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SUPPLEMENTARY INFORMATION

Spectroscopy of hydroxyphenyl benzazoles in solution and human serum albumin: Detecting flexibility, specificity and high affinity of the warfarin drug binding site

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Förster's resonance energy transfer

The distance between the donor (W214) and acceptor (HBX probes) can be calculated according to Förster's theory for resonance energy transfer (FRET).[1-3] The efficiency of energy transfer, *E*, is related to the distance (R_{DA}) between the donor and acceptor by

$$E = \frac{R_0^6}{R_0^6 + R_{DA}^6} = 1 - \left(\frac{F}{F_0}\right) \quad (1)$$

where R_0 is the Förster distance (critical distance) when the efficiency of energy transfer is 50%. F and F_0 are the fluorescence intensities of HSA in the presence and absence of the ligand, respectively. The value of R_0 can be calculated from

$$R_0 = 0.211(\kappa^2 d^{-4} \phi_D J)^{1/6} \quad (2)$$

where κ^2 is the spatial orientation factor between the emission dipole of the donor and the absorption dipole of the acceptor, d is the refractive index of the medium, ϕ_D is the fluorescence quantum yield of the donor, and J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor and is given by

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4 \frac{\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (3)$$

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength λ, and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength λ. In the present case, the values of εs are given in the caption of Figure S1 for the HBX ligands.

J can be evaluated by integrating the overlapped portion of the spectra in Figure S1. If both the donor and acceptor are tumbling rapidly and are free to assume any orientation, then the dipole orientation factor, κ^2, can adapt the value 2/3.[2] The value of κ^2 represents a major factor in the analysis of the energy transfer efficiencies. This factor reflects the angle between the emission transition dipole of the donor and the transition absorption dipole of the acceptor. Depending upon the relative orientation of donor and acceptor this factor can range from 0 to 4. For head-to-tail parallel transition dipoles $\kappa^2 = 4$, and for parallel dipoles $\kappa^2 = 1$. Since the sixth root is taken to calculate the distance, variation of κ^2 from 1 to 4 results in a 26% change in R_{DA}. Compared to $\kappa^2 = 2/3$, the calculated distance can be in error by no more than 35%. However, if the dipoles are oriented perpendicular to one another, $\kappa^2 = 0$, which would result in serious errors in the calculated distance. A detailed discussion about this parameter is presented in reference 1.

In the present case, $d = 1.36$ and $\phi_D = 0.15$.[4] Using the aforementioned parameters, we calculated the values summarized in Table S1 for 1:1 molar ratio of HSA:HBX (0.05 mM). The estimated donor-to-acceptor distances are all less than 7 nm, indicating a major contribution from a static quenching interaction between the donor and acceptor according to Förster's nonradiative energy transfer theory.[1-3]

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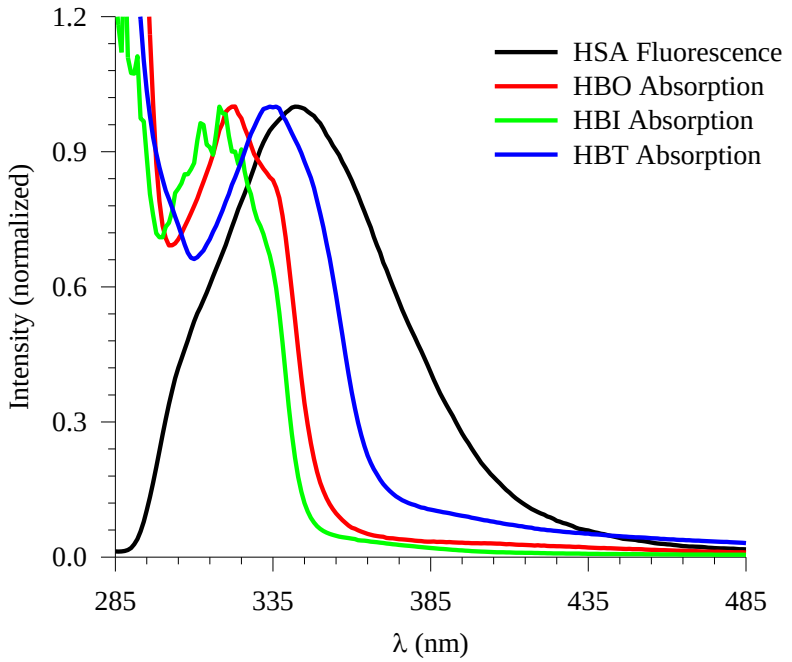

Fig. S1 Overlap of the fluorescence spectrum of HSA (λ_{ex} = 280 nm) with the absorption spectra of HBXs as indicated in the graph. The molar absorption coefficient values were: ε_{323} = 10000 $M^{-1}cm^{-1}$ for HBO, ε_{318} = 9980 $M^{-1}cm^{-1}$ for HBI, and ε_{334} = 10339 $M^{-1}cm^{-1}$ for HBT. All solutions were prepared in 50 mM phosphate buffer of pH 7.2. The concentration of HSA and HBX was 0.05 mM.

Table S1. Calculated parameters for the HSA-HBX complexes using FRET theory[a]

	W214-HBO	W214-HBI	W214-HBT
Efficiency of Energy Transfer (%*E*)	69	85	83
Donor-Acceptor Critical Distance (R_0 (Å))	22	21	21
Donor-Acceptor Apparent Distance (R_{DA} (Å))	19	16	17
Energy Transfer Rate Constant (k x 10^{-8} (s^{-1}))	3.4	8.7	7.3

[a] The calculations are based on the spectra shown in Figure 4 in the paper.

References

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