Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Table S1 Residues identified in the binding cavity for proflavine binding to BSA and HSA as obtained by means of the CavityPlus software. The polar, hydrophobic, basic (positively charged) and acidic (negatively charged) residues are indicated in green, orange, blue, and red, respectively

Protein	Subdomain	Residues in the Cavity
BSA	IIB	R; V; C; K, N; Y; Q; E; A; K; D; L; E; L; E; E; C; C; A, K; P
HSA	IIB - IIA	D; Y; V; E; P; Q; N; P; E; A; R; M

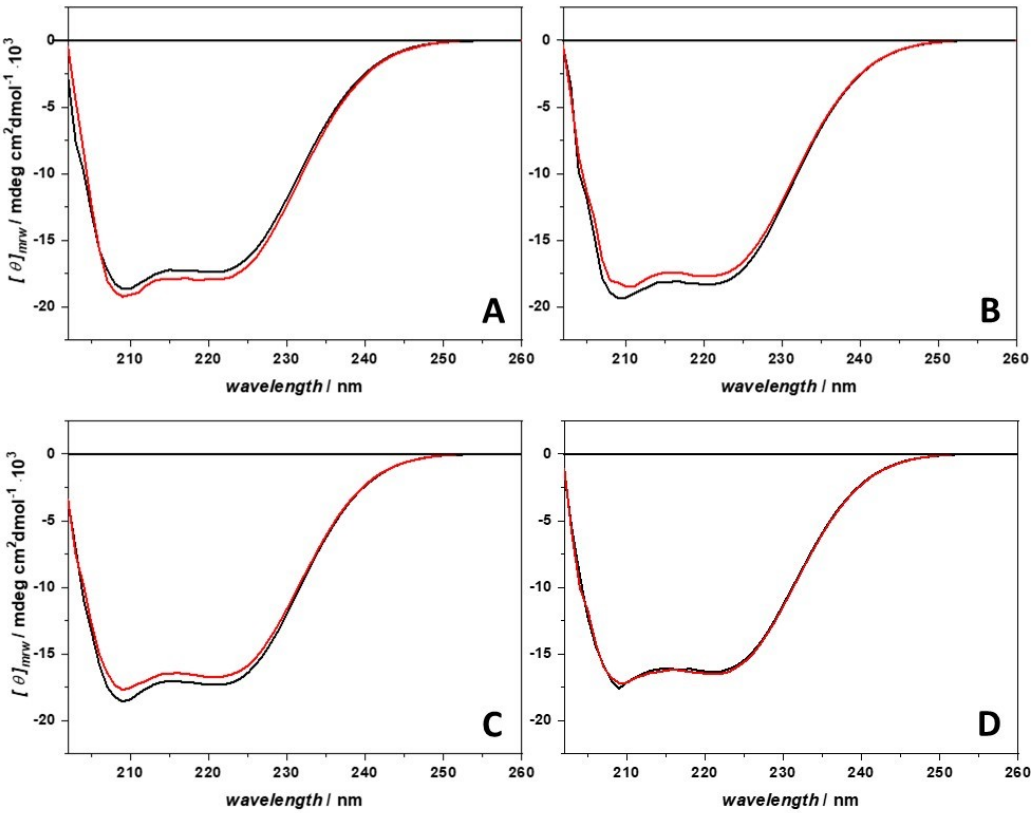

Figure S1. Circular dichroism spectra of BSA in buffer containing 0.1 M TMAO (A) and 0.1 M betaine (B). Panels C and D report the spectra of HSA in the buffer containing 0.1 M TMAO and 0.1 M betaine, respectively. Black and red lines represent the spectra of the proteins in the absence and in the presence of the ligand proflavine, respectively. All the experiments were performed in 20 mM Tris-HCl buffer, pH 7.4, at the temperature of 25 °C.

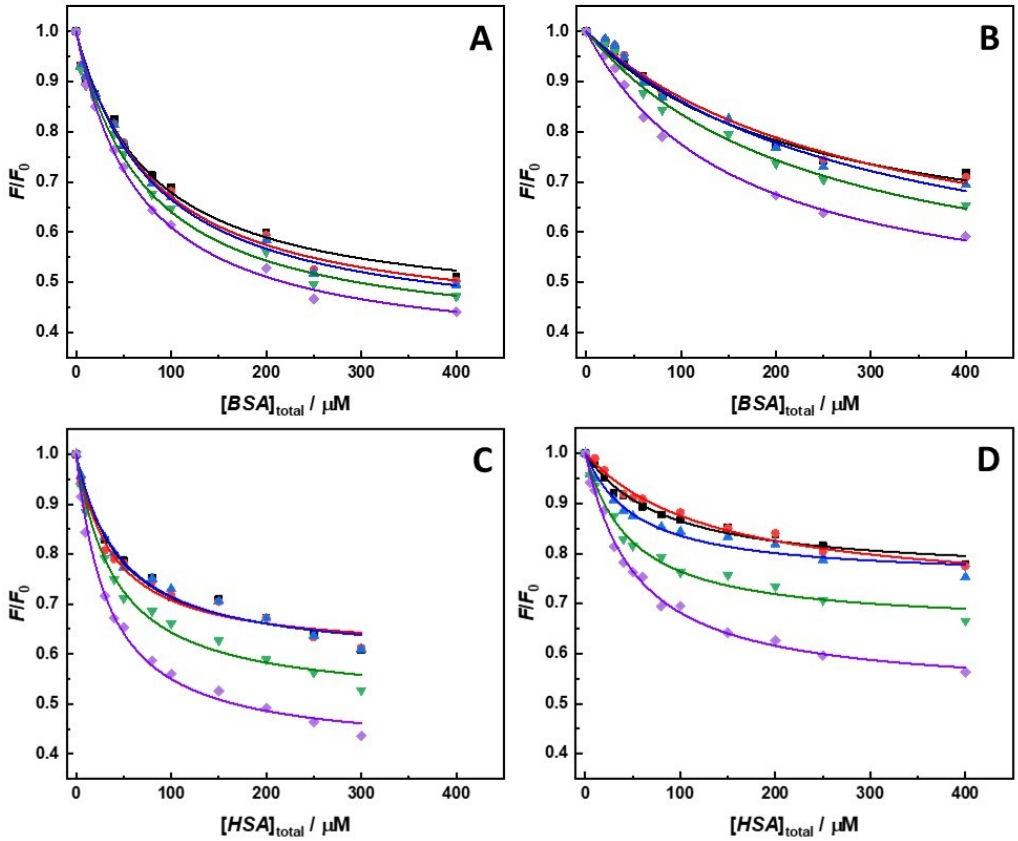

Figure S2. Binding isotherms measured by means of HHP-fluorescence spectroscopy for the complex formation between proflavine and (A and B) BSA and (C and D) HSA in 20 mM Tris-HCl buffer, pH 7.4, in the presence of 0.5 M TMAO (A and C) and 0.5 M glycine betaine (B and D), at the pressures of 1 bar (black squares), 500 bar (red circles), 1000 bar (blue triangles), 1500 bar (green reversed triangles), and 2000 bar (violet diamonds). The solid lines represent the best fit of the experimental data according to a 1:1 binding model. All experiments were performed at the temperature of 25 °C.

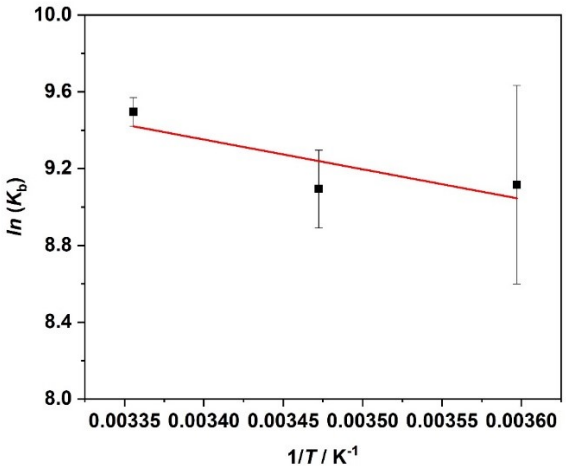

Figure S3. Plot of ln(K_b) *vs.* 1/T for the binding of proflavine to HSA in 20 mM Tris-HCl buffer, 0.5 M TMAO, pH 7.4. The slope of ln(K_b) *vs.* 1/T is equal to -$\Delta H_b°/R$, which allowed us to determine the enthalpy change of binding, ΔH_b. The value obtained was ΔH_b = 12.9 ± 8.0 kJ mol^{-1}.