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Non-covalent interaction of 2,6-dialkyl-substituted BODIPY with human serum albumin

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Methods and materials

Materials

Dimethyl sulfoxide (DMSO) pure (Panreac, Barcelona) was used without further purification. Bovine and Human Serum Albumins (Sigma, M = 69 kDa) were used without further purification. All biological experiments were performed in phosphate buffer (PBS) at pH = 7.42.[S1] BODIPY **1**–**8** were synthesized and identified according to the published procedure.[S2]

Spectral analysis method of BODIPY – HSA systems

Electronic absorption spectra of the protein-dye systems with BODIPY **1** – **8** were obtained on a SOLAR CM 2203 spectrometer (SOLAR, Belarus). Steady-state and time-resolved fluorescence studies of the systems with BODIPY **1**–**8** were performed using a high-performance FluoTime 300 spectrometer (PicoQuant, Germany) with a LDH-P-C-450 as the excitation source. The instrument response function (IRF) of the spectrometer was measured using the scattered light signal of a dilute suspension of colloidal silica (LUDOX®). Fluorescence decay curves were measured at the maximum of the emission peaks, and the fluorescence lifetime was obtained by reconvolution of the decay curves using the EasyTau 2 software package (PicoQuant, Germany).

Anisotropy studies

On the FluoTime 300 instrument, spectra with different light polarizations were obtained for each luminophore-protein system in order to obtain a quantitative value of anisotropy. Spectra with VV, VH, HV, HH light polarization were recorded (where V is the vertical polarization of light, H is the horizontal polarization of light). The anisotropy value *r* was calculated using the equations:

$$\frac{I_{HV}}{I_{HH}} = \frac{S_V}{S_H}\frac{I_\perp}{I_\perp} = \frac{S_V}{S_H} = G$$

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where I_{HV} - is the fluorescence intensity with HV polarization, I_{HH}– is the fluorescence intensity with HH polarization, I_{VV} – is the fluorescence intensity with VV polarization, I_{VH} – is the fluorescence intensity with VH polarization

Molecular docking of BODIPY–SA systems

An automated molecular docking procedure was performed using the Autodock 4.2 [S3] software package to identify potential binding sites of BODIPY **6**–**8** with SA. Geometric optimization of dyes was performed based on density functional theory (DFT) with ωB97X-D functional[S4] and 6-311++G(d,p) basis set[S5,S6] in the gas phase. The Lamarck genetic algorithm was used to find the protein-dye complex with the minimal free energy.[S7] The protein was considered a rigid molecule, while the dye structure was flexible. The structures of BSA and HSA were obtained from a protein database (4f5s and 1ao6, respectively) with charge distribution characteristics at pH 7 (www.rcsb.org). To assess the possible interactions of BODIPY with SA, blind docking was first performed without assigning a specific binding site. Blind docking calculations were performed for a 126 × 126 × 126 Å grid with the SA molecule in the center and a spacing of 0.690 Å. Each docking experiment was obtained from 50 individual runs, which were to terminate after 25 million energy evaluations. The BODIPY - SA complexes with close binding energy values were sorted into groups according to the RMSE (root mean square deviation of the atomic positions between this conformation and the cluster reference). For each complex, the conformation with the lowest energy was considered the most stable. The UCSF Chimera software package[S8] was used to analyze and visualize the molecular docking results.

Appendix

Table S1. Energy parameters of the BODIPY – SA interaction

System	Δ*E*, kJ/mol	System	Δ*E*, kJ/mol
8 - BSA	-17.87	**1** - HSA	-22.43
7 - BSA	-18.87	**7** - HSA	-22.51
2 – BSA	-22.89	**5** – HSA	-22.97
1 - BSA	-23.72	**8** - HSA	-23.47
6 - BSA	-24.06	**4** – HSA	-23.60
5 – BSA	-24.23	**3** - HSA	-23.93
3 – BSA	-25.56	**2** – HSA	-24.39
4 – BSA	-27.57	**6** - HSA	-25.23

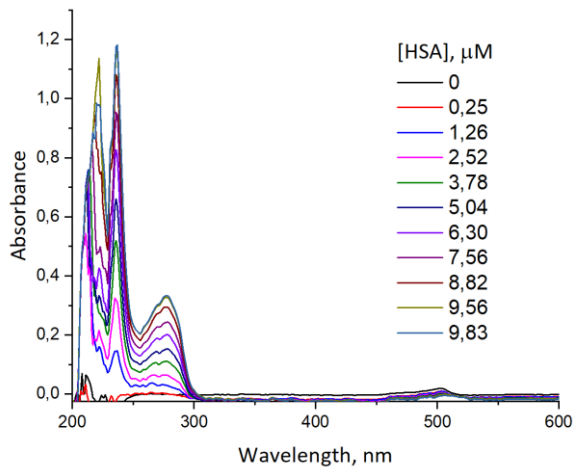
Table S2. K_{SV} values for BODIPY – HSA systems in the temperature range 298-308K

BODIPY	**Temperature, K**	$\boldsymbol{K_{SV} \cdot 10^5}$**, L/mol***
1	298	1.64
	303	1.62
	308	1.36
2	298	0.34
	303	0.48
	308	0.52
3	298	0.28
	303	0.25
	308	0.36
4	298	0.26
	303	0.18
	308	0.43
5	298	0.25
	303	0.25
	308	0.25
6	298	-
7	298	-
8	298	-

* where the K_{SV} value was obtained from the equation:

$$F_0/F = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q],$$

where F_0 and F are the fluorescence intensities of BODIPY in the absence and presence of SA, respectively; K_{SV} is the Stern-Volmer constant; [Q] is the concentration of SA; k_q is the bimolecular quenching rate constant; τ_0 is the BODIPY fluorescence lifetime.



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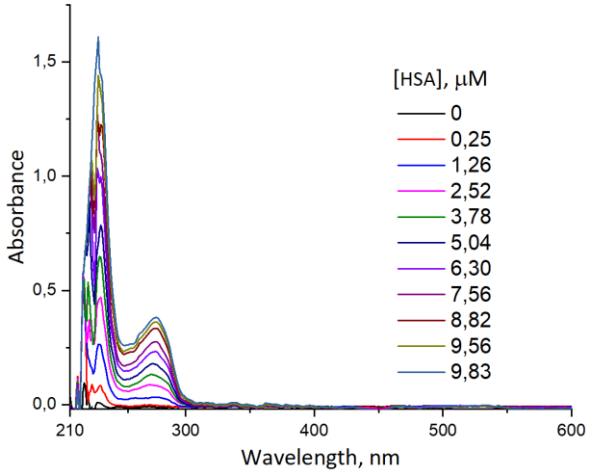

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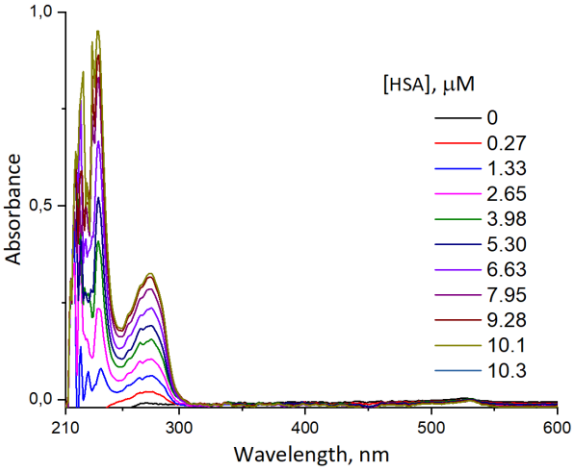

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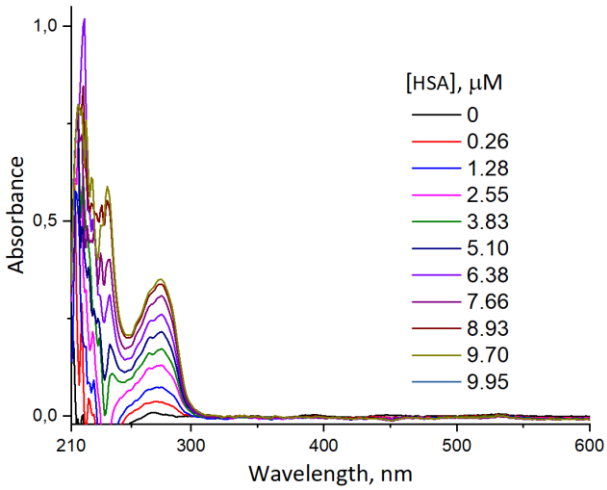

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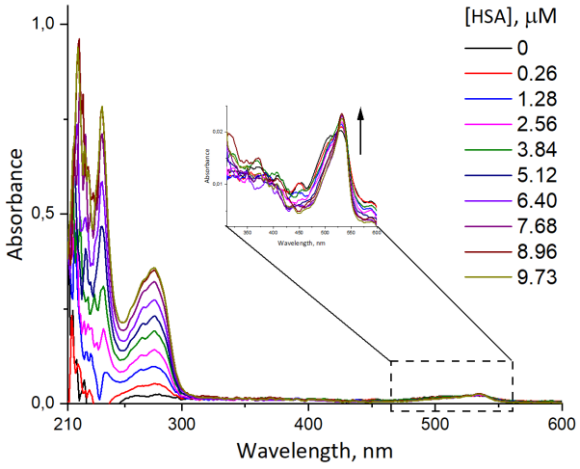


e

Figure S1. Absorption spectra of BODIPY – HSA systems with different HSA concentrations (C_{BODIPY} = 0,3-0,4 μM) in PBS-DMSO mixtures: BODIPY **1** (*a*), BODIPY **2** (*b*), BODIPY **3** (*c*), BODIPY **4** (*d*), BODIPY **5** (*e*)

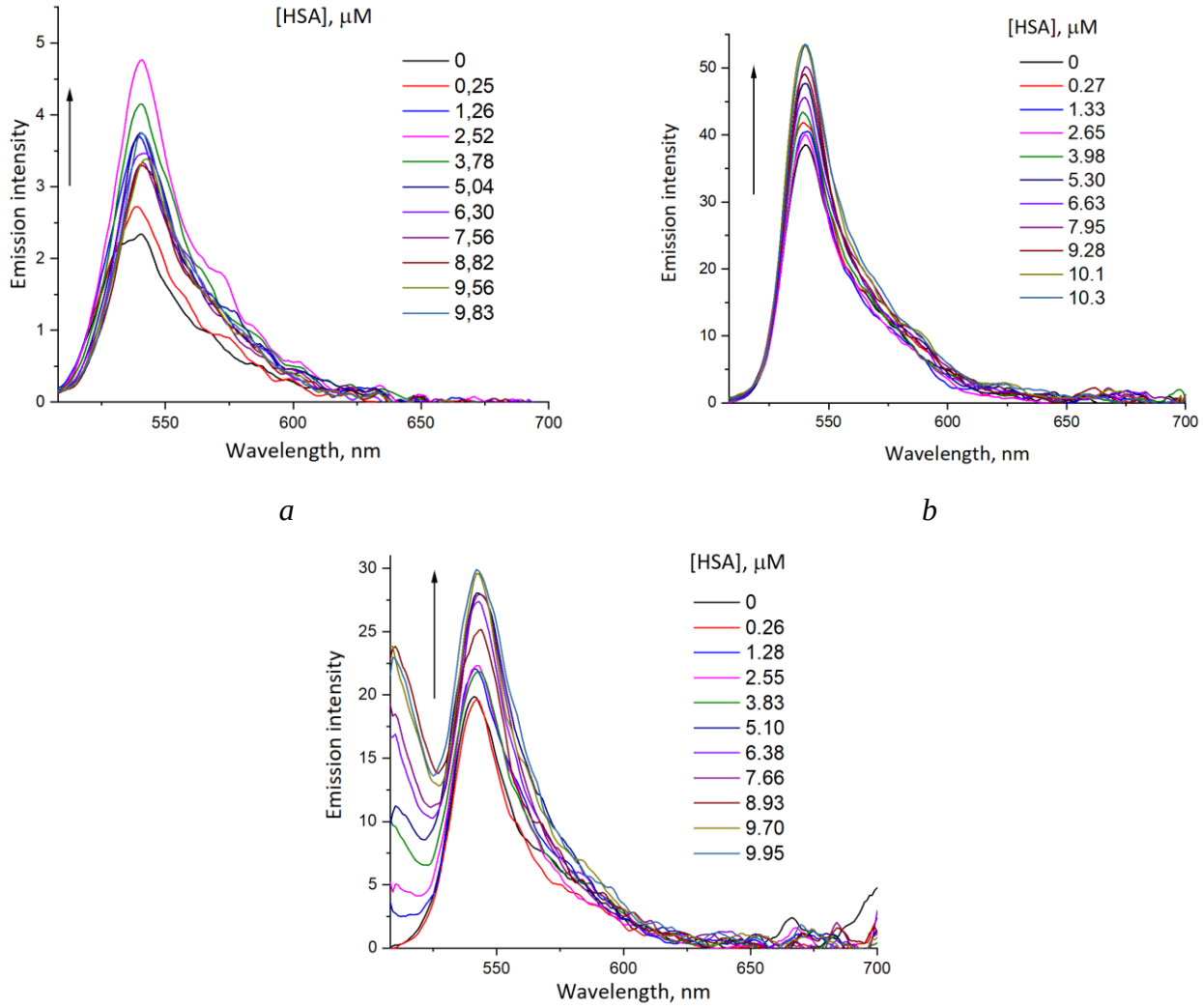
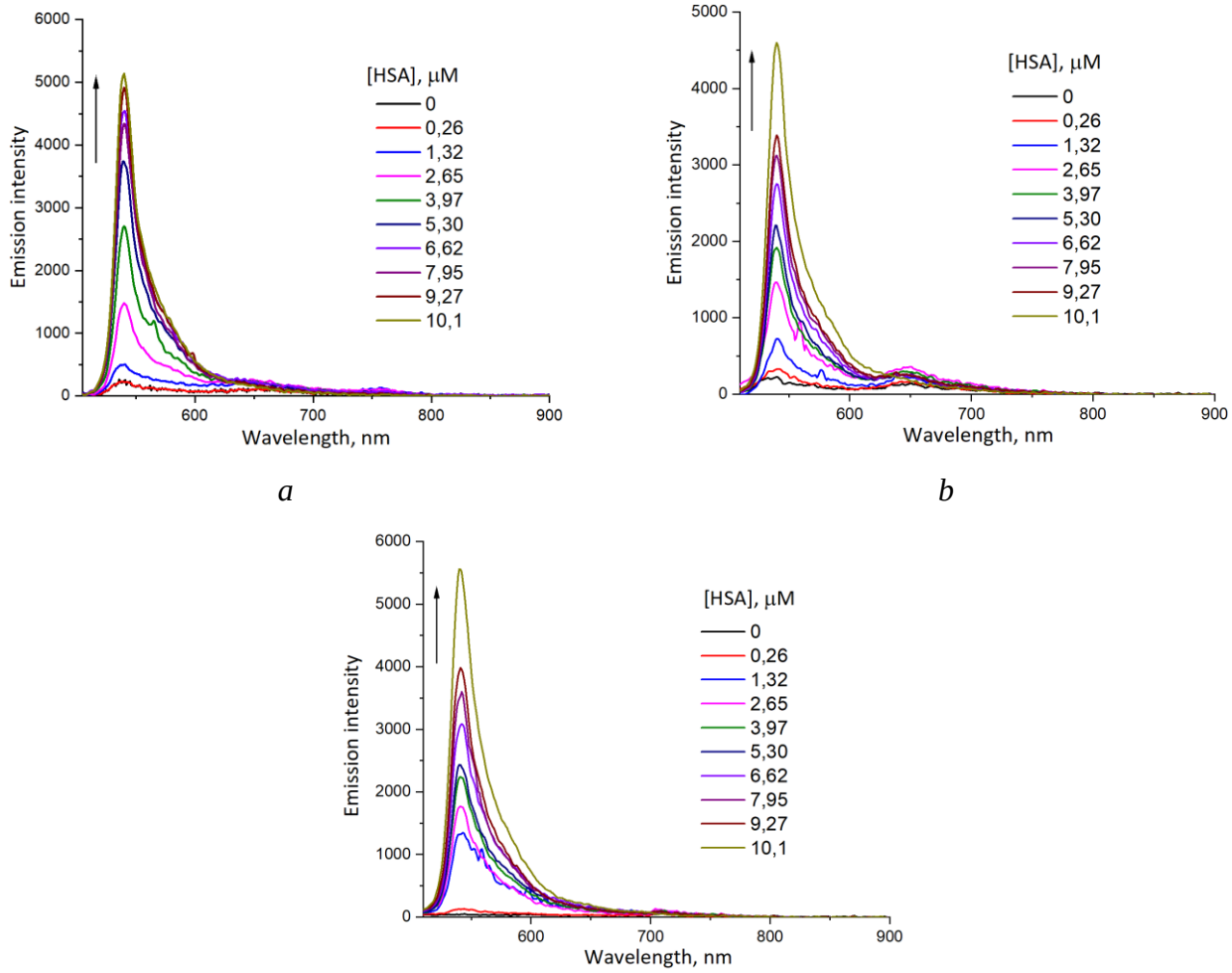


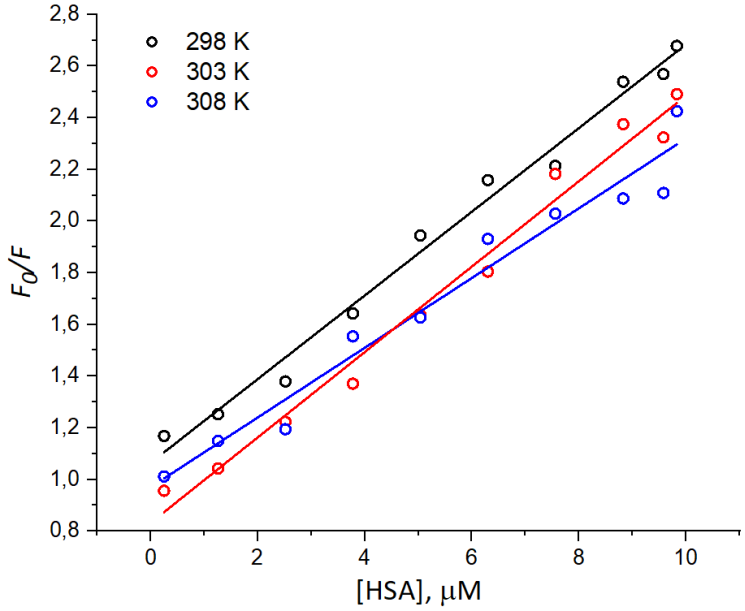
Figure S2. Emission spectra of BODIPY – HSA systems with different HSA concentrations (C_{BODIPY} = 0,3-0,4 μM) in PBS-DMSO mixtures: BODIPY **2** (*a*), BODIPY **3** (*b*), BODIPY **4** (*c*)



a *b*

c

Figure S3. Emission spectra of BODIPY – HSA systems with different HSA concentrations (C_{BODIPY} = 0,3-0,4 μM) in PBS–DMSO mixtures: BODIPY **5** (*a*), BODIPY **6** (*b*), BODIPY **7** (*c*)



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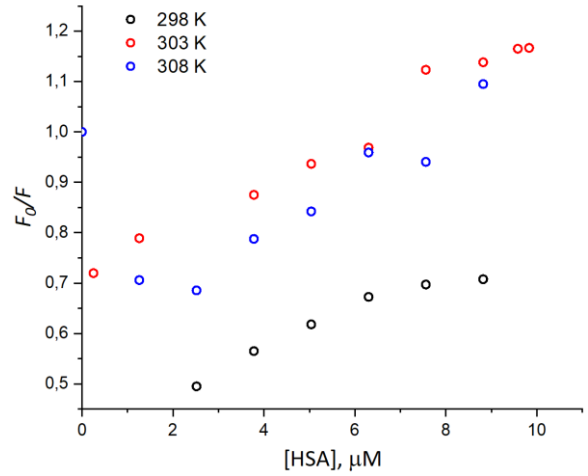

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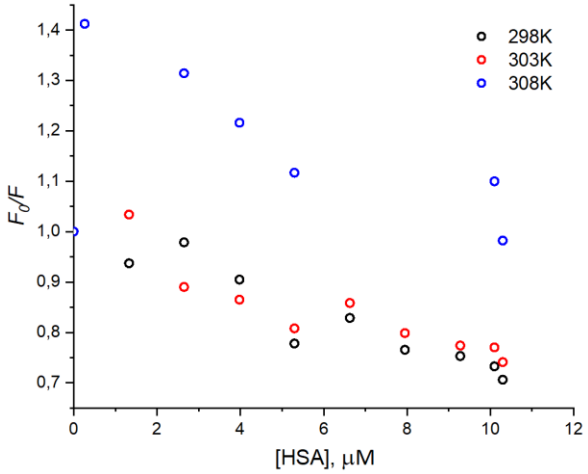

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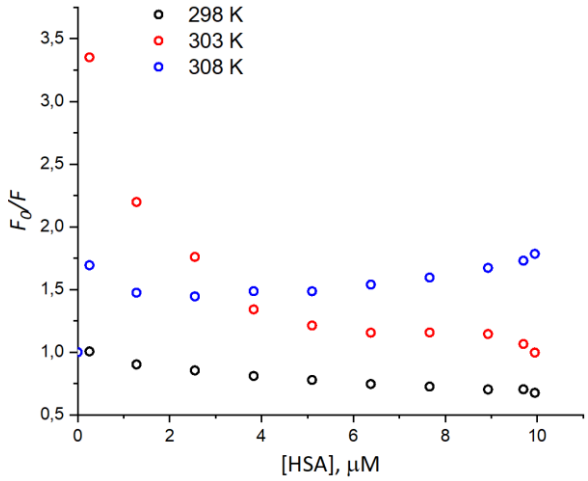

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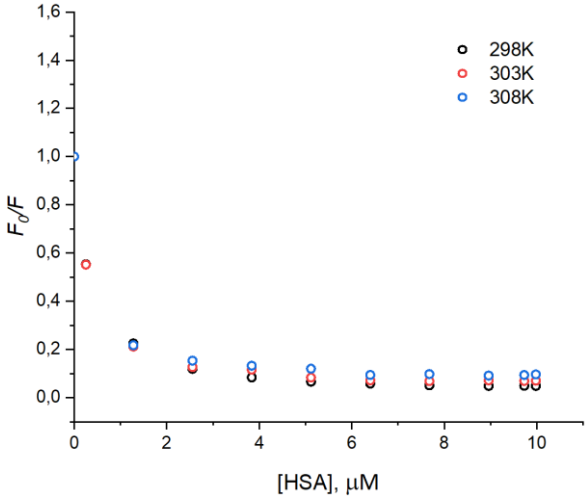

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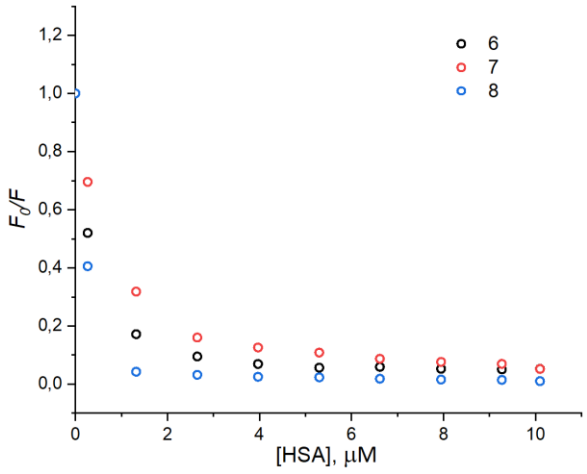
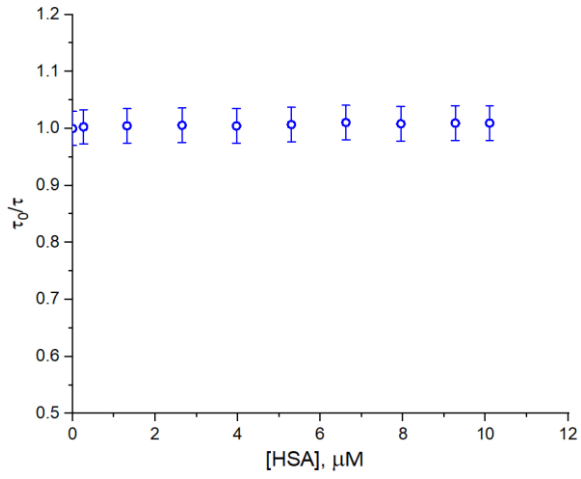
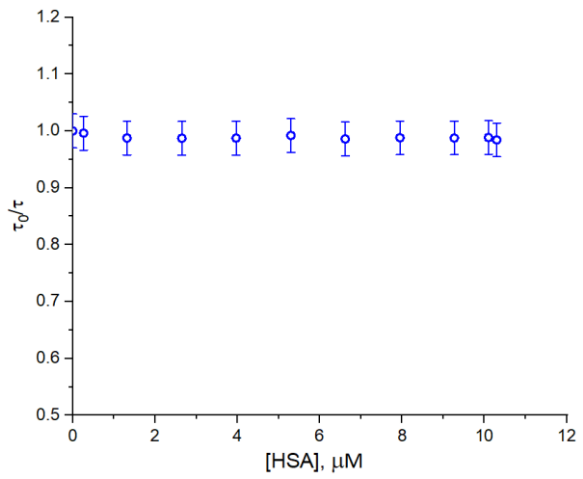


f

Figure S4. Stern-Volmer dependence for BODIPY **1** (*a*), BODIPY **2** (*b*), BODIPY **3** (*c*), BODIPY **4** (*d*), BODIPY **5** (*e*) with HSA in the temperature range of 298-308K; Stern-Volmer dependences for BODIPY **6-8** with HSA (*f*) at a temperature of 298K


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
τ0/τ
0
2
4
6
8
10
12
[HSA], μM

a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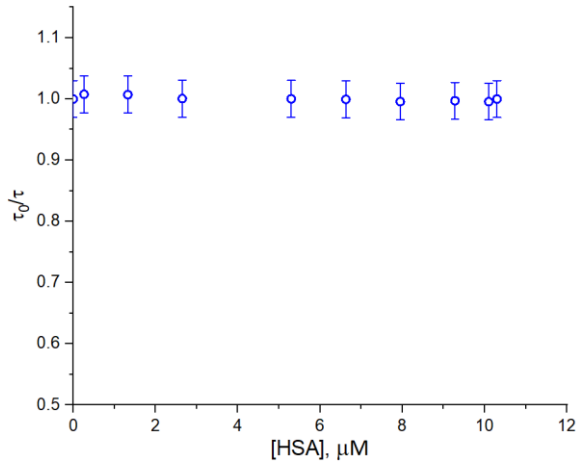
1.1
1.0
0.9
0.8
0.7
0.6
0.5
τ0/τ
0
2
4
6
8
10
12
[HSA], μM

b


1.1
1.0
0.9
0.8
0.7
0.6
0.5
τ0/τ
0
2
4
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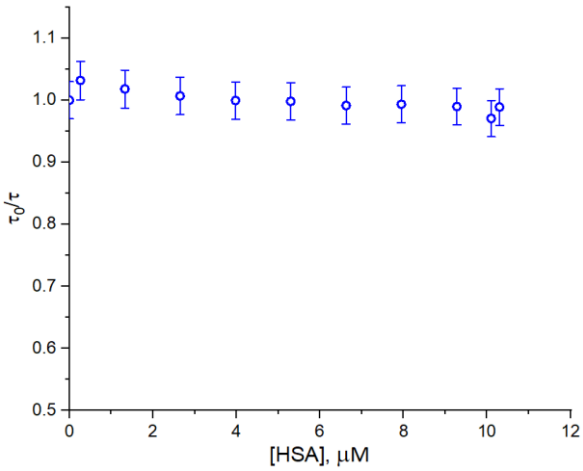
12
[HSA], μM

c


1.1
1.0
0.9
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0.7
0.6
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τ0/τ
0
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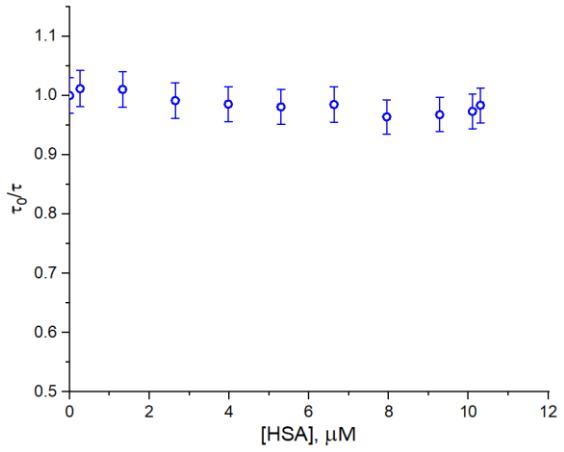
10
12
[HSA], μM

d


1.1
1.0
0.9
0.8
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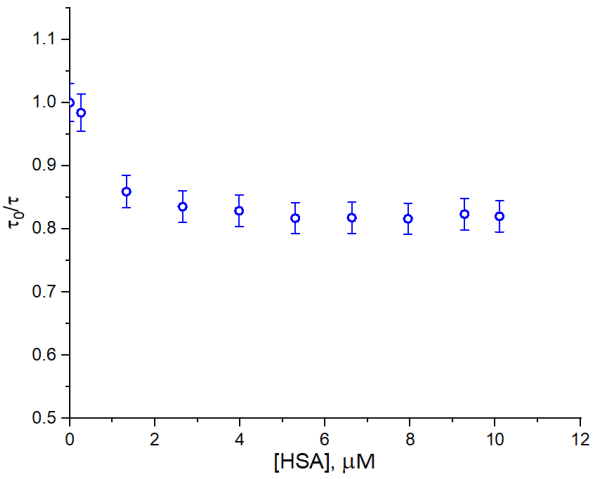
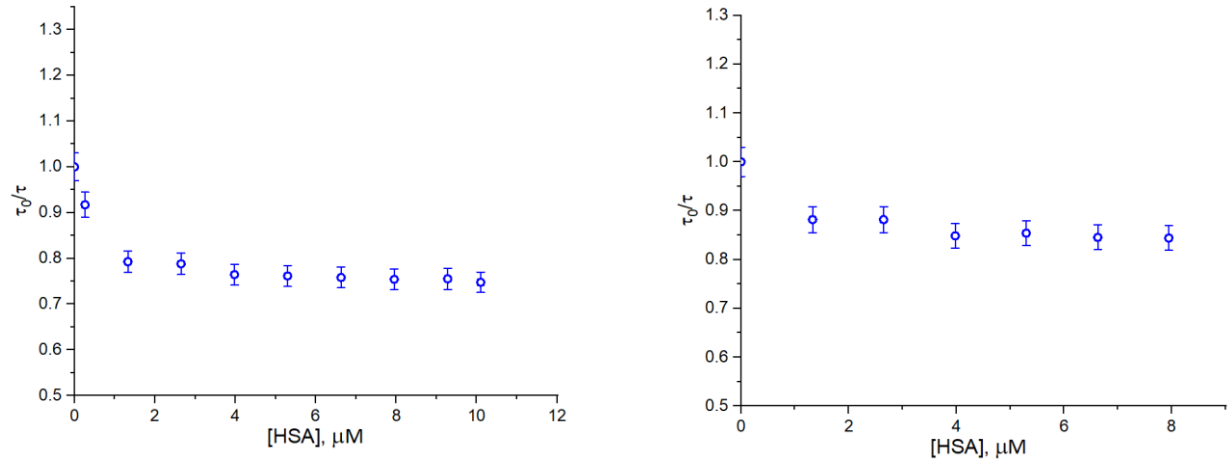
τ0/τ
0
2
4
6
8
10
12
[HSA], μM

e

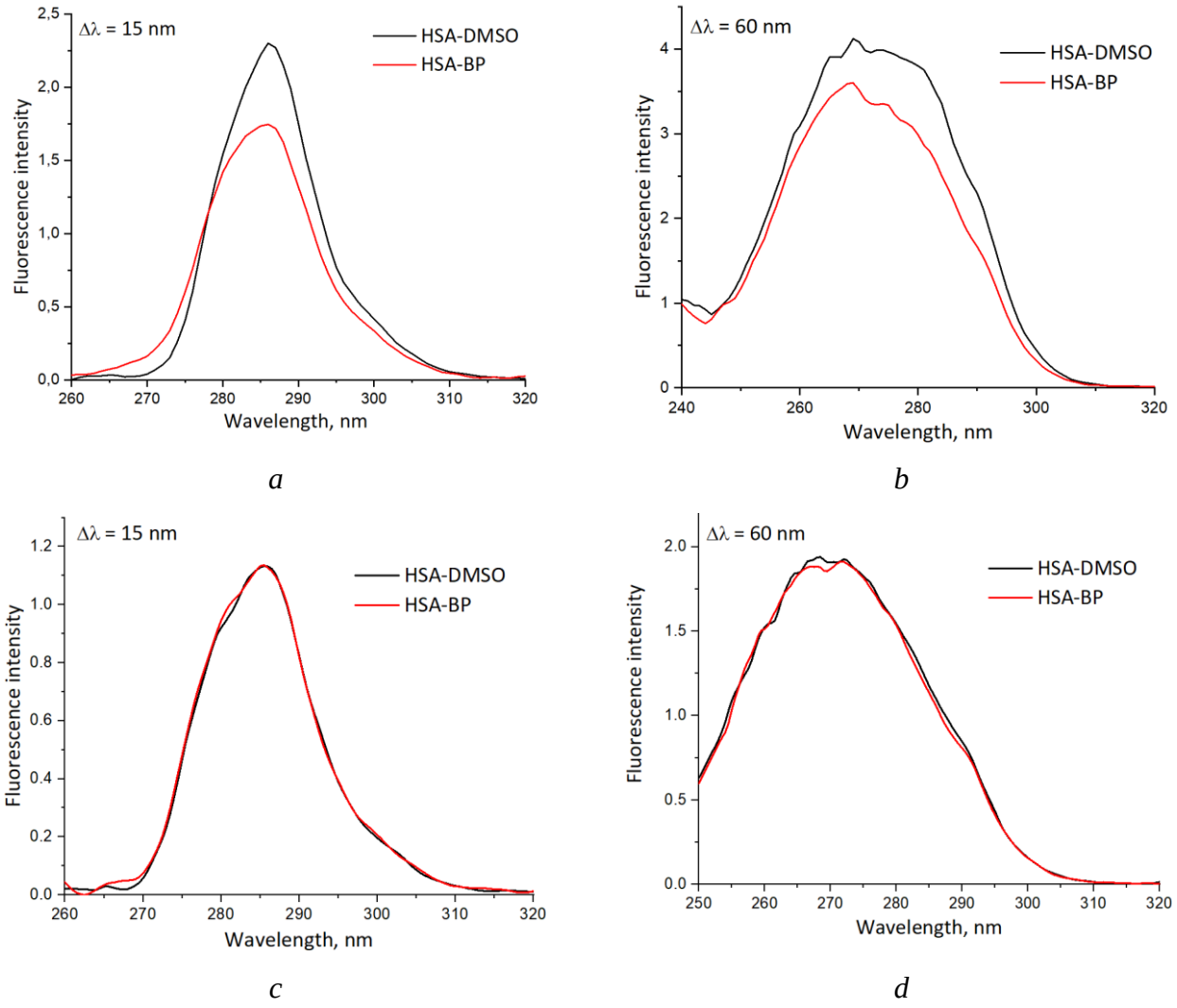

1.1
1.0
0.9
0.8
0.7
0.6
0.5
τ0/τ
0
2
4
6
8
10
12
[HSA], μM

f



g *h*

Figure S5. Dependences of τ_0/τ on [HSA] for BODIPY **1** (*a*), BODIPY **2** (*b*), BODIPY **3** (*c*), BODIPY **4** (*d*), BODIPY **5** (*e*), BODIPY **6** (*f*), BODIPY **7** (*g*), BODIPY **8** (*h*)



a *b*

c *d*

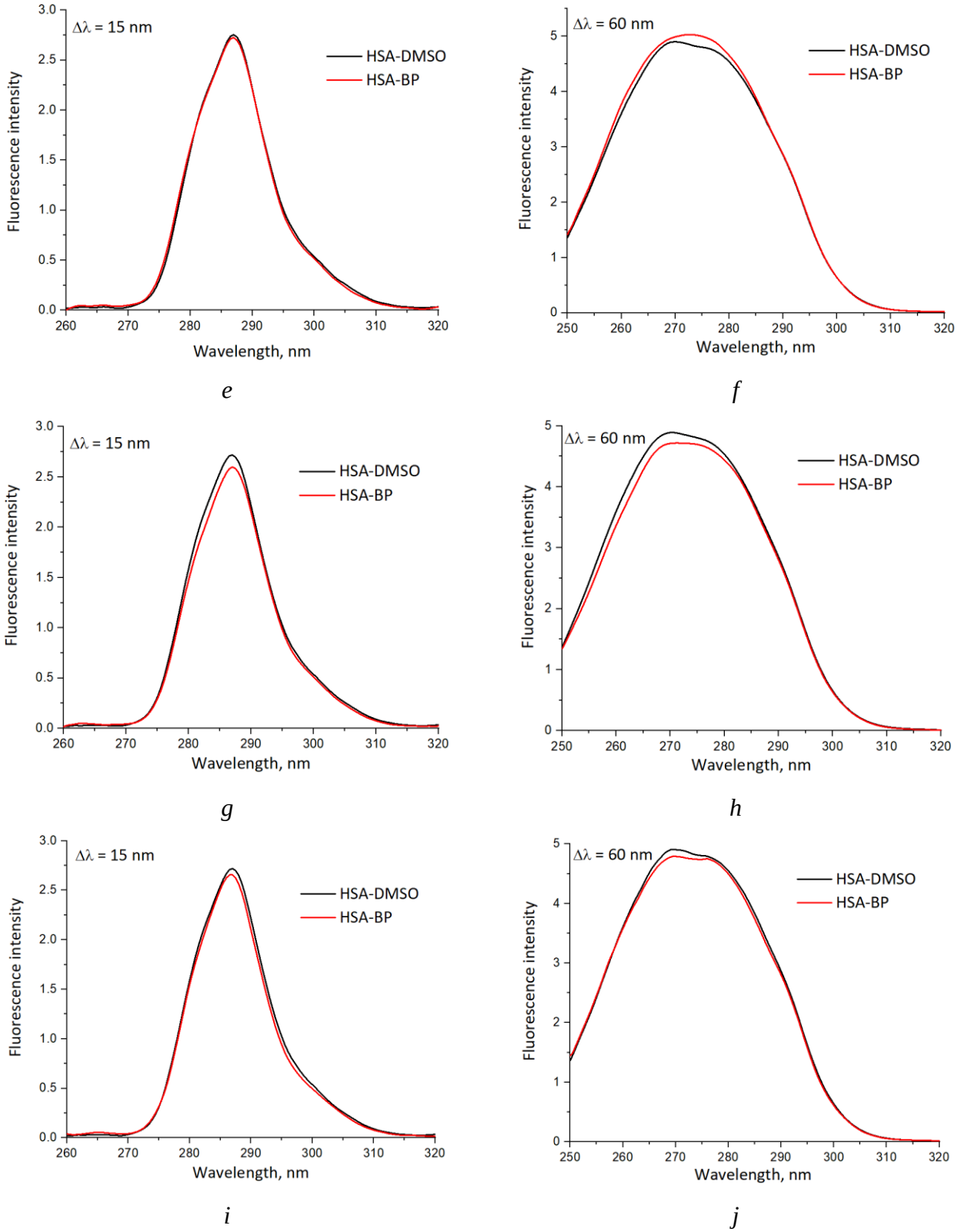


Figure S6. Synchronous spectra of BODIPY **1** (*a*, *b*), BODIPY **2** (*c*, *d*) BODIPY **3** (*e*, *f*), BODIPY **4** (*g*, *h*), BODIPY **5** (*i*, *j*) with HSA

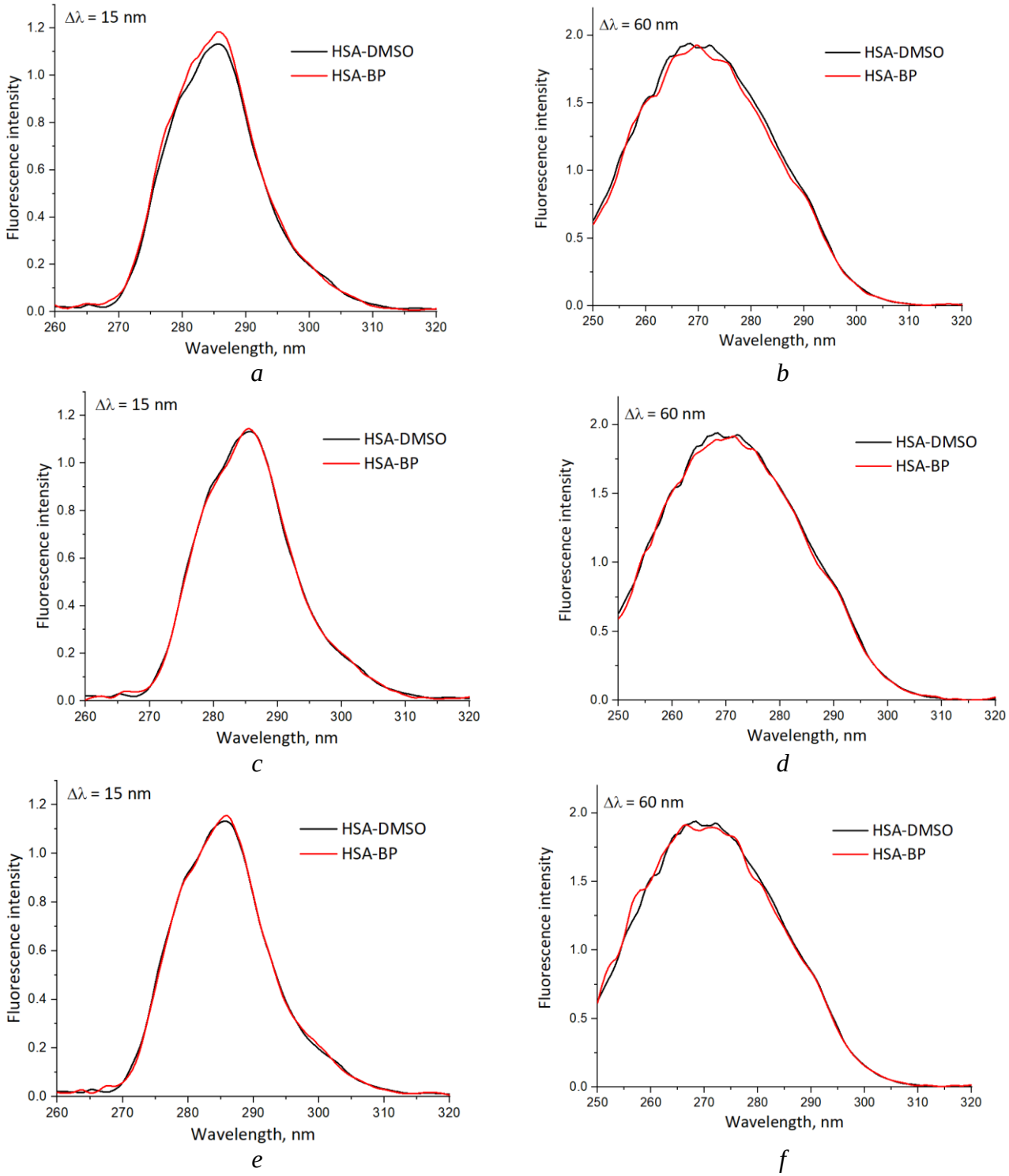


Figure S7. Synchronous spectra of BODIPY **6** (*a*, *b*), BODIPY **7** (*c*, *d*), BODIPY **8** (*e*, *f*) with HSA

References

S1 A. Kumagai, R. Ando, H. Miyatake, P. Greimel, T. Kobayashi, Y. Hirabayashi, T. Shimogori and A. Miyawaki, *Cell*, 2013, **153**, 1602; https://doi.org/10.1016/j.cell.2013.05.038.

S2 S.L. Yutanova, M.B. Berezin, A.S. Semeikin, E.V. Antina, G.B. Guseva and A.I. V'yugin, *Russ J Gen Chem*, 2013, **83**, 545; https://doi.org/10.1134/S1070363213030237.

S3 G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell and A.J. Olson, *J. Comput. Chem.*, 2009, **30**, 2785; https://doi.org/10.1002/jcc.21256.

S4 J.-D. Chai and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615; https://doi.org/10.1039/B810189B.

S5 A.D. McLean and G.S. Chandler, *J. Chem. Phys.*, 1980, **72**, 5639; https://doi.org/10.1063/1.438980.

S6 R. Krishnan, J.S. Binkley, R. Seeger and J.A. Pople, *J. Chem. Phys.*, 1980, **72**, 650; https://doi.org/10.1063/1.438955.

S7 F.J. Solis and R.J.-B. Wets, *Mathematics of OR*, 1981, **6**, 19; https://doi.org/10.1287/moor.6.1.19.

S8 E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605; https://doi.org/10.1002/jcc.20084.