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Supplementary Material

A novel hydrazide Schiff base self-assembled nanoprobe for selective detection of human serum albumin and its applications in renal disease surveillance

Zhi-Gang Wang[a,1], Xiao-Jing Yan[a,1], Hai-Bo Liu[b], De-Long Zhang[c], Wei Liu[d], Cheng-Zhi Xie[a,*], Qing-Zhong Li[e], Jing-Yuan Xu[a,*]

[a]Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University, Tianjin 300070, P. R. China.

[b]Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, P. R. China.

[c]Department of Pharmacy, Tianjin Santan Hospital, Tianjin 300193, P. R. China.

[d]The Second Hospital of Tianjin Medical University, Tianjin 300211, P.R. China.

[e]The Laboratory of Theoretical and Computational Chemistry, School of Chemistry and Chemical Engineering, Yantai University, Yantai 264005, P.R. China.

[1]These authors contributed equally.

Corresponding author. E-mail address: xiechengzhi@tmu.edu.cn (C.-Z. Xie), xujingyuan@tmu.edu.cn (J.-Y. Xu)

Fig. S1. ^{1}H-NMR spectrum of probe **NPC** in DMSO-d$_6$.

Fig. S2. ^{13}C-NMR spectrum of probe **NPC** in DMSO-d$_6$.

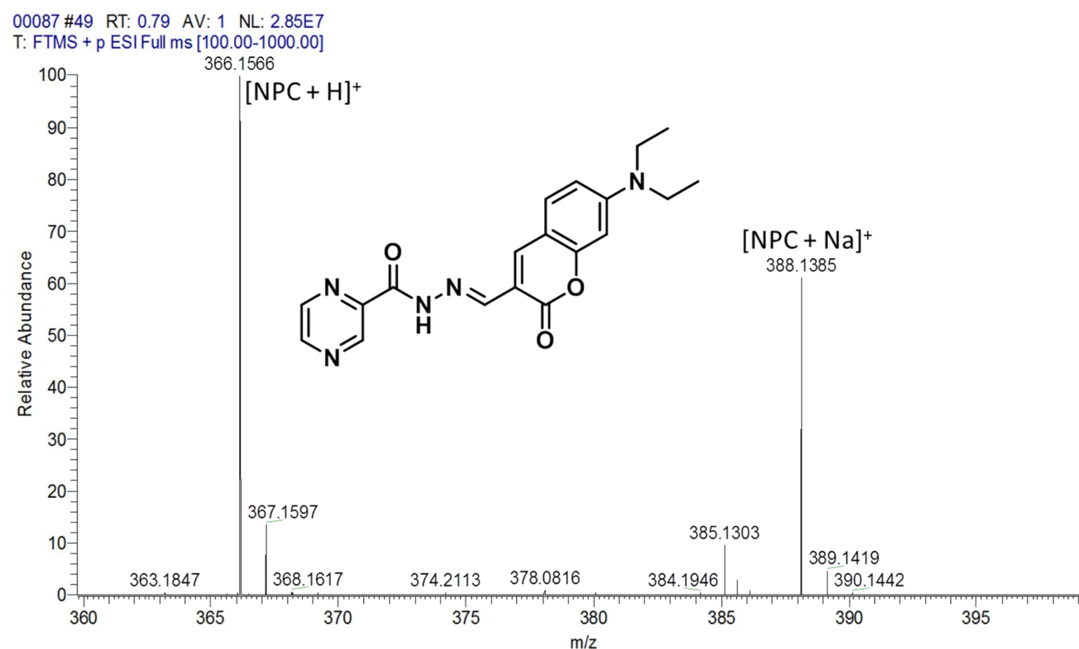


Fig. S3. ESI-MS spectrum of probe **NPC**.

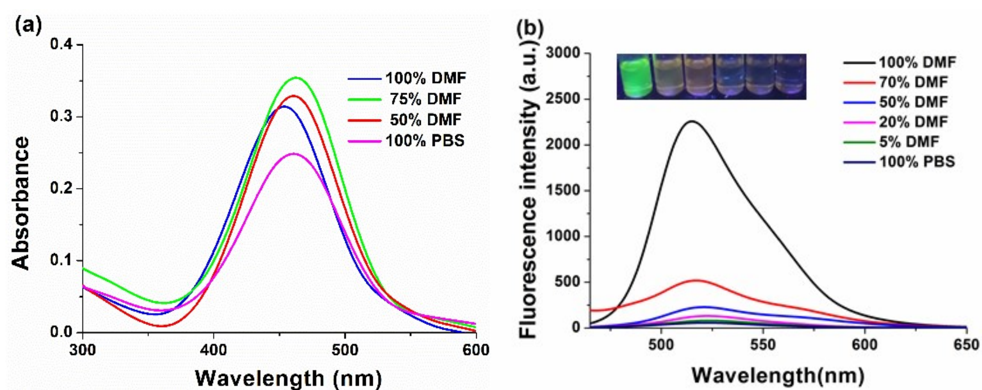


Fig. S4. (a) UV-vis absorption spectra of **NPC** (5 μM) in DMF/PBS buffer solution (pH 7.4) with different DMF fractions. (b) The fluorescence emission spectra of **NPC** (5 μM) in DMF/PBS buffer solution (pH 7.4) with different DMF fractions. Inset: photograph for corresponding color changes of **NPC** in solutions with different DMF fractions under 365 nm UV light. (λ_{ex} = 450 nm, slit: 5 nm/5 nm).

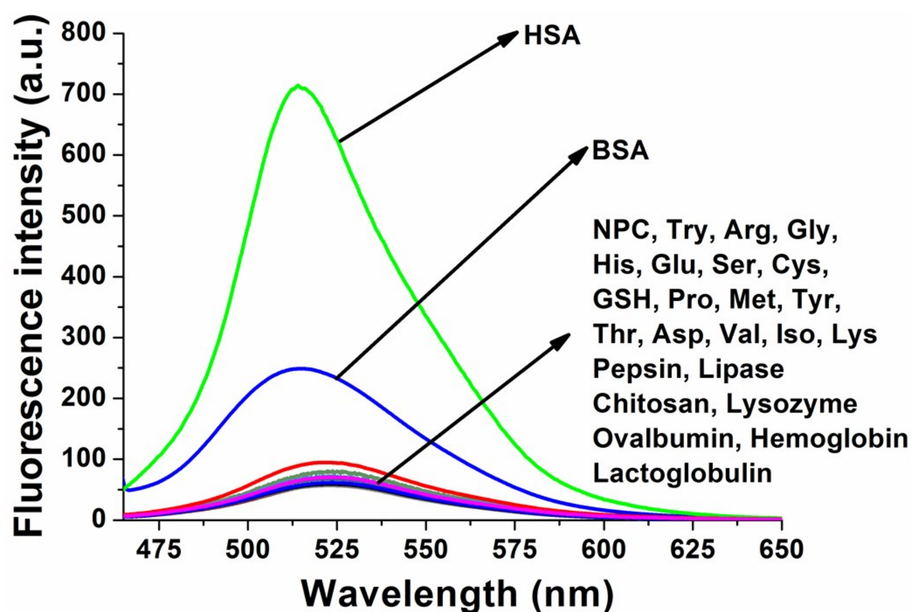


Fig. S5. The fluorescence emission spectra of **NPC** (5 μM) upon addition of 1 equiv. HSA, other proteins (5 equiv. for BSA) and various amino acid in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, slit: 5 nm/5 nm).

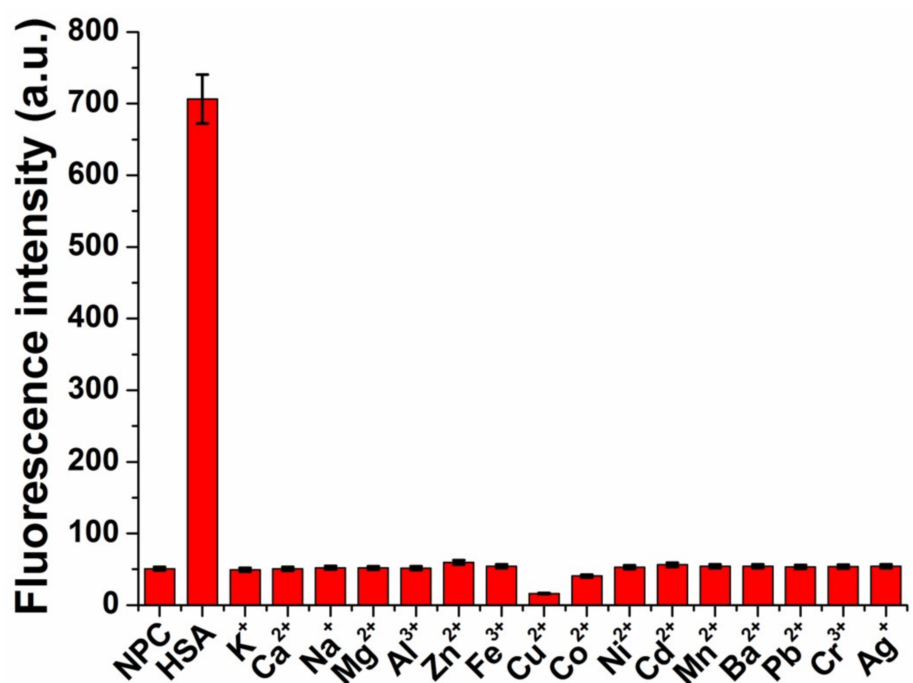


Fig. S6. The selectivity of **NPC** (5 μM) to 1 equiv. HSA from various metal ions (5 μM) in PBS (pH 7.4) buffer solution. (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

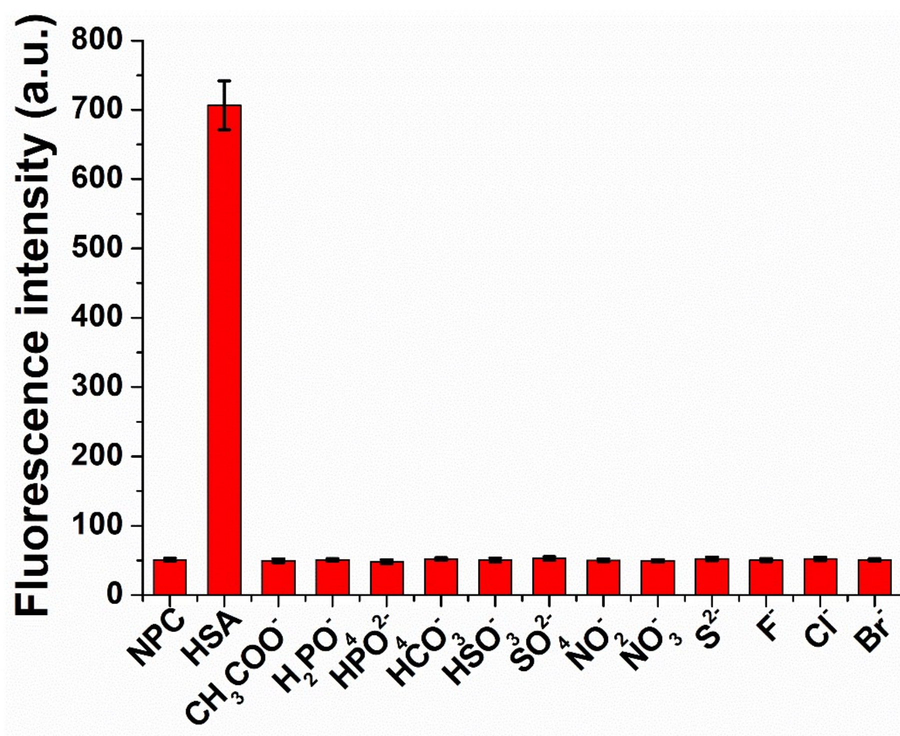


Fig. S7. The selectivity of **NPC** (5 μM) to 1 equiv. HSA from various anions (5 μM) in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

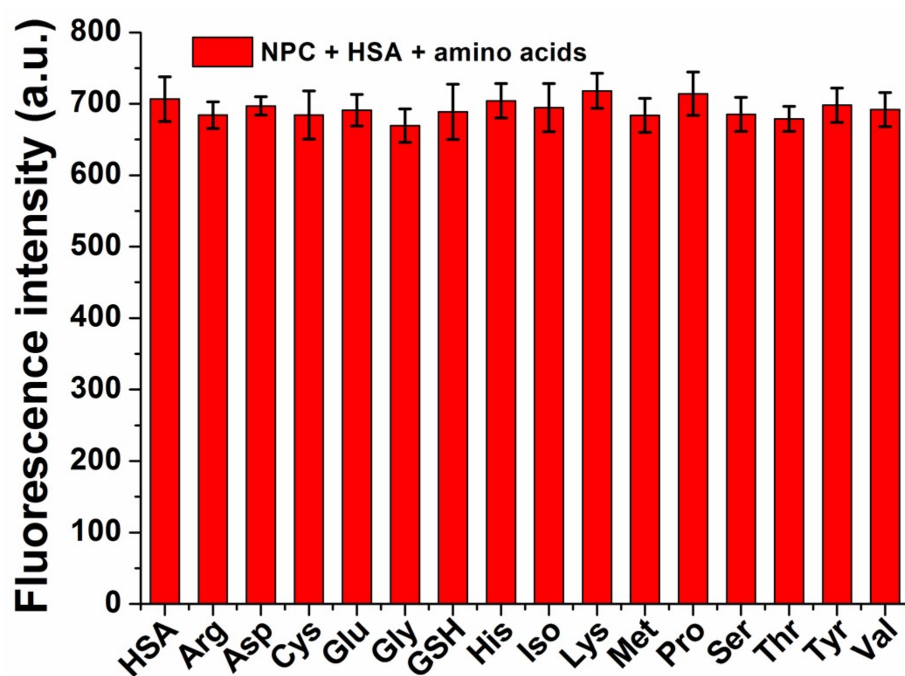


Fig. S8. The fluorescence responses of **NPC** (5 μM) to 1 equiv. HSA with the addition of various amino acids (5 μM) in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

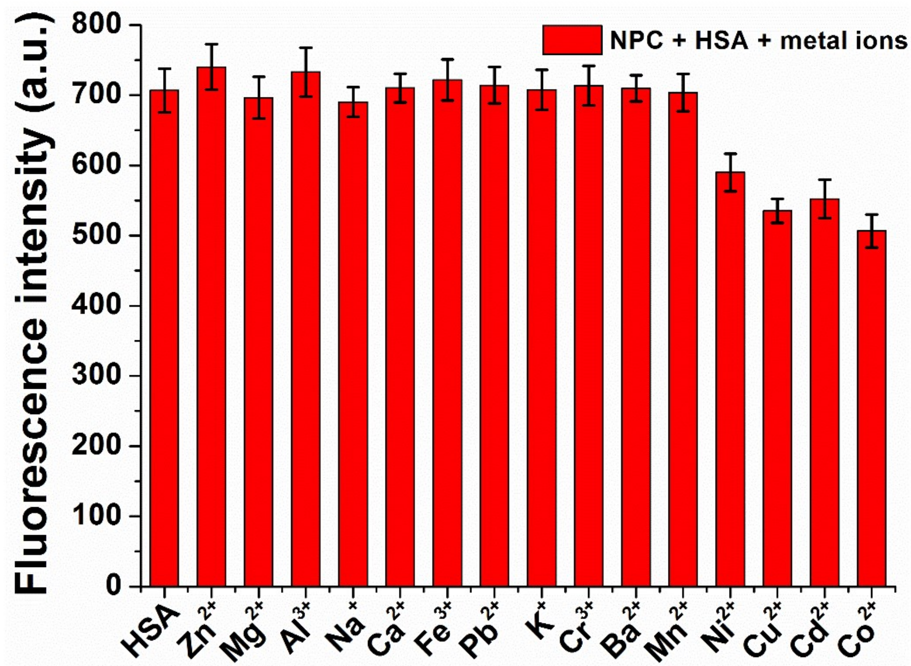


Fig. S9. The fluorescence responses of **NPC** (5 μM) to 1 equiv. HSA with the addition of various metal ions (5 μM) in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

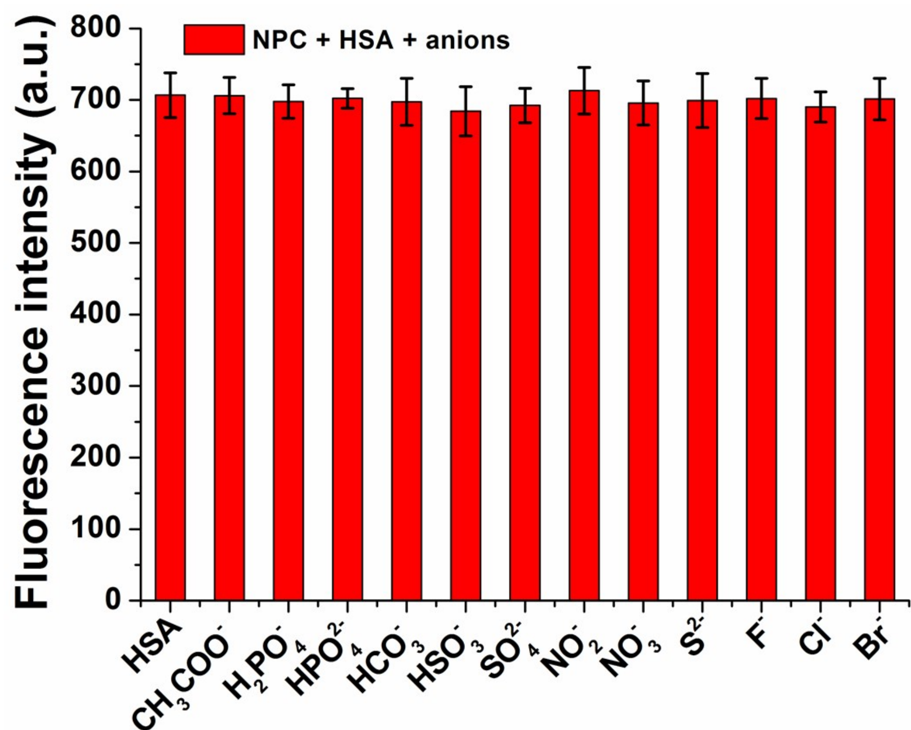
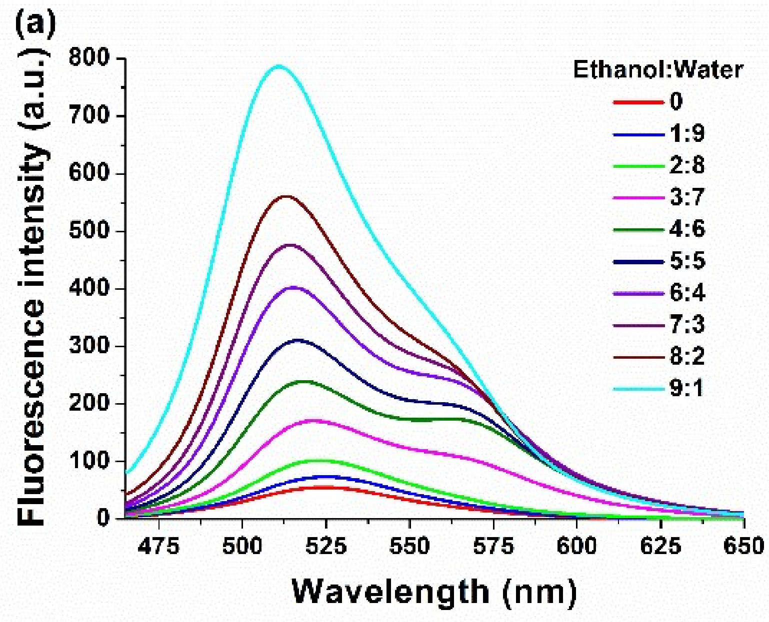
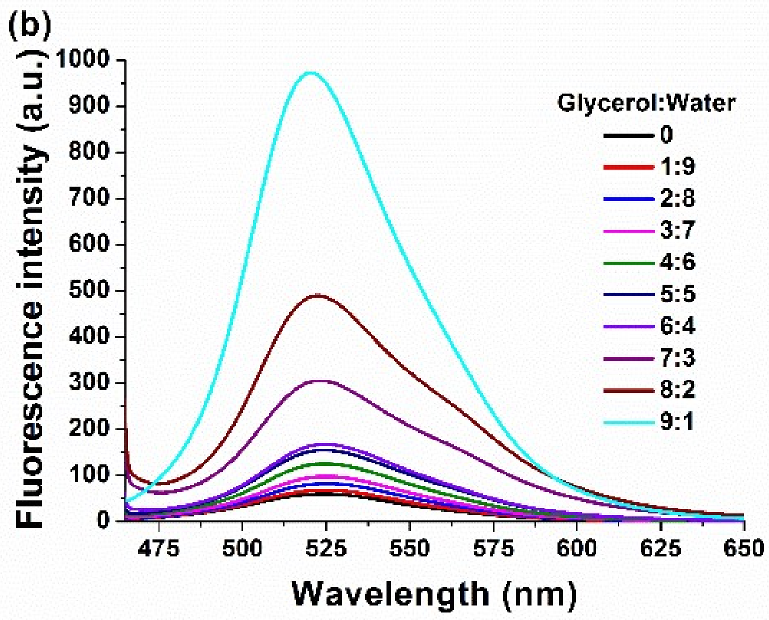


Fig. S10. The fluorescence responses of **NPC** (5 μM) to 1 equiv. HSA with the addition of various anions (5 μM) in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).


(a)
Fluorescence intensity (a.u.)
Wavelength (nm)
Ethanol:Water
0
1:9
2:8
3:7
4:6
5:5
6:4
7:3
8:2
9:1
0
100
200
300
400
500
600
700
800
475
500
525
550
575
600
625
650


(b)
Fluorescence intensity (a.u.)
Wavelength (nm)
Glycerol:Water
0
1:9
2:8
3:7
4:6
5:5
6:4
7:3
8:2
9:1
0
100
200
300
400
500
600
700
800
900
1000
475
500
525
550
575
600
625
650

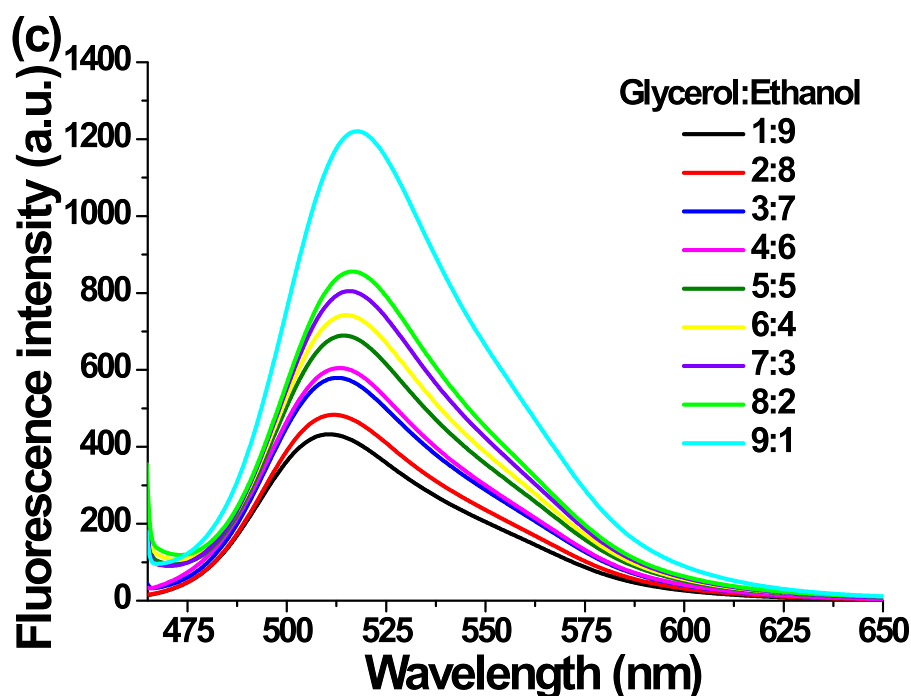


Fig. S11. The fluorescence spectra of **NPC** (5 μM) in different volume ratio of ethanol/water (a), glycerol/water (b) and methanol-glycerol (c) from 1:9 to 9:1 for studying the influence of polarity and viscosity on **NPC**, respectively (λ_{ex} = 450 nm, slit: 5 nm/5 nm).

Time-resolved fluorescence analysis

During the time-resolved fluorescence analysis, the curves generated for fluorescence lifetime intensity decay were fitted to the following equation to estimate the lifetime of the samples.

$$R(t) = \sum_{i=1}^{n} \alpha_i \exp(-\frac{t}{\tau_i})$$

Where, α_i denotes the pre-exponential factors of the i_{th} component and τ_i is its relative lifetime and n is the number of distinct decay components. Again, the mean lifetimes (τ_m) of the samples were calculated using the following equation.

$$\tau_m = \alpha_1 \tau_1 + \alpha_2 \tau_2$$

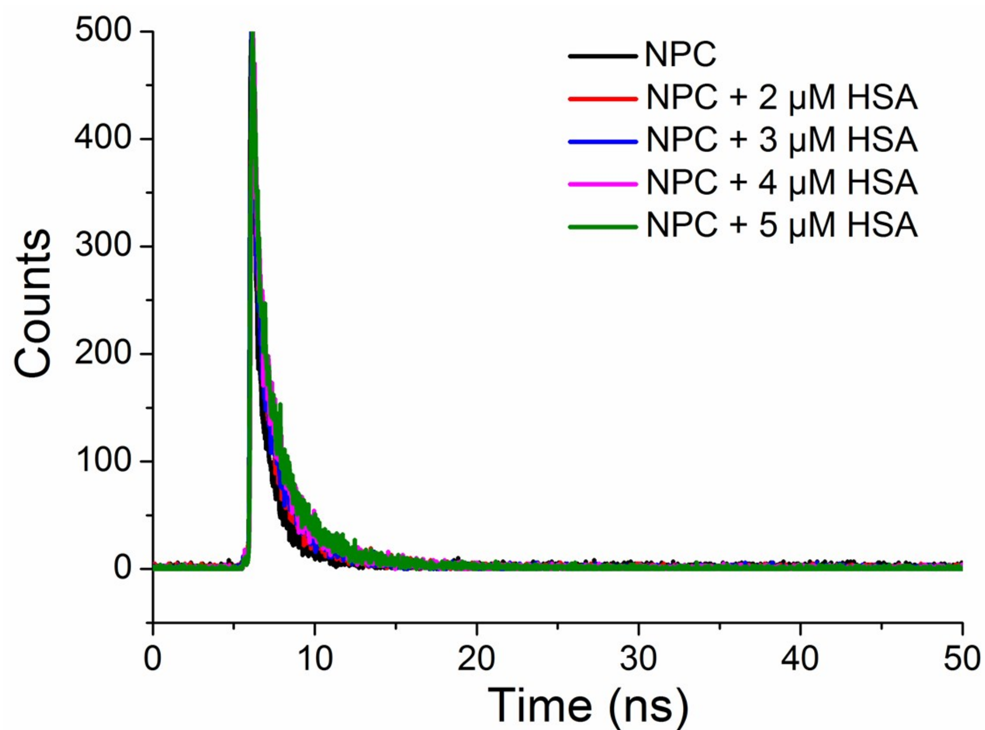


Fig. S12. Time-resolved fluorescence decay spectra of NPC (5 μM) in the absence and with the incremental addition of HSA (0−5 μM) (λ_{ex} = 450 nm, λ_{em} = 513 nm).

Table S1. Fluorescence lifetime decay parameters of NPC (5 μM) with gradual addition of HSA.

System	[HSA] (μM)	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_m (ns)	χ^2
NPC-HSA	0	0.29	23.35	1.44	76.65	1.17	1.082
	2	0.44	25.15	2.00	74.85	1.61	1.034
	3	0.39	20.08	2.00	79.92	1.68	1.012
	4	0.33	14.09	1.92	85.91	1.70	1.080
	5	0.37	15.35	2.06	84.65	1.80	1.000

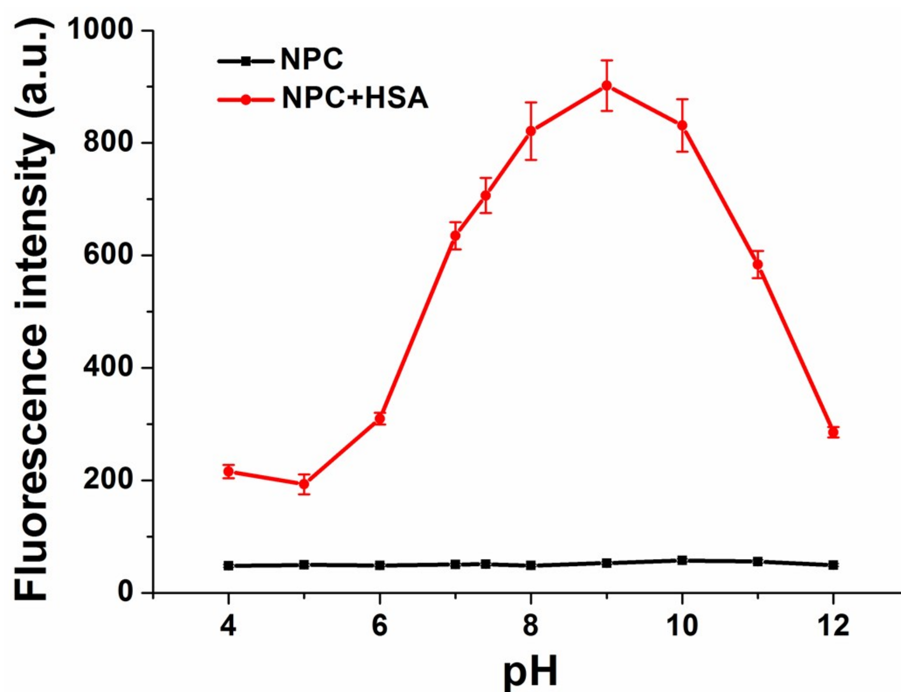


Fig. S13. The influence of pH value in the range of 2–12 on the fluorescence spectra of **NPC** (5 μM) in the absence and presence of 1 equiv. HSA in PBS (pH 7.4) buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

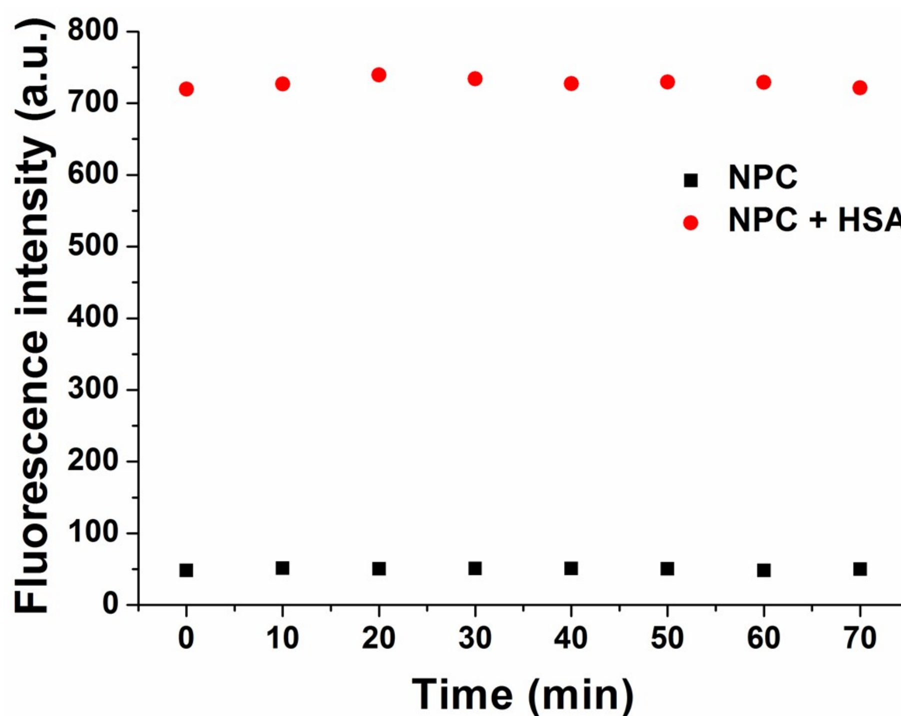


Fig. S14. The fluorescence intensity of **NPC** (5 μM) in the absence and presence of 1 equiv. HSA during 70 min in PBS (pH 7.4) buffer solution for stability measurement (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

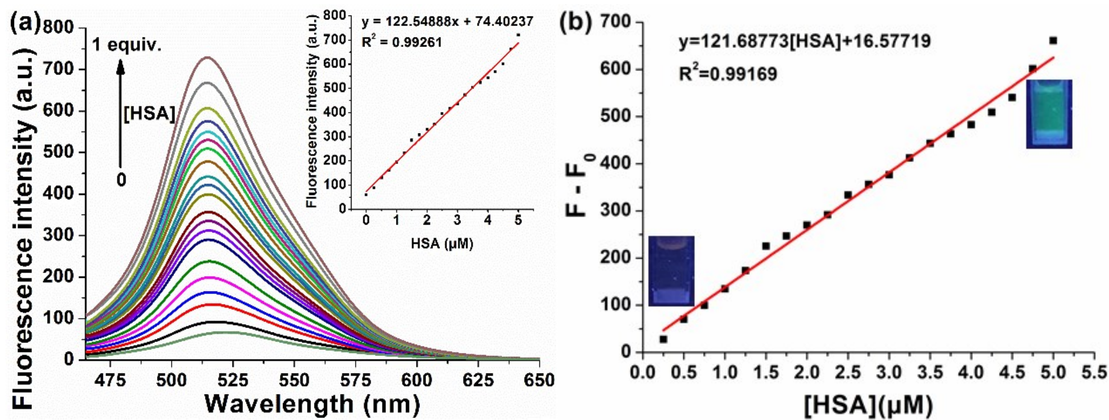


Fig. S15. (a) The fluorescence emission spectra of **NPC** (5 μM) with the addition of different concentrations of HSA (0–1 equiv.) in PBS buffer solution (pH = 7.4). (b) The linear relationship between fluorescence intensity changes of probe **NPC** (5 μM) at 513 nm and HSA concentrations in the range of 0–5 μM. (λ_{ex} = 450 nm, slit: 5 nm/5 nm).

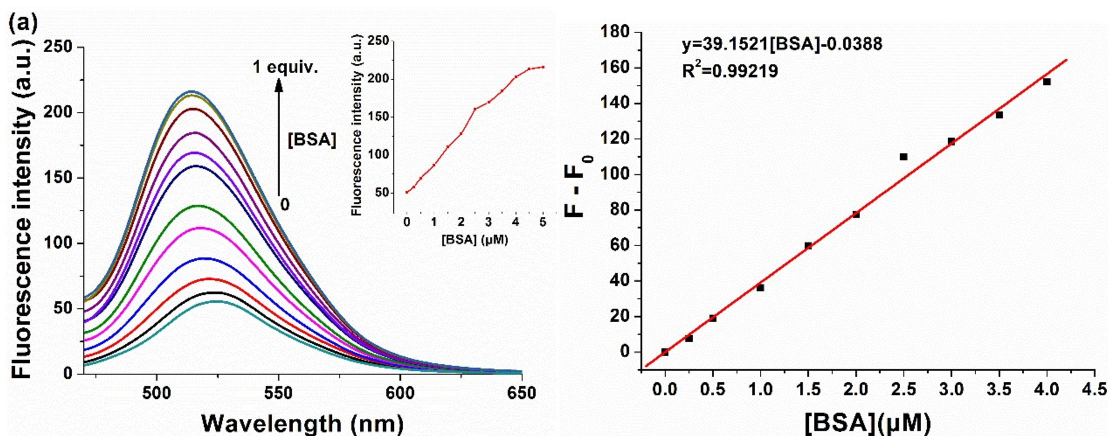


Fig. S16. (a) The fluorescence emission spectra changes of probe **NPC** (5 μM) in PBS (pH 7.4) buffer solution after gradually increasing the concentrations of BSA (0–5 μM). The inset shows the fluorescence intensity of **NPC** at 517 nm vs. BSA concentrations. (b) The fluorescence intensity changes of **NPC** (5 μM) at 517 nm as a linear function of BSA (0–4 μM) in PBS (pH 7.4) buffer solution. The inset shows the corresponding color changes of **NPC** with the increasing concentrations of BSA (λ_{ex} = 450 nm, slit: 5 nm/5 nm).

HSA binding studies

To better understand the interaction of **NPC** with HSA, the fluorescence quenching data was analyzed with the linear Stern–Volmer equation:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q]$$

where F_0 and F represent the fluorescence intensity of HSA in the absence and presence of **NPC** which acted as quencher, k_q is the quenching rate constant, τ_0 denote the average lifetime of the HSA without quencher (about 10^{-8} s), [Q] is the concentration of probe **NPC** and K_{SV} is the Stern–Volmer quenching constant. As shown in Fig. S19a, the fluorescence intensity of HSA was gradually quenched by probe **NPC** and K_{SV} can be obtained from the slope of F_0/F *vs.* [Q] (Fig. S19a inset). From the k_q values, we can know the quenching process of HSA by **NPC** is a static or dynamic quenching process. The number of binding sites and the binding constant of **NPC** with HSA could be determined from the Scatchard equation:

$$\log[(F_0-F)/F] = \log K_b + n\log[Q]$$

where K_b and n stand for the binding constant and binding sites, respectively. And the K_b and n can be obtained from the intercept and slope of $\log[(F_0-F)/F]$ *vs.* log[Q], respectively.

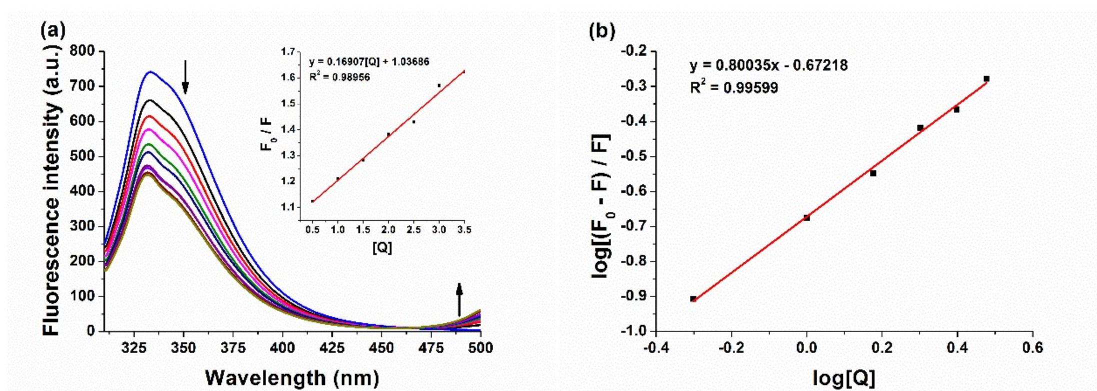


Fig. S17. (a) The fluorescence quenching of HSA (2 μM) with the addition of probe **NPC** (0–4 μM) in PBS (pH 7.4) buffer solution. Inset: Stern–Volmer plot of the fluorescence quenching of HSA induced by probe **NPC**. (b) The Modified Stern-Volmer plot of the fluorescence quenching of HSA induced by probe **NPC** in PBS (pH 7.4) buffer solution. (λ_{ex} = 295 nm, λ_{em} = 332 nm, slit: 5 nm/5 nm).

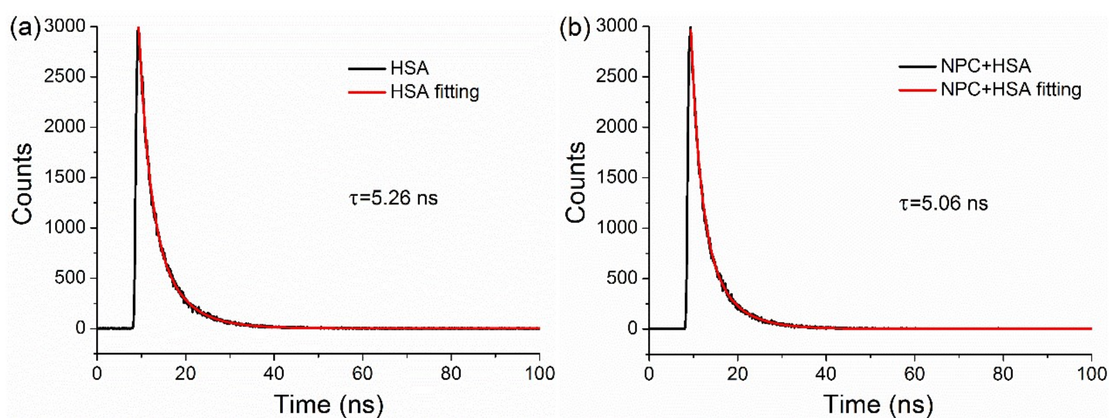


Fig. S18. Fluorescence lifetime tests (black line) and corresponding fittings (red line) of HSA in the absence (a) and the presence (b) of NPC in PBS (10 mM, pH 7.4). λ_{ex} = 295 nm, λ_{em} = 332 nm.

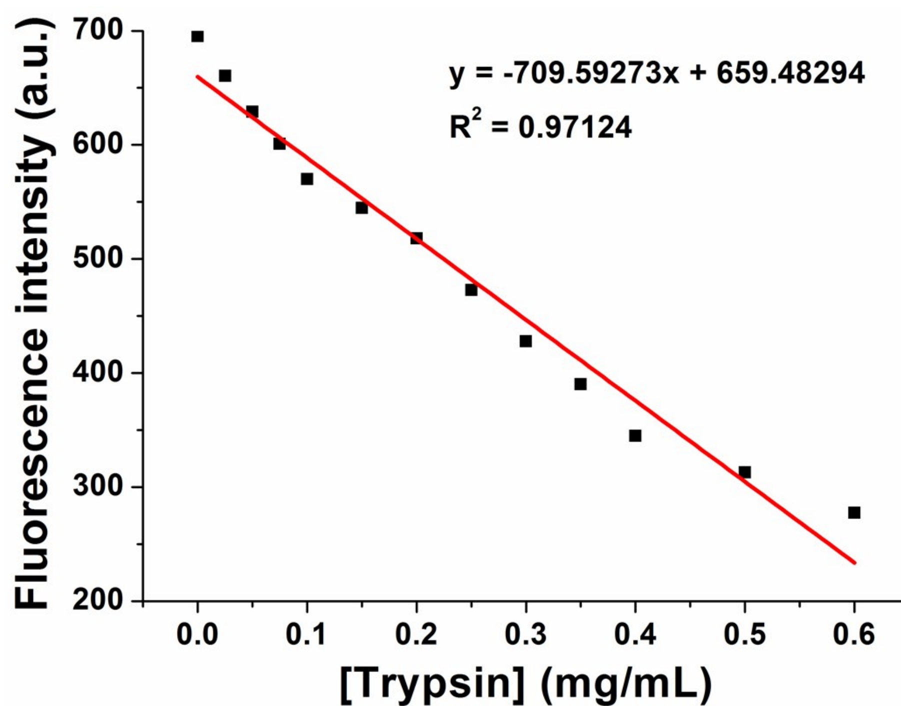


Fig. S19. The linear relationship of the fluorescence intensity with trypsin concentrations (0–0.65 mg/mL) in PBS buffer solution (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).

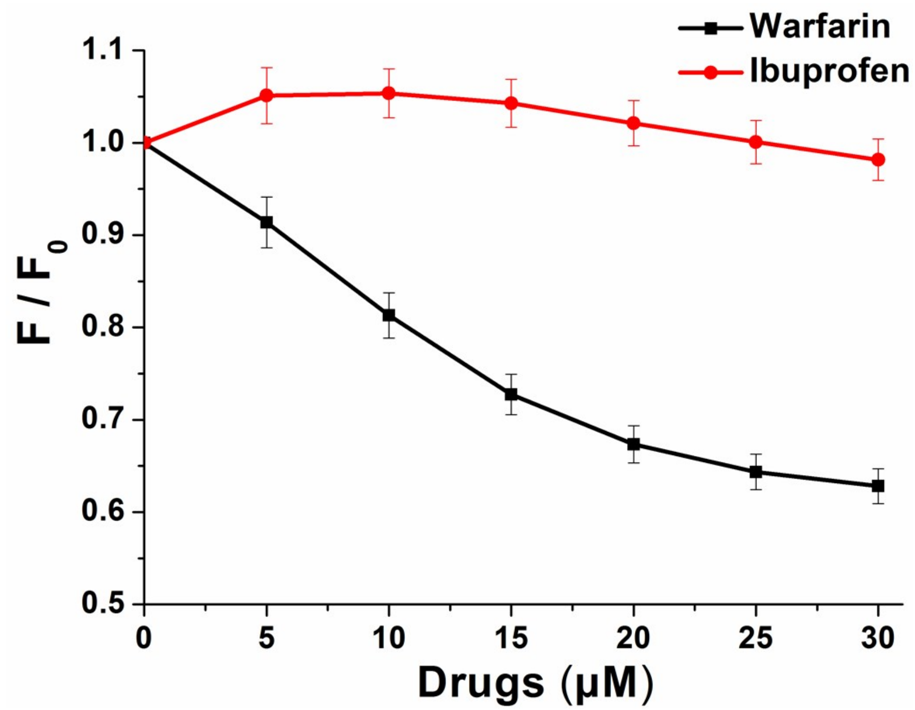


Fig. S20. The competitive displacement effect of site-specific drugs warfarin and ibuprofen for **NPC**-HSA complex (5 μM **NPC** and 5 μM HSA) (λ_{ex} = 450 nm, λ_{em} = 513 nm, slit: 5 nm/5 nm).