Electronic Supplementary Information (ESI)

Engineering a hierarchically micro-/nanostructured Si@Au-based artificial enzyme with improved accessibility of active sites for enhanced catalysis

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1. Additional Experimental Section

1.1. Characterization

The Ultraviolet-visible (UV-Vis) absorption spectra were recorded using a P4 UV-Vis spectrophotometer. Fourier transform infrared (FTIR) spectroscopy was carried out on a Bruker Tensor 27 FTIR spectrometer. A field emission scanning electron microscope (SEM) (JSM 7800F Prime) was applied to determine the morphology of as-prepared microspheres. The SEM samples were prepared by depositing a dilute ethanol dispersion of the as-prepared microspheres onto the surface of a silicon wafer. Transmission electron microscopy (TEM) (JEM-2100F) equipped with an energy-dispersive X-ray spectrum was applied to determine the structural and chemical characteristics of the as-prepared microspheres. TEM images and high-angle annular dark-field scanning TEM (HAADF-STEM) were recorded using a JEM-2100F high-resolution transmission electron microscope operating at 200 kV. The TEM samples were prepared by dispersing synthesized products in ethanol by sonication and then depositing onto a holey carbon film supported by copper grids. X-ray photoelectron spectra (XPS) were collected on a Kratos Axis Ultra X-ray photoelectron spectrometer (Thermo Scientific ESCALAB Xi+). The crystalline structures of the as-prepared microspheres were evaluated by X-ray diffraction (XRD) analysis on an Ultima IV X-ray diffractometer with Ni-filtered Cu Kα radiation. The operation voltage and current were kept at 40 kV and 40 mA. N_2 adsorption-desorption isotherms were recorded on a Micromeritics 3Flex automated sorption analyzer. The pore size was determined following the Barrett-Joyner-Halenda (BJH) method.

1.2. Fabrication of smooth silica microspheres with low surface thiol density using a post-grafting method

Smooth silica microspheres (SSM) were prepared according to the previously reported method with slight modifications.[1] Briefly, 12.8 mL of ethanol and 2.3 mL of ammonium hydroxide (28%) were added sequentially to 5 mL of water. After stirring for 30 min, 2.26 mL of tetraethyl orthosilicate was added dropwise over 1 min. The reaction was stirred at room temperature for 24 h. The products were collected by

centrifugation at 5000 rpm for 3 min and washed three times with ethanol and deionized water to remove the residual reaction products, respectively. The obtained pure solid product was then put into a vacuum drying oven for 12 h at 37 °C to finally obtain 0.55 μm smooth silica microspheres.

The sample (20 mg) was dispersed in toluene (15 mL) under nitrogen. After stirring for 1 h at 110 °C, a certain amount of MPTMS was added and the mixture was refluxed for 18 h under nitrogen.[2] The product was centrifuged, washed with toluene and ethanol three times, and then dried under vacuum at 50 °C. The sample prepared by the post-grafting method was called smooth silica microspheres low surface thiol density (SSM-L).

1.3. Fabrication of smooth silica microspheres with high surface thiol density using the one-pot synthesis without CTAB

Smooth silica microspheres with high surface thiol density (SSM-H) were prepared according to the previously reported method with slight modifications.[3] Briefly, 0.25 g of PVA is rapidly dissolved in 5 mL of water by sonication. Keeping the stirring speed constant, 8 mL of methanol and 2 mL of ammonia (5.6%) were added sequentially to the solution. After stirring for 15 min, a certain amount of MPTMS was added dropwise over 15 s. The reaction was stirred at room temperature for 24 h. The products were collected by centrifugation at 5000 rpm for 3 min and washed three times with ethanol and deionized water to remove the residual reaction products, respectively. The obtained pure solid product was then put into a vacuum drying oven for 12 h at 37 °C to finally obtain smooth silica microspheres with high surface thiol density (SSM-H).

1.4. Ellman's assay

The Ellman reagent method is a typical technical method used to measure the surface density of sulfhydryl groups.[4] In a typical procedure, $DTNB^{2-}$ solution was made by dissolving sodium acetate (41 mg) and $DTNB^{2-}$ (7.96 mg) in water (10 mL). To prepare the blank sample, $DTNB^{2-}$ solution (100 μL) was added to Tris buffer solution (200 μL, 1 M, pH 8) and then diluted in water (1700 μL). The nanoparticle solution (20 μL, 2.5 mg mL^{-1}) and $DTNB^{2-}$ solution (100 μL) were added to Tris buffer solution (200 μL, 1 M, pH 8) and then diluted in water (1680 μL), mixed carefully using a pipette, and then

incubated at room temperature for 5 min. The mixture was centrifuged and the absorbance of the supernatant was measured at 412 nm. The quantity of the surface thiol groups was calculated by dividing absorbance by the extinction coefficient of the reagent (13600 $M^{-1}cm^{-1}$).

1.5. Peroxidase activity assessment of Au-containing system

To study the peroxidase like study of Au-containing system for TMB oxidation, we used a total of 2 mL solution and all the absorption measurements were carried out using P4 UV-Visible spectrophotometry. In a 5 ml centrifuge tube, we separately added (1 mL) aqueous solution of Au-containing system (SoS-0.55, SSM-L@Au-7.8, SSM-H@Au-7.6 and SoS-0.55@Au-7.3). (500 µL) H_2O_2 and (500 µL) TMB were added to the centrifuge tube and react for 2 minutes. Afterwards, the reaction solution was centrifuged for 1 minute and the reaction solution was aspirated for UV spectroscopy. Control samples were made by replacing the Au-containing system with DI water.

1.6. Peroxidase activity assessment of SoS-0.55@Au-s

The peroxidase-like activity of the synthesized SoS-0.55@Au-s was measured based on the steady-state kinetic study. The catalytic ability of SoS-0.55@Au-s was measured by P4 UV-Visible spectrophotometry. All assays were carried out at room temperature in quartz cuvettes [path length (l) = 1.0 cm] using sodium acetate buffer (100 mM, pH 4) as the reaction buffer. The absorbance of the produced blue color at 652 nm represents the released TMB diimine product.[5] In a typical experiment, SoS-0.55@Au-s (400 µg) was dispersed in sodium phosphate (1000 µL) and then placed in the cuvette. H_2O_2 (500 µL) and TMB (500 µL) were added to the cuvette and the increase of absorbance at 652 nm was immediately measured as a function of time (5 s intervals) for 2 min. The relative activity was defined as the absorbance of SoS-0.55@Au-s to that of SoS-0.55-@Au-7.3.

1.7. Estimation of kinetic parameters

To measure the kinetic parameters for SoS-0.55@Au-7.3, kinetic assays were conducted using SoS-0.55-@Au-7.3 (40 µg) dispersed in sodium phosphate (200 µL) and H_2O_2 (final concentration of 50 mM) as the reaction media at 35 °C. Various concentrations of TMB were added to the cuvette and the absorbance at 652 nm was

instantly measured as a function of time with intervals of 5 s for 2 min. The obtained "absorbance versus time" plots were then used to calculate the slope at the initial point ($slope_{initial}$) of each reaction. The initial reaction velocity (v) was calculated by dividing $slope_{initial}$ by ($\varepsilon_{TMB\text{-}652nm} \times l$), where $\varepsilon_{TMB\text{-}652nm}$ represents the molar extinction coefficient of TMB at 652 nm which equals to 3.9×10^4 M^{-1} cm^{-1}.[6]

Nonlinear regression of the Michaelis-Menten equation [$v = V_{max} \times [S] / (K_m + [S])$] was used to fit the plots of v against TMB concentrations ([S]). Lineweaver-Burk plot was created from the Michaelis Menten equation to obtain the kinetic parameters, K_m and V_{max}. V_{max} and K_m are the highest reaction rate and the Michaelis constant, respectively.[7]

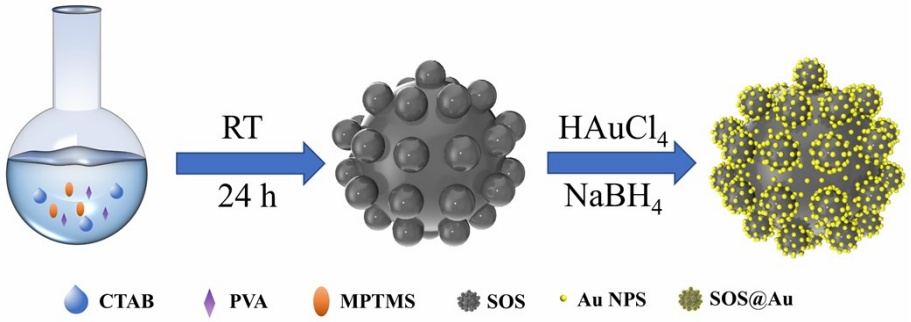
1.8. Reusability assay

The reusability of SoS-0.55@Au-7.3 was studied by repeating the use of artificial enzymes to catalyze the oxidation reaction of TMB. After each cycle (2 min), the absorbance was read and the artificial enzymes were then separated from the reaction system by centrifugation, washed three times with buffer solution to remove all of the substrate and products from the samples, and applied in the next activity measurement with fresh substrates. The relative activity was defined as the observed absorbance of each cycle to the absorbance of the first cycle.

1.9. H_2O_2 detection assay

Various concentrations of H_2O_2 in sodium acetate buffer (100 mM, pH 3) were reacted with 200 μg mL^{-1} SoS-0.55@Au-7.3 and 1 mM TMB in a total volume of 200 μL. After incubated at 40 ℃ for 15 min, the reaction mixture was treated as the previous description.

2. Additional Scheme



Scheme S1 The schematic representation of the synthesis SoS@Au. The illustration is not drawn to scale.

3. Additional Figures

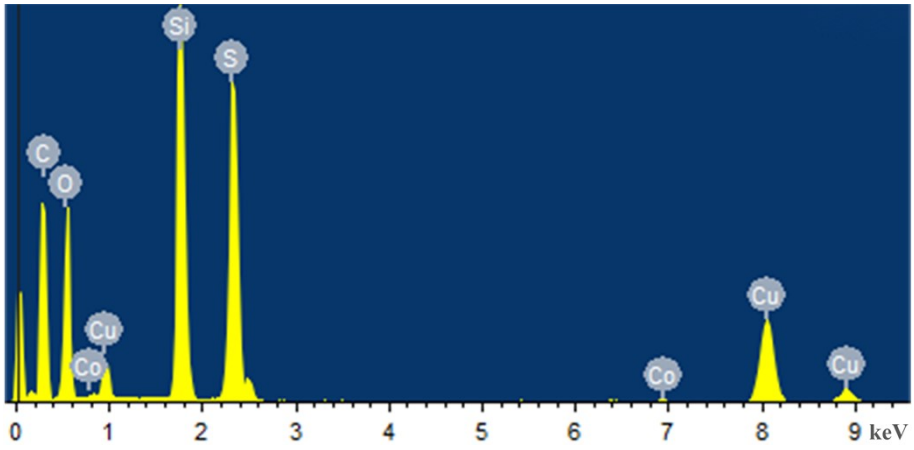


Fig. S1 The EDS spectra of SoS silica microspheres.

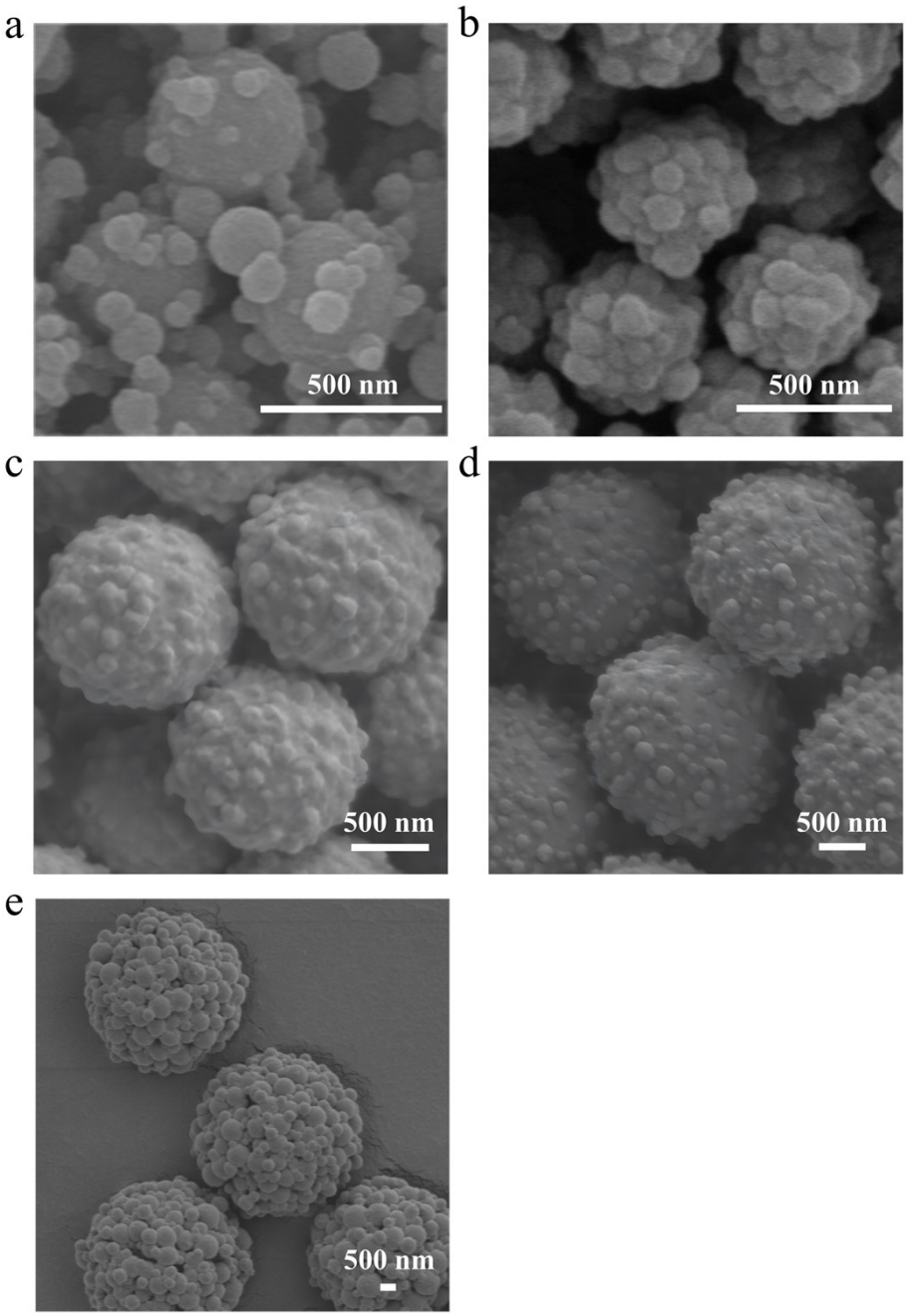
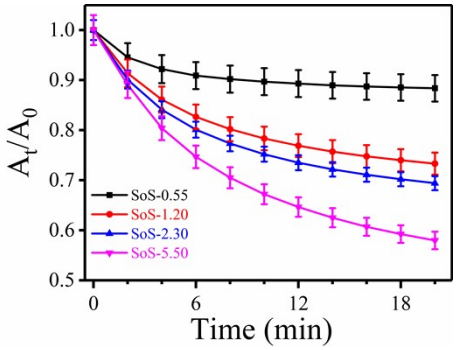


Fig. S2 The effect of the MPTMS concentration on the size and morphology of SoS silica microspheres: the SEM images of the SOS silica microspheres prepared using 9.1 mM MPTMS (a), 12.6 mM MPTMS (b), 36.1 mM MPTMS (c), 72.2 mM MPTMS (d), and 180.5 mM MPTMS (e).



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146 **Fig. S3** The relative absorbance (A_t/A_0) of SoS-X (X = 0.55, 1.20, 2.30, and 5.50 μm)
147 silica microspheres dispersed in water as a function of settling time, where A_0 and A_t
148 represent the absorption values of the microsphere solution after settling for 0 and t
149 min.

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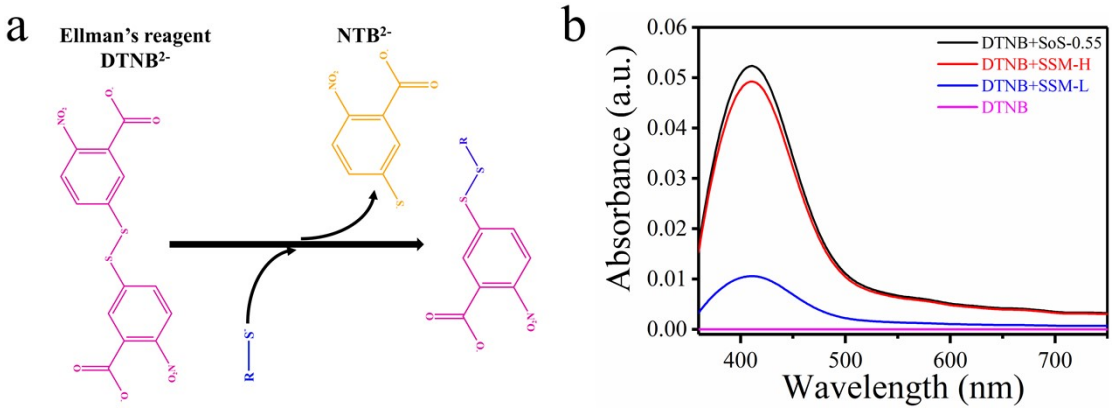
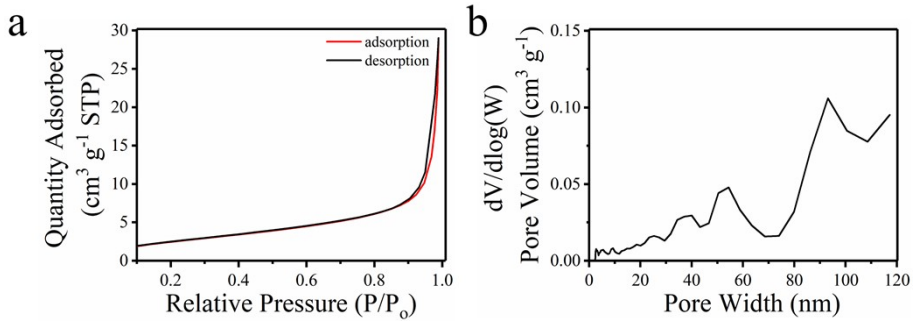


Fig. S4 (a) Schematic diagram of the Elman test method for the determination of thiol groups and (b) the absorbance spectra of the reaction product NTB^{2-} of $DTNB^{2-}$ in different reaction systems.



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157 **Fig. S5** (a) N_2 sorption isotherms and (b) the pore size distribution curve of SoS-0.55

158 silica microspheres.

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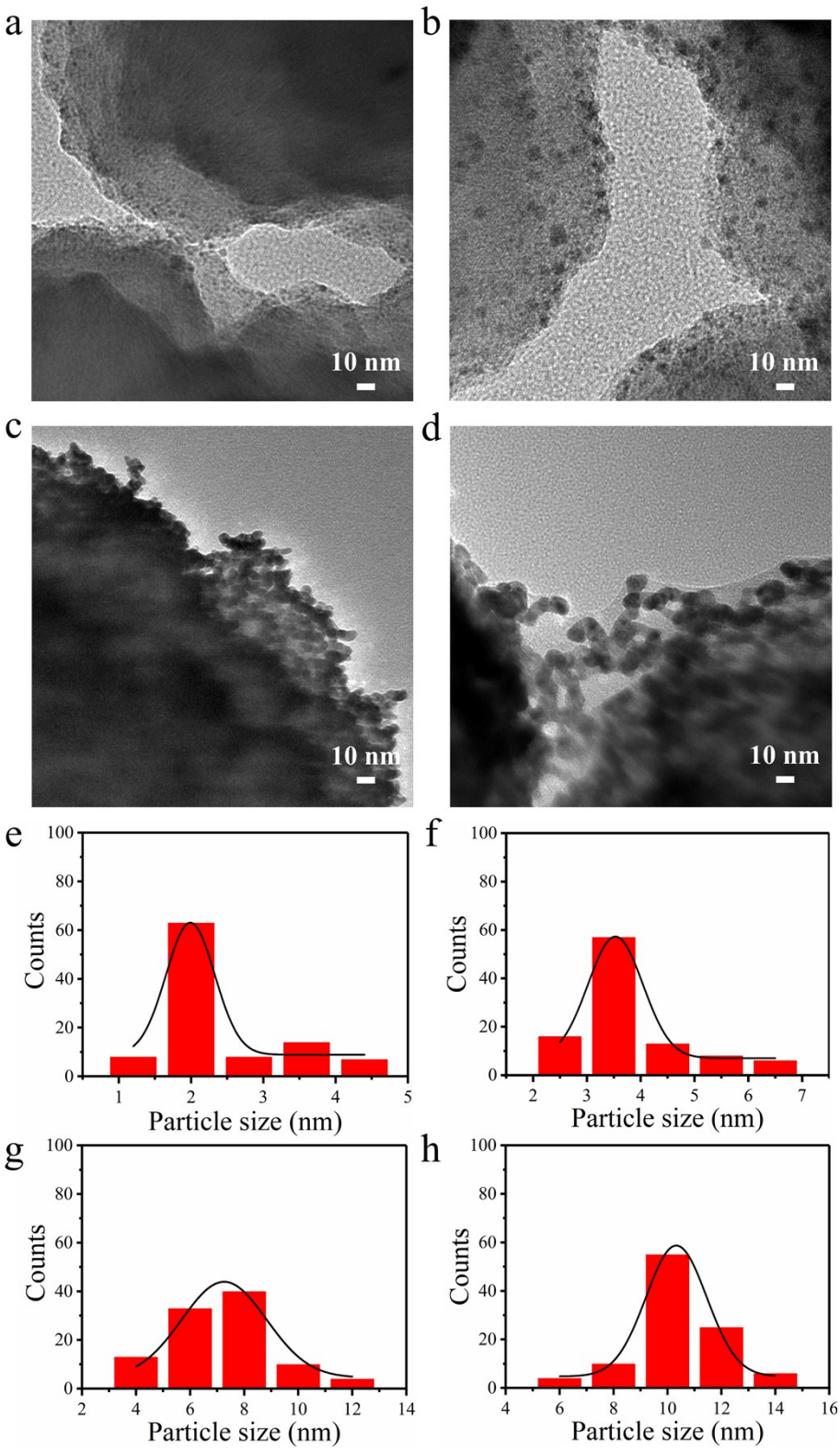


Fig. S6 (a-d) Selected high-magnification TEM images of SoS-0.55@Au-s (s = 2.1, 3.5, 7.3 and 10.3 nm), the scale bar is 10 nm. (e-h) The size distribution histogram of SoS-0.55@Au-s, the total number of AuNPs counted for the histogram was 100.

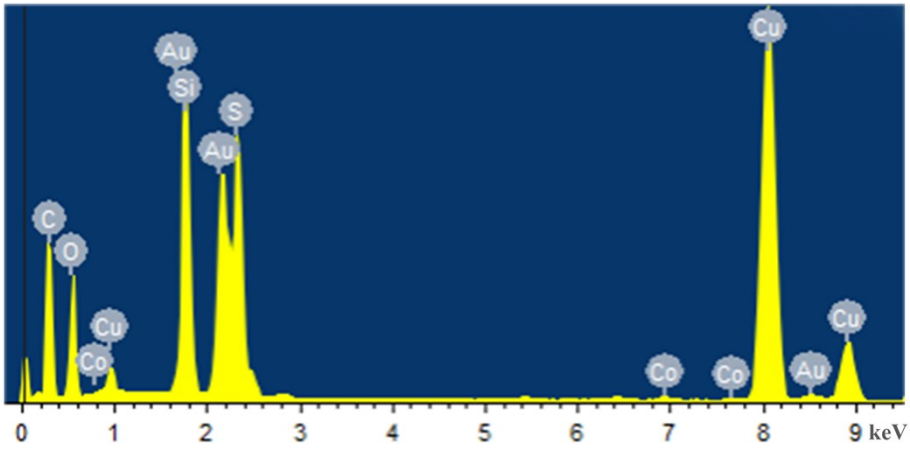
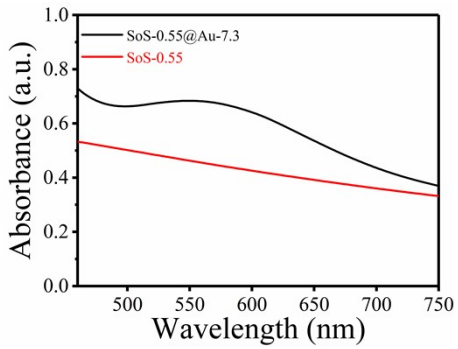


Fig. S7 The EDS spectra of SoS-0.55@Au-7.3.



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168 **Fig. S8** UV-Vis absorption spectra of SoS-0.55@Au-7.3 (black curve) and SoS-0.55

169 silica microspheres (red curve).

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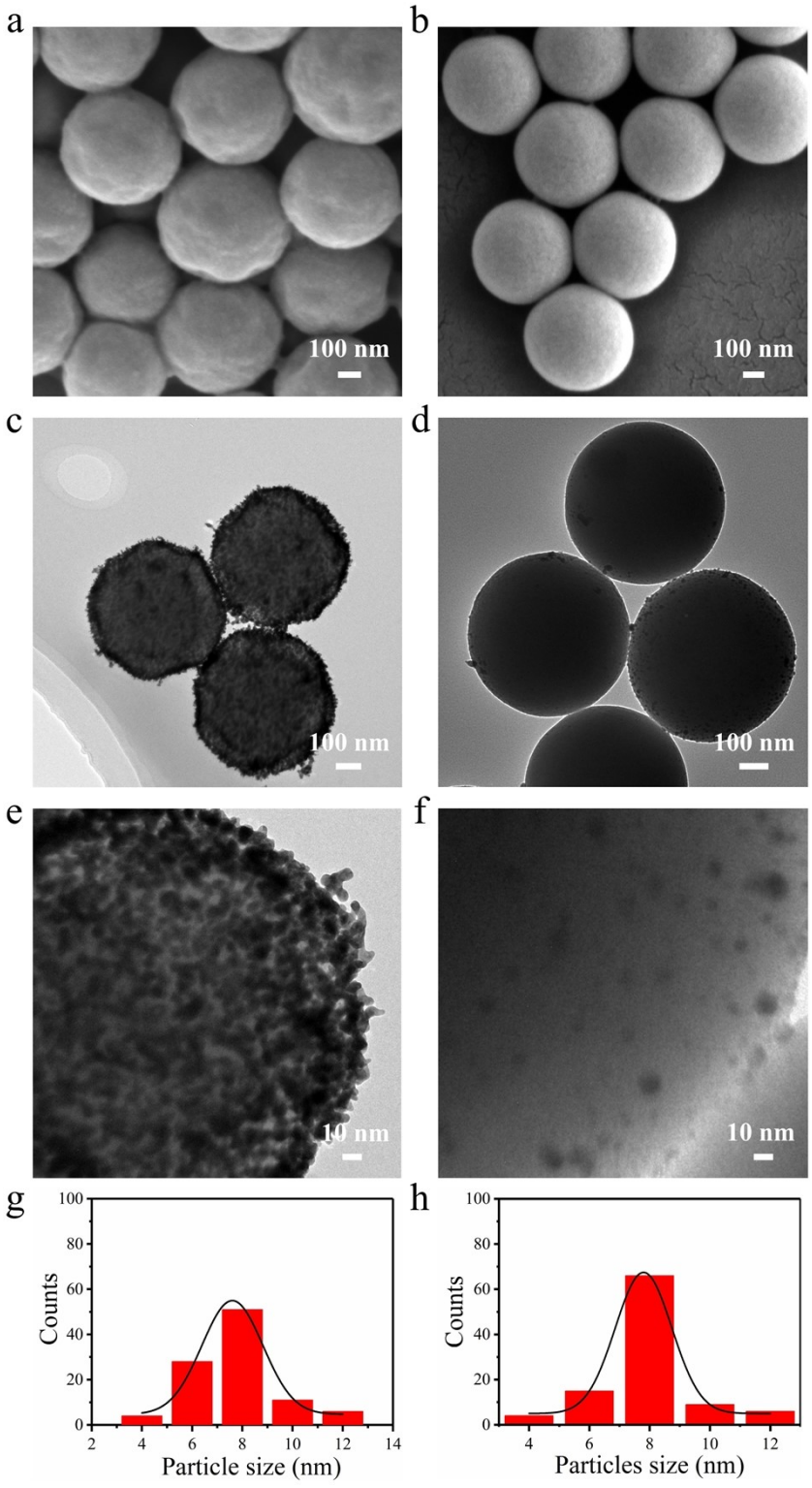
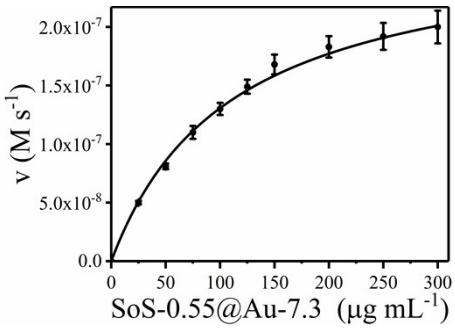


Fig. S9 SEM images of SSM-H (a) and SSM-L (b). TEM images of SSM-H@Au-7.6 (c) and SSM-L@Au-7.8 (d). High-resolution TEM images of SSM-H@Au-7.6 (e) and SSM-L@Au-7.8 (f). The size distribution histogram of SSM-H@Au-7.6 (g) and SSM-L@Au-7.8 (h). The total number of AuNPs counted for the histogram was 100.



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177 **Fig. S10** The rates of TMB oxidation by H_2O_2 using variable concentrations of SoS-

178 0.55@Au-7.3 in the presence of 0.5 mM TMB and 50 mM H_2O_2: 0, 25, 50, 75, 100,

179 125, 150, 200, 250, 300 μg mL^{-1}, the total volume of the reaction mixture: 2 mL. All

180 the experiments were conducted in sodium acetate buffer (100 mM, pH 4).

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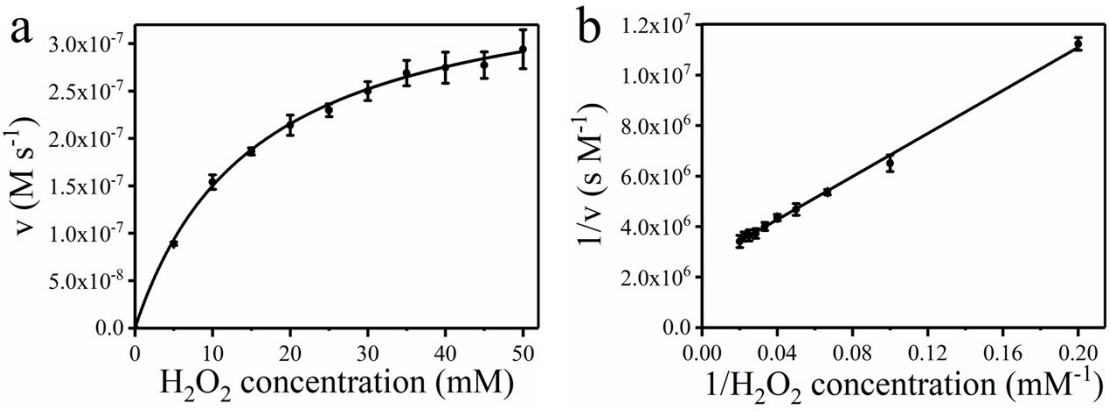

Fig. S11 (a) Steady-state kinetic assay of SoS-0.55@Au-7.3. Experiments were performed in 0.1 M sodium acetate buffer (pH 4) with 200 μg mL^{-1} SoS-0.55@Au-7.3, 0.05 mM TMB and varied concentrations of H_2O_2 at 35 °C. (b) Lineweaver-Burk plot of the inverse of the initial rate vs. the inverse of the substrate concentration for estimating the kinetic parameters (K_m and V_{max}) of SoS-0.55@Au-7.3 using H_2O_2 as the substrate.

4. Additional Tables

Table S1. Detailed synthesis parameters for SoS-X silica microspheres and SSM-H.

Samples	MPTMS	PVA	CTAB	5.6% $NH_3{\cdot}H_2O$	Size
SoS-0.50	9.1 mM	0.25 g	0.2 g	2 mL	0.50 μm
SoS-0.55	12.6 mM	0.25 g	0.2 g	2 mL	0.55 μm
SoS-1.20	36.1 mM	0.25 g	0.2 g	2 mL	1.20 μm
SoS-2.30	72.2 mM	0.25 g	0.2 g	2 mL	2.30 μm
SoS-5.50	180.5 mM	0.25 g	0.2 g	2 mL	5.50 μm
SSM-H	12.6 mM	0.25 g	0 g	2 mL	0.55 μm

Table S2. Partial elemental content of SoS-0.55@Au-s (s = 2.0, 3.5, 7.3 and 10.3 nm), SSM-H@Au-7.6 and SSM-L@Au-7.8.

Samples	Si	S	Au
SoS-0.55@Au-2.0	11.08 wt %	5.82 wt %	1.97 wt %
SoS-0.55@Au-3.5	10.48 wt %	6.23 wt %	8.06 wt %
SoS-0.55@Au-7.3	11.38 wt %	6.44 wt %	14.50 wt %
SoS-0.55@Au-10.3	10.91 wt %	6.83 wt %	20.47 wt %
SSM-H@Au-7.6	11.26 wt %	6.15 wt %	11.28 wt %
SSM-L@Au-7.8	25.19 wt %	1.22 wt %	2.61 wt %

Table S3. Detailed synthesis parameters for SoS-0.55@Au-s (s = 2.0, 3.5, 7.3 and 10.3 nm), SSM-H@Au-7.6 and SSM-L@Au-7.8.

No.	Samples	Dispersing solvent (DI water)	$HAuCl_4$ (100 mM)	$NaBH_4$ (100 mM)
1	SoS-0.55@Au-2.1	14.4 mL	0.1 mL	0.5 mL
2	SoS-0.55@Au-3.5	13.2 mL	0.3 mL	1.5 mL
3	SoS-0.55@Au-7.3	12.0 mL	0.5 mL	2.5 mL
4	SoS-0.55@Au-10.3	10.8 mL	0.7 mL	3.5 mL
5	SSM-H@Au-7.6	12.0 mL	0.5 mL	2.5 mL
6	SSM-L@Au-7.8	12.0 mL	0.5 mL	2.5 mL

203 **Table S4.** Comparison of kinetic parameters of HRP and various materials.

Nanozyme	K_m（mM）	V_{max}（M s^{-1}）	Ref.
HRP[6]	0.041	4.3×10^{-8}	*ACS Nano* **2012,** *6*, 3142-3151.
Ferromagnetic nanoparticles[8]	0.098	3.44×10^{-8}	*Nat. Nanotechnol.* **2007,** *2*, 577-583.
Graphene oxide[9]	0.024	3.45×10^{-8}	*Adv. Mater.* **2010,** *22*, 2206-2210.
Cobalt nanoflakes[10]	2.020	4.74×10^{-8}	*Anal Bioanal Chem* **2017,** *409*, 4225-4232.
C-Dot[11]	0.039	3.61×10^{-8}	*Chem. Commun.* **2011,** *47*, 6695-6697.
Mesoporous silica-supported gold nanoparticles[5]	0.041	12.7×10^{-8}	*Adv. Mater.* **2015,** *27*, 1097-1104.
Dendritic fibrous nano-silica supported gold nanoparticles[12]	0.220	17.1×10^{-8}	*J. Mater. Chem. B* **2018,** *6*, 1600-1604.
T-DMSNs@Au[13]	0.0407	25.9×10^{-8}	*ACS Appl. Mater. Interfaces* **2019,** *11*, 13264-13272.
SoS-0.55@Au-7.3	0.033	34.6×10^{-8}	This work

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206 **Table S5.** Comparison of different materials for H_2O_2 detection.

Nanozyme	Reaction substrate	Detection limit of H_2O_2 (μM)	Ref.
α-$AgVO_3$ microrods[14]	TMB	2.0	*Microchim. Acta* **2017,** *185*, 1-8.
$Co_3(PO_4)_2$•8 H_2O[15]	TMB	4.4	*Colloids Surf., A* **2022,** *647*, 129031-129038.
CeO_2/NT-TiO_2@0.1[16]	TMB	3.2	*ACS Appl. Mater. Interfaces* **2015,** *7*, 6451-6461.
N-doped TiO_2[17]	TMB	2.5	*J. Colloid Interface Sci.* **2017,** *505*, 1147-1157.
GO-PtNi[18]	TMB	5.0	*Mater. Today Chem.* ***2018,*** *7,* 35-39.
SoS-0.55@Au-7.3	TMB	1.6	This work

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