Supplementary Material (ESI) for Chemical Communications

Artificial Amphiphilic Scaffolds for the Selective Sensing of Protein Based on Hydrophobicity

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SUPPORTING INFORMATION

Experimental section

Materials. Commercially available 5-aminonaphthalen-1-ol and alkyl halide were obtained from Sigma, USA, while BSA was purchased from Fluka Germany and used as supplied. Other chemicals were of reagent grade and used without further purification.

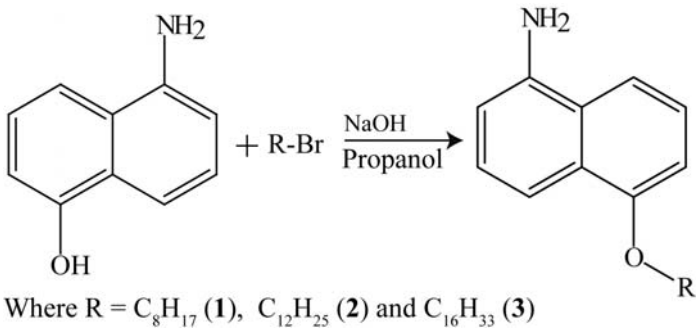
Methods. The absorption spectra were recorded on a *Varian Cary50 Bio* UV-Visible spectrophotometer using 10 mm path length quartz cuvettes at 298 K, in the range of 200-450 nm wavelengths. Fluorescence measurements were carried on a *Carry eclipse* spectrofluorometer using 10 mm path length quartz cuvettes with the slit width of 5 nm at 298 K. The compounds were excited at 320 nm wavelength. Background intensities of the buffer blanks in which BSA omitted were subtracted from each sample spectrum to cancel out any contribution due to the solvent. Time-resolved intensity decays and anisotropy decays were measured using a *Life Spec II* spectrofluorimeter (Edinburgh instrument) at 298 K. For time resolved anisotropy, the measurement was carried out by exciting the samples by 375 nm laser at room temperature. While for lifetime measurement the sample was excited by *Pico-quant* 342 nm LED light source and the decay was measured through 50 ns time scale at a time resolution of 0.0122 ns/channel. The decay curves were analyzed by FAST software using discrete exponential method, provided by Edinburgh instrument along with the fluorescence instrument. Steady state anisotropy measurement was performed in *FSP920* spectrofluorimeter (Edinburgh instrument).

Preparation of Enzyme/Protein and Compound Solution. A 1.0 mg/mL protein/enzyme solution was prepared by dissolving the protein/enzyme in the appropriate buffers. Phosphate buffer of pH 7.0 was used for α-amylase (from hog pancreas, Fluka), BSA (Fluka) and AMG (from *Aspergillus niger*, Fluka). As α-amylase was sparingly soluble in buffer solution, the resulted solution was stirred for 20 minutes, followed by centrifugation at 5000 rpm for 20

minutes to collect the supernatant for further analysis. The compound stock solutions were prepared in DMSO because of their poor solubility in water.

Successive Addition of Protein/Enzyme Solution to Compound solutions. For interaction study of compounds with protein, a 3.0 mL aqueous solution of compound (5.0 μM) was titrated with different concentrations of protein/enzyme (ranging from 0 to 275 μg/mL), where the total volume of DMSO did not exceed 5%. The presence of 5% DMSO induces no major structural changes to protein/enzyme. Each solution was mixed thoroughly before spectral measurements at room temperature.

*Synthesis of the compounds (**1**-**3**).* 5-(alkoxy)naphthalen-1-amine were prepared following the literature method (Scheme S1).[1] To a solution of 5-aminonaphthalen-1-ol (1 mol) in 25 ml of dry *n*-propanol crushed sodium hydroxide (5 mol) was added. The reaction mixture was stirred for 1 h at RT followed by the addition of corresponding alkyl halide (1.2 mol). In order to complete the reaction, the mixture was refluxed for another 6 h. The products obtained were re-crystallized from ethanol and characterized by NMR, IR and melting point.



Scheme S1. Synthesis of the compounds (**1**-**3**).

Characterization. NMR spectra were recorded on a *Varian* FT-400 MHz instrument. The chemical shifts were recorded in parts per million (ppm) scale using tetramethylsilane (TMS) as a reference at 298 K. FT-IR spectra were recorded at 4 cm^{-1} resolution with 10 scan with a

Perkin Elmer-Spectrum One FT-IR Spectrometer from 4000 to 450 cm^{-1}. A background spectrum was measured for pure KBr.

Compound 1 (5-(octyloxy)naphthalen-1-amine): IR analysis: 3434-3100, 2919, 1724 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.892 (t, *J* = 6, 3H), 1.359 (m, 10 H), 1.915 (m, 2H), 4.113 (t, *J* = 6.4, 2H), 6.808 (m, ArH), 7.273 (m, ArH), 7.361 (m, ArH), 7.725 (m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.291, 22.854, 26.461, 29.451, 68.368, 104.921, 110.603, 113.196, 124.849, 141.970 and 155.545. Deep brown color, viscous liquid.

Compound 2 (5-(dodecyloxy)naphthalen-1-amine): IR analysis: 3410-3100, 2900, 1720 cm^{-1}; 1H NMR (400 MHz, $CDCl_3$): δ 0.864 (t, *J* = 6.1, 3H), 1.356 (m, 18H), 1.887 (t, *J* = 6.8, 2H), 4.114 (t, *J* = 6.2 2H), 6.811 (m, ArH), 7.275 (m, ArH), 7.336 (m, ArH), 7.771 (m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.253, 23.901, 27.044, 30.153, 68.321, 105.213, 112.321, 115.546, 125.326, 142.361 and 156.954. Deep brown color, semi solid

Compound 3 (5-(hexadecyloxy)naphthalen-1-amine): IR analysis: 3436-3100, 2922, 1721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.809 (t, *J* = 6.4, 3H), 1.187 (m, 30 H), 1.841 (t, *J* = 6.8, 1H), 4.041 (t, *J* = 6.4, 2H), 6.805 (m, ArH), 7.209 (m, ArH), 7.321 (m, ArH), 7.741 (m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.642, 23.921, 28.621, 30.267, 67.8541, 106.321, 113.215, 115.985, 126.532, 143.258 and 155.274. Light brown color solid, Melting point: 58 ^{o}C.

Calculation of quantum yield (Φ). For quantum yield calculation, 2-aminopyridine (Φ = 0.6) in 0.1 N H_2SO_4 was used as the standard. The quantum yields of fluorescence were calculated using the equation 1.[2]

$$\Phi_u = \frac{A_s F_u n_u^2}{A_u F_s n_u^2} \Phi_s \qquad (1)$$

Where, A_s and A_u are the absorbance of standard and unknown, respectively. F_s and F_u are the areas of fluorescence peaks of the standard and unknown and n_s and n_u are the refractive indices of the solvents used for the standard and unknown, respectively. Φ_s and Φ_u are the fluorescence quantum yields of the standard and unknown compound.

Calculation of lifetime and mean lifetime (τ_m). The generated curves for intensity decay was fitted in the equation 2 to calculate the lifetime values defined as

$$I(t) = \sum_i \alpha_i \exp(\frac{-t}{\tau_i}) \qquad (2)$$

Where, α_i is the initial intensity of the decay component *i*, having a lifetime τ_i. While the mean lifetime (τ_m) of the compound in different experimental condition was calculated using the following the equation[3]

$$\tau_m = \frac{\sum_i \alpha_i \tau_i}{\sum_i \alpha_i} \qquad (3)$$

Calculation of anisotropy (r). Steady state anisotropy (r) was defined by equation 4

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \qquad (4)$$

Where I_{VV} and I_{VH} are the intensities obtained with the excitation polarizer orientated vertically, and the emission polarizer oriented vertically and horizontally, respectively. The G factor is defined as,

$$G = \frac{I_{HV}}{I_{HH}} \qquad (5)$$

The calculated time-resolved anisotropy was represented by the following function:[4]

$$r(t) = \beta_r \exp(\frac{-t}{\tau_r}) \qquad (6)$$

The fitting parameters β_r and τ_r are the amplitudes and correlation times for the exponential terms involved in the deconvoluted time-resolved anisotropy function. The data were fitted to

a single exponential and the goodness of the fit was examined by random distribution of weighted residuals and χ^2 value (Table S1).

Calculation of association constant (K_a). The intrinsic binding constant (K_a) was determined from the half reciprocal plot of [BSA]/$\Delta\varepsilon_{ap}$ vs. [BSA], where $\Delta\varepsilon_{ap} = [\varepsilon_a - \varepsilon_f]$ and $\Delta\varepsilon = [\varepsilon_b - \varepsilon_f]$. The apparent extinction coefficient, ε_a, is obtained by calculating $A_{[obsd]}$/[compound]. ε_b and ε_f correspond to the extinction coefficient of bound form of compound and free compound respectively. The data were fitted to equation 4 with a slope equal to $1/\Delta\varepsilon$ and a y-intercept equal to $1/\Delta\varepsilon K_a$. ε_b was determined from $\Delta\varepsilon$, and K_a was obtained from the ratio of the slope to the y-intercept.[5]

$$\frac{[BSA]}{\Delta\varepsilon_{app}} = \frac{[BSA]}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon K_a} \qquad (7)$$

Calculation of change in free energy (*ΔG*). Change in free energy (*ΔG*) associated with the complexation between compound and BSA, was determined using the equation 8,[6]

$$\Delta G = -2.303RT\log K_a \qquad (8)$$

Calculation of non-radiative decay rate constant (k_{nr}). In order to calculate the non-radiative decay rate constant (k_{nr}), the equation 9 was used,[7]

$$k_{nr} = k_r\left[\frac{1}{\Phi} - 1\right] \qquad (9)$$

Where k_r is radiative decay rate constant, Φ = fluorescence quantum yield of compound. Radiative decay rate constant k_r can be determined using the equation 10,

$$k_r = \frac{\Phi_f}{\tau_f} \qquad (10)$$

Where τ_f is lifetime of compound and Φ_f is fluorescence quantum yield of the compound.

Table S1. Photophysical properties of compounds[a] in presence and absence of bovine serum albumin (BSA).[b]

Sample	λ_{em} (nm)[c]	Lifetime (ns)		Mean lifetime (τ_m) ns	Correlation time (ns)
Compound **1**	420	0.25	_	_	0.91
Compound **2**	405	0.15	_	_	1.42
Compound **3**	398	0.11	_	_	1.53
Compound **1** + BSA	400	1.21	5.13	6.11	12.9
Compound **2** + BSA	396	1.01	4.23	5.61	7.94
Compound **3** + BSA	396	0.27	3.24	3.95	4.19

[a]5 μM of Compound; [b]275 μg/mL of BSA; [c]Excitation wavelength was 320 nm; [d]Quantum yield relative to 2-aminopyridine as standard.[2]

Table S2. Physical properties of the different protein/enzyme.[a]

Protein/Enzyme	Molecular weight (Da)	No. of amino acid residues	No. of aliphatic side chains (Ala, Ile, Leu & Val)	Aliphatic Index[b]
BSA	69323.4	607	Ala-47, Ile-15, Leu-65 & Val-38	77.30
Amylase	55345.1	496	Ala-31, Ile-24, Leu-25 & Val-42	69.33
Proteinase K	28934.9	129	Ala-33, Ile-11, Leu-14 & Val-19	66.52
Lysozyme	14300.1	279	Ala-11, Ile-5, Leu-9 & Val-7	66.59
AMG	12283.3	112	Ala-7, Ile-5, Leu-6 & Val-9	67.86

[a]Walker, John M. *The Proteomics Protocols Handbook*; Humana Press Inc: Totowa, NJ, 2005; [b]Ikai, A. J. *J. Biochem.* **1980**, *88*, 1895.

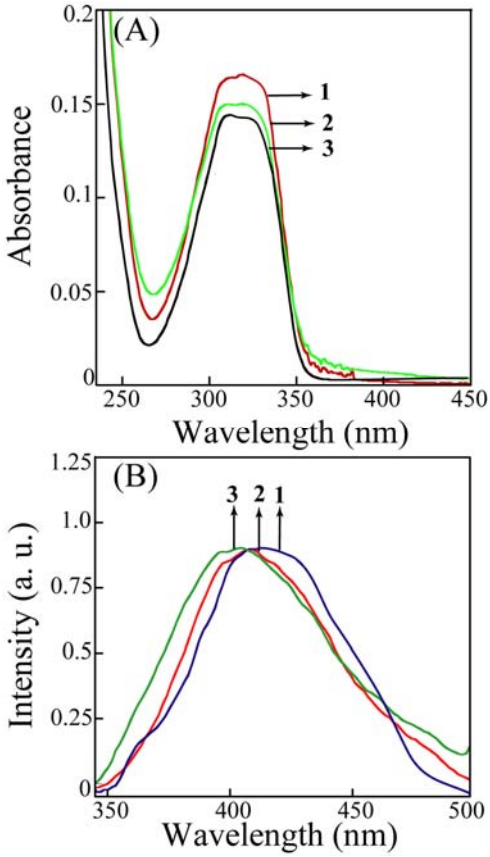


Figure S1. (A) UV-visible absorption and (B) Emission spectra of the compounds (**1-3**) in an aqueous buffer solution of pH 7.0

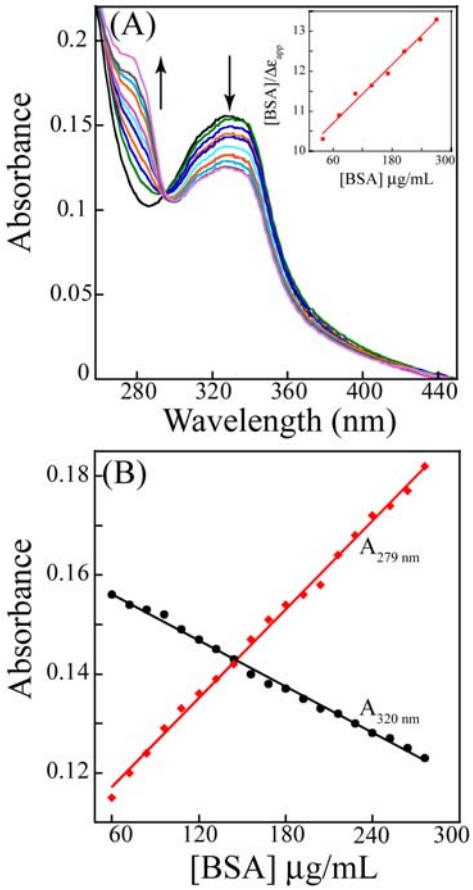


Figure S2. (A) Absorption spectra of Compound **2** (5 µM) as a function of the BSA concentration and (B) Variation of absorbance of the compound **2** (5 µM) at 320 nm and 279 nm as a function of the BSA concentration (from 0 to 275 µg/mL)

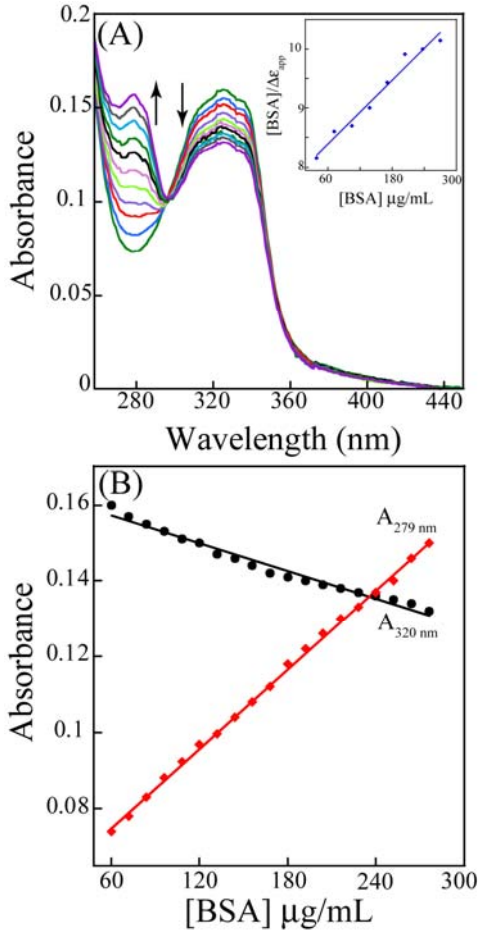


Figure S3. (A) Absorption spectra of Compound **3** (5 µM) as a function of the BSA concentration and (B) Variation of absorbance of the compound **3** (5 µM) at 320 nm and 279 nm as a function of the BSA concentration (from 0 to 275 µg/mL)

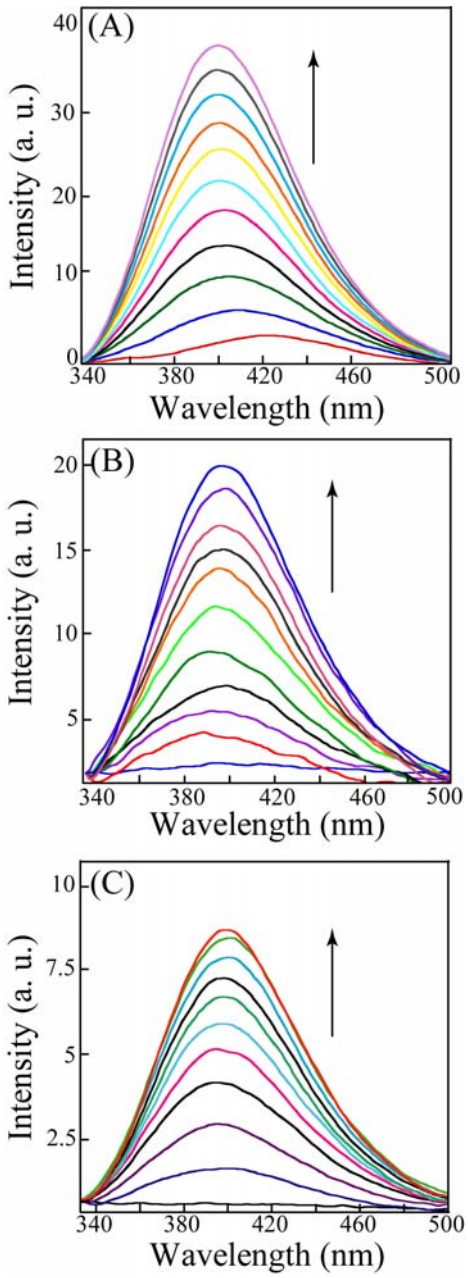


Figure S4. Emission spectra of (A) Compound **1,** (B) Compound **1** and (C) Compound **3** with increasing concentration of BSA ranging from 0 to 275 μg/mL in an aqueous buffer of pH 7.0. The compound concentration used was 5 μM and the λ_{exc} was 320 nm.

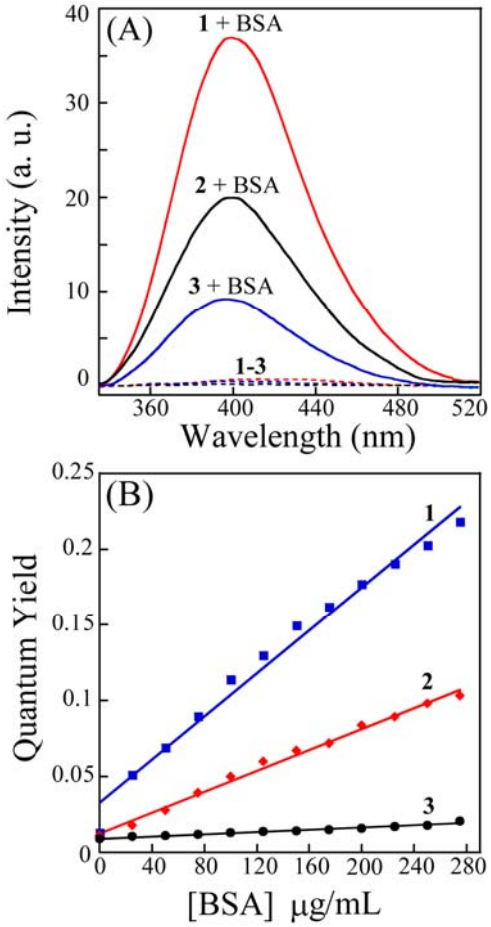


Figure S5. (A) Emission spectra of Compound **1, 2**, **3, 1** + BSA**, 2** + BSA and **3** + BSA in an aqueous buffer of pH 7.0. The compound concentration used was 5 μM and BSA used was 275 μg/mL. (B) Fluorescence quantum yield of the compounds (5 μM) as a function of the BSA concentration (from 0 to 275 μg/mL).

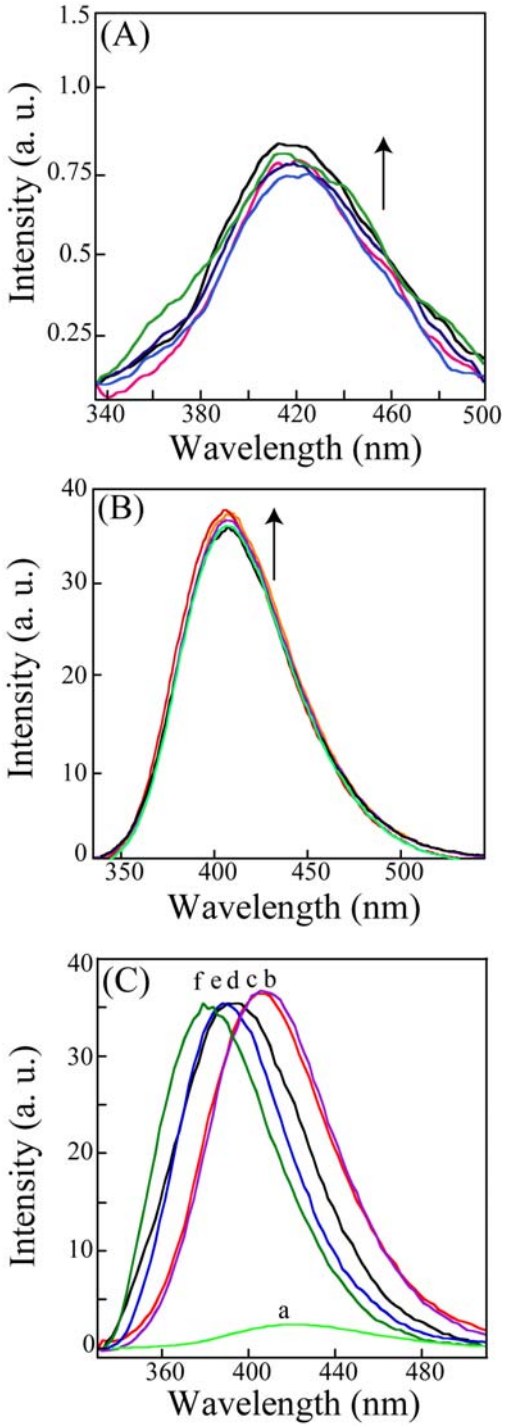


Figure S6. (A) Emission spectra of Compound **1** with increasing concentration of trytophan from 0 to 275 μg/mL in an aqueous buffer of pH 7.0; (A) Emission spectra of 5-aminonaphthalen-1-ol (the parent compound) with increasing concentration of BSA from 0 to 275 μg/mL in an aqueous buffer of pH 7.0 and (C) Effect of different solvent on the emission

spectra of the compound **1**; Where Trace a-f: Water, DMSO, Ethanol, THF, Hexane and Toluene. The compound concentration used was 5 μM and the λ_{exc} was 320 nm.

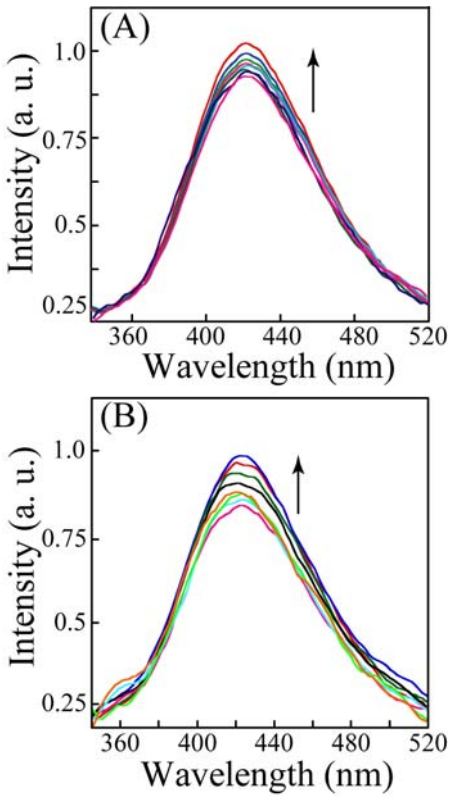


Figure S7. Emission spectra of (A) Compound **1** with increasing concentration of α-amylase (0 to 275 μg/mL) and (B) Compound **1** with increasing concentration of Lysozyme (0 to 275 μg/mL) in an aqueous buffer of pH 7.0. The compound concentration used was 5 μM and the λ_{exc} was 320 nm.

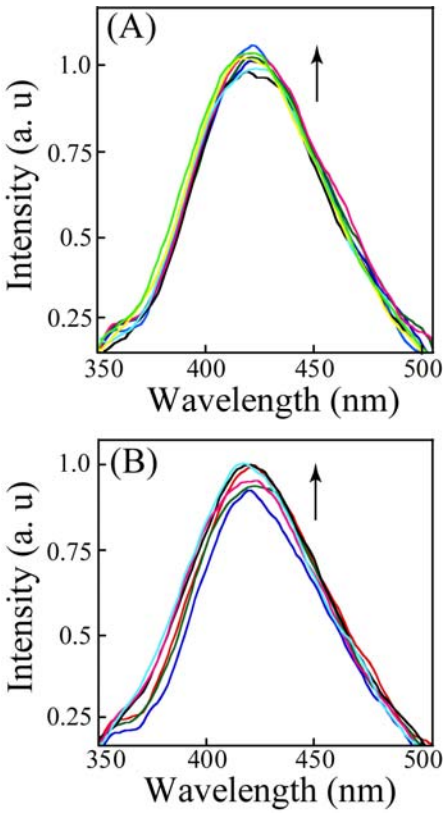


Figure S8. Emission spectra of (A) Compound **1** with increasing concentration of Proteinase K (0 to 275 μg/mL) and (B) Compound **1** with increasing concentration of AMG (0 to 275 μg/mL) in an aqueous buffer of pH 7.0. The compound concentration used was 5 μM and the λ_{exc} was 320 nm.

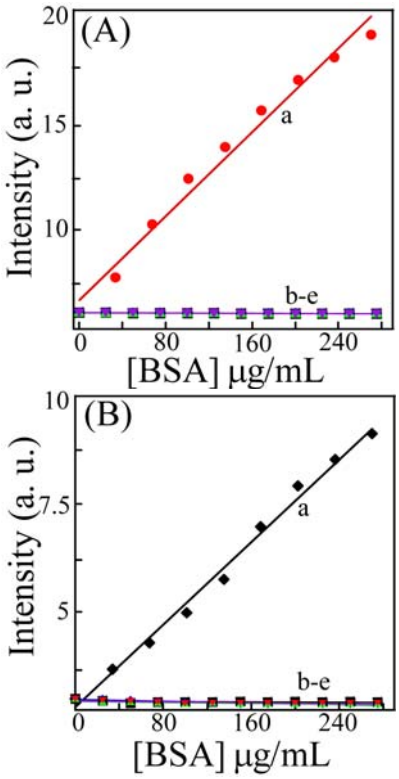


Figure S9. Variation of fluorescence intensity of (A) Compound **2** and (B) Compound **3** at 400 nm as a function of different protein/enzyme concentration (from 0 to 275 µg/mL). Where, Trace a: BSA, Trace b-e: α-amylase, Lysozyme, Proteinase K and AMG.

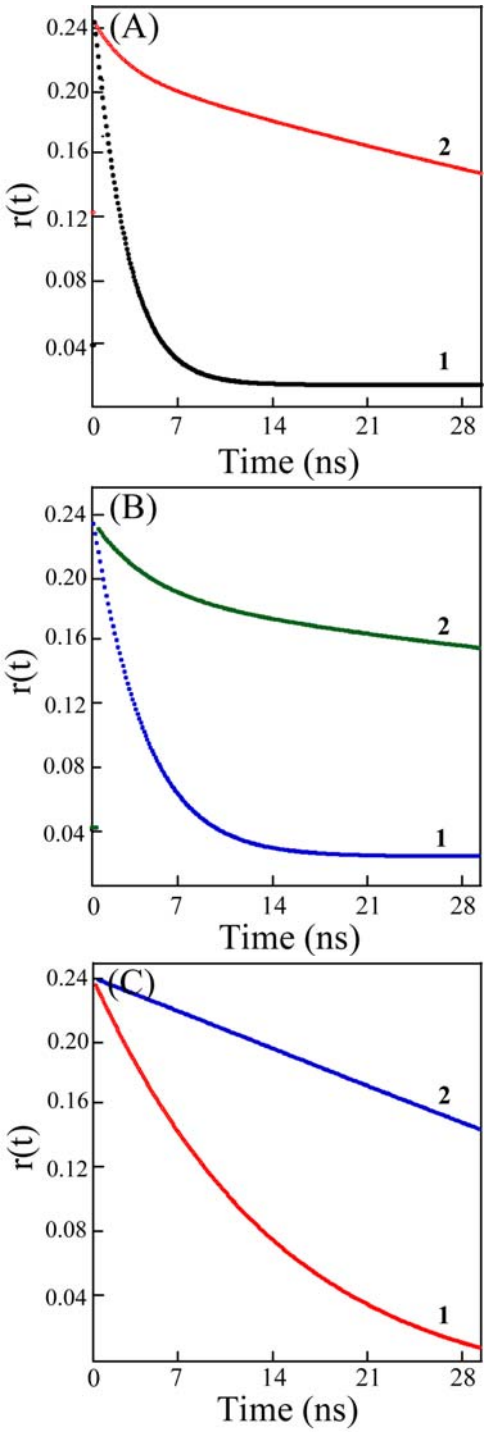


Figure S10. Anisotropy decay (fit curve) of (A) Compound **1** alone and with BSA; (B) Compound **2** alone and with BSA and (C) Compound **3** alone and with BSA. Where Trace 1: Compound only and Trace 2: Compound with BSA. The emission monochromators were set to 400 nm, while the concentration of the compound and BSA used were 5 μM and 275 μg/mL respectively.

References

(1) K. Ward Jr., *J. Am. Chem. Soc*., 1935, **57**, 914.

(2) D. F. Eaton, *Pure & Appl. Chem.,* 1988, **60**, 1107.

(3) R. Swaminathan, G. Krishnamoorthy and N. Periasamy, *Biophys J.,* 1994, **67**, 2013.

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