Supporting Information

Probing the interaction between human serum albumin and 9-hydroxyphenanthrene: a spectroscopic and molecular docking study

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Photophysical properties of HSA and 9-OHPhe

The singlet energies of HSA and 9-OHPhe are 101.22 and 85.55 kcal·mol^{-1}, respectively.

3.2. Fluorescence quenching mechanism of HSA-9-OHPhe system

Stern–Volmer equation:

$$F_0/F = 1 + K_{SV}[Q] = 1 + K_q\tau_0[Q] \qquad \text{(S1)}$$

F_0 and F are the relative fluorescence intensities of HSA in the absence and presence of 9-OHPhe; $[Q]$ is the concentration of 9-OHPhe, τ_0 is the average fluorescence lifetime of HSA in the absence of 9-OHPhe (6.09×10^{-9} s), K_{SV} is the Stern-Volmer quenching constant and K_q is the quenching rate constant.

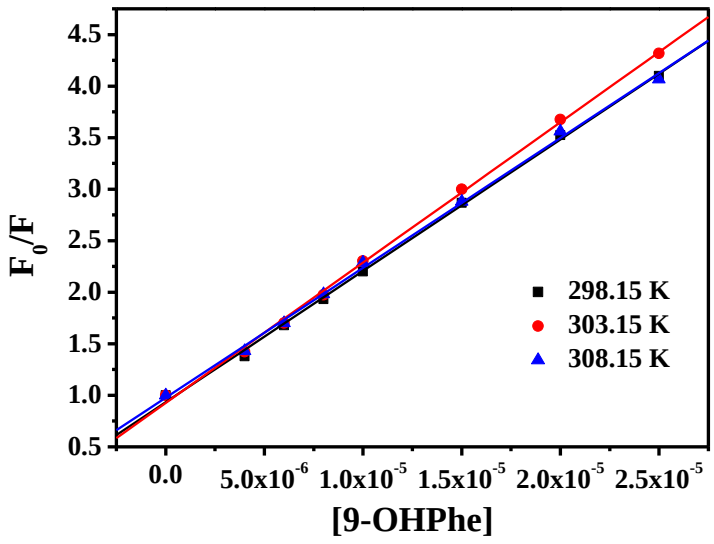

Figure S1. Stern-Volmer plots for the quenching of HSA by 9-OHPhe at three temperatures

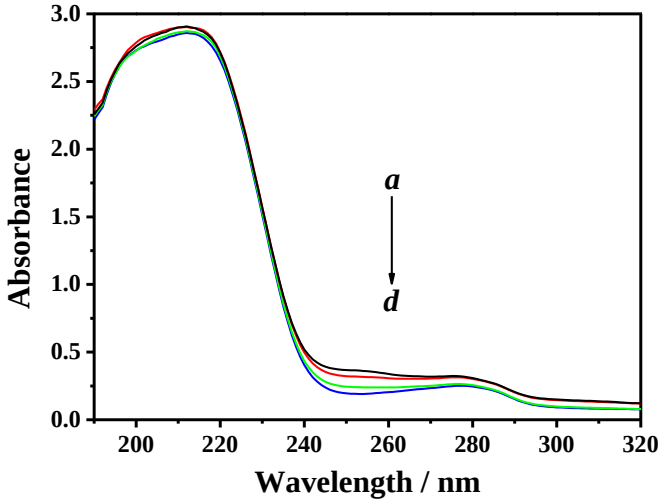

Figure S2. The UV–visible absorption spectra of HSA in the presence of 9-OHPhe at different concentrations. T = 298.15 K; a ~ d: [HSA]=5.0 × 10^{-6} mol·L^{-1}, [9-OHPhe] = (0, 2, 4, 6) × 10^{-6} mol·L^{-1}

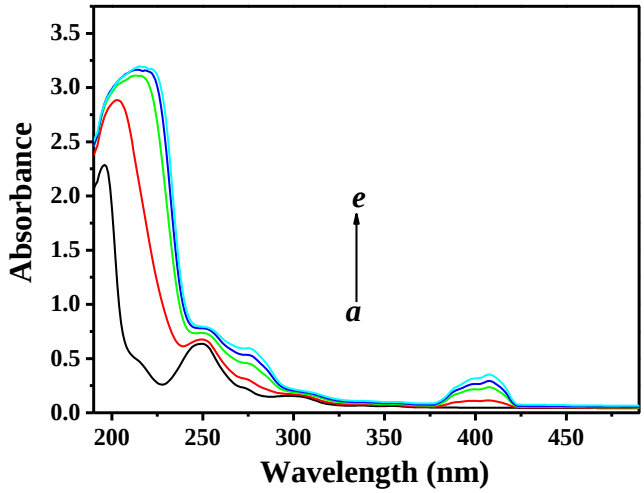

Figure S3. The UV–visible absorption spectra of 9-OHPhe in the presence of the increasing concentrations of HSA. T = 298.15 K; a ~ e: [9-OHPhe]=2.0 × 10^{-5} mol·L^{-1}, [HSA] = (0, 2, 6, 8, 10) × 10^{-6} mol·L^{-1}

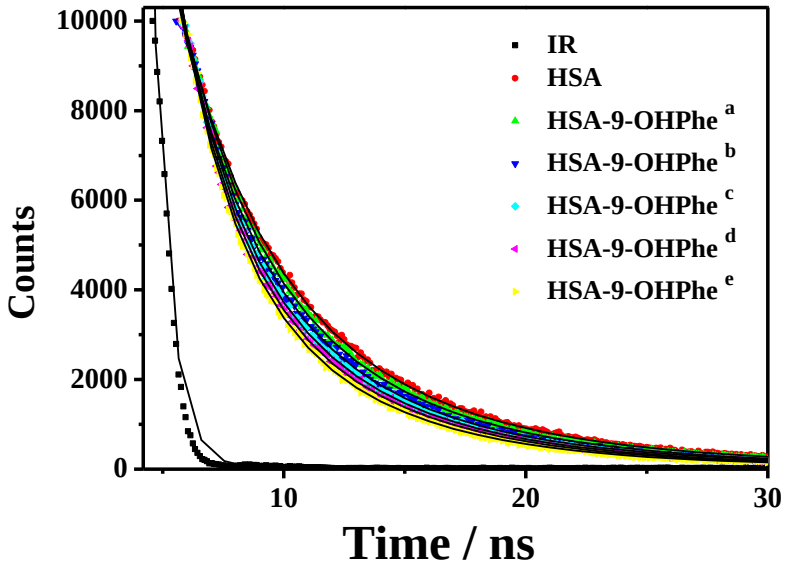

Figure S4. The fitting lines for the fluorescence lifetime decay plots of the HSA-9-OHPhe system. [HSA]= 5.0×10^{-6} mol·L^{-1}; [9-OHPhe], a-e: (5.0, 10.0, 15.0, 20.0, 25.0) $\times 10^{-6}$ mol·L^{-1}

Table S1 The fluorescence lifetime of HSA in the presence of various concentrations of 9-OHPhe

System	τ_{av} (ns)	χ^2
HSA	5.39	0.9995
HSA-9-OHPhe [a]	5.00	0.9996
HSA-9-OHPhe [b]	4.98	0.9992
HSA-9-OHPhe [c]	4.57	0.9995
HSA-9-OHPhe [d]	4.40	0.9994
HSA-9-OHPhe [e]	4.22	0.9995

[HSA]= 5.0×10^{-6} mol·L^{-1}; [9-OHPhe], a-e: (0, 5.0, 10.0, 15.0, 20.0, 25.0) $\times 10^{-6}$ mol·L^{-1}**;** The reduced χ^2 value approaching 1 suggests a good fit.

3.3. Energy transfer from HSA to 9-OHPhe

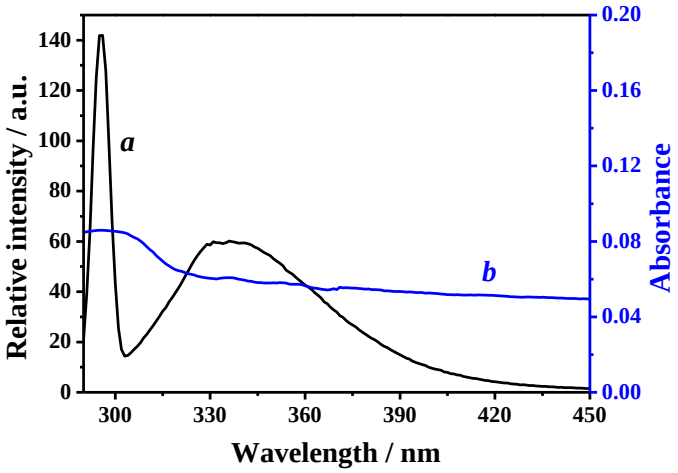

Figure S5. The overlap of the fluorescence emission spectrum of HSA (a) and the absorption spectrum of 9-OHPhe (b). [HSA]=[9-OHPhe] =5.0×10^{-6} mol·L^{-1}; T = 298.15 K; λ_{ex} = 295.0 nm, Ex. / Em. Slit =5.0 / 5.0 nm

The efficiency of energy transfer (E) between the donor (HSA) and acceptor (9-OHPhe) can be calculated by applying equation (S2):

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \qquad \textbf{(S2)}$$

In the equation (S2), F and F_0 are the fluorescence intensity of HSA in the presence and absence of 9-OHPhe. r is the distance between the donor and acceptor. R_0 is the Förster radius, the critical distance at which the transfer efficiency equals 50%, which is given by the following equation:

$$R_0^6 = 8.79 \times 10^{-25} K^2 n^{-4} \phi J \qquad \textbf{(S3)}$$

Where K^2 is the factor describing the relative orientation (2/3), n is the refractive index of medium (1.336), ϕ is the fluorescence quantum yield of HSA (0.15), and the

overlap integral J expresses the degree of spectral overlap between the donor emission and the acceptor absorption, which is calculated by the following equation：

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F(\lambda)d\lambda} \quad \textbf{(S4)}$$

$F(\lambda)$ is the fluorescence intensity of the donor in the wavelength ranges from λ to $\lambda+\Delta\lambda$ *and* $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at the wavelength λ.

3.4. Binding site number (*n*) of HSA with 9-OHPhe

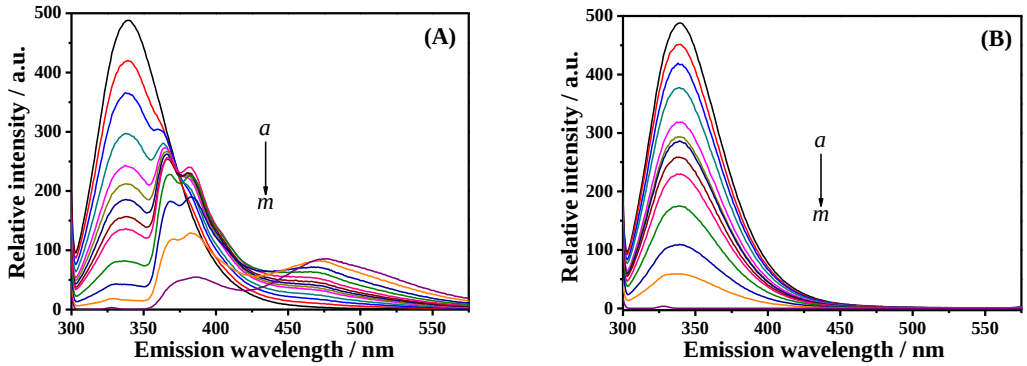

Figure S6. Fluorescence emission spectra of HSA in the presence (A) and absence (B) of 9-OHPhe. [HSA]+[9-OHPhe]= 2.0×10^{-5} mol ·L^{-1}; T = 298.15 K; λ_{ex} = 295.0 nm, Ex. / Em. Slit =5.0 / 10.0 nm; a ~ m: [HSA]= (20, 18, 16, 14, 12, 11, 10, 9, 8, 6, 4, 2, 0) × 10^{-6} mol·L^{-1}

3.5. Binding constant (K_b) of HSA with 9-OHPhe at different temperatures

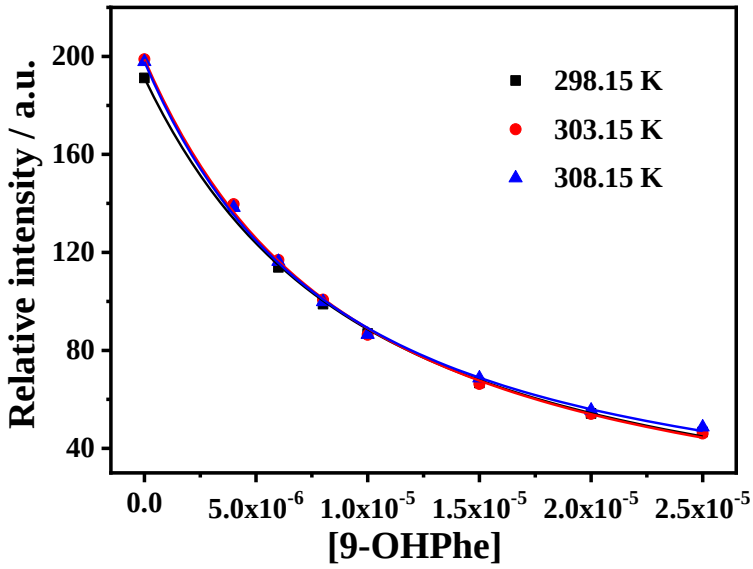

Figure S7. The fitting plots for the fluorescence quenching of HSA by 9-OHPhe at three temperatures

3.6. Thermodynamics analysis

Van't Hoff equations:

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad \text{(S5)}$$

$$\Delta G = \Delta H - T\Delta S \qquad \text{(S6)}$$

where T is the experimental temperature (i.e. 298.15, 303.15, 308.15 K), R is the universal constant of gases (8.314 J $mol^{-1}\cdot K^{-1}$). K_b is the binding constant at each temperature, obtained in **Section 3.5**.

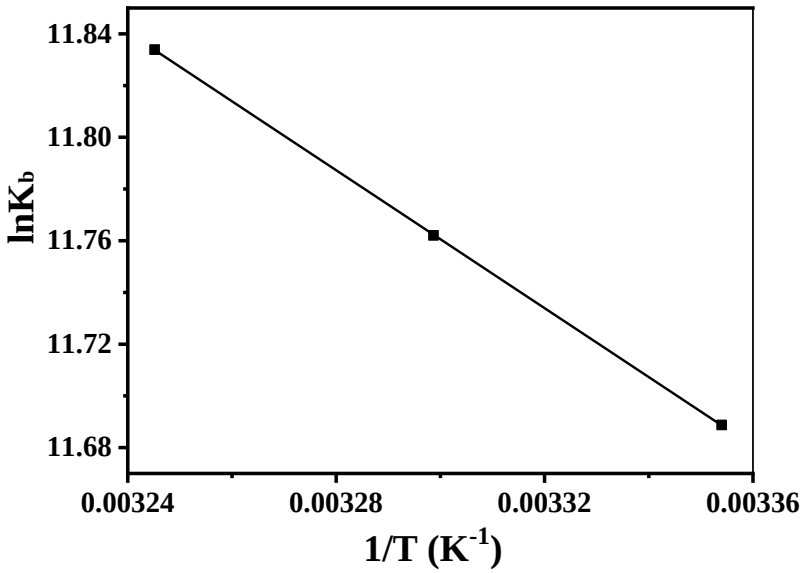

Figure S8. Fitting line of Van't Hoff equation for 9-OHPhe-HSA system

3.7. Molecular docking

Table S2 Specific information on the interaction forces between 9-OHPhe and HSA

	Index	Residue	Distance (Å)	Ligand Atom	Residue Atom [a]
Hydrophobic Interactions	1	LEU134	3.15	C3	CD2
	2	TYR138	3.37	C3	CG
	3	LEU139	3.17	C11	CD2
	4	ILE142	3.52	O15	CG2
	5	ILE142	3.71	C14	CD1
	6	LEU154	3.44	C13	CD2
	7	PHE157	3.80	C13	CG
	8	ALA158	3.41	C11	N
	9	TYR161	3.26	O15	CZ
	10	TYR161	3.56	C9	CE1
Hydrogen Bonds	Index	Residue	Distance (Å)	Donor Atom [a]	Acceptor Atom [a]
	1	LEU135	1.86	O15 (Ligand)	O (Residue)
	Index	Residue	Distance (Å)	Angle (°)	Ligand Atoms [a]
π-Stacking	1	TYR161	4.19	19.75	C6, C7, C2, C3, C4, C5

[a] The first one character of the atom name consists of the chemical symbol for the atom type. All the atom names beginning with "C" are carbon atoms; "N" indicates a nitrogen and "O" indicates oxygen. The next character is the remoteness indicator code, which is transliterated according to: "D" stands for (~) "δ"; "E" ~ "ε"; "G" ~ "γ"; "Z" ~ "ζ".