Supporting information

Spheres-on-Sphere Silica Microspheres as Matrix for Horseradish Peroxidase Immobilization and Detection of Hydrogen Peroxide

Zhen Lei,[a,c] Xia Liu,[a,c] Lina Ma,[a] Dianjun Liu,[a] Haifei Zhang*[b] and Zhenxin Wang*[a]

[a]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, P. R. China.

[b]Department of Chemistry, University of Liverpool, Oxford Street, Liverpool, L69 7ZD, UK.

[c]University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing, 100049, P. R. China.

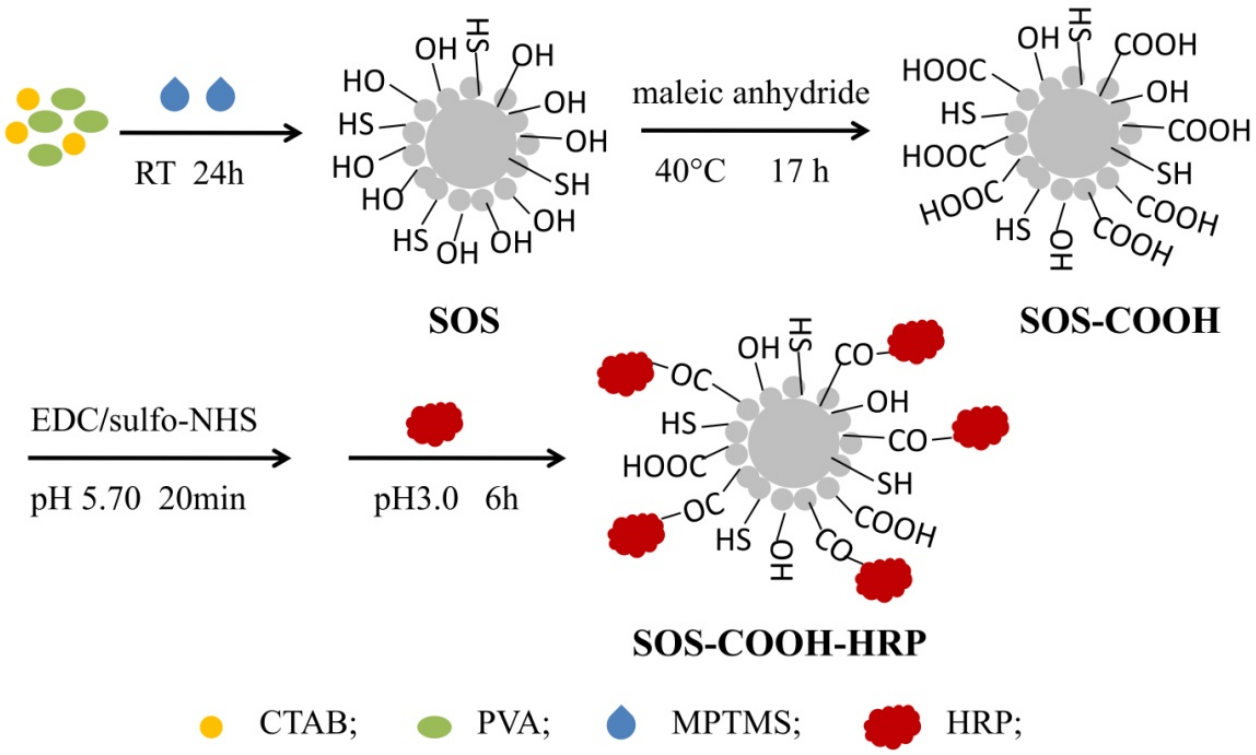
CORRESPONDING AUTHOR INFORMATION

E-mails: Zhanghf@liverpool.ac.uk (HZ) and wangzx@ciac.ac.cn (ZW).

Additional experimental section

Preparation of human serum sample. The serum sample was freshly prepared before use by literature reported methods.[S1,S2] Generally, frozen human plasma was thawed in a 37° C water bath. Then, 0.43 μL recombinant tissue factor (rTF) solution (11.6 nM), 4 μL procoagulant phospholipids (PL) solution (1mM) and 17 μL $CaCl_2$ solution (17 mM) were added in this order into 1 mL human plasma and mixed thoroughly. After incubation at 37° C for 30 min, the mixture was centrifuged at 4500 rpm for 10 min at 37° C. The supernatant (serum) was taken out, and diluted with PBS buffer (100 mM, pH 7.0) for subsequent experiment.

Additional Schemes S1



Scheme S1. The schematic representation of the synthesis SOS-COOH-HRP. The illustration is not drawn to scale.

Additional Figures S1-S4

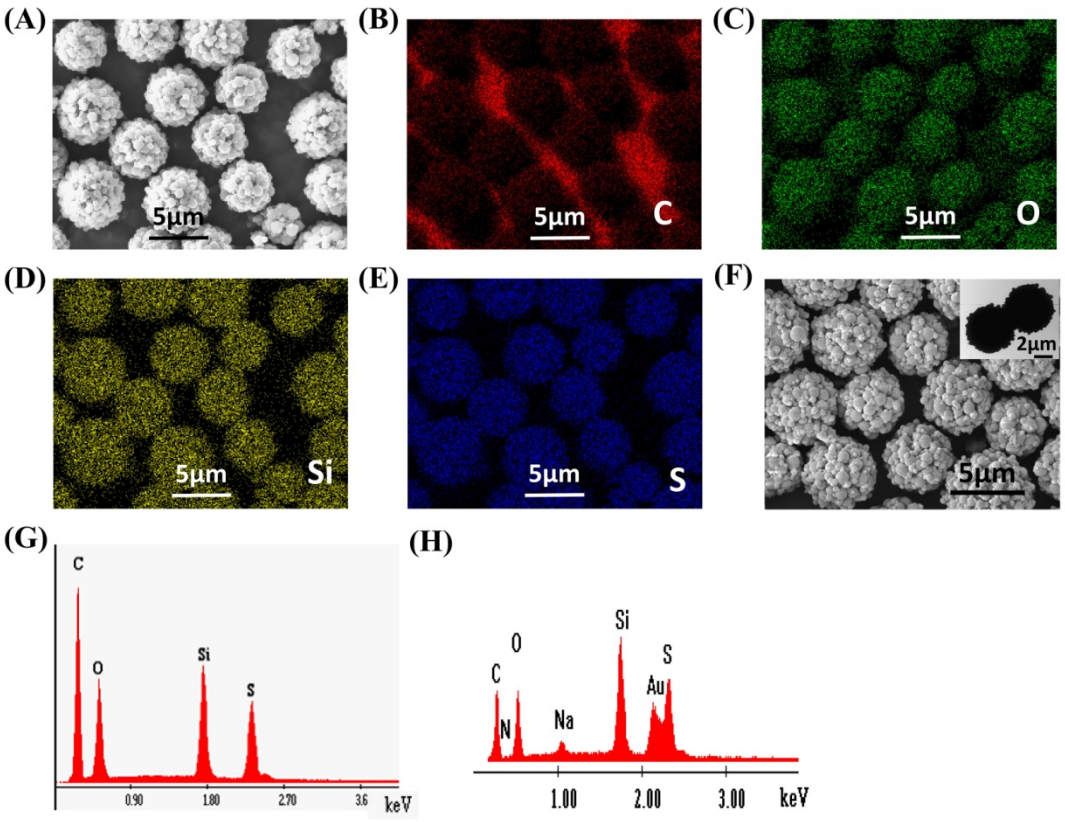


Figure S1. SEM image of the as-prepared SOS-COOH microspheres (A) and corresponding EDX mapping images (B, C, D, E); (F) SEM and TEM (inset of F) micrographs of the SOS-COOH-HRP, Energy–dispersive X-ray analysis (EDX) of the SOS-COOH microspheres (G) and the SOS-COOH-HRP (H).

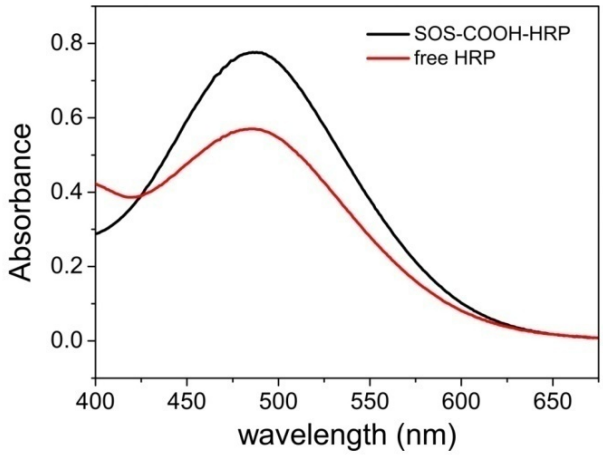


Figure S2. UV-Visible spectra of the catalytic oxidation of PSA and 4-AAP in the presence of H_2O_2 by the same concentration of free HRP and SOS-COOH-HRP under the same reaction conditions.

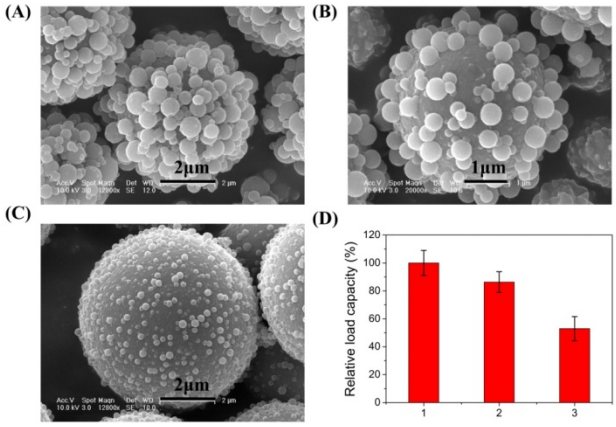


Figure S3. SEM images of three SOS-COOH microspheres with different nanospheres coverage on the surface (A, B, C). (D) Relative HRP load capacity of the three SOS-COOH microspheres; and the numbers 1, 2 and 3 are correspondent to microspheres as indicated in A, B, C, respectively.

The surface nanosphere coverage of SOS-COOH microsphere is strongly dependent on the concentration of ammonium hydroxide in the reaction mixture, i.e., the surface nanosphere coverage is decreased by increasing the concentration of ammonium hydroxide.

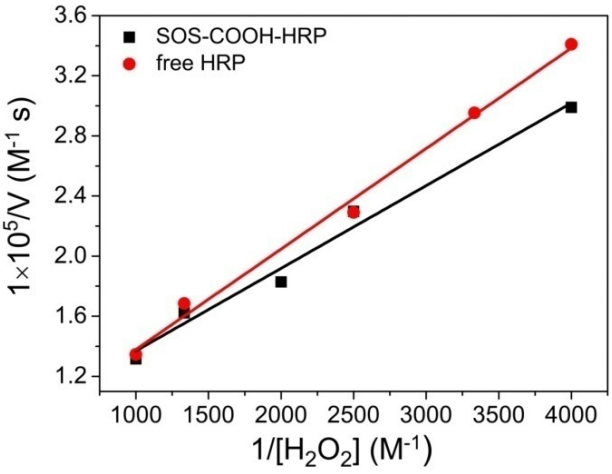


Figure S4. Double reciprocal plots of activity of SOS-COOH-HRP and free HRP with the varying concentrations of H_2O_2.

Table S1. Tolerance of coexisting substance (CS) in the determination of 500 μM H_2O_2

Coexisting substances	Tolerance [CS/H_2O_2]	Relative error (%)	Coexisting substances	Tolerance [CS/H_2O_2]	Relative error (%)
K^+	100:1	0.63	CH_3COO^-	200:1	-1.46
Mg^{2+}	100:1	-3.64	Glucose	100:1	-1.09
Ca^{2+}	100:1	-5.10	Surcose	100:1	-1.18
Pb^{2+}	100:1	-1.45	Maltose	100:1	-0.73
Fe^{3+}	100:1	-6.28	L-Gly	100:1	1.09
NH^{4+}	100:1	-3.91	L-Glu	100:1	2.19
NO^{3-}	100:1	0.64	L-Arg	100:1	4.19
SO_4^{2-}	100:1	-3.64	L-Phe	100:1	1.09
F^-	100:1	-3.92			

Table S2. Recovery rate of detecting spiked H_2O_2 in 10% human serum

Spiked [H_2O_2] (μM)	10	50	100	250	500	750	1000
Recovery rate (%)	102.75	98.75	108.35	94.45	95.60	94.67	96.96
RSD (%)	6.41	3.62	2.56	5.43	2.83	1.51	1.57

Additional References

S1 S. Centi, S. Tombelli, M. Minunni and M. Mascini, *Anal. Chem.*, 2007, **79**, 1466–1473.

S2 S. A. Smith, P. C. Comp and J. H. Morrissey, *J. Thromb. Haemostasis*, 2006, **4**, 820–827.