

Effects of Heme Site (FA1) Ligands Bilirubin, Biliverdin, Hemin, and Methyl Orange on the Albumin Binding of Site I Marker Warfarin: Complex Allosteric Interactions

SUPPLEMENTARY MATERIAL

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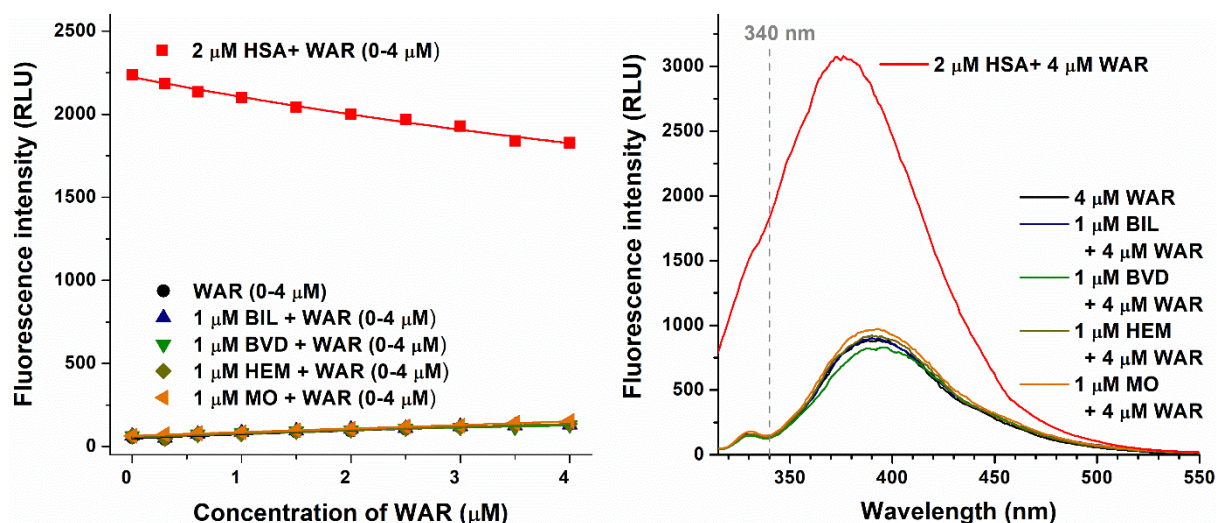


Figure S1: Fluorescence emission signal (at 340 nm) and representative emission spectra of warfarin in the absence and presence of Heme site ligands. **Left panel:** Fluorescence emission signal of albumin (HSA, 2 μM) in the presence of increasing concentrations warfarin (WAR, 0-4 μM) (**red**). Fluorescence emission signal of WAR (0-4 μM) without (**black**) and with bilirubin (BIL, 1 μM ; **blue**), biliverdin (BVD, 1 μM ; **green**), hemin (HEM, 1 μM ; **dark yellow**), and methyl orange (MO, 1 μM ; **orange**) in PBS (pH 7.4; $\lambda_{\text{ex}} = 295 \text{ nm}$, $\lambda_{\text{em}} = 340 \text{ nm}$). **Right panel:** Fluorescence emission spectrum of HSA in the presence of WAR (**red**). Fluorescence emission spectrum of WAR without (**black**) and with BIL (**blue**), BVD (**green**), HEM (**dark yellow**), and MO (**orange**) in PBS (pH 7.4; $\lambda_{\text{ex}} = 295 \text{ nm}$).

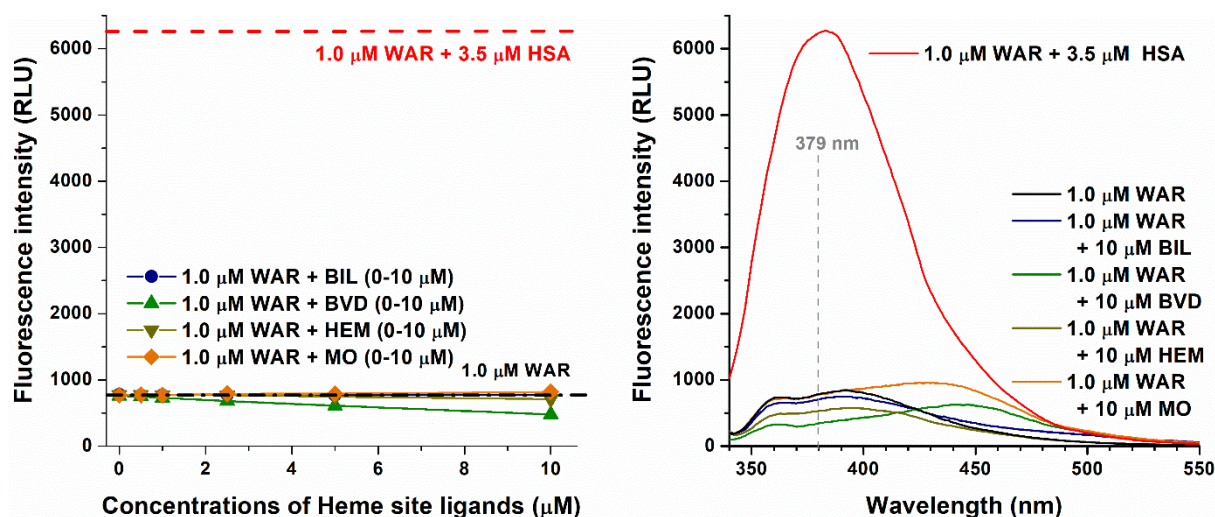


Figure S2: Fluorescence emission signal (at 379 nm) and representative emission spectra of warfarin in the absence and presence of Heme site ligands. **Left panel:** Fluorescence emission signal of warfarin-HSA complex (**red dashed line**), warfarin (WAR) alone (**black dash-dotted line**), and WAR in the presence of increasing concentrations of bilirubin (BIL; **blue**), biliverdin (BVD; **green**), hemin (HEM; **dark yellow**) and methyl orange (MO; **orange**) in PBS (pH 7.4; $\lambda_{\text{ex}} = 317 \text{ nm}$, $\lambda_{\text{em}} = 379 \text{ nm}$). **Right panel:** Fluorescence emission spectra of WAR-HSA complex (**red**), WAR alone (**black**), and WAR in the presence of BIL (**blue**), BVD (**green**), HEM (**dark yellow**), and MO (**orange**) in PBS (pH 7.4; $\lambda_{\text{ex}} = 317 \text{ nm}$).

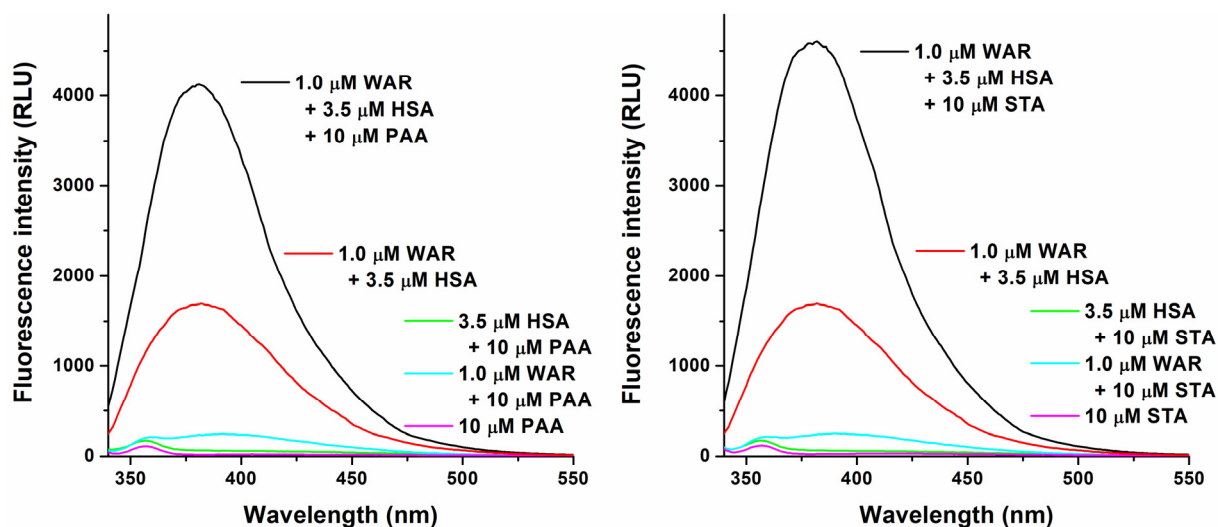


Figure S3: Representative emission spectra of palmitic acid and stearic acid without and with warfarin, albumin, and warfarin + albumin. Emission spectra of palmitic acid (PAA, 10 μM ; *left panel*) and stearic acid (STA, 10 μM ; *right panel*) in the absence and presence of warfarin (WAR, 1.0 μM), albumin (HSA, 3.5 μM), and WAR-HSA complex in PBS (pH 7.4; $\lambda_{\text{ex}} = 317$ nm).