Electronic Supplementary Information (ESI) for

A Highly Sensitive SERS-based Platform for Zn(II) Detection in Cellular Media

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Experimental methods

Reagents

All the chemicals were analytical grade and used without any further purification. Silver nitrate and (3-Mercaptopropyl)trimethoxysilane were purchased from Alfa Aesar Chemicals Co., Ltd. Acetonitrile and N'N-Bis(2-hydroxybenzylidene)-4-aminophenyl disulfide (HBA) were purchased from Sigma-Aldrich Co. All metal ion solutions were prepared from their nitrate salts and purchased from Chemical Reagent Beijing Co., Beijing, China. Distilled and deionized water from a Milli-Q-plus system was used throughout this work.

Silver Nanoparticle Films

Detailed synthesis of the silver nanoparticle films has been outlined elsewhere.[1] Briefly, glass slides were cleaned with piranha solution (H_2SO_4:H_2O_2 = 3:1) at 90~95 ℃ for 1h before immersion in 2% 3-mercaptopropyltriethoxysilane (in toluene) for 8h. 200 μL of 10% NaOH solution was added dropwise to a silver nitrate solution (440 mg/50 mL). 2 mL of 10% ammonia was dropwise added until the solution became transparent. After the resulting Tollens' reagent was cooled in an ice-water bath, 300 μL of 25% glutaraldehyde was added and the solution stirred for 30s, turning yellow indicating silver nanoparticles have grown. The solution was then rapidly heated to 90 ℃ with the substrate kept in the reactor for 4 min without stirring before removal and rinsing and storage in ethanediol.

Sensor preparation

The above substrate was soaked in HBA (in acetonitrile) solution with the concentration of 10^{-4} M for 2h to form a densely assembled monolayer of half-HBA molecules. After exhaustive rinsing with ethanol and drying with nitrogen, the substrate containing the adsorbed HBA was soaked in Zn (II) solutions (pH 7.0,10 mM Tris–HCl) for 50 min and then was submitted directly to SERS measurements.

Preparation of HeLa cells

HeLa Cells were obtained from the Chinese Academy of Medical Sciences (CAMS) and all the reagents for culture were purchased from HyClone. HeLa cells were cultured in DMEM including 10% fetal bovine serum, 1% penicillin and 1% streptomycin at 37 ℃ in 5% CO_2 and then transferred to a glass-bottomed dish and incubated for 24 h before cell experiments. Cell concentration was *ca*. 8.28 $\times 10^6$. cells/mL. Hela cell disruption and addition of Zn(II): HeLa cells were disrupted, centrifuged at 8000 r•$min^{-1}$for 3 min and diluted with different concentrations of Zn (II) in TBS buffer. Hela cell incubation with Zn(II) before disruption: HeLa cells were incubated with Zn (II) for 30 min, washed with TBS twice, then disrupted and diluted to 1ml with TBS buffer. Note: The cell number in each culture dish is 8.28×10^6. Assuming the diameter of Hela cell is 12 μm, then the total volumn of cells in each dish is ~7.5 μL. It was diluted by 133 times to 1 mL with TBS buffer.

Measurements

Scanning electron microscopy (SEM) characterization was undertaken using a Hitachi S-4800 at 30kV. UV-vis spectra were recorded by a Shimadzu UV-3600Plus Spectrometer. SERS spectra were recorded on a DXR Smart Raman Spectrometer (Thermo Fisher, 633 nm, 2.0 mW laser excitation, 10 s integration time, room temperature).

Theoretical calculations

The M062X density functional was employed to obtain all structures.[2] The geometry optimizations were performed using a mixed basis set for all atoms. Lanl2dz basis set for Zn and Ag and the 6-311++G(d,p) basis set for nonmetal atoms.[3,4] All calculations are performed with SMD solvent model in water solution via using the GAUSSIAN 09 package.[5]

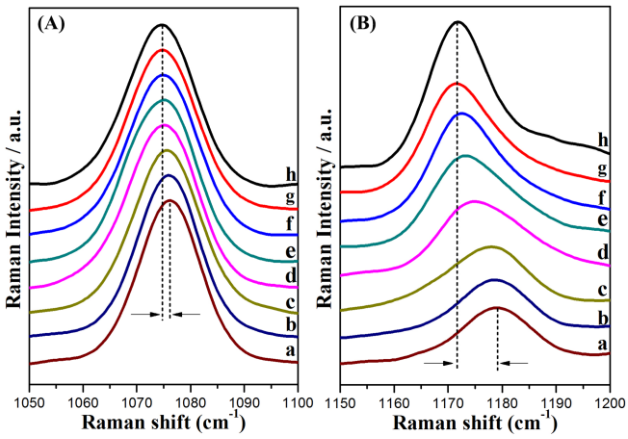

Figure S1. Raman spectra of an Ag-HBA substrate (A) at 1075 cm^{-1} and (B) at 1172 cm^{-1} after exposure to Zn(II) with concentrations of 10^{-6}, 10^{-8}, 10^{-10}, 10^{-12}, 10^{-14},10^{-16}, 0 M (a–g, respectively)

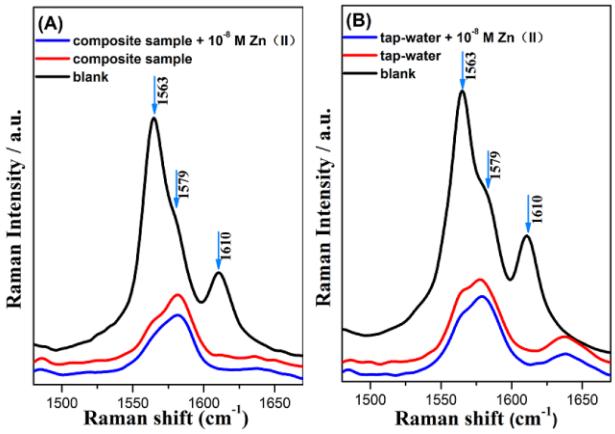

Figure S2. Application of our sensor to the detection (A) of Zn(II) in composite samples of several metal ions, and (B) in tap water samples. (A) Black curve: distilled water with 10mM Tris–HCl buffer only. Red curve: composite sample containing 3.0 nM of Zn(II), 1.0 nM of Na(I), K(I), Cd(II), Pb(II), Ag(II), Ni(II), at pH 7.0 buffered by 10 mM Tris–HCl. Blue curve: the composite sample further spiked with 10^{-8} M Zn(II). (B) Black curve: distilled water with 10mM Tris–HCl buffer only. Red curve: tap water diluted 1000 times with 10mM Tris–HCl buffer stock solution. Blue curve: diluted tap water sample further spiked with 10-8 M Zn(II).

Table S1. Assignment of the prominent peaks in the SERS spectrum of HBA

Wavenumber (cm^{-1})	Band assignments
1075	ν(CS), 7a (a_1)
1172	δ(CH), 9b (b_2)
1358	ν(CH) + ν(CC), 3 (b_2)
1455	ν(CC) + δ(CH), 19 (b_2)
1563	ν(CC), 8a (b_2)
1579	ν(CC), 8a (a_1)
1610	ν(CN), ρ(NC_7H_6O)

ν=stretching; δ= bending;ρ=rocking. For ring vibrations, the corresponding vibrational modes of benzene and the symmetry species under C_{2v} symmetry are indicated.

Table S2. The calculated binding energy of different coordination modes of Zn(II) to Ag-HBA. Note: All calculations are performed with SMD solvent model in water solution via using the GAUSSIAN 09 package

Structure	Binding Energy ΔG (kcal/mol)
Ag–HBA + Zn(II)	−9.5
Ag–HBA + Zn(II) + $2H_2O$	2.0
Ag–HBA + Zn(II) + $4H_2O$	10.2
2Ag–HBA + Zn(II)	−31.0
2Ag–HBA + Zn(II) + $2H_2O$	−19.8

These structures have different charges. Ag–HBA (1) is neutral. Ag–HBA + Zn(II) (2), Ag–HBA + Zn(II) + $2H_2O$ (3), Ag–HBA + Zn(II) + $4H_2O$ (4), 2Ag–HBA + Zn(II) + $2H_2O$ (5) and 2Ag–HBA + Zn(II) (6) have two positive charges. The calculated binding energies of Ag–HBA and zinc ions for various configurations are included in Table S2. One or two halves of the surface-bound HBA can chelate Zn(II), forming structures with binding energies of 9.5 and 31.0 kcal/mol, respectively. Direct coordination of water molecules with the 1:1 complex is not spontaneous at room temperature. However coordination of water to the 2:1 complex is spontaneous though mildly destabilizing. The calculated structures are depicted in Figure S3.

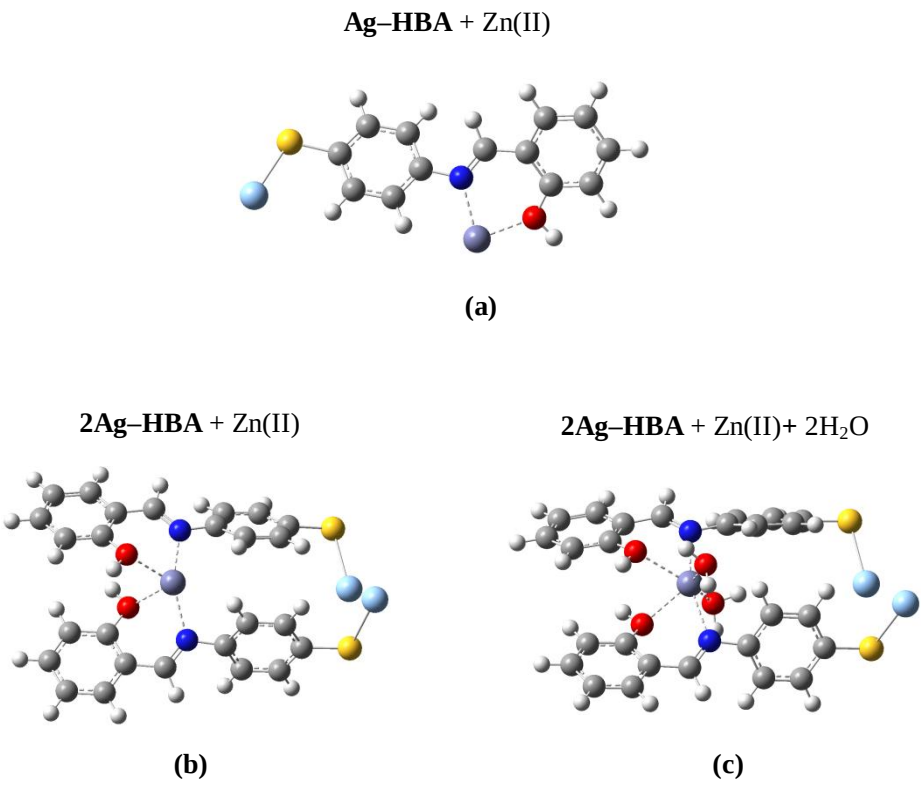

Figure S3. The calculated structures of the complexes between Zn(II) and the Ag-HBA.

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