Supporting Information

Domain Specific Association of Phenanthrene-Pyrene Based Synthetic Fluorescent Probe with Bovine Serum Albumin: Spectroscopic and Molecular Docking Analysis

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Figure S1. ¹H-NMR spectrum of PPI in DMSO-d₆ in Bruker 300 MHz instrument.
Figure S2. $^{13}$C-NMR spectrum of PPI in DMSO-$d_6$ in Bruker 300 MHz instrument.

Figure S3. Mass spectrum of PPI in CH$_3$CN.
Figure S4. IR spectrum of PPI.
**Figure S5.** Job’s plot for the binding of PPI to BSA. Fluorescence quenching of BSA was monitored at 342 nm exciting at 295 nm.

**Figure S6.** ANS displacement study for the quenching of BSA emission by PPI at different BSA-ANS ratio (1:0, 1:1 respectively).
Figure S7. Representative emission profile of BSA (alone), BSA+PPI (1:1) and PPI (alone). $\lambda_{ex} = 295$ nm.

Figure S8. Modified Stern-Volmer plot of PPI induced quenching of BSA (10 µM) fluorescence at 298 K.

Figure S9. A) Representative time-resolved fluorescence decay spectra of PPI (10 µM) in the absence and presence of increasing concentration of BSA. Spectra 1-4 corresponds to the BSA concentration 0 µM (red circle), 10 µM (blue circle), 20 µM (olive green circle) and 30 µM (purple circle), respectively at 25 °C. B) Fluorescence decay spectra of BSA (10 µM) in the absence and presence of increasing concentration of PPI. Spectra 1-4 corresponds to the PPI concentration 0 µM (red circle), 5 µM (blue circle), 10 µM (olive green circle) and 20 µM (purple circle), respectively at 25 °C. C) Inset of Figure B: plot shows variation of average lifetime ($\langle \tau \rangle$) of BSA as a function of PPI concentration. IRF represents the instrument response function.
Table S1. Binding constants ($K_b$), number of binding sites (n) and thermodynamic parameters for the PPI-BSA system at different temperatures.

| Temp. (K) | $K_b$ (10$^5$ M$^{-1}$) | n      | $\Delta H^\circ$ (kJ mol$^{-1}$) | $\Delta S^\circ$ (J mol$^{-1}$ K$^{-1}$) | $\Delta G^\circ$ (kJ mol$^{-1}$) |
|----------|--------------------------|--------|-----------------------------------|------------------------------------------|----------------------------------|
| 288      | 16.08 ± 0.08             | 1.19 ± 0.03 | – 41.93                          | – 26.60                                  | – 34.27                         |
| 298      | 9.64 ± 0.11              | 1.16 ± 0.02 | – 41.93                          | – 26.60                                  | – 34.01                         |
| 308      | 5.15 ± 0.16              | 1.12 ± 0.03 | – 41.93                          | – 26.60                                  | – 33.74                         |

Table S2. Time-resolved fluorescence lifetime decay parameters of PPI (10 µM) with increasing concentration of BSA at 25 ºC.

| System    | Concentration of BSA (µM) | $\tau_1$ (ns) | $a_1$ (%) | $\tau_2$ (ns) | $a_2$ (%) | $<\tau>$ (ns) | $\chi^2$ b |
|-----------|---------------------------|---------------|-----------|---------------|-----------|----------------|------------|
| PPI-BSA   | 0                         | 4.18          | 100       | -             | -         | -              | 1.063      |
| PPI-BSA   | 10                        | 1.84          | 67.40     | 3.58          | 32.60     | 2.40           | 1.029      |
| PPI-BSA   | 20                        | 1.42          | 41.23     | 2.81          | 58.77     | 2.23           | 1.002      |
| PPI-BSA   | 30                        | 1.34          | 42.34     | 2.70          | 57.66     | 2.12           | 1.008      |

* $\alpha_i$ denotes the pre-exponential factor corresponding to the $i$th decay component $\tau_i$ and $\chi^2$ represents the goodness of fit.

Table S3. Time-resolved fluorescence lifetime decay parameters of BSA (10 µM) with increasing concentration of PPI at 25 ºC.

| System     | Concentration of PPI (µM) | $\tau_1$ (ns) | $a_1$ (%) | $\tau_2$ (ns) | $a_2$ (%) | $<\tau>$ (ns) | $\chi^2$ b |
|------------|---------------------------|---------------|-----------|---------------|-----------|----------------|------------|
| BSA-PPI    | 0                         | 3.69          | 22.12     | 6.68          | 77.88     | 6.01           | 1.021      |
| BSA-PPI    | 5                         | 3.25          | 25.13     | 6.50          | 74.87     | 5.68           | 1.088      |
| BSA-PPI    | 10                        | 3.11          | 28.13     | 6.33          | 71.87     | 5.42           | 1.138      |
| BSA-PPI    | 20                        | 2.52          | 31.52     | 6.07          | 69.48     | 5.01           | 1.081      |

* $\alpha_i$ denotes the pre-exponential factor corresponding to the $i$th decay component $\tau_i$ and $\chi^2$ represents the goodness of fit.
Experimental methods

UV-Visible Absorption Spectroscopy.

All the UV-Vis spectra were collected with proper background correction. The absorption titrations were performed by keeping BSA concentration fixed at 5 µM with successive increasing the concentration of the PPI solution within the range 0-25 µM.

Fluorescence Spectroscopic Studies.

A fixed amount of PPI (20 µM) was titrated with gradual increasing the concentration of BSA in the range 0-70 µM where PPI was excited at 380 nm. Whereas, in another experiment a fixed amount of BSA (10 µM) was titrated with increasing concentration of PPI within the range 0-20 µM to measure the intrinsic fluorescence of BSA. Here, protein molecule BSA was excited at 295 nm. The titrations were carried out under constant stirring condition.

Determination of Steady-state Fluorescence Anisotropy.

Steady-state fluorescence anisotropy values provides valuable information about the nature of the surrounding of a fluorescent probe. Fluorescence anisotropy value is determined by the rotational motion of the fluorophore molecule. Rotational motion depends upon size, shape and rigidity of the fluorophore in solution. An increase in the rigidity of the surrounding environment of a fluorescent probe results in enhancement of anisotropy value. The higher anisotropy value indicates the lower rotational diffusion of fluorophore in solution.

Fluorescence anisotropy (r) measurements were carried out on a PTI QM-40 spectrofluorimeter by the following equation described by Larsson et al.

\[
r = \frac{I_{VV} - G I_{VH}}{I_{VV} + 2G I_{VH}}
\]

(Eq. S1)

Where, the polarizer positions were set at (0°,0°), (0°,90°), (90°,0°), and (90°,90°) to get \(I_{VV}\), \(I_{VH}\), \(I_{HV}\), \(I_{HH}\) for excitation and emission signals respectively. \(G\) factor is defined as

\[
G = \frac{I_{HV}}{I_{HH}}
\]

(Eq. S2)

Where, \(I_{HV}\) and \(I_{HH}\) are respectively the vertical and horizontal component of emission polarizer, keeping the excitation polarizer horizontal. \(G\) depends on slit widths and monochromator wavelength. The excitation and emission wavelengths were fixed at 380 and 475 nm respectively. The excitation and emission slit width were fixed at 3 nm.

Circular Dichroism (CD) Spectra.

All the reported CD spectra were recorded in the wavelength range 195 – 300 nm under constant purged with nitrogen and a scan speed of 100 nm min\(^{-1}\). Here, a fixed concentration of BSA (0.75 µM) was titrated with the increasing concentration of PPI from 0 µM to 4 µM in Tris-HCl buffer solution of pH 7.4 at 25 °C. Each CD spectrum was an average of five scans and the baseline correction was performed with Tris–HCl buffer signal.

Fluorescence Lifetime Measurements.

The TCSPC measurements were carried out in 10 mM Tris-HCl buffer solution of pH 7.4 for the fluorescence decay of PPI in the absence and in the presence of increasing concentration of BSA at 25 °C. Further, the fluorescence decay of BSA was performed in the absence and in the presence of increasing concentration of PPI to assess the interaction with PPI. The instrument response function (IRF) was ascertained experimentally by using dilute micellar solution of SDS in water as light signal scatterer.
During the TCSPC measurements the photoexcitation was fixed at 370 nm for PPI, and at 300 nm for BSA. The fluorescence decay data were collected by using equation (S3):

\[ F(t) = \sum_{i} \alpha_i \exp\left(-\frac{t}{\tau_i}\right) \]  

(Eq. S3)

Where, \( \alpha_i \) represents the ith pre-exponential factor and \( \tau_i \) denotes the decay time of component i. The decay time is mentioned to as the lifetime of the excited species. The average lifetimes \( \langle \tau \rangle \) for the fluorescence decay profiles were calculated by using the following equation:\textsuperscript{53}

\[ \langle \tau \rangle = \frac{\sum_i \alpha_i \tau_i}{\sum_i \alpha_i} \]  

(Eq. S4)

In case of time-resolved anisotropy decay experiment anisotropy decay function \( r(t) \) was formed by using the following equation:\textsuperscript{54}

\[ r(t) = \frac{I_\parallel(t) - GI_\perp(t)}{I_\parallel(t) + 2GI_\perp(t)} \]  

(Eq. S5)

Where, \( I_\parallel(t) \) and \( I_\perp(t) \) represent fluorescence decays gained for parallel and perpendicular emission polarizer, respectively with respect to the vertical excitation polarizer. \( G \) denotes the correction factor for the detector sensitivity of the instrument.

References.

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