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for

Boosting the smartphone-assisted on-site monitoring capacity for nitroxynil by using synergistic fluorescent sensing system

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1. Materials and methods

1.1 Materials and instruments

All chemicals and solvents were purchased from Energy Chemical China without further purification. DNSA was directly purchased from Sigma-Aldrich and used without purification. Quantum dots were purchased from BeiDa JuBang and used directly. Human serum albumin (HSA, No. 9731) and amino acids were all purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 0.1 mM, pH ~ 7.4) was purchased J&K scientific. UV–Vis absorption and fluorescence spectra were tested by a Thermo-Fisher Evolution 220 and Thermo-Fisher Lumina fluorometer, respectively. Fluorescence lifetimes were obtained by Horiba DeltaFlex with a 392 nm Laser NanoLED. Fluorescent images were captured by a smartphone (iPhone XR) in a dark box (Ultraviolet Analyzer). The Lab values of points in each color range were determined by the ImageJ software or a phone application Color Desk.

1.2 Test of spectroscopic properties

The stock solution of DNSA with concentration of 10 mM were prepared in DMSO. The stock solution was stored in the dark environment at room temperature. The stock solution of albumin (ALB) with concentration of 0.5 mM was prepared in water. The stock solution of Quantum Dot (QD) with concentration of 0.1 μM were prepared in water. DNSA@HSA (10 μM) was prepared by mixing 2 μL of DNSA stock solution and 40 μL of HSA stock solution into 2 mL of PBS buffer (0.1 mM, pH = 7.4).

DNSA@HSA–QD was prepared by mixing 6 μL of QD stock solution into 2ml DNSA@HSA (10 μM). The stock solutions of nitroxynil (NIT), ractopamine, 3-hydroxytyramine hydrochloride, phenothiazine, diethylcarbamazine, nicarbazin and mebendazole were prepared in DMSO at a concentration of 40 mM. The fluorescent titration experiment was carried out by titrating different concentrations (0 - 40 μM) of NIT into 2 mL DNSA@HSA – QD solution with a 10 s shaking. For drug-displacement experiments, two site-specific drugs Warfarin (DS1) and Ibuprofen (DS2) were added proportionally into the DNSA@HSA complex solution, and the emission spectra were measured after shaking for 10 s.

1.3 Molecular docking

The 3D geometry of each ligand (DNSA and NIT) was energy minimized in Chem3D by working with mm2. The ligand-free crystal structures of HSA (PDB ID: 4K2C) was taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Flexible ligand docking was performed by AutoDock 4.2 molecular docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm. The Autogrid was used to calculate Grids. The grid spacing was 0.375 Å as default. 20 docking runs with 25,000,000 energy evaluations were performed. The output from AutoDock was rendered with PyMol. The 2D diagrams were produced by a ligplot software.

1.4 Limit of detection

The limit of detection (LOD) was calculated by using $3\sigma/k$ rule based on NIT titration experiments. Where σ is the standard deviation of blank measurement for three times and k is the slope of the fitting line of fluorescent signal and the concentration of analyte.

1.5 Calculation of binding constant

For DNSA, the titrating concentration ranges of ALB for DNSA (10 μM) were 0 ~ 10 μM. The excitation wavelength was 405 nm. The reaction time was 10 s. The apparent binding constant K_a of DNSA to ALB was estimated from fluorescence titration data using the Benesi-Hildebrand equation:

$$\frac{F_{max} - F_0}{F - F_0} = \frac{1}{K_a \times [ALB]} + 1$$

where F_0 is the fluorescence intensity of DNSA in the absence of ALB, F is the intensity recorded in the presence of added ALB, F_{max} is intensity in the presence of added the maximum of ALB. The K_a value was calculated to be the ratio of intercept / slope.

For NIT, different concentrations (0 ~ 10 μM) of NIT solution were added into an ALB solution (10 μM). Samples were shaken for 10 s before fluorescent tests. The excitation wavelength was 280 nm. Based on fluorescence titration data, the Benessy-Hildebrand equation (adapted from quenching rate) was used to estimate the binding constant K_a of NIT and ALB:

$$\frac{Q_{max} - Q_0}{Q - Q_0} = \frac{1}{K_a \times [NIT]} + 1$$

Where, Q_0 is the fluorescence quenching rate of ALB without adding NIT ($Q_0 = 0$), Q is the fluorescence quenching rate recorded when adding NIT, and Q_{max} is the fluorescence quenching rate when adding the maximum NIT. The K_a value is calculated as the ratio of intercept / slope.

1.6 RGB values and Delta E

The RGB values on the fluorescence images were recorded by Color Desk ver 1.2.5. CIE LAB coordinates (L*a*b*) were converted by the RGB value via Color Desk application on smartphone. The Delta E values representing changes in color difference were calculated with the following equation.

$$\text{Delta E} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

1.7 The 3D-printed miniaturized testing system

The 3D-printed miniaturized testing system included a smartphone as the signal analyzer, a quartz cell placed into a holder as the reaction vessel, a handhold UV flashlight (365 nm) as the excitation source, and a small dark box as the imaging device. The imaging device was readily fabricated by 3D-printing technology using (polyamide fiber). The sample holder was designed based on the size of the standard quartz cell (12.5 mm × 12.5 mm × 45 mm). The quartz cell was added with 2 mL of DNSA@HSA and DNSA@HSA-QD in PBS (0.1 mM, pH ~ 7.4) as the working

solution. The testing samples containing different concentrations of NIT were added into the working solution and were vigorously vortexed for 10 s before capturing. Finally, the fluorescence of quartz cell was captured and analyzed by a smartphone using the application of Color Desk.

1.8 Analysis in real samples

Fresh meat samples (muscle, liver and kidney) were purchased from the local supermarket without any pretreatment. Each sample (0.5 g) was put in a centrifuge tube (15 mL), and then was added 10 mL of methanol. The mixture was whirled by a vortex mixer for 2 min, sonicated for 15 min, and then centrifuged at 7,000 rpm for 30 min. At last, the supernatant of the centrifuged sample was filtered through a syringe filter, transferred into 20 mL volumetric flask, and spiked with NIT as the testing sample. DNSA@HSA (10 μM) in PBS buffer (0.1 mM, pH ~ 7.4) was used as the sensor solution. Different volumes of testing sample were added into the sensor solutions (2 mL) in quartz cells, which were then measured by a fluorometer. Each solution was measured parallel for 3 times. The recovery was calculated based on the following equation: Recovery (%) = $(c_{detected} - c_{initial})/c_{spiked} \times 100\%$. The $c_{detected}$ was the NIT concentration obtained from the standard curve. The $c_{initial}$ was the NIT concentration in the water sample before spiking treatment. The c_{spiked} was the NIT concentration in the testing sample after spiking treatment.

2. Supporting Figures and Table

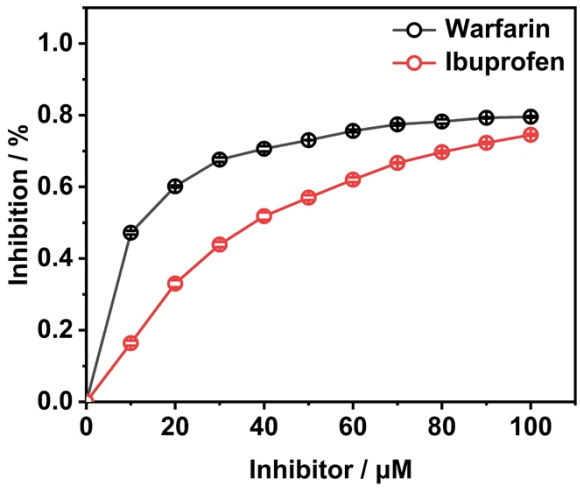

Fig. S1 Site-specific displacement experiments for DNSA@HSA (10 μM) by adding two drug site indicators (Warfarin for drug site 1 and Ibuprofen for drug site 2)

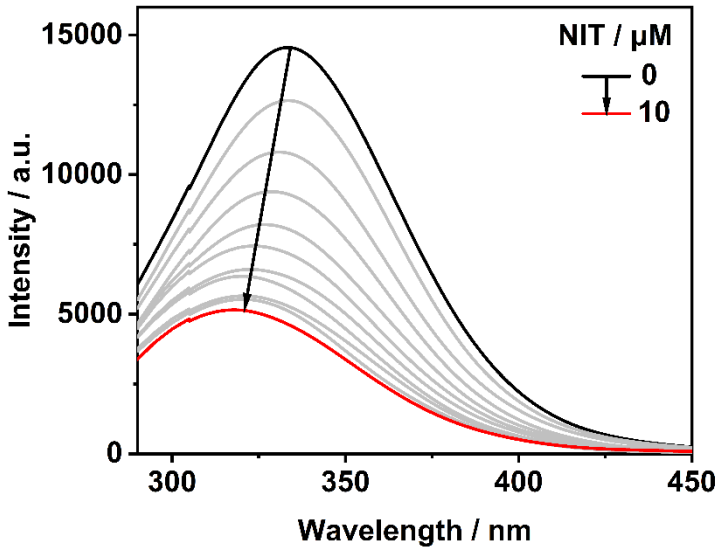

Fig. S2 Fluorescent spectra of HSA (10 μM) in the presence of NIT (0 - 10 μM) in PBS buffer (0.1 mM, pH ~ 7.4). λ_{ex} = 280 nm.

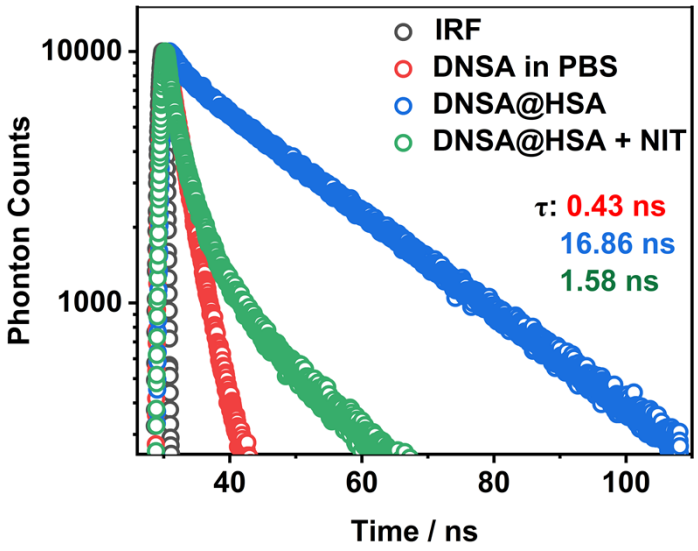

Fig. S3 Fluorescent decay curves of DNSA, DNSA@HSA in the absence and presence of NIT in PBS buffer (0.1 mM, pH ~ 7.4). [DNSA] = [DNSA@HSA] = 10 μM. [NIT] = 40 μM. The excitation wavelength of laser was 392 nm. IRF: instrument response function.

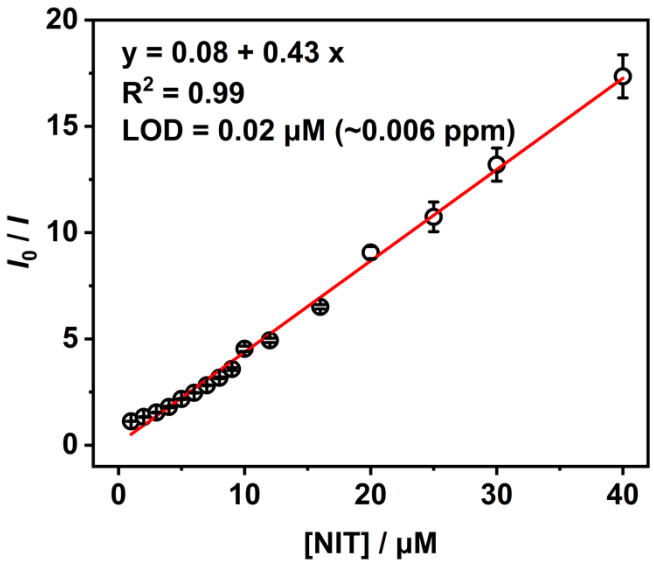

Fig. S4 Relationship between the concentration of NIT and the intensity ratio (I_0 / I) of DNSA@HSA. The red line represented the linearly fitting region.

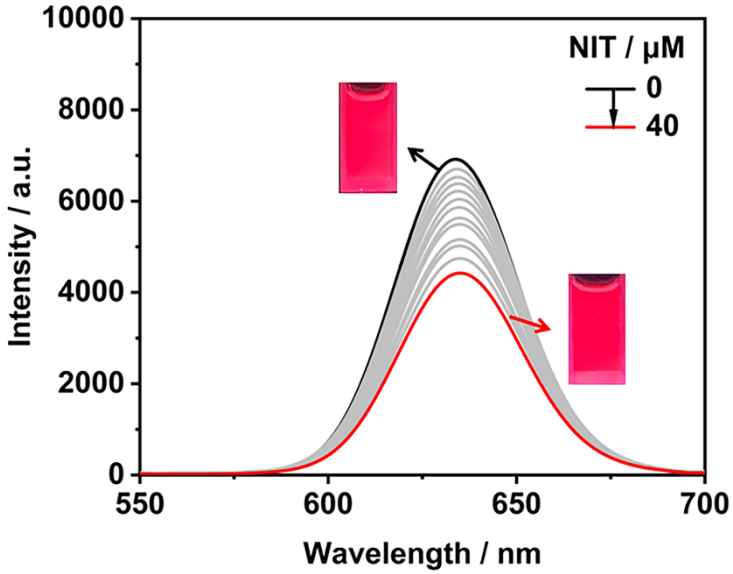

Fig. S5 Fluorescent spectra of QDs (0.3 nM) in a PBS buffer with increasing concentration of NIT. λ_{ex} = 405 nm. Inset: photos of QD in the absence and presence of NIT under a handheld UV lamp (365 nm).

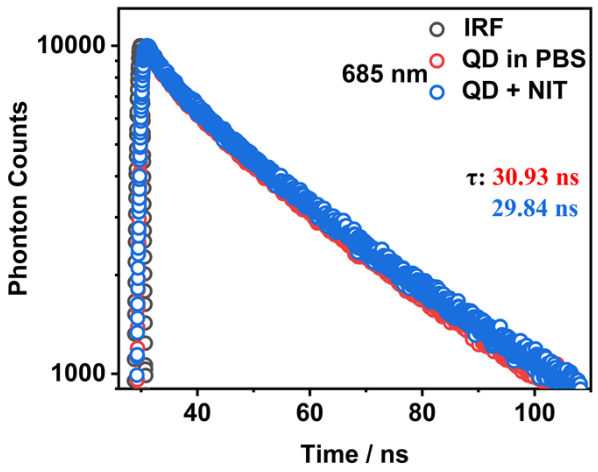

Fig. S6 Fluorescent decay curves of QDs in the absence and presence of NIT in PBS buffer (0.1 mM, pH ~ 7.4). [QD] = 0.3 nM. [NIT] = 40 μM. The excitation wavelength of laser was 392 nm. IRF: instrument response function.

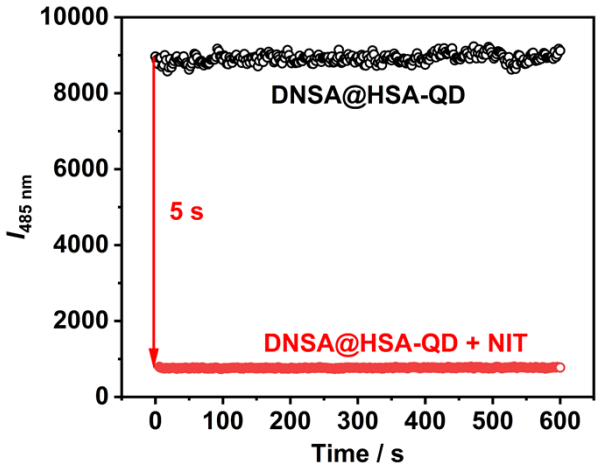

Fig. S7 Time-dependent intensity (485 nm) of DNSA@HSA - QD in the presence and absence of NIT (40 μM) in PBS (0.1 mM, pH ~ 7.4). [DNSA@HSA] = 10 μM. [QD] = 0.3 nM. λ_{ex} = 405 nm.

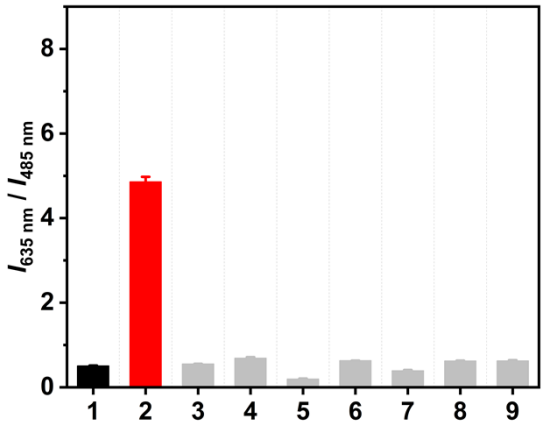

Fig. S8 Intensity ratios ($I_{635\ nm}/I_{485\ nm}$) of DNSA@HSA - QD with addition of 40 μM NIT and 40 μM other six veterinary medicines (ractopamine, 3-hydroxytyramine hydrochloride, phenothiazine, diethylcarbamazine, nicarbazin, mebendazole) and nitro compound (nitrobenzene). [DNSA@HSA] = 10 μM. [QD] = 0.3 nM. λ_{ex} = 405 nm.

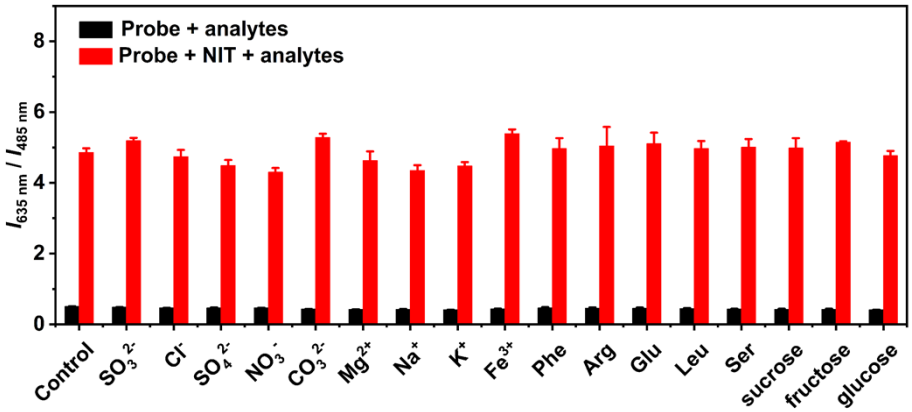

Fig. S9 Intensity ratios ($I_{635\ nm}/I_{485\ nm}$) of DNSA@HSA - QD in the presence (red bars) and absence (black bars) of NIT coexisting with ions (SO_3^{2-}, Cl^-, SO_4^{2-}, NO_3^-, CO_3^{2-}, Mg^{2+}, Na^+, K^+, Fe^{3+}), amino acids (Phe, Arg, Glu, Leu, Ser), saccharides (sucrose, fructose, glucose). [DNSA@HSA] = 10 μM. [QD] = 0.3 nM. [analytes] = [NIT] = 40 μM. λ_{ex} = 405 nm. Error bars = ± SD.

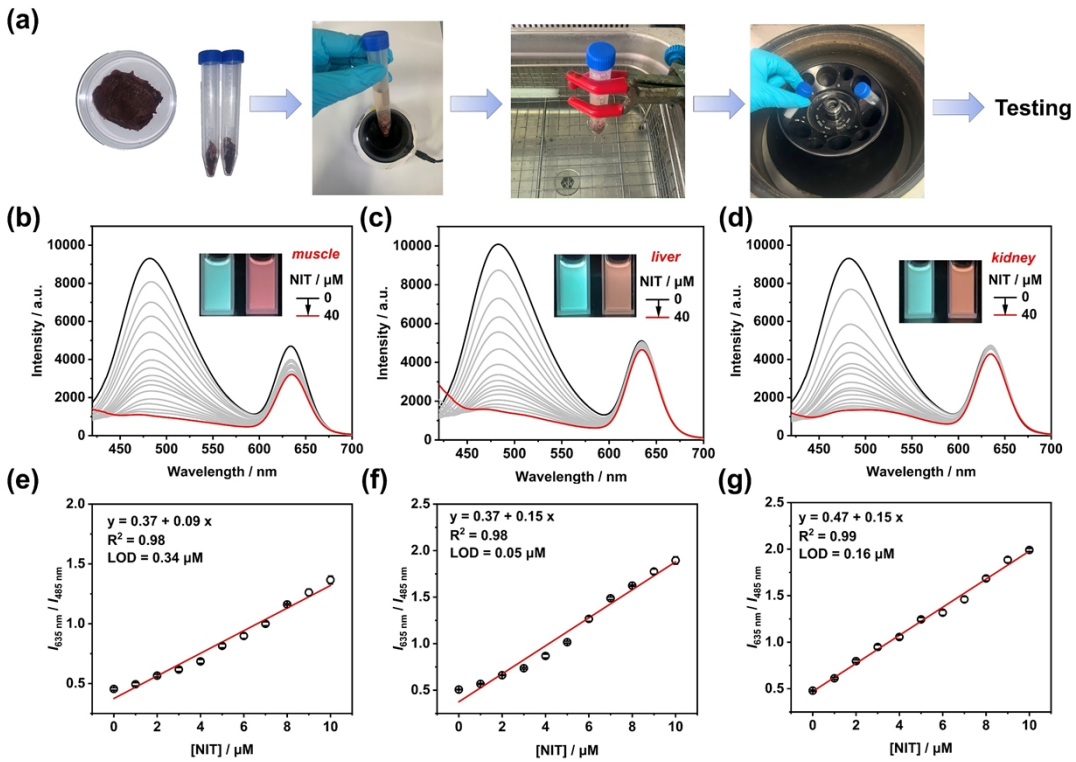

Fig. S10 Analysis of real food samples (muscle, liver, and kidney). (a) Illustration of

the procedure for the analysis of real food samples. (b) – (d) fluorescence spectra and (e) – (g) relationship between intensity ratio ($I_{635\ nm}/I_{485\ nm}$) of DNSA@HSA - QD and the concentration of NIT (0 – 40 μM). [DNSA@HSA] = 10 μM. [QD] = 0.3 nM.

Table S1 Determination of NIT in real samples

Samples	Added (μM)	Detected (μM)	Recovery (%)	RSD (%)
Muscle	0	<LOD	-	-
	1.00	1.11	111.43	2.93
	2.00	2.18	108.80	6.32
	3.00	2.75	91.52	5.95
	4.00	3.57	89.21	2.85
	5.00	4.95	98.94	3.69
Liver	0	<LOD	-	-
	1.00	1.15	114.85	4.33
	2.00	1.90	95.17	2.32
	3.00	2.60	86.59	1.38
	4.00	3.51	87.81	1.07
	5.00	4.31	86.14	1.35
Kidney	0	<LOD	-	-
	1.00	0.95	94.82	7.22
	2.00	2.18	108.77	4.34
	3.00	3.19	106.18	5.05
	4.00	3.90	97.60	3.72
	5.00	5.16	103.29	2.73
Water	0	<LOD	-	-
	1.00	1.04	103.99	0.55
	2.00	2.26	113.16	6.59
	3.00	2.88	95.93	2.42
	4.00	4.07	101.67	6.92
	5.00	4.92	98.49	2.68