Electronic Supplementary Material (ESI) for Analyst.

Supporting information for

A Single-Component yet Multifunctional Tongue-Mimic Sensor Array for Upconversion Fluorescent Biosensing

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Contents:

1. Experimental Section

Materials and instruments. Rare earth oxides (Yb_2O_3, Tm_2O_3, Gd_2O_3, Yb_2O_3, Er_2O_3, and Nd_2O_3, 99.99%) were purchased from Diyang Chemical Co. Ltd. (Shanghai, China). Yttrium oxide (Y_2O_3, 99.99%), glutathione, and D-cysteine ($_{D}$-Cys) hydrochloride monohydrate were ordered from Aladdin Reagent Co. Ltd. (Shanghai, China). Oleic acid (OA, 90%), 1-octadecene (ODE, 90%), dopamine hydrochloride (DA) and homocysteine (Hcys) were purchased from Sigma-Aldrich (St. Louis, MO). Oleyl amine (OM, 90%), L-cysteine ($_{L}$-Cys) and ascorbic acid (AA) were bought from Adamas Reagent Co. Ltd. (Shanghai, China). Trifluoroacetic acid (TFA) was ordered from Macklin Biochemical Co. Ltd. (Shanghai, China). Sodium trifluoroacetic was obtained from Alfa Aesar. Then, 10×Tris-HCl buffer (100 mM, pH 8.5) was prepared using metal-free reagents in ultrapure water.

The upconversion fluorescence measurements were performed using a fluorescence spectrophotometer (Hitachi F7000, Japan) assembled with 980- and 808-nm laser accessory. UV−vis absorption measurements were obtained on the TECAN microplate reader. Transmission electron microscopy (TEM) photos were taken by a JEOL-2100F microscope (Japan). Fourier transform infrared spectroscopy (FTIR) spectroscopic measurements were obtained using a Nexus 670 optical bench (Nicolet, USA).

Synthesis of the Muti-emission Ln-UCNPs. The $NaGdF_4$:Yb,Er@$NaYF_4$@$NaYF_4$: Yb,Tm@$NaYbF_4$:Nd@$NaYF_4$ nanoparticle was synthesized as follows:

a. Preparation of RE(CF_3COO)$_3$ (RE=Y, Gd, Yb, Er, Tm, and Nd). 2 mmol of rare earth oxide was added to a 50 mL teflon reactor containing 14 mL DI water. Then a 14 mL amount of TFA was slowly added to the reactor. The reactor was highly sealed and the slurry was maintained at 80℃ for 24 h to form a clear solution. After cooling down to room temperature, a rotary evaporator was used to remove the water and TFA. Finally, the product was recrystallized twice and kept at 80℃ for 24 h.

b.Synthesis of OA-capped UCNPs. Precursors (1 mmol CF_3COONa, 0.78 mmol $(CF_3COO)_3Gd$, 0.2 mmol $(CF_3COO)_3Yb$, and 0.02 mmol $(CF_3COO)_3Er$) were added to a 100 mL three-necked flask containing 10 mmol OA, 10 mmol OM, and 20 mmol ODE. The slurry was heated to 120 °C for 90 min to remove water and oxygen. Then, a clear solution formed and was heated to 310 °C for 15 min. The whole heating process was under the protection of N_2 atmosphere to isolate oxygen and water. After cooling down to room temperature, a 8 mL amount of ethanol was added to precipitate the nanoparticles. The final products were separated by centrifugation at 12000 rpm for 15 min. Nanoparticles were dispersed in 10 mL of cyclohexane. Then, a 5 mL amount of as-prepared nanoparticle colloidal solution, 0.5 mmol CF_3COONa, 0.39 mmol $Gd(CF_3COO)_3$, 0.1 mmol $Yb(CF_3COO)_3$, and 0.01 mmol of $Er(CF_3COO)_3$ and solvents (20 mmol of OA and 20 mmol of ODE) were added into the 100 mL three-necked flask to react at 310 ℃ for 30 min, and aftertreatments are the same as those in the previous step. The second shell contain 2.5 mL amount of product in the first step, 1 mmol CF_3COONa and 1 mmol $Y(CF_3COO)_3$; the third shell is 10 mL amount of as-prepared Er@Y colloidal solution, 1 mmol CF_3COONa, 0.49 mmol $Y(CF_3COO)_3$, 0.5 mmol $Yb(CF_3COO)_3$, and 0.01 mmol $Tm(CF_3COO)_3$; the fourth shell is 5 mL amount of as-prepared Er@Y@Tm colloidal

solution, 0.5 mmol CF_3COONa, 0.25 mmol $Nd(CF_3COO)_3$; and the shell contain 5 mL amount of as prepared Er@Y@Tm@Y colloidal solution, 0.25 mmol CF_3COONa, 0.25 mmol $Y(CF_3COO)_3$. The final products were collected for further characterizations.

c.Preparation of water-dispersible ligand-free UCNPs. The as-prepared Ln-UCNPs (200 mg) were dispersed in 20 mL DI water. The solution was acidified with 0.1 M HCl solution in order to maintain the pH at 2~3. The carboxylate group of OA ligand was protonated during this reaction. After intensely agitated overnight, the reaction was entirely finished. The aqueous solution was re-extracted with diethyl ether at least three times to remove the oleic acid and the combined organic extracts were re-extracted with water. Moreover, the combined water layers were re-extracted by diethyl ether. The ligand-free Ln-UCNPs in water layers were isolated by centrifugation after precipitation with acetone. Finally, the nanoparticles were readily dispersed in water for further use.

Assay for Antioxidants Using Ln-UCNPs as a Single-Component Sensor Array. First, 0.5 mg/mL UCNPs was dispersed with Tris-HCl buffer (100 mM, pH 8.5), and 100 μM DA was added to react for 40 min under 37°C to form UCNPs@PDA. Sensitivity experiments were conducted by mixing several concentrations of GSH, Cys, Hcys or AA with DA into the above solution and incubating for 40 min. For selectivity and competitivity assays, antioxidant mixtures were tested. They were excited at 980 nm or 808 nm, and the fluorescent spectra were recorded. The resulting responses of the sensor (F/F_0), where F and F_0 are the fluorescence intensity in the presence and absence of antioxidants, respectively, were recorded at emission peaks of 368, 484 and 550 nm. Each group had five replicates to react with UCNPs@PDA in identical conditions. The raw data matric (3 sensor elements × 4 antioxidants × 5 repeats) was obtained. Principal component analysis (PCA) was used to process the multivariate pattern data.

Identification of unknown samples in biofluids. We prepared a series of complex samples by spiking different concentrations of GSH to artificial cerebrospinal fluid (aCSF): (1) 4 μM; (2) 8 μM; (3) 12 μM; (4) 18 μM. Meanwhile, three test samples were prepared as a comparison: (A) 6 μM; (B) 10 μM; (C) 15 μM. To prepare the artificial cerebrospinal fluid (aCSF) solution [1], 124.0 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl2, 2.0 mM $MgSO_4$, 1.25 mM KH_2PO_4, 26 mM $NaHCO_3$ and 11.0 mM glucose were dissolved in distilled water. In addition, 1 μM, 3 μM, 5 μM and 7 μM GSH samples were set in artificial urine with three test samples of 2 μM, 4 μM and 6 μM. The artificial urine was prepared according to the literature [2]: NaCl (55 mM), KCl (67 mM), Mg_2SO_4 (3.2 mM), Ca_2SO_4 (2.6 mM), Na_2SO_4 (29.6 mM), Na_2HPO_4 (19.8 mM), $C_4H_7N_3O$ (9.8 mM) and $CO(NH_2)_2$ (310 mM). Both biological fluids were diluted by 10 times using deionized water.

Measurement of $_D$-Cys by DTNB (5,5'- Dithiobis (2-nitrobenzoic acid)) method. DTNB (50 μL of 0.4 mg/mL solution) was mixed with 250 μL aliquots of different concentrations of Hcys directly in phosphate buffer (pH= 8.0). After incubation for 15 min at room temperature, UV spectra for each sample were recorded from 350 to 600 nm, and a standard curve based on absorbance intensity at 412 nm was obtained as a reference.

2. Supplementary Figures

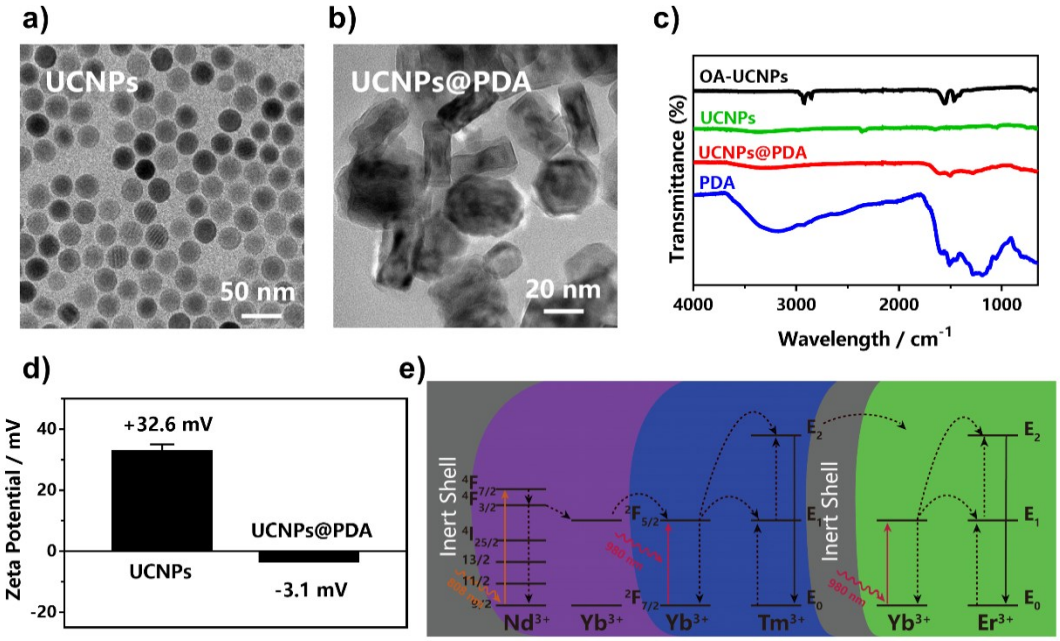

Figure S1. TEM image of (a) UCNPs and (b) UCNPs@PDA. (c) FTIR spectrum of the OA-UCNPs (black line), ligand-free UCNPs (green line), UCNPs@PDA (red line) and PDA (blue line). (d) Zeta potential diagram of UCNPs and UCNPs@PDA. (e) Illustration of the composition of the UCNPs and its energy migration.

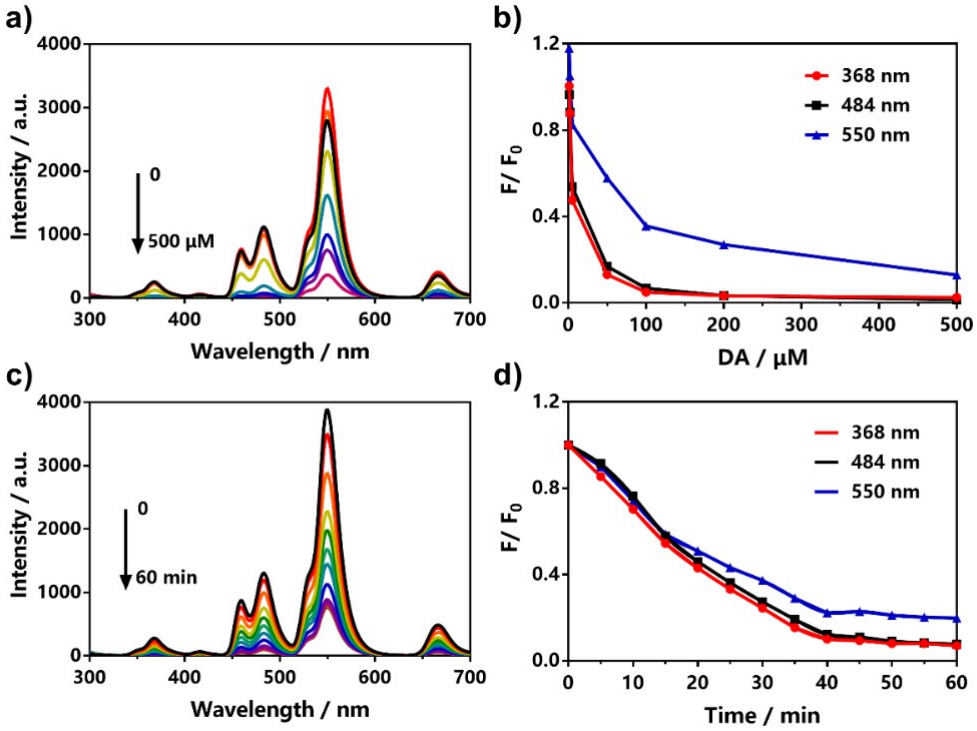

Figure S2. (a) Upconversion emission spectra of UCNP@PDA correlated with DA of varied concentrations (0, 1, 2, 5, 50, 100, 200 and 500 μM), and (b) corresponding plot of emission peaks at 368 nm, 484 nm, and 550 nm. (c) Upconversion emission spectra of UCNP@PDA challenged with

100 μM DA within 1 h, and (d) corresponding plot of emission peaks at 368 nm, 484 nm, and 550 nm. Where F and F_0 are the fluorescence intensity of the UCNPs before and after additions of DA.

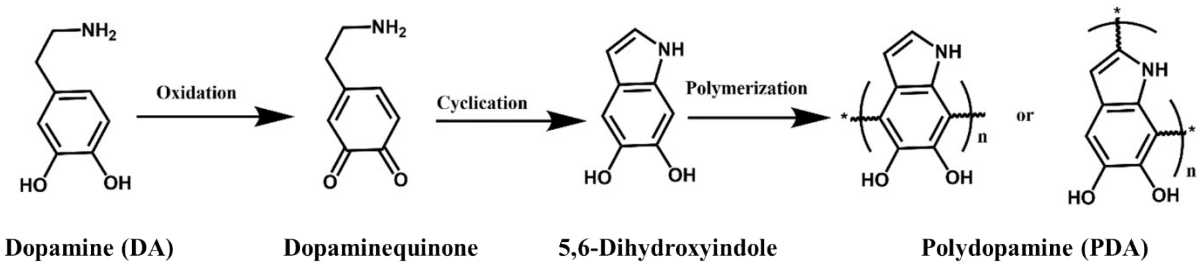

Figure S3. The chemical reaction process of polydopamine formation.

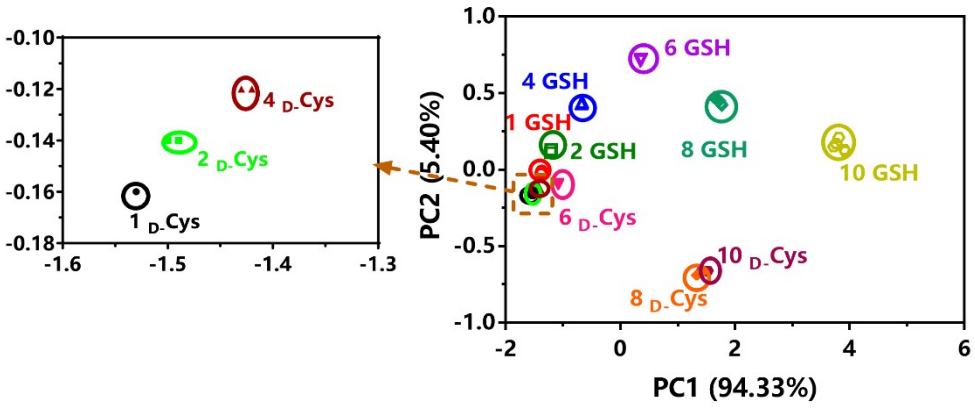

Figure S4. Canonical score plots derived from upconversion fluorescent F/F_0 patterns of the single-component UCNPs@PDA nanoprobe toward various concentrations of $_D$-Cys and GSH.

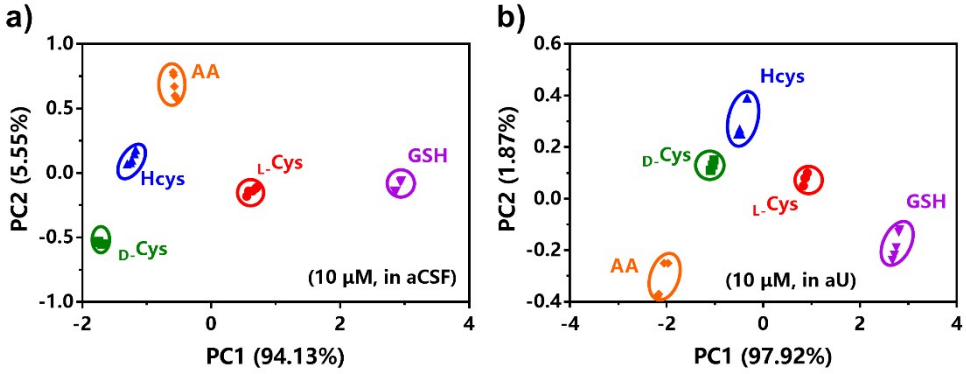

Figure S5. Canonical score plots derived from upcoversion fluorescent patterns of the presented nanoprobe toward antioxidants indicated in artificial cerebrospinal fluid (a) and urine (b).

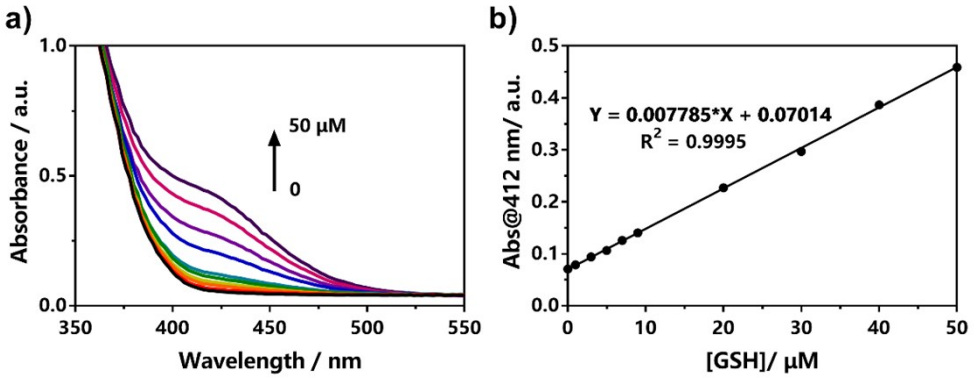

Figure S6. (a) UV spectra of the DNTB method for detection of GSH. (b) Plot of absorbance at 412 nm as a function of the increasing concentrations of GSH.

3. References

1 V. Ranc, Z. Markova, M. Haiduch, R. Prucek, L. Kvitek, J. Kaslik, K. Safarova and R. Zboril, *Anal. Chem.,* 2014, **86,** 2939−2946

2 X. Gu, H. Wang, Z.D. Schultz and J.P. Camden, *Anal. Chem.,* 2016, **88,** 7191−7197.