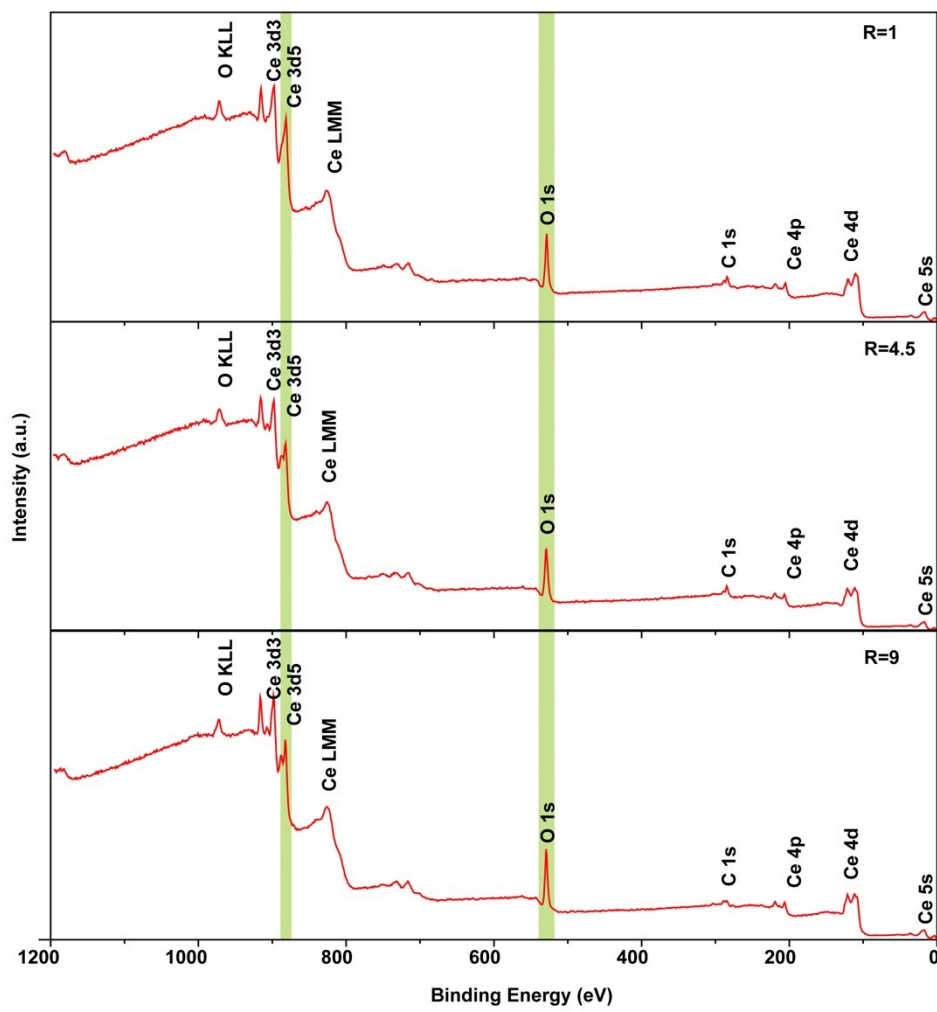
Supporting Information

Figures
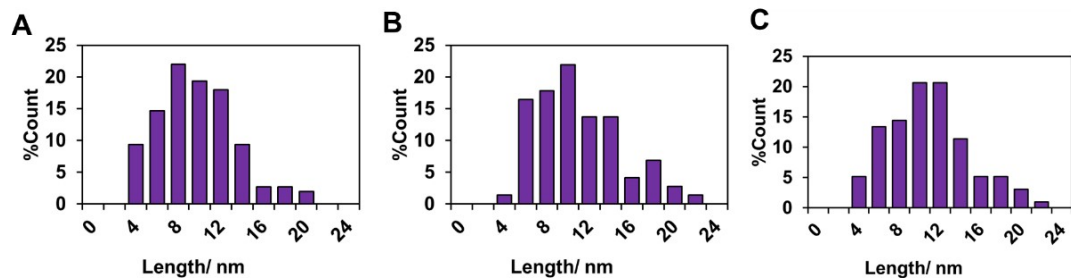
Fig. S1. Width distribution of CeO$_{2-X}$ nanorods based on the TEM image analysis.

A) R=1, B) R=4.5, C) R=9. The total number of nanoparticles counted was 150.

Fig. S2. UV-vis spectra of phenol red bromination assay.

Fig. S3. XPS survey spectra of CeO$_{2-X}$ nanorods. The Ce 3d and O 1s were highlighted (green).

Tables.

Table S1. Size distributions and zeta potential of the CeO$_{2-x}$ nanorods/nanotubes with different aspect ratios.

Ratio (R = Length/width)	R=1	R=4.5	R=9
Length (nm)	7.5 ± 2.5	46.0 ± 17.9	86.9 ± 40.1
Width (nm)	11.2 ± 4.7	11.9 ± 4.0	12.0 ± 2.5
Zeta potential (mV)	-14.6 ± 0.6	-13.6 ± 0.6	-13.6 ± 2.0

Table S2. Content of the Ce(III) and Ce(IV) surface sites in the CeO$_{2-x}$ nanorods/nanotubes.

	% Ce(III)	% Ce(IV)
R=1	33.4	66.6
R=4.5	47.1	52.8
R=9	33.2	66.8

Table S3. The kinetic parameters for the CeO$_{2-x}$ nanoparticles.

R ratio	Substrate	K_m (mM)	V_{max} (mM min^{-1})
R=1	Br$^-$	206.9 × 10^3	3.35
	H$_2$O$_2$	408.8	1.21
R=4.5	Br$^-$	17.9 × 10^3	2.53
	H$_2$O$_2$	35.0	2.21
R=9	Br$^-$	13.54 × 10^3	1.42
	H$_2$O$_2$	41.81	0.96

Parameters obtained using the Lineweaver-Burk linearization.