

Specific enzymatic synthesis of 2,3-diaminophenazine and copper nanoclusters used for dual-emitting ratiometric and naked-eye visual fluorescence sensing of choline

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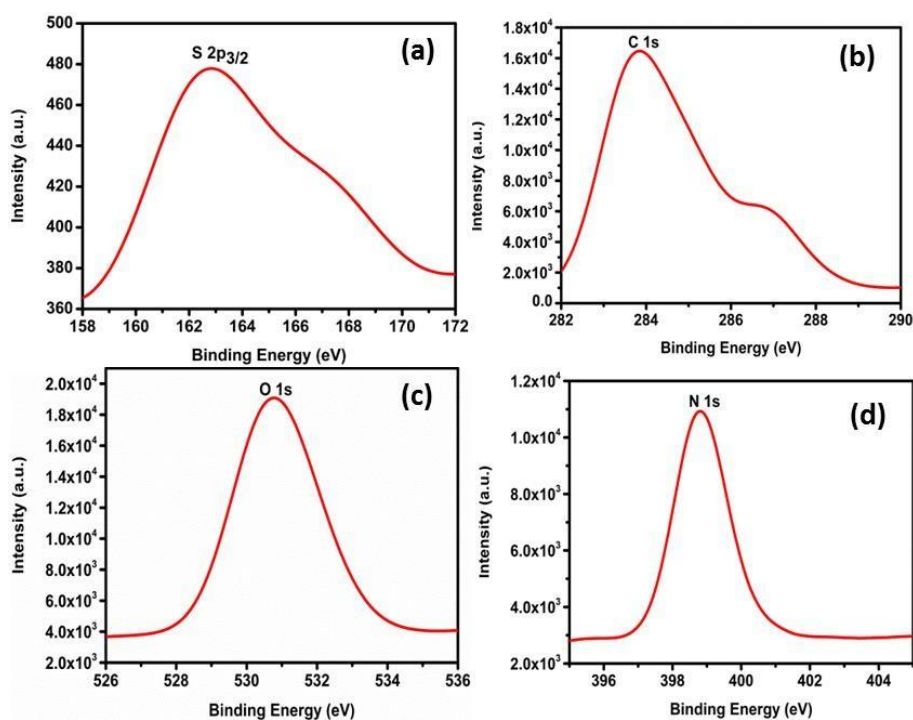


Fig. S1. The high-resolution XPS spectra of CuNCs, splitting from S2p (a), C1s (b), O1s (c), and N1s (d).

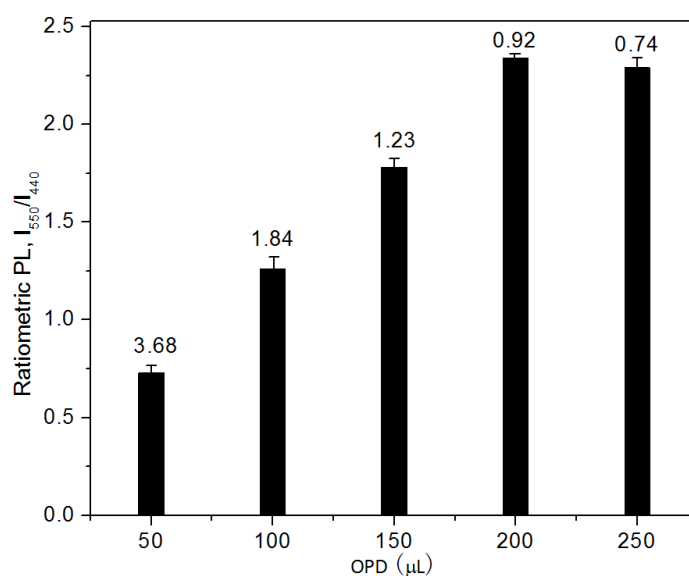
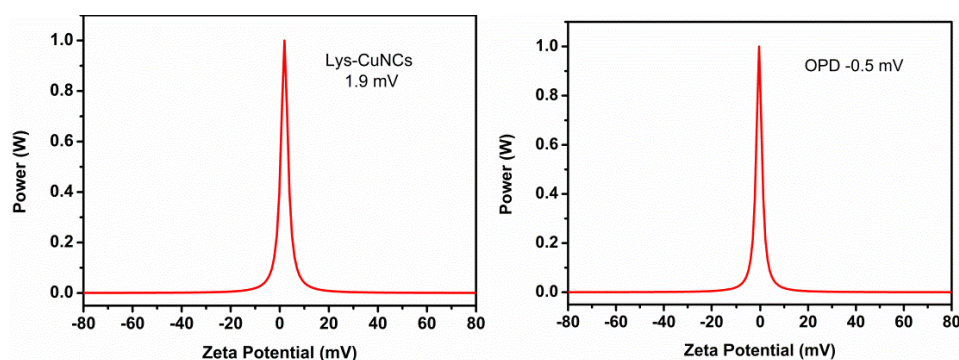


Fig. S2. The relationship of ratiometric FL (I_{550}/I_{440}) intensities *versus* the coexisting volumes of OPD in a mixture solution. The mixture solution containing CuNCs ($40 \mu\text{L}$, 1.0 mg mL^{-1}), HRP (1 mg mL^{-1}), choline oxidase (0.075 U mL^{-1}) and choline ($80 \mu\text{M}$). OPD (2 mM) was added into the mixture solution. The finally added contents of OPD were regulated from 50, 100, 150, 200 to 250 μL . The corresponding mass concentration ratios of CuNCs and OPD were calculated to be 3.68, 1.84, 1.23, 0.92 and 0.74, respectively. FL emission spectra of these resulting mixture solutions in the coexistence of OPD were measured repeatedly, and the corresponding values of I_{550}/I_{440} were calculated and expressed as the average values of six measurements.



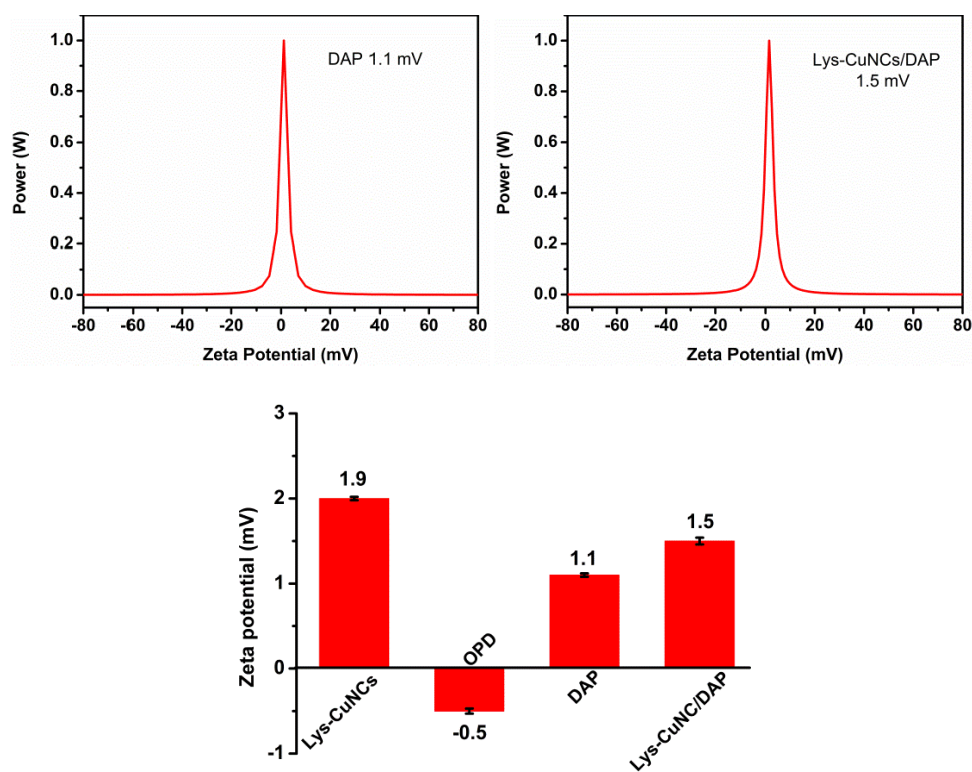


Fig. S3. Zeta potential measurements and average Zeta potentials of OPD, DAP, lysozyme-CuNCs before and after the addition of DAP.

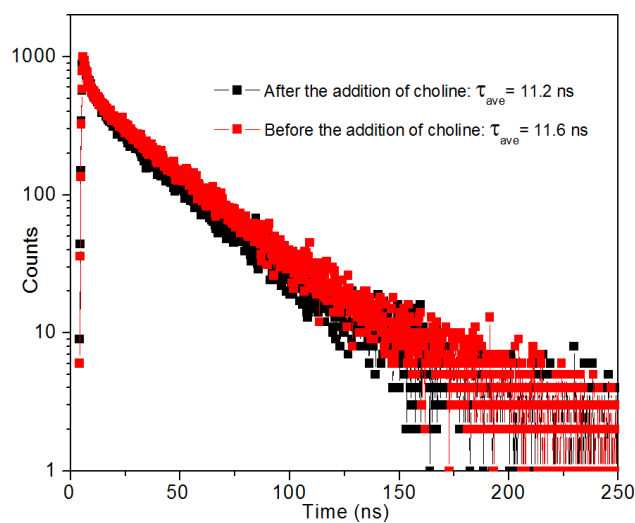


Fig. S4. The time-resolved fluorescence decay curves of CuNCs-based mixture sensing system before and after the addition of choline (80 μ M). The measured samples were excited at 335 nm and the emission wavelength was set at 440 nm.

Table S 1. A brief comparison of different analysis methods for choline detection.

Analysis method	Linear detection range	LOD	Ref.
Electrochemical sensor	1~100 μM	1.12 μM	Xie et al. 2017
Electrochemical sensor	300~5100 μM	82.5 μM	Thiagarajan et al. 2016
Electrochemical sensor	1~800 μM	0.3 μM	Zhang et al. 2014
Electrochemical sensor	5~200 μM	–	Pal et al. 2014
Chemiluminescence	1.25×10^{-3} ~94.5 μM	5×10^{-4} μM	Chen et al. 2016
Colorimetric method	1~180 μM	0.4 μM	Nirala et al. 2018
Fluorescent assay	0.5~10 μM	0.027 μM	Valekar et al. 2018
Liquid chromatography tandem mass spectrometry	0.048~ 9.6×10^4 μM	0.029 μM	Liu et al. 2016b
Ratiometric FL biosensor	0.1~80 μM	0.025 μM	This work

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Table S 2. Detection of [choline] in real human serum and liquid milk samples based on the developed CuNCs-based ratiometric FL biosensor of choline.

^a Samples	Spiked / μ M	^b Detected / μ M	Recovery /%	^c RSD /%
Liquid milk #1	0	Not found	—	—
Liquid milk #2	5	5.13 \pm 0.09	102.60	1.75
Liquid milk #3	20	19.80 \pm 0.29	99.00	1.46
Liquid milk #4	40	38.70 \pm 0.81	96.75	2.09
Human serum #1	0	Not found	—	—
Human serum #2	5	5.15 \pm 0.14	103.00	2.72
Human serum #3	20	20.57 \pm 0.31	102.85	1.51
Human serum #4	40	40.83 \pm 0.84	102.07	2.06

Note: ^a Samples were prepared by 10-fold diluting real samples with PBS. ^b Detected results were expressed as the average of six repeated measurements \pm standard deviation (SD). ^c Relative standard deviation (RSD) results were defined as (SD/mean) \times 100%.