Electronic Supplementary Material (ESI) for ChemComm.

ESI of

Harnessing a simple ratiometric fluorescent probe for albumin recognition and beyond †

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1. Experimental section

1.1 materials and instruments

All biological analytes including human serum albumin (HSA), tyrosinase, EC3.1.1.1, globulin, lysozyme, trypsin, lipase, RNA, DNA, glucose, lactose, creatinine, cysteine (Cys), homocysteine (Hcy), glutathione (GSH) lysine (Lys), tryptophan (Trp), histidine (His), and methionine (Met) were purchased from Sigma-Aldrich without further purification. All chemicals and solvents were purchased from Energy Chemical China without further purification. Phosphate buffered saline (PBS, 1×, pH 7.4) was purchased J&K scientific. Fluorescence spectra were recorded by a Thermo Lumina Fluorescent spectrometer.

1.2 General testing method

The stock solution of HMM, DCN, HNP, and DNP was prepared in DMSO with a concentration of 10 mM and restored in the refrigerator for further usage. For the test of spectroscopic properties, a volume of 10 μL of 10 mM flavonoids stock solution was injected into 2 mL solution to yield a detection solution with a concentration of 5 μM. The mixture was shaken for 30 sec and then measured by spectroscopic measurements at room temperature. The excitation wavelength is 365 nm. For Job's plot experiments, the emission spectra were measured in the different ratios of DNP and HSA with the total concentration at 10 μM. For indicator displacement experiments, two site-specific drugs Warfarin (DS1) and Ibuprofen (DS2) were added proportionally into the complex DNP@HSA solution, and the emission spectra were measured after shaking for 1 min. For fluorescent response time experiments, the intensity decay curves were measured in the solution of fluorescent probe DNP with or without HSA. For fluorescence titration experiments, small aliquots of HSA solution were successively added into the solution of HFH and allowed to shake for at least 30

sec every time before measurement. The optical spectra data were plotted using Origin graphing software (OriginLab Origin 2022B).

1.3 Molecular docking

The 3D geometry of flavonoids was constructed using the Gaussian viewer, then optimized at the level of B3LYP/6-31g* with PCM implicit water solvent model. The structure of HSA (PDB code: 4k2c) was obtained from the Brookhaven protein data bank (http://www.rcsb.org/pdb). The R-value and the resolution of the file were 0.213 and 3.23 Å, respectively. The 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 first step is to set the docking box of the molecules and the HSA ligands. Then used Autogrid to calculate grids. Set the parameters as follows: 60 grid points per dimension, grid point interval: 0.375 Å, the number of runs: 20 times, the maximum energy evaluation: 2500000 times. The output from AutoDock was rendered with PyMol and the ligand site analysis was assisted with LigPlus.

1.4 Procedure for sensing HSA in real serum samples

The standard serum sample (from 1 μL to 10 μL) was dropped in the 1 mL PBS solution with the concentration of DNP at 5 μM. After sharking for 30 sec, the mixture was incubated for 1 min at room temperature and then was measured by fluorometer at room temperature.

1.5 BCG method

The human serum was added in BCG solution and transported into 96-well plate. The signals were collected by a Microplate Reader using ultraviolet-visible

spectrometry at 630 nm. The concentrations of HSA were calculated by following equation (1):

$$\frac{A}{A_0} = \frac{C}{C_0} \quad (1)$$

Where A and A_0 are the absorption of sample and standard sample, respectively. C and C_0 are HSA concentration in serum and standard serum, respectively.

1.6 Portable device testing system

The standard serum sample was diluted 40 times with 1× PBS buffer. Different volumes of serum sample were added to the quart cell so that the final volume of working solution is 2 mL with 5 μM DNP. Then, the fluorescence of quart cell was captured and analyzed by a smartphone using the application of Color Recognition.

1.7 RGB and ΔE analysis

The image of solution was captured by the camera of iPhone 13 (Wide Angle Camera, ISO 3200, 26 mm, ƒ1.6) under 365 nm UV lamp (10 w). For RGB analysis, photos were imported into the APP Color Recognition of smartphone. The plot of the RGB value versus the added volume of standard serum sample was obtained by the Origin software. For Delta E (ΔE) analysis, the ΔE values was calculated based on CIELAB (L a* b*) coordinates.

2. Supporting Figures and Tables

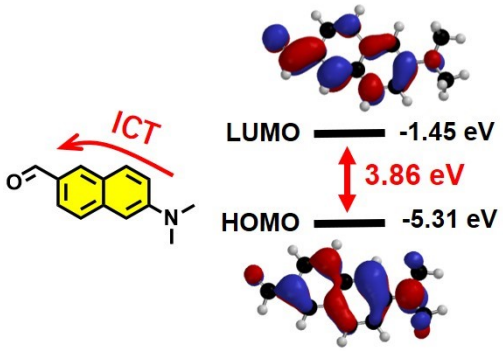


Fig. S1. The intramolecular charge transfer in DNP. Electron distributions in HOMO and LUMO energy levels were given by a Gaussian software.

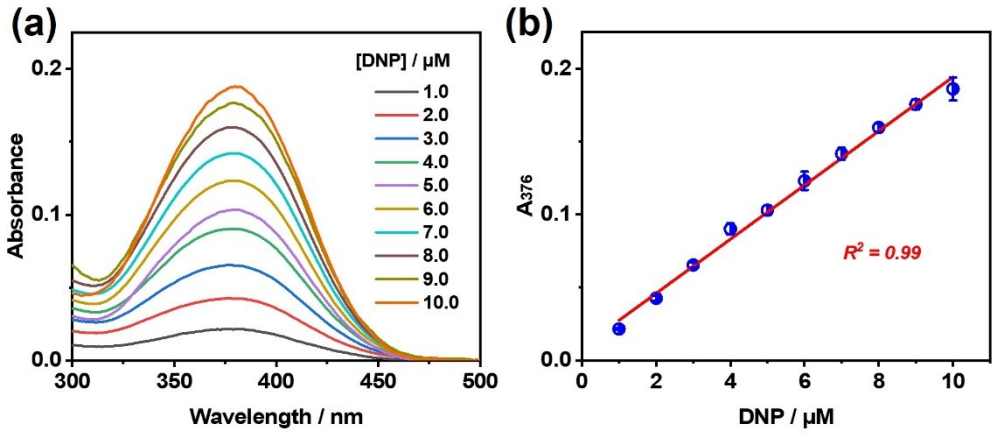


Fig. S2. (a) The absorption spectra of DNP with different concentrations in water. (b) Relationship between the maximum absorbance of DNP and concentration.

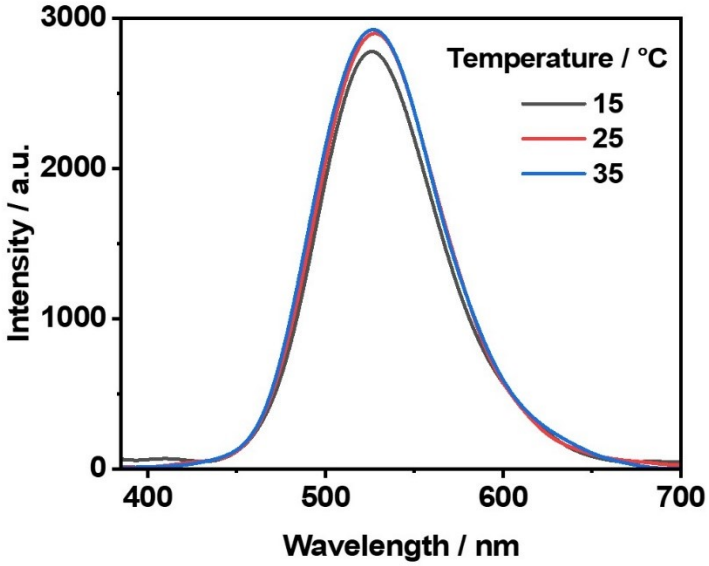


Fig. S3. The fluorescence spectra of DNP in PBS buffer with different temperatures. λ_{ex} = 365 nm.

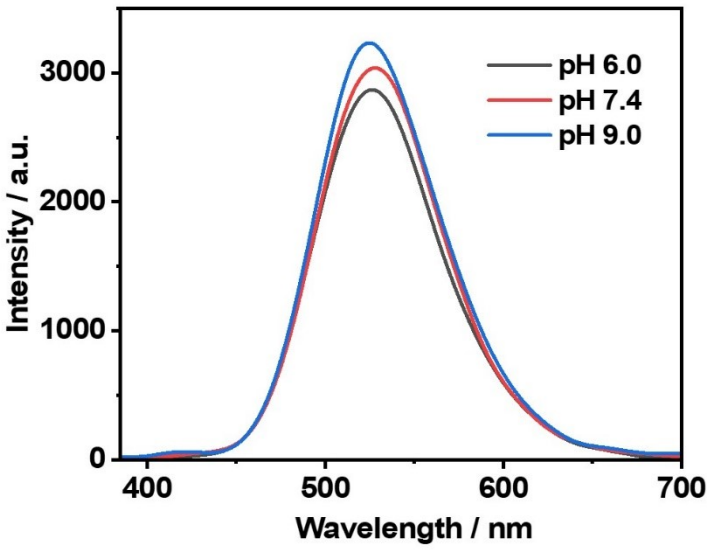


Fig. S4. The fluorescence spectra of DNP in PBS buffer with different pH. λ_{ex} = 365 nm.

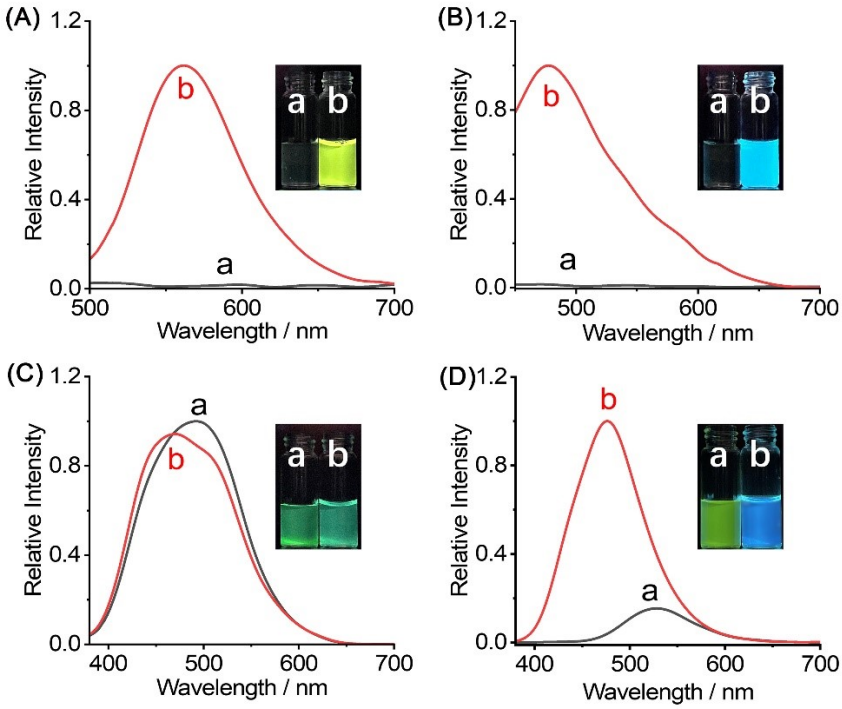


Fig. S5. The fluorescence spectra of (A) HMM, (B) DCN, (C) HNP, and (D) DNP in the absence (a) and presence (b) of HSA. Inset photography: the emission color of probes under 365 nm UV light.

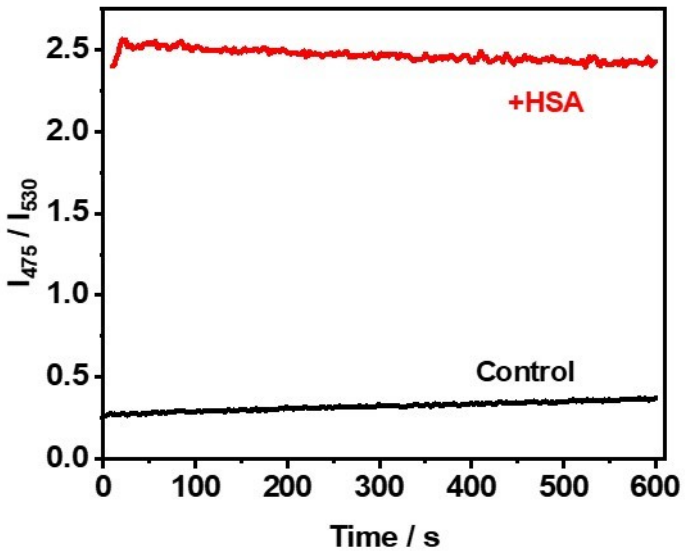


Fig. S6. Time-dependent intensity ratio of DNP (5 μM) in the absence and presence of HSA (5 μM). λ_{ex} = 365 nm.

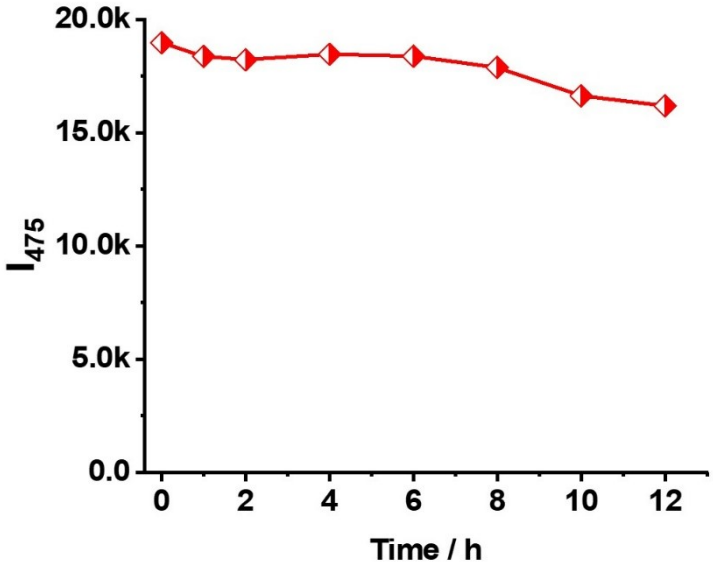


Fig. S7. The fluorescence intensity of DNP@HSA (5 μM) at 475 nm within 12 hours under 365 nm UV light.

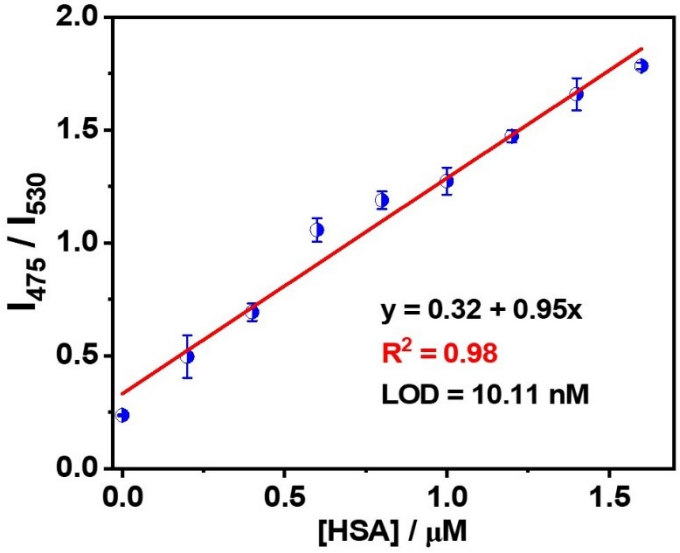


Fig. S8. The dependence of intensity ratio of DNP (5 μM) on the concentration of HSA (0-1.6 μM). λ_{ex} = 365 nm. Error bars = ±SD, n = 3. LOD = 3δ/k, δ was obtained based on ten times detection for blank sample.

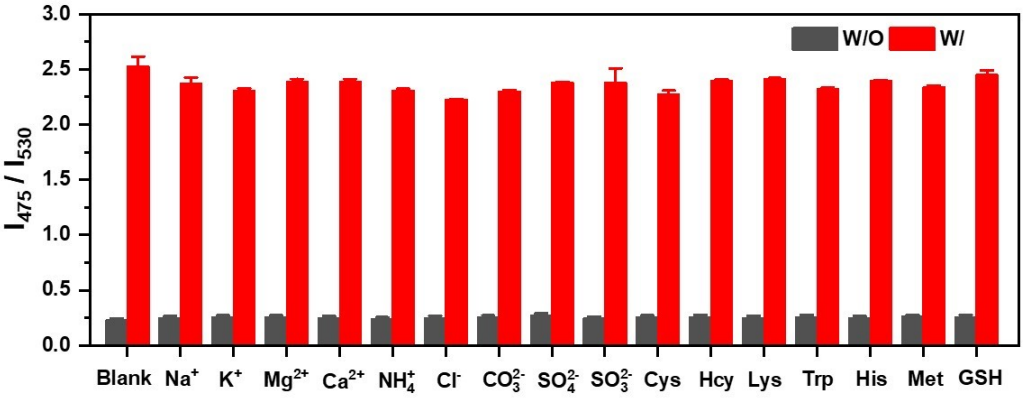


Fig. S9. Intensity ratios of DNP (black bar, 5 μM) and complex DNP@HSA (red bar, 5 μM) various species: inorganic salts and amino acids. [ions] = [amino acids] = 5 μM, λ_{ex} = 365 nm. Error bars = ±SD, n = 3.

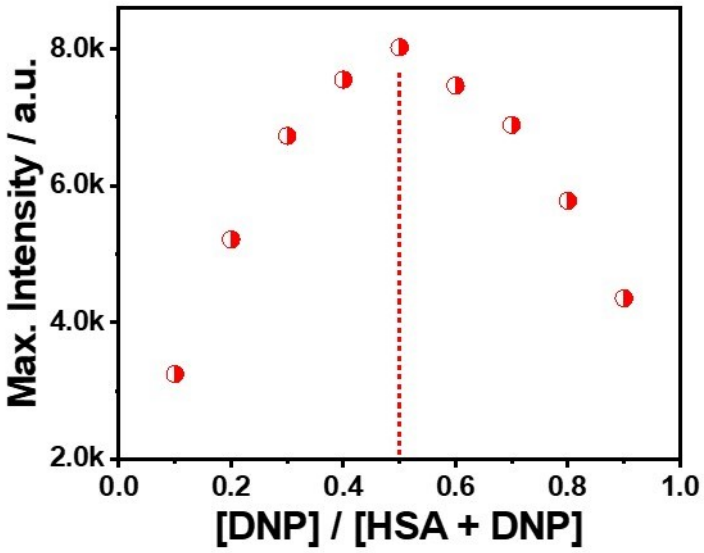


Fig. S10. The Job's plot analysis by using maximum fluorescence intensity. [DNP + HSA]

= 5 μM. λ_{ex} = 365 nm.

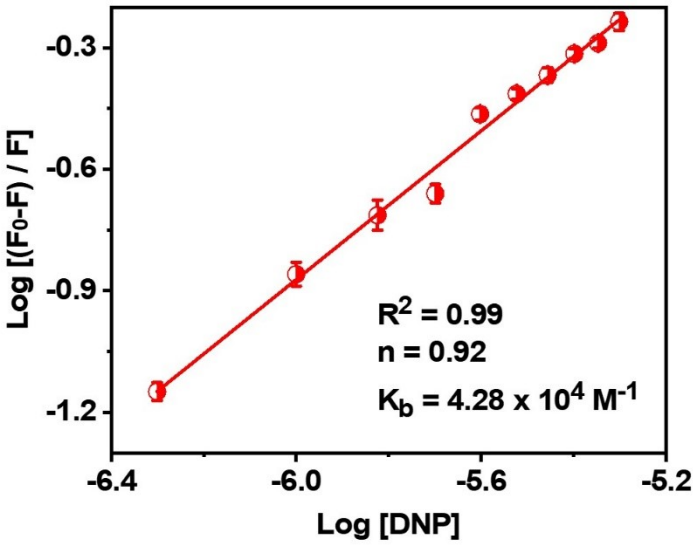
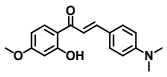
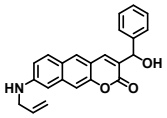
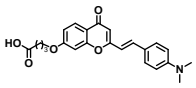
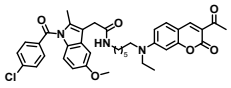
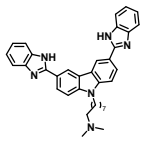
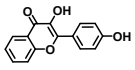
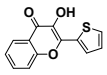
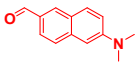


Fig. S11. The Stern-Volmer plot of binding interaction between DNP and HSA in PBS buffer. Where F_0 and F are the fluorescence intensities in absence and presence of DNP.

Fig. S12. The RGB analysis process of portable device.

Table S1. Reported ratiometric fluorescent probes for HSA detection.

Probe	Synthetic route	Signal change(nm)	LOD μg/mL	Response time	Mechanism	Ref.
 **4MC**	simple	620/520	1.08	< 5 min	DSE	**1**
 **rHSAp**	complicated	580/490	0.2	< 1 min	EC	**2**
 **SC2**	complicated	614/562	3.38	< 1 min	EC	**3**
 **ADC-IMC-6**	complicated	575/475	4.65	> 5 min	EC	**4**
 **Compound 1**	complicated	400/454	0.27	120 min	EC	**5**
 **HFH**	simple	450/520	1.28	< 5 sec.	IPT	6
 **STF**	simple	430/515	3.56	< 5 sec	EC	7
 **DNP**	No	470/525	0.59	< 5 sec	EC	This work

Note: DSE: dual-state-emission; EC: environmental change (polarity or viscosity); IPT: intermolecular proton transfer.

Table S2 The photophysical properties of DNP in different solvents.

Solvents	Polarizability factor Δ*f*	$E_T(30)$ (kcal/mol)	Abs (nm)	ε ($\times 10^5$ $M^{-1}cm^{-1}$)	E_m (nm)	τ (ns)	ϕ (%)
THF	7.58	37.4	363	0.141	440	1.932	63.1
DCM	0.217	40.7	372	0.117	445	2.242	97.8
DMF	0.309	43.2	372	0.139	464	2.416	97.5
ACN	0.305	45.6	368	0.151	473	2.379	79.6
MeOH	0.309	55.4	373	0.145	500	0.771	30.4
H_2O	0.321	63.1	376	0.086	525	0.211	7.10

3. References

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