Supporting Information

Methyl 3-(2-formyl-5-methoxynaphthalen-1-yl)propanoate as a fluorescent probe for folding and binding studies of human serum albumin

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1. Materials and general methods:

All reactions were carried out in oven dried glassware with magnetic stirring. All solvents were purified and dried according to standard methods prior to use. Probe Methyl 3-(2-formyl-5-methoxynaphthalen-1-yl)propanoate (MFMNP) were prepared by our previous reported methods.[1] 1H spectra were recorded on BRUKER 400 MHz in CDCl3 and 13C NMR spectra were recorded on 100 MHz in CDCl3 using TMS or residual solvent signals as internal standard. Data for 1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved, coupling constant(s) in Hz, integration). Data for 13C NMR are reported in terms of chemical shift (δ, ppm). High resolution mass spectra (HRMS) were obtained by the ESI (Q-TOF) ionization sources. Routine monitoring of reactions was performed using precoated silica gel TLC plates from E-Merck. All the chromatographic separations were carried out by using silica gel (Acme's, 100-200 mesh). The probe molecule was synthesized by following the detailed procedure given below. The HSA protein, surfactant SDS, urea, tris-HCl buffer, spectroscopic grade water and acetonitrile were purchased from SRL India chemical supplier. The concentration 0.01 M of this buffer used throughout the experiments to maintain the pH 7.03. For the measurement of absorption, a LABINDIA UV 3200 XE UV/vis spectrophotometer was used, and for the emission study, PerkinElmer fluorescence spectrophotometer FL 6500 instruments were used to study the samples. The excited state fluorescent lifetime measurement was carried out on Horiba Jobin Yvon Deltaflex, Springer New York, 2006.

Quantum yield (ϕ) of a fluorophore is calculated by the conventional optical method, based on eq. S1.[2]

$$\Phi_2 = \Phi_1 \times \frac{\int F_2(\lambda)d\lambda}{\int F_1(\lambda)d\lambda} \times \frac{A_1(\lambda)}{A_2(\lambda)} \times \frac{{n_2}^2}{n_1^2}$$

eq. S1

Suffixes 1 and 2 refer to standard and MFMNP, respectively. Here quantum yields, integrated fluorescent intensities, absorbances, and refractive indexes of the medium are denoted by ϕ, $\int F(\lambda)d\lambda,$. $A(\lambda)\ and\ n$, respectively.

As per the requirement, the emission data were fitted at double or triple exponential function monitoring 400 nm wavelength best on eq. S2.[3]

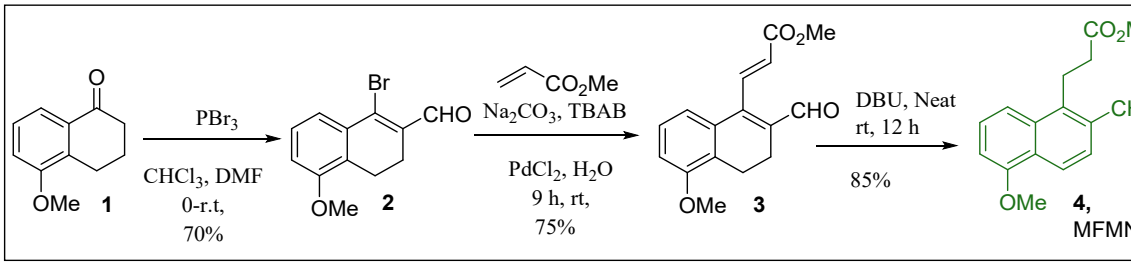
$$F(t) = a_0 + a_1 e^{(-t/\tau_1)} + a_2 e^{(-t/\tau_2)} + a_3 e^{(-t/\tau_3)} \quad \text{eq. S2}$$

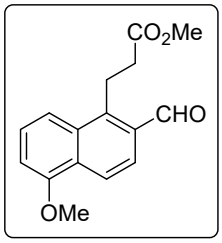
The time shift between sample decay and IRF (Instrument Response Function) is denoted by τ_1, τ_2, and τ_3 are different lifetime components of different characteristic excited states with amplitudes a_1, a_2, and a_3, respectively. The average fluorescence lifetime is given by eq. S3.

$$\tau_{av} = <\tau> = \sum_i a_i \tau_i / \sum_i a_i \quad \text{eq. S3}$$

2. General procedure for the synthesis of Methyl 3-(2-formyl-5-methoxynaphthalen-1-yl)propanoate (MFMNP):

We prepared compound MFMNP following our previous literature procedure.[1]

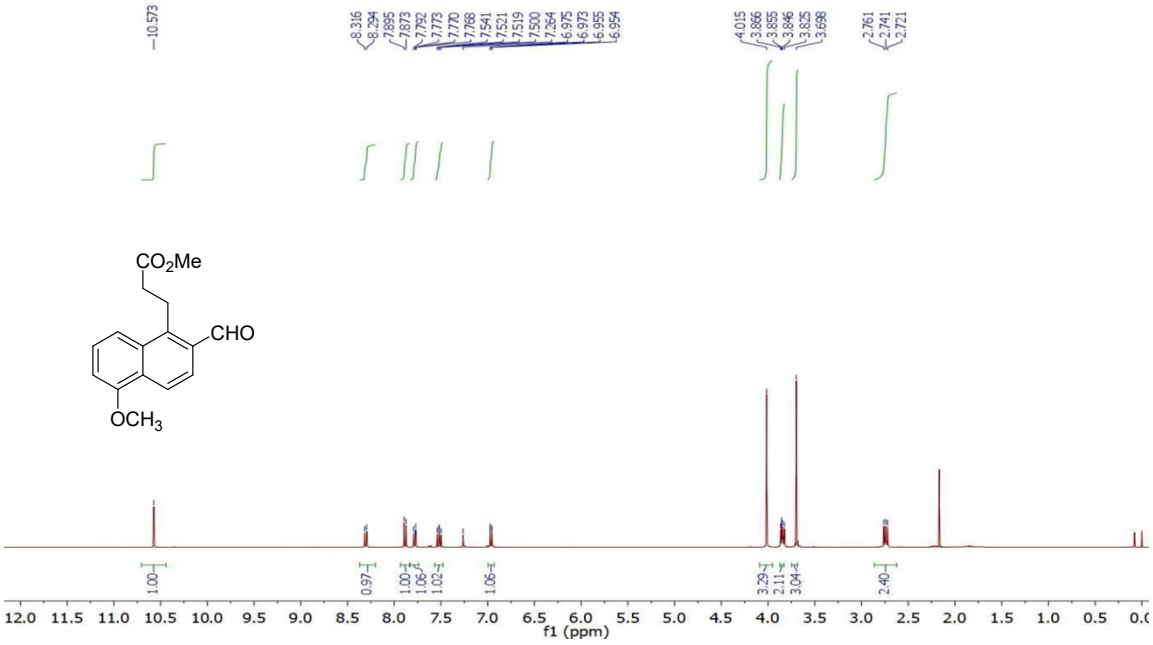


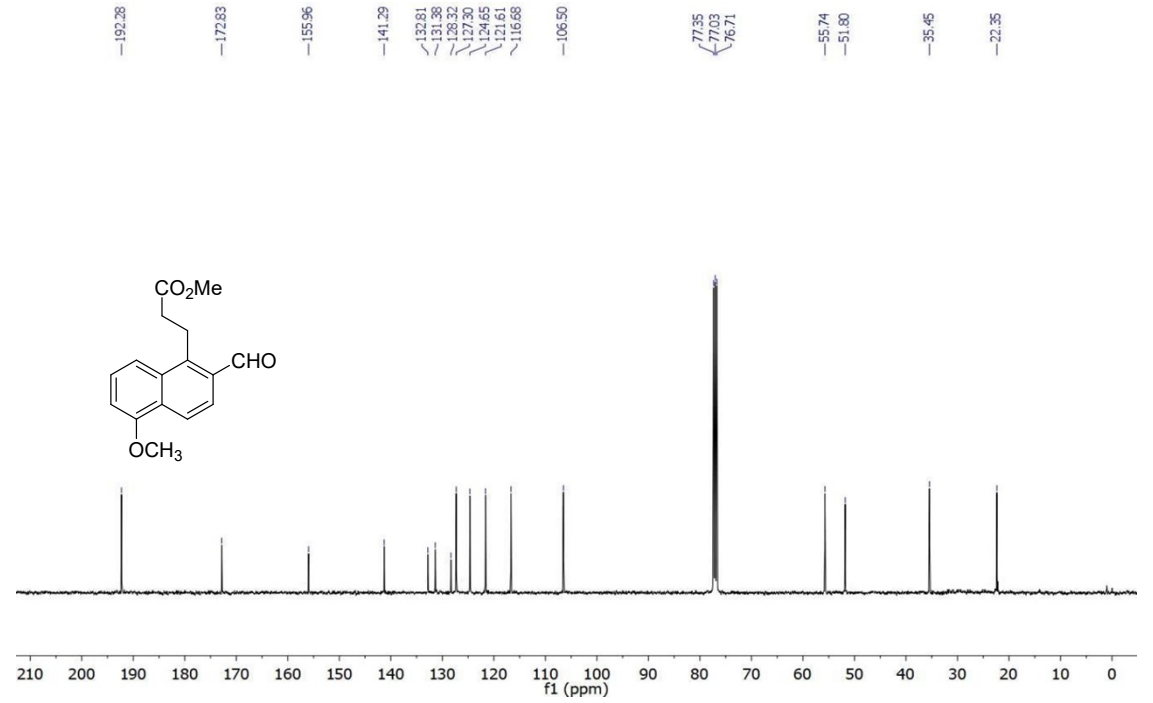


Scheme S1. Preparation of MFMNP from α-tetralone.

Methyl 3-(2-(1H-benzo[d]imidazol-2-yl)naphthalen-1-yl)propanoate (MFMNP) (112.5 mg, 0.413 mmol, 1.0 equiv.) was taken and DBU (6.288 mg, 0.0413 mmol, 0.1 equiv.) was added, the reaction mixture was stirred for 12 hours at room temperature. After 12 hours the resulting reaction mixture was dried under reduced pressure. The crude residue was purified by silica gel column chromatography (10% EtOAc in hexane) The compound was obtained as a yellowish oily product in 85% yield.1H NMR (400 MHz, Chloroform-d) δ: 10.57 (s, 1H), 8.30 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.83–7.74 (m, 1H), 7.52 (dd, J = 8.7, 7.7 Hz, 1H), 6.96 (dd, J = 7.8, 0.7 Hz, 1H), 4.02 (s, 3H), 3.87–3.83 (m, 2H), 3.70 (s, 3H), 2.86–2.62 (m, 2H);13C NMR (100 MHz, Chloroform-d) δ: 192.3, 172.8, 156.0, 141.3, 132.8, 131.4, 128.3, 127.3, 124.7, 121.6, 116.7, 106.5, 55.7, 51.8, 35.5, 22.4; HRMS cald. for $C_{16}H_{16}O_4$: $(M+Na)^+$ 295.0947, found: 295.0940.

3. ^{1}H and ^{13}C NMR of MFMNP





4. Effect of DMSO on MFMNP and two-dimensional interaction diagrams of the HSA-MFMNP complex:

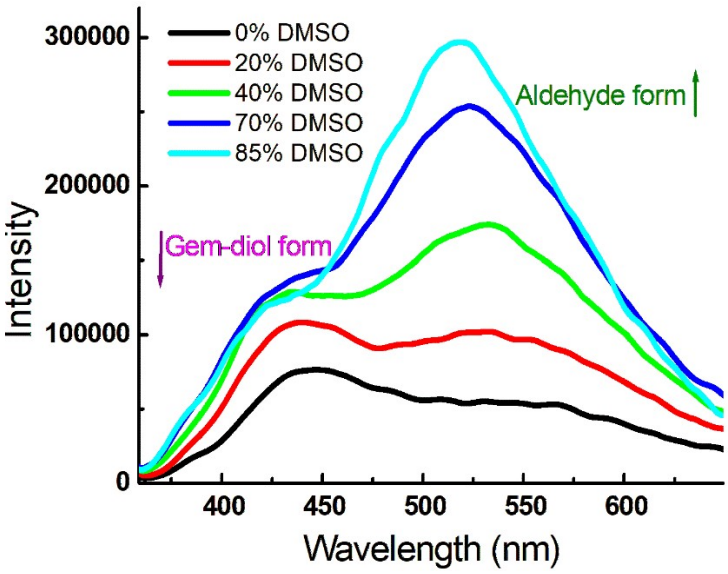
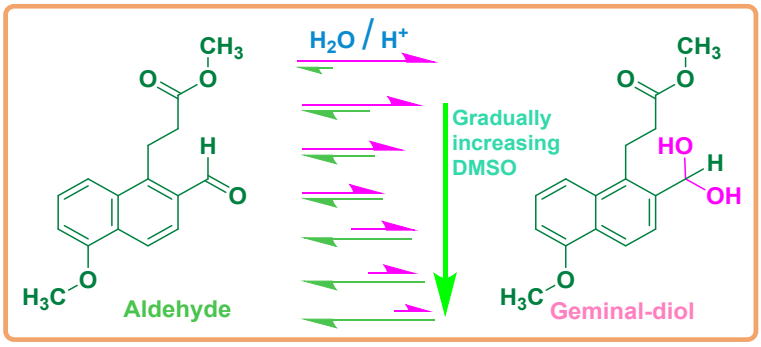


Figure S1. The emission spectra (λ_{exc} = 350 nm) of MFMNP in a mild acidic medium (aqueous) treated with gradually adding DMSO. (Extremely diluted AcOH is used to acidify the solution.)



Scheme S2. Schematic representation of DMSO effect on gem-diol equilibrium.

5. The emission spectra of 550 nm band of MFMNP vs. SDS concentration (mM) to determine the CMC of SDS.

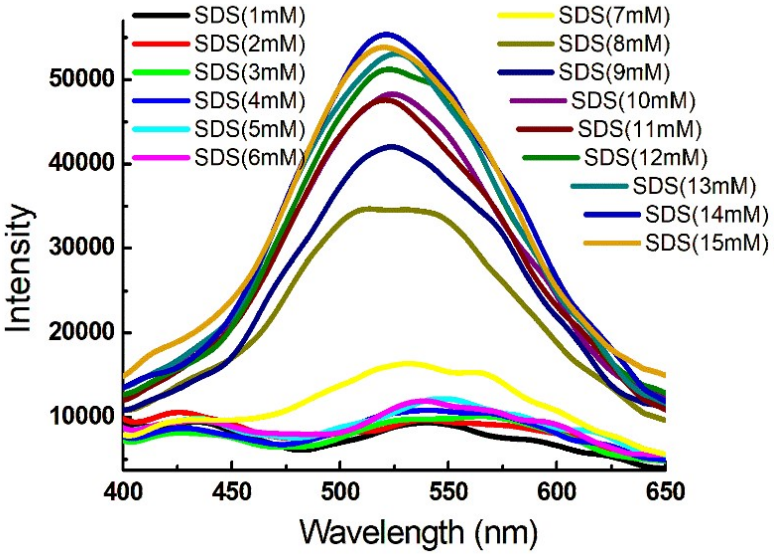


Figure S2. The emission spectra (λ_{exc} = 350 nm) of 550 nm band of MFMNP vs. SDS concentration (mM) to determine the CMC of SDS. (Concentration of the MFMNP is 3.62 μM.).

6. Benesi-Hildebrand spectra of MFMNP and HSA interaction

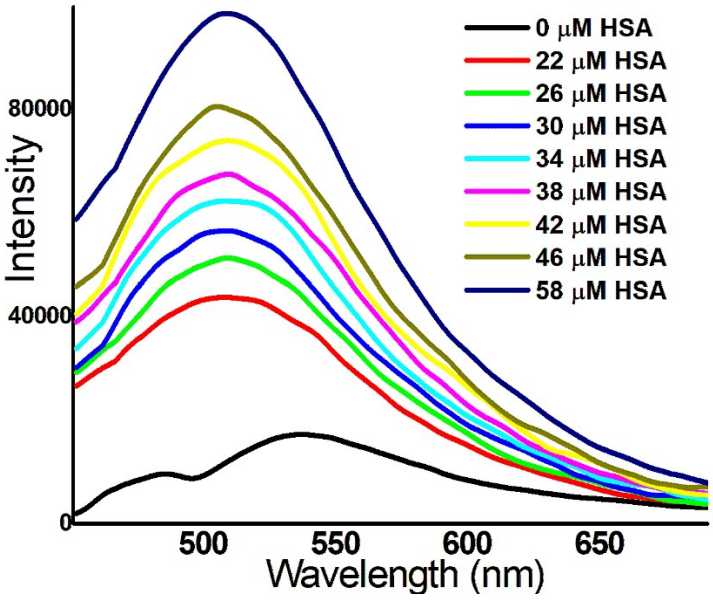


Figure S3. Benesi-Hildebrand spectra (λ_{exc} = 350 nm) of MFMNP and HSA interaction in 0.01 M Tris-HCl solvent (concentrations of HSA 22, 26, 30, 34 38, 42, 46 and 58 μM and concentration of the probe is 1.6 μM).

7. Emission spectra of FRET (HSA to MFMNP)

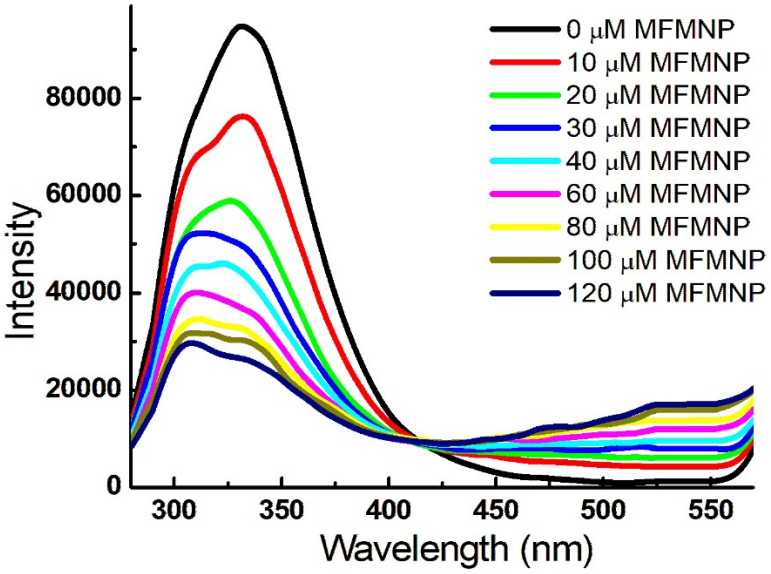
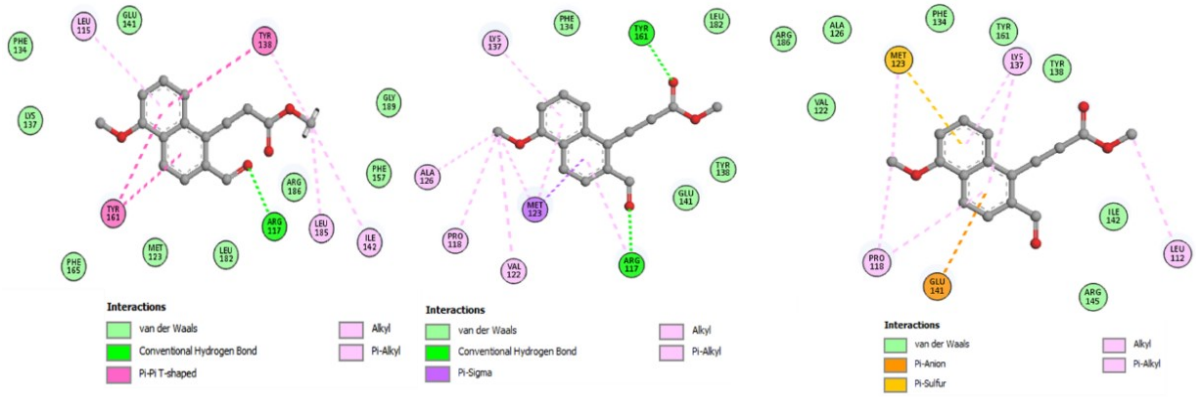


Figure S4. Fluorescence spectra of HSA (entire range) as a function of gradual increasing concentration of MFMNP (λ_{exc} = 280 nm) in aqueous Tris-HCl buffer solution (0.01M). (Concentration of HSA is 5.17 μM)

8. Two-dimensional interaction diagrams of the HSA-MFMNP



(i) (ii) (iii)

Figure S5. Two-dimensional interaction diagrams of the HSA-MFMNP complex at three time points: (i) 0 ns, (ii) 50 ns, and (iii) 100 ns.

9. Emission spectra of MFMNP in various solvents

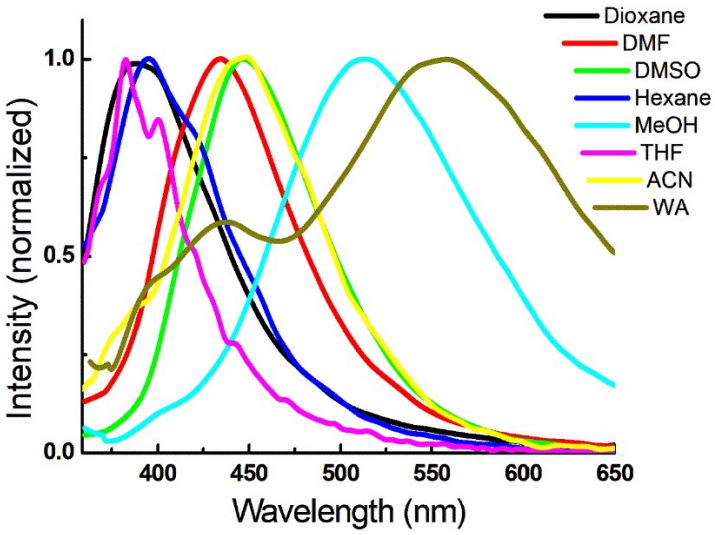


Figure S6: Emission spectra (normalized) of MFMNP in various solvents having different polarity

10. Absorption and emission spectra of MFMNP in different viscosity media

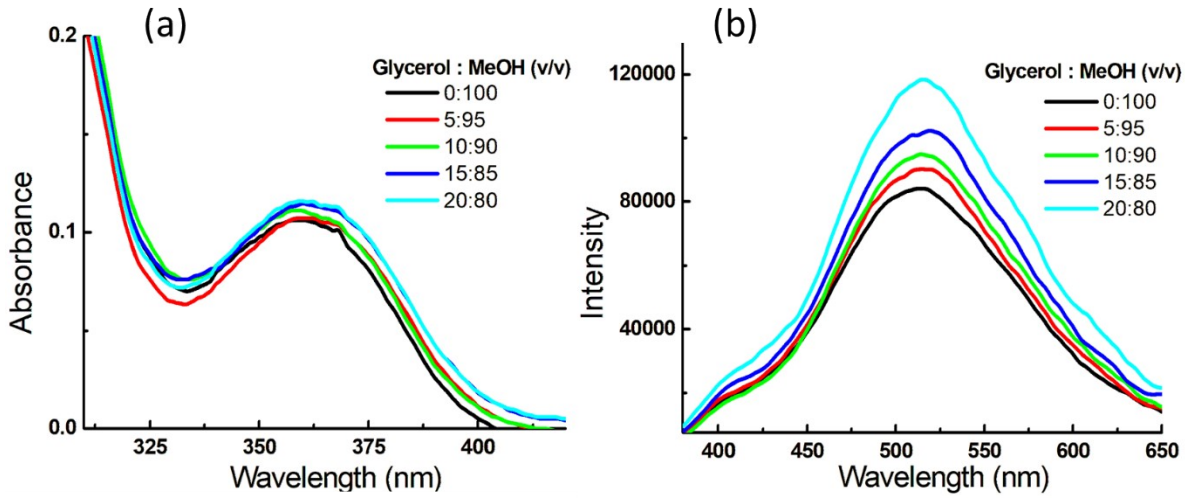


Figure S7: (a) Absorption and (b) Emission spectra (λ_{exc} = 350 nm) of MFMNP in various media having different viscosity

11. Emission spectra of MFMNP-HSA-probe system treated with warfarin and ibuprofen separately.

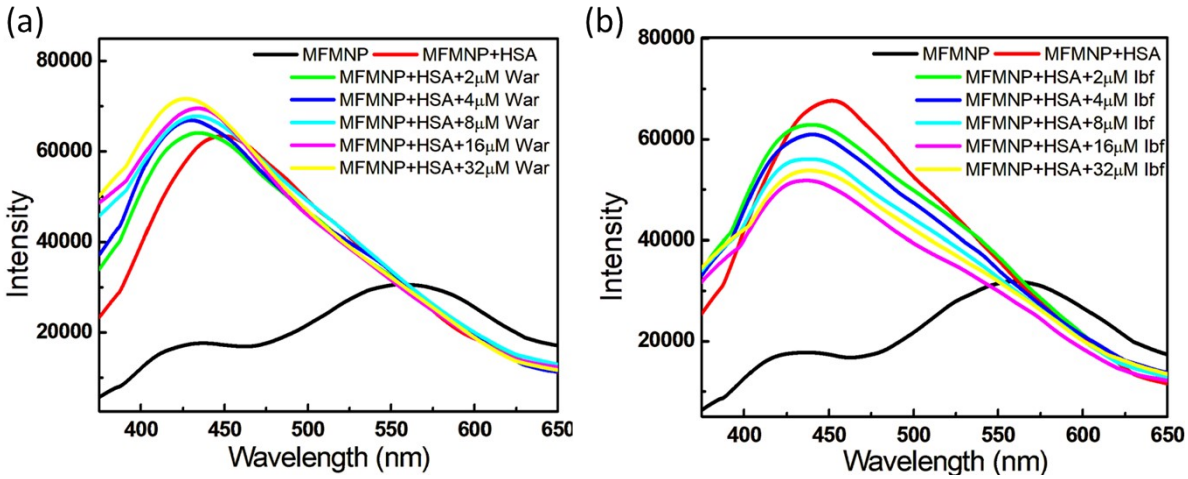


Figure S8: Emission spectra (λ_{exc} = 350 nm) of MFMNP-HSA- probe system treated with gradual addition of (a) warfarin and (b) ibuprofen.

12. References:

1 M. Sau, S. Dubey, J. Shaoo, G. Shaoo, P. Trivedi, A. Jana, S. Samanta and T. Das, *EurJOC.*, 2022, **2022**, e202201188.

2 J. Hu and C. Zhang, *Anal. Chem.*, 2013, **85**, 2000-2004.

3 P. Kumar and H. B. Bohidar, *J. Fluoresc.*, 2012, **22**, 865-870.