Supporting Information

Potential Fluorescent Probe Ethyl 3-(2-(1H-benzo[d]imidazol-2-yl)-5 methoxynaphthalen-1-yl)propanoate for Real-Time Monitoring of Human Serum Albumin Dynamics and Cancer Cell Imaging

Shreya Roy,[a] Pragya Trivedi,[b] Titas K. Mukhopadhyay,[a] Sapana Dubey,[a] Krishna Prasad Bag,[a] Sipra Ghosh,[c] Subhojit Das[a] Subrata Mahanta,[a] Tapas Das,*[a]

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a Department of Chemistry
National Institute of Technology Jamshedpur
Jamshedpur-831014, India
tapas.chem@nitjsr.ac.in

b Department of Chemistry
CSIR-Indian Institute of Chemical Technology Hyderabad
Hyderabad- 500007, India

c Metal Extraction and Recycling Division, CSIR-National Metallurgical Laboratory Jamshedpur

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 3-(2-(1H-benzo[d]imidazol-2-yl)-5 methoxynaphthalen-1-yl)propanoate (EBINP) was prepared by our previously reported method.[1] ^{1}H spectra were recorded on BRUKER 400 MHz in $CDCl_3$ and ^{13}C NMR spectra were recorded on 100 MHz in $CDCl_3$ using TMS or residual solvent signals as an internal standard. Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved), and coupling constants (in Hz). Data for ^{13}C 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 of 0.01 M of this buffer was used throughout the experiments to maintain the pH of 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. The dynamic light scattering (DLS) and zeta potential experiment ware carried out on Anton Paar Litesizer 500 instrument.

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 to a double or triple exponential function monitoring the 400 nm wavelength based on eq. S2.[3]

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

eq. S2

The time shift between sample decay and IRF (Instrument Response Function) is denoted by τ_1, τ_2, and τ_3 which 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.

$$\tau_{av} = <\tau> = \sum_i a_i\tau_i / \sum_i a_i$$

eq. S3

For DLS Stokes-Einstein equation S4 is used.[4]

$$D_h = \frac{k_b T}{3\pi\eta D_t}$$

eq. S4

Where D_h is the hydrodynamic diameter, k_b is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity of the dispersant and D_t is translational diffusion coefficient.

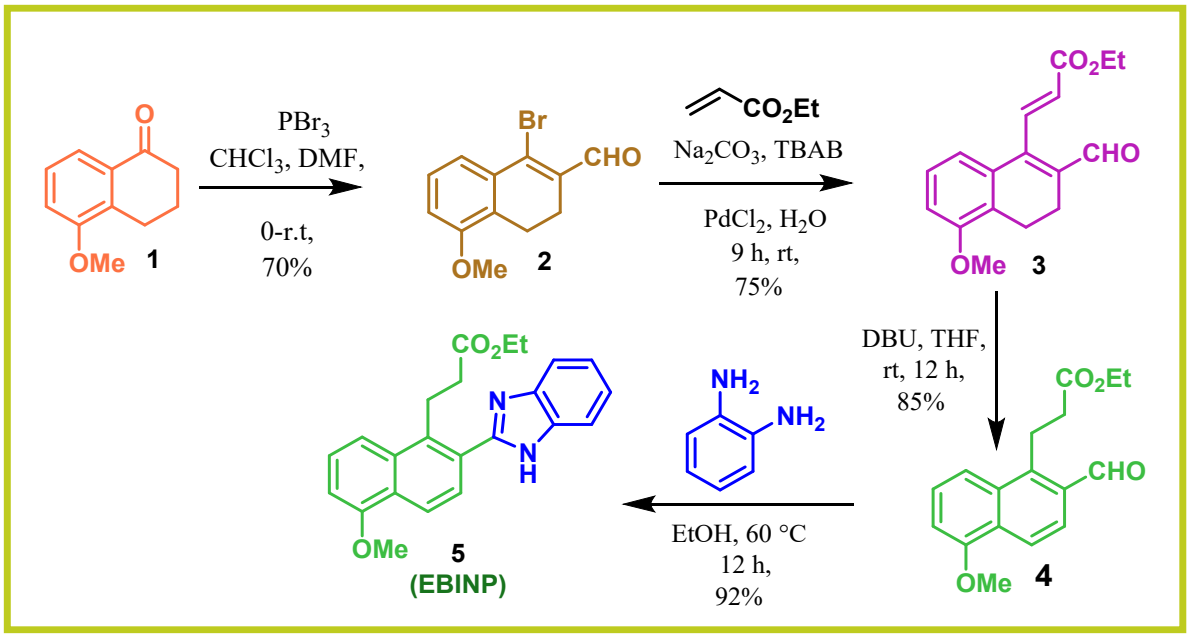
For Zeta potential (ζ) measurement, Henry equation S5 is used.[5]

$$\zeta = \frac{3\eta\mu_e}{2\varepsilon\varepsilon_0 f(Ka)} \quad \text{eq. S5}$$

Where η is the dynamic viscosity, μ_e is the electrophoretic mobility, ε is the relative permittivity of the medium, ε_0 is the permittivity of a vacuum and $f(Ka)$ is the Henry's function, which is typically assumed to be 1.5 (Smoluchowski approximation) for aqueous solutions.

2. General procedure for the synthesis of Ethyl 3-(2-(1H-benzo[d]imidazol-2-yl)naphthalen-1-yl)propanoate (EBINP):

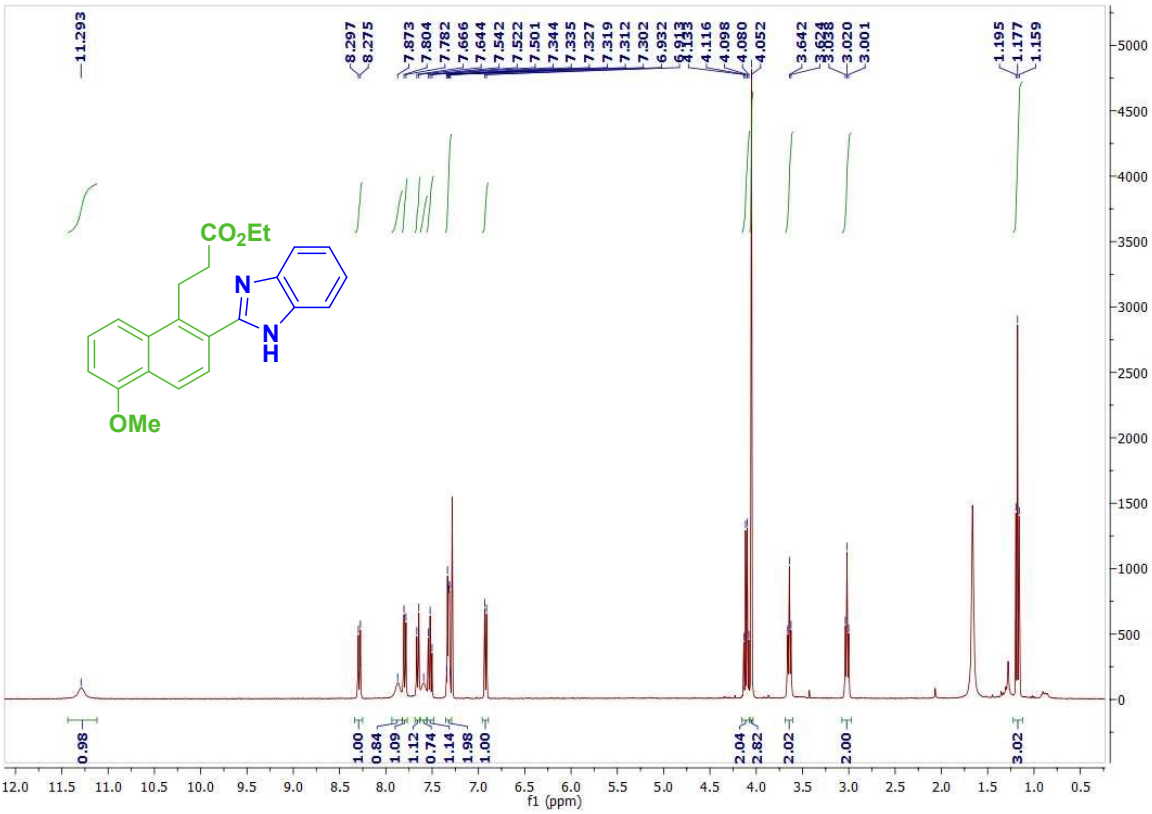
We prepared compound **5** following our previous literature procedure.[1]

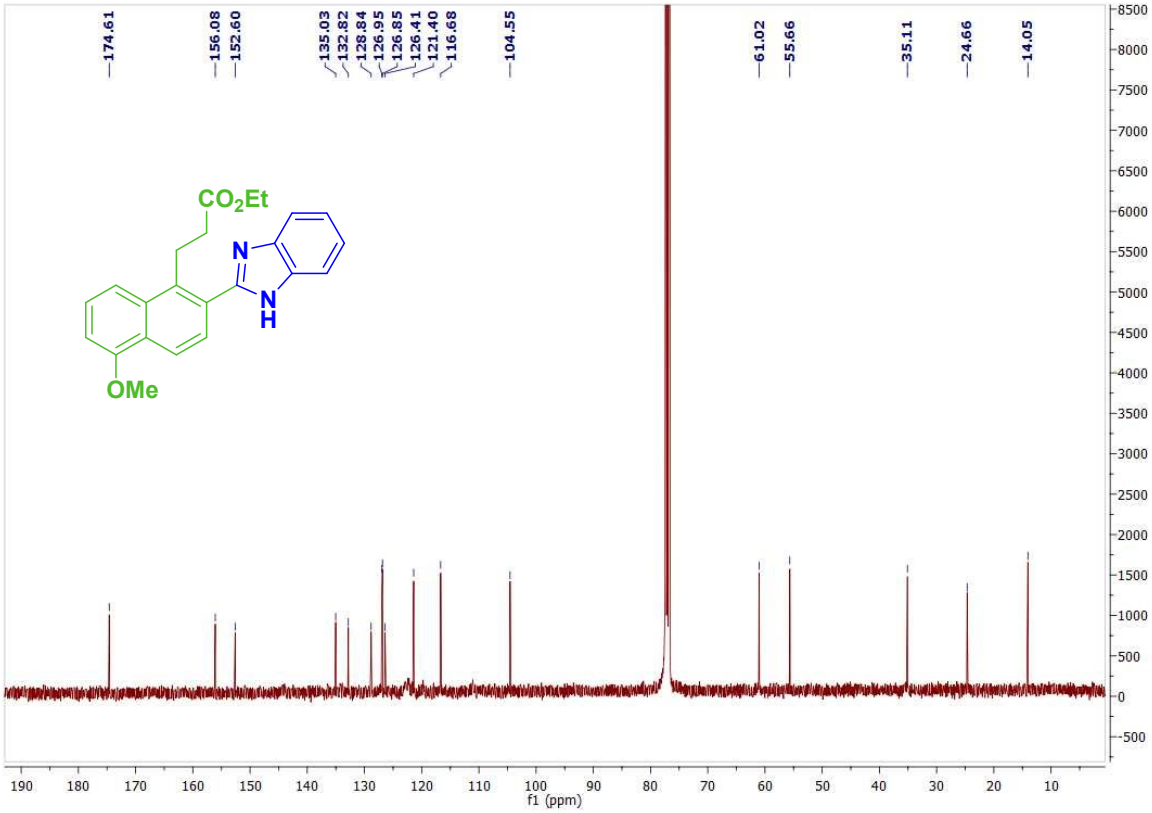


Scheme S1. Preparation of EBINP from 5-OMe substituted α-tetralone.

Ethyl 3-(2-(1H-benzo[d]imidazol-2-yl)-5-methoxynaphthalen-1-yl)propanoate (EBINP) (50 mg, 0.1334 mmol, 1.0 equiv.) was taken in a pressure tube, 1.5 ml EtOH was added as a solvent and benzene-1,2-diamine (14.5 mg, 0.1334 mmol, 1.0 equiv.) was added. The reaction mixture was stirred for 12 hours at 60 ºC temperature. After 12 hours the resulting reaction mixture was dried under reduced pressure. The reaction mixture was diluted with 20 ml of EtOAc and the organic phase was separated from the aqueous phase. The aqueous phase was extracted from EtOAc (2 x 15 ml), the combined organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (15% of EtOAc in hexane) to obtain an off-white amorphous solid in 92% yield (60 mg). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 11.93 (s, 1H), 8.08 (dd, *J* = 6.4, 3.2 Hz, 2H), 7.73 (s, 2H), 7.62 (dd, *J* = 6.4, 3.3 Hz, 3H), 7.35–7.29 (m, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 2.96 (t, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 1.32 (s, 9H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 174.6, 152.8, 133.7, 133.6, 133.2, 131.9, 128.6, 128.0, 126.3, 126.3, 125.2, 125.1, 81.7, 36.3, 27.9, 24.2, 19.3. **HRMS cald**. For $C_{25}H_{26}N_2O_2$: $(M+H)^+$ 386.1994; found: 386.2074

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


11.293
8.297
8.275
7.873
7.804
7.782
7.666
7.644
7.542
7.522
7.501
7.344
7.335
7.327
7.319
7.312
7.302
6.932
6.913
4.133
4.116
4.098
4.080
4.052
3.642
3.624
3.038
3.020
3.001
1.195
1.177
1.159
CO2Et
N
N
H
OMe
0.98
1.00
0.84
1.09
1.12
0.74
1.14
1.98
1.00
2.04
2.82
2.02
2.00
3.02
f1 (ppm)


174.61
156.08
152.60
135.03
132.82
128.84
126.95
126.85
126.41
121.40
116.68
104.55
61.02
55.66
35.11
24.66
14.05
CO2Et
N
N
H
OMe
f1 (ppm)

4. DLS and Zeta potential Graphs:

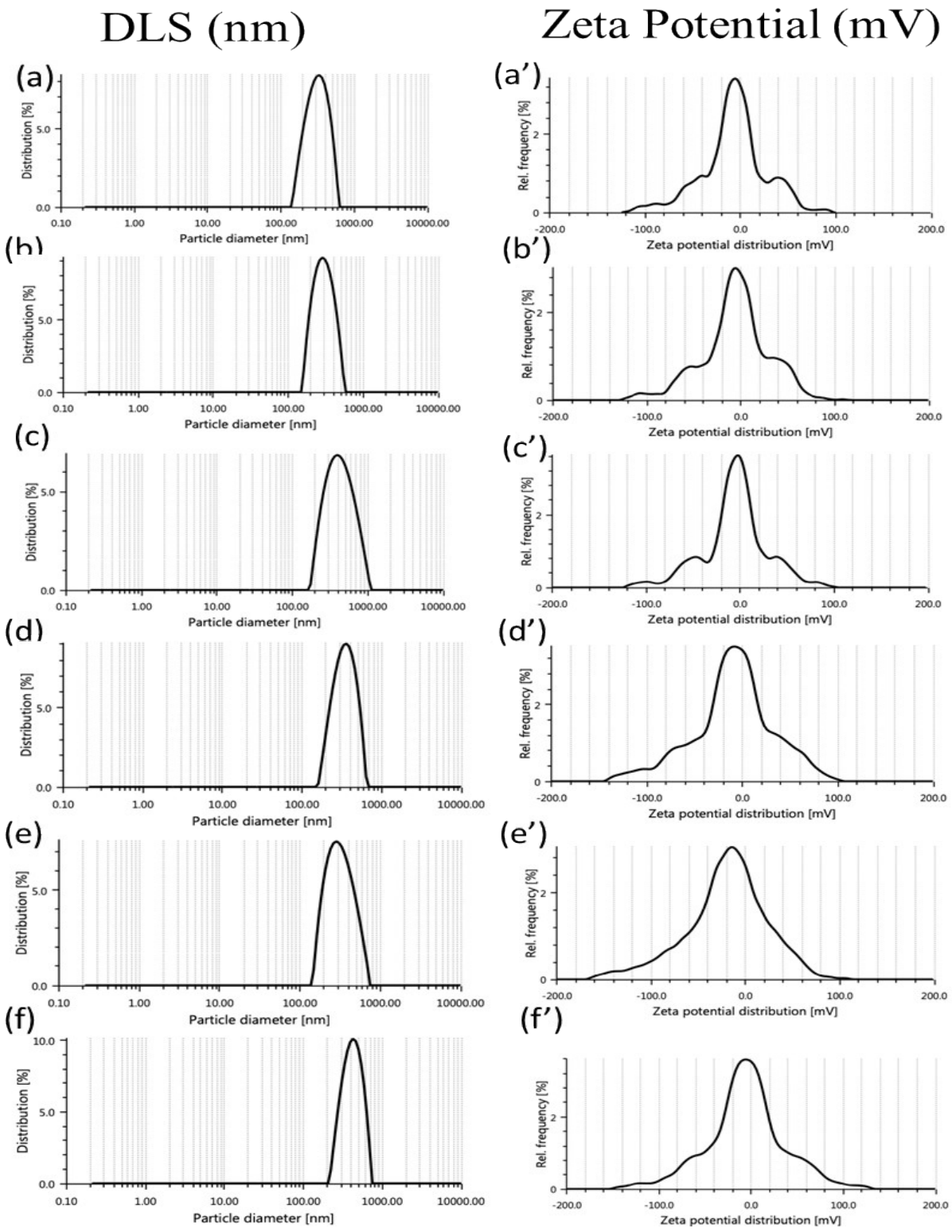


Figure S1: Representative (a–f) DLS size distributions and (a'–f') zeta potential profiles for the following systems: (a, a') pure HSA; (b, b') HSA + EBINP; (c, c') HSA + SDS (4 mM,

below CMC); (d, d') HSA + SDS (4 mM, below CMC) + EBINP; (f, f') HSA + SDS (7.9 mM, just before CMC) + EBINP and HSA + SDS (8.5 mM, just after CMC) + EBINP.

5. References:

1 S. Roy, T. K. Mukhopadhyay, M. Sau, S. Dubey, G. Gulfisha, K. Basudev, S. Nayak, G. Sahoo, S. Mahanta, T. Das, *New J. Chem.* **2025**, *49*, 11767–11777.

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

4 F. Varenne, H. Hillaireau, J. Bataille, C. Smadja, G. Barratt and C. Vauthier, *Colloids Surf., A*, **2019**, *560*, 418–425.

5 M. Sharma, in *Applications of Targeted Nano Drugs and Delivery Systems*, Elsevier, **2019**, pp. 499–550