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Supporting Information

Determination of Enantiomeric Compositions by Transient Absorption Spectroscopy using proteins as chiral selectors

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Supporting Tables

Table S1. Percentage of free, site I- and site II-bound *(S)*- and *(R)*-FBPMe in the presence of PSA at different FBPMe/PSA ratios.

	t (μs)	**FBPMe/PSA Molar ratios**					
		1:1.25	**1:1**	**1.5:1**	**2:1**	**3:1**	**4:1**
(R)-FBPMe	τ_{FREE} = 1.5	-	-	22	30	44	57
	τ_{SITE-I} = 95.3	75	77	57	50	31	22
	$\tau_{SITE-II}$ = 9.5	25	23	21	20	25	21
(S)-FBPMe	τ_{FREE} = 1.5	-	-	27	33	55	59
	τ_{SITE-I} = 41.2	53	53	35	33	21	18
	$\tau_{SITE-II}$ = 6.0	47	47	38	34	24	23

Table S2. Percentage of free, site I- and site II-bound *(S-)* and *(R)*-FBP in the presence of PSA at different FBP/PSA ratios.

	t (μs)	**FBP/PSA Molar ratios**					
		1:1.25	**1:1**	**1.5:1**	**2:1**	**3:1**	**4:1**
(R)-FBP	τ_{FREE} = 1.5	-	-	26	42	60	75
	τ_{SITE-I} = 6.8	44	45	30	24	14	10
	$\tau_{SITE-II}$ = 45.0	56	55	44	36	26	15
(S)-FBP	τ_{FREE} = 1.5	-	-	33	50	66	75
	τ_{SITE-I} = 6.5	44	44	30	20	10	10
	$\tau_{SITE-II}$ = 44.5	56	56	37	30	24	14

Table S3. Optical rotation (O.R.), standard deviation (σ) and % coefficient of variance (C.V.) of mixtures containing different percentages of (*S*)- and (*R*)-FBP in MeCN solutions at 2.5×10^{-5} M total concentration.

% *(S)*-FBP	**O.R.[a,b]**	s [b]	**C.V.[b] %**
100	-0,0031	0,0048	-152
90	-0,0014	0,0056	-393
80	0,0012	0,0031	260
70	-0,0020	0,0033	-167
60	0,0017	0,0034	198
50	-0,0020	0,0014	-67
40	0,0023	0,0014	62
30	0,0035	0,0029	84
20	-0,0010	0,0045	-446
10	-0,0023	0,0025	80
0	-0,0026	0,0026	-102

[a] Measured in a JASCO polarimeter P-1030 with a quartz cell of 10 cm pathway. [b] No reliable values for the were obtained, indicating that polarimetry does not provide accurate results in the same range of minute concentrations of the LFP based method.

Supporting Figures

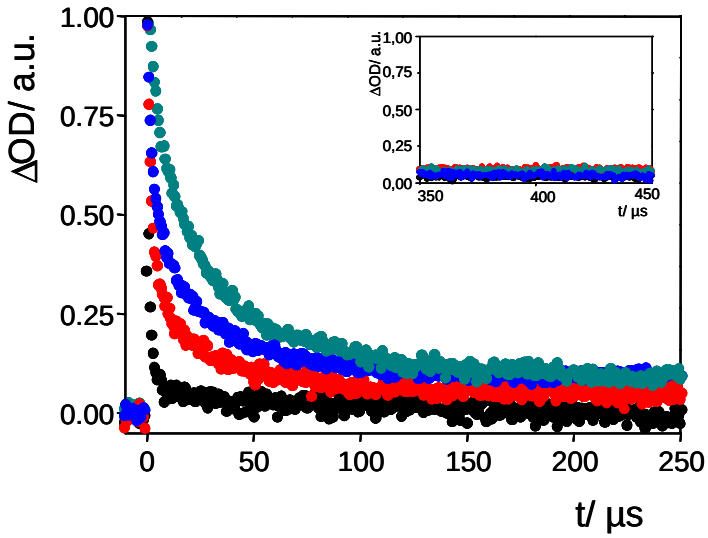


Figure S1. Laser flash photolysis (λ_{exc} = 266 nm) of *(S)*-FBPMe (black) and *(S)*-FBPMe/PSA at different molar ratios, 4:1 (red), 2:1 (blue) and 1:1.25 (cyan). Normalized decays monitored at 360 nm. The concentration of *(S)*-FBPMe was $2.5 \cdot 10^{-5}$ M in all cases. Inset: Traces obtained at longer decay times.

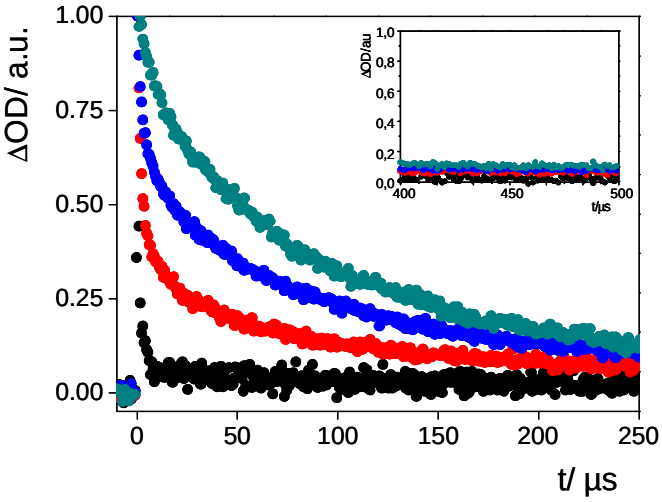


Figure S2. Laser flash photolysis (λ_{exc} = 266 nm) of *(R)*-FBPMe (black) and *(R)*-FBPMe/PSA at different molar ratios, 4:1 (red), 2:1 (blue) and 1:1.25 (cyan). Normalized decays monitored at 360 nm. The concentration of *(R)*-FBPMe was $2.5 \cdot 10^{-5}$ M in all cases. Inset: Traces obtained at longer decay times.

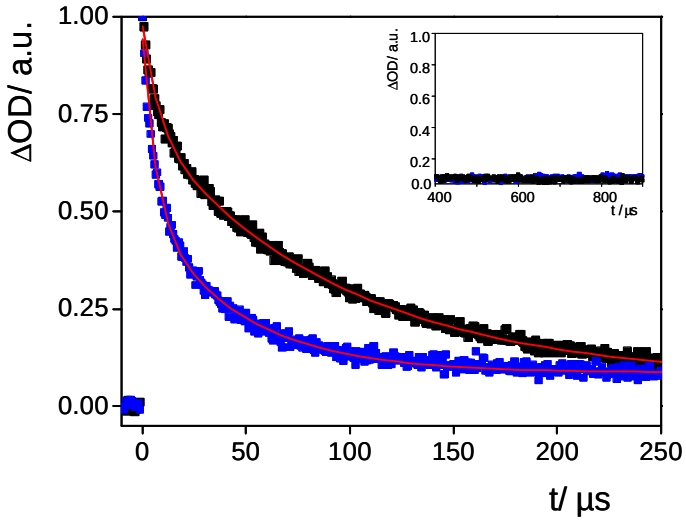


Figure S3. Laser flash photolysis (λ_{exc} = 266 nm) of *(R)*-FBPMe/PSA (black) and *(S)*-FBPMe/PSA (blue) at 1:1 molar ratio, showing a high stereodifferentiation in the triplet lifetimes. Normalized decays monitored at 360 nm. The concentration of FBPMe was $2.5 \cdot 10^{-5}$ M in all cases. Fittings are shown in red. Inset: Traces obtained at longer decay times

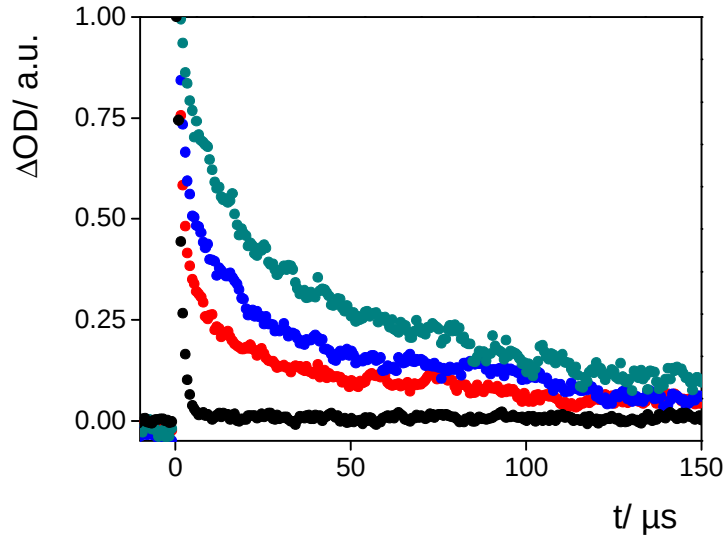


Figure S4. Laser flash photolysis (λ_{exc} = 266 nm) of *(S)*-FBP (black) and *(S)*-FBP/PSA at different molar ratios, 4:1 (red), 2:1 (blue) and 1:1.25 (cyan). Normalized decays monitored at 360 nm. The concentration of *(S)*-FBP was $2.5 \cdot 10^{-5}$ M in all cases.

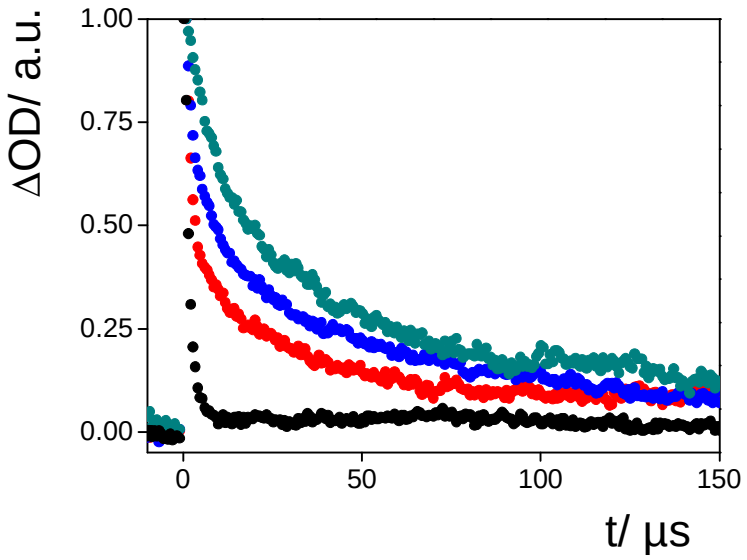


Figure S5. Laser flash photolysis (λ_{exc} = 266 nm) of *(R)*-FBP (black) and *(R)*-FBP/PSA at different molar ratios, 4:1 (red), 2:1 (blue) and 1:1.25 (cyan). Normalized decays monitored at 360 nm. The concentration of *(R)*-FBP was $2.5 \cdot 10^{-5}$ M in all cases.

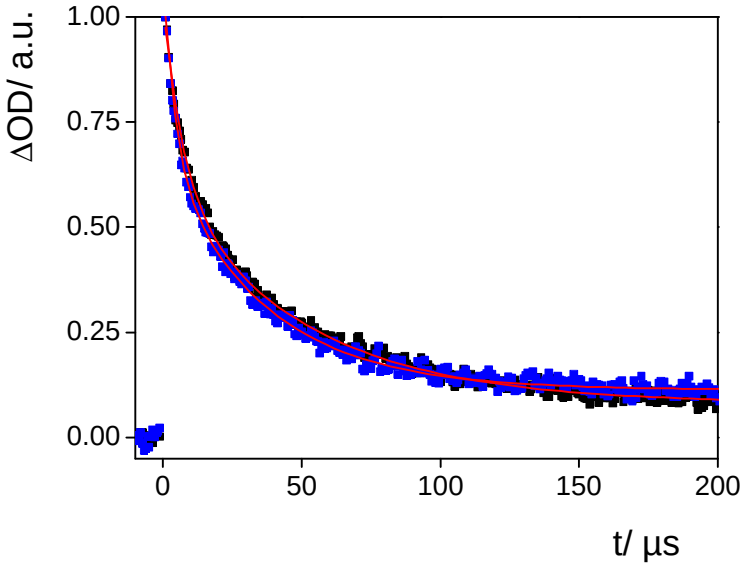


Figure S6. Laser flash photolysis (λ_{exc} = 266 nm) of *(R)*-FBP/PSA (black) and *(S)*-FBP/PSA (blue) at 1:1 molar ratio, showing no stereodifferentiation in the triplet lifetimes. Normalized decays monitored at 360 nm. The concentration of FBP was $2.5 \cdot 10^{-5}$ M in all cases. Fittings are shown in red.

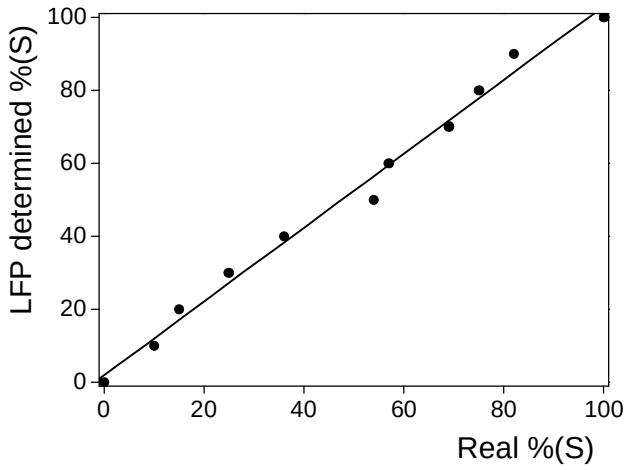
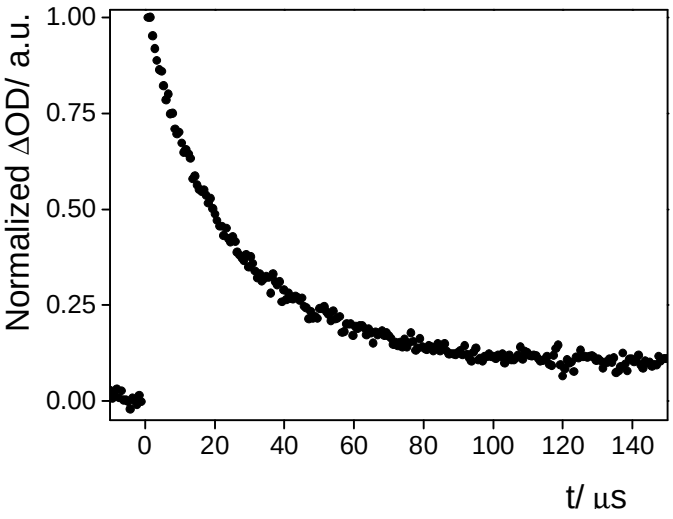


Figure S7. LFP-determined against known real percentages of *(S)*-FBP in the presence of BSA, together with the linear fit of the experimental points.



τ^{S}_{I} = 8.5 μs, τ^{S}_{II} = 31.6 μs, A_I^S/A_{II}^S = 0.333

τ^{R}_{I} = 7.5 μs, τ^{R}_{II} = 25.4 μs, A_I^R/A_{II}^R = 0.370

Real	LFP determined
70	67
50	51
40	42

Figure S8. Laser flash photolysis (λ_{exc} = 266 nm) of *(S)*-NPX/*(R)*-NPX/HSA at 0.5:0.5:1 molar ratios. Normalized decay monitored at 420 nm. The total concentration of NPX was $4 \cdot 10^{-5}$ M. From the lifetimes and preexponential factors found for the separate enantiomers, real *vs.* LFP-determined percentages of *(S)*-NPX were compared for three (*S*)/(*R*) mixtures (table on the right).

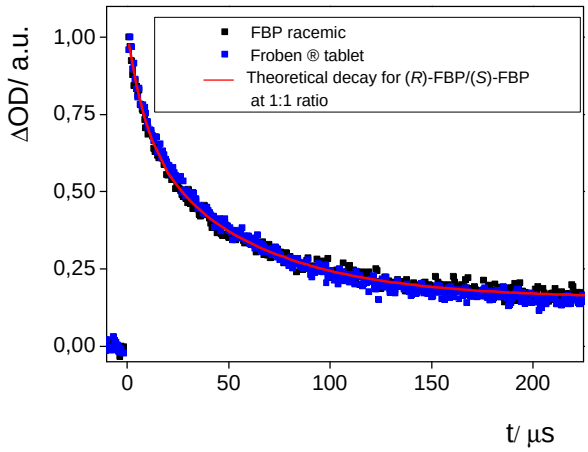


Figure S9. Decays at 360 nm of the LFP experiment (λ_{exc} = 266 nm) for racemic FBP/BSA and Froben®/BSA, compared with the fitting for a 50:50 enantiomeric composition. A tablet of Froben® 50 (Abbott Laboratories Limited) weights 200 mg; 50 of them correspond to racemic flurbiprofen, while the other 150 are different excipients: glucose, sacarose, lactose, maize starch, povidone, magnesium estearate, estearic acid, titanium dioxide, sandarac varnish, carnauba wax, colloidal silicon dioxide, black ink, etc.

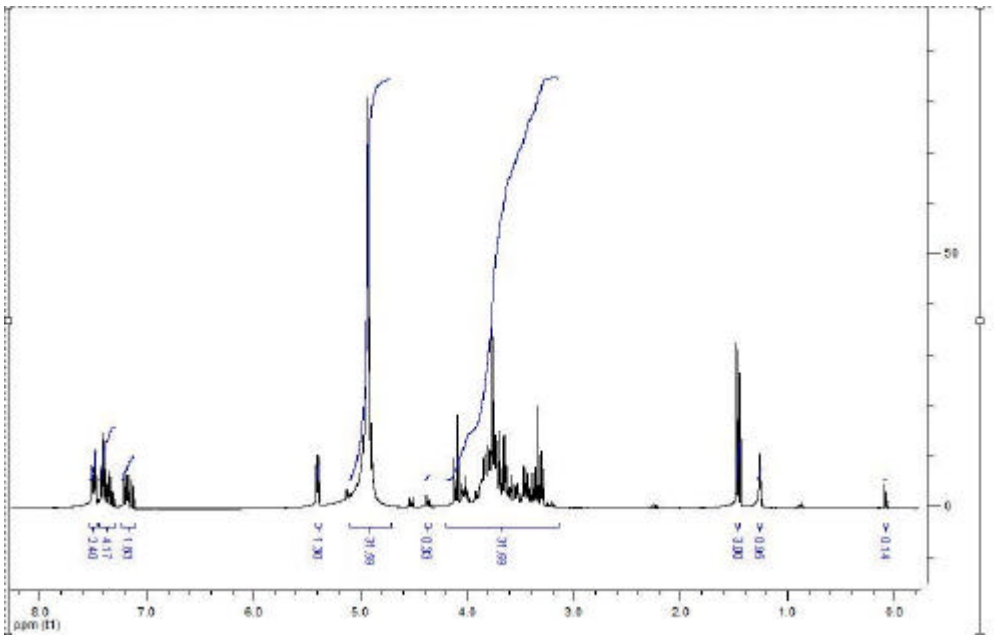


Figure S10. ^{1}H-RMN 300 MHz spectrum of Froben 50 ® in CD_3OD. A tablet was dissolved in 80 mL of CH_3OH, filtered, and the solvent evaporated in vacuo to afford a white powder. Then, 10 mg of this solid were dissolved in CD_3OD (0.7 mL), and the resulting solution was submitted to NMR spectroscopy.

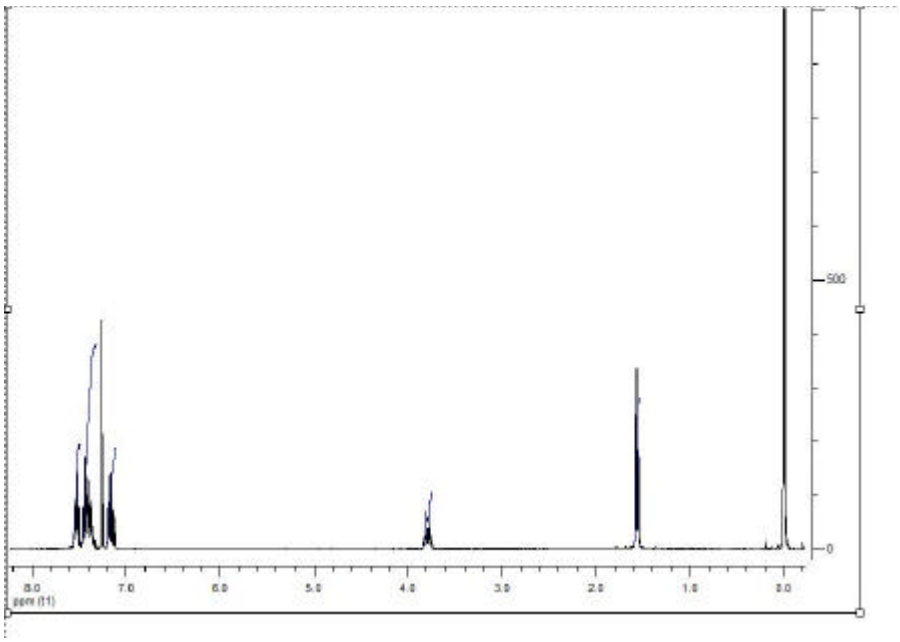


Figure S11. ^{1}H-RMN 300 MHz spectrum of racemic FBP (10 mg in 0.7 ml of $CDCl_3$). for comparison with Froben ®.

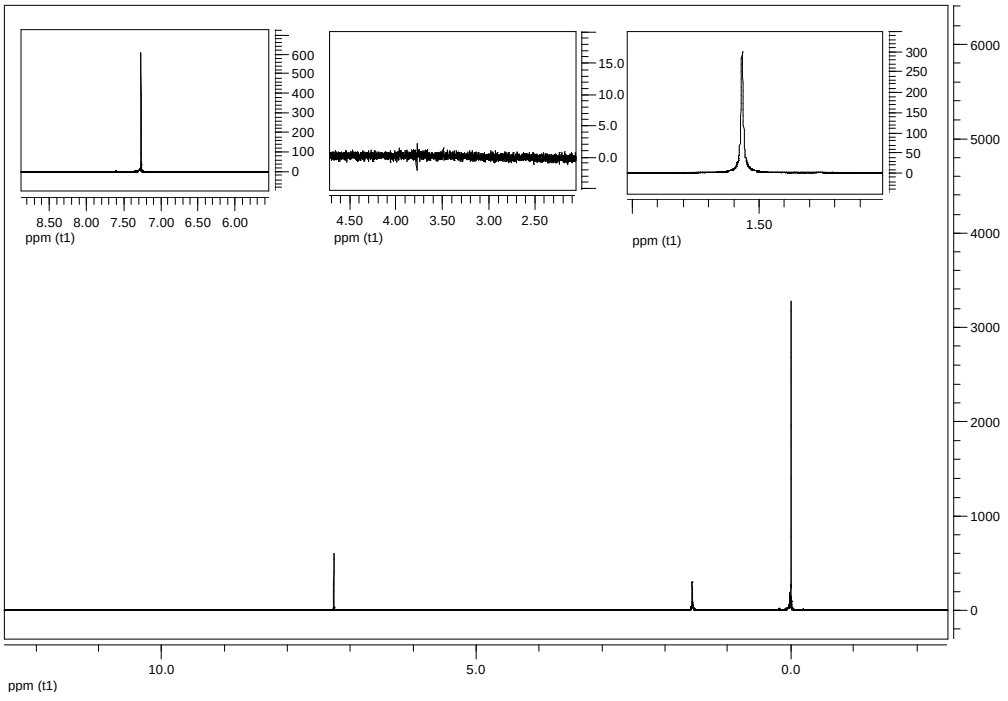


Figure S12. ^{1}NMR Spectrum (300 MHz) of the amount of FBP required for the LFP experiment (1 10^{-7} mol) in 0.7 mL of $CDCl_3$. No signals of the drug (see magnifications in the insets) were detected at this concentration.

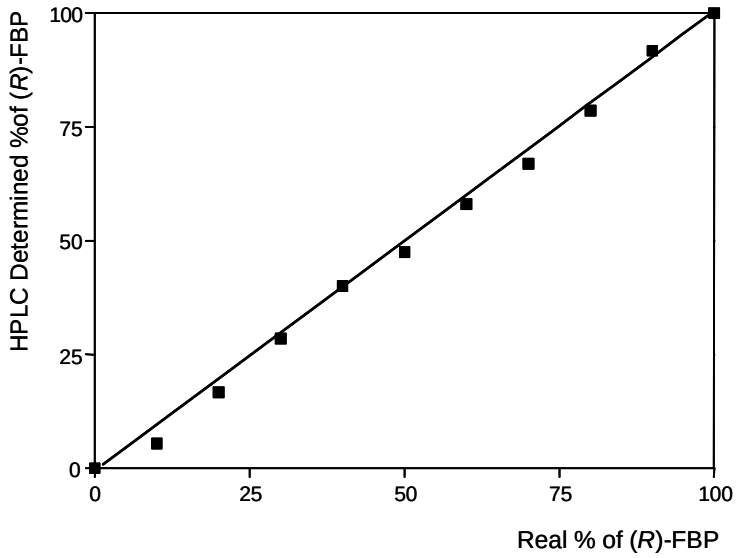


Figure S13. HPLC-determined compositions of different mixtures of (*S*)- and (*R*)-FBP against known real values, together with the linear fit of the experimental points.The same amount of FBP required for the LFP experiment (1 10^{-7} mol) was dissolved in 300 µL of *tert*-butyl methyl ether. An aliquot of 200 µL of this solution was injected in a HPLC system (reverse phase) provided with a chiral column (Kromasil 100 TBB 5 µm 25 cm ×1 cm). The eluent employed was *tert*-butyl methyl ether/hexane/acetic acid (60:40:0.1) with a flux of 1.5 mL/min. The detection system was a chiral polarimeter (JASCO OR-1590). The time to record a full chromatogram was *ca*. 25 min.

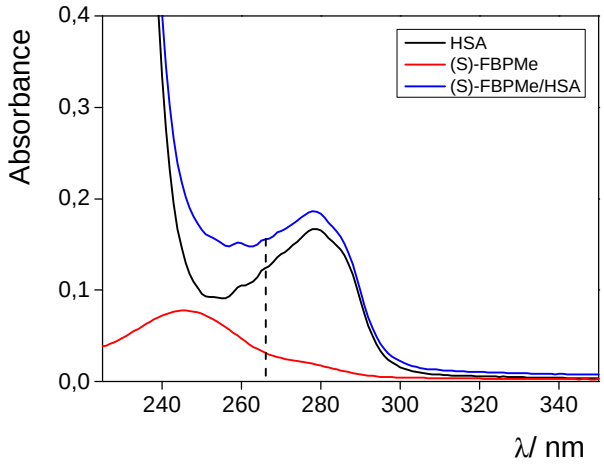


Figure S14. UV absorption spectra of (2.5×10^{-5} M) solutions of (*S*)-FBPMe, HSA and 1:1 (*S*)-FBPMe/HSA in PBS. In the complex, at 266 nm, 17% of the incident light is absorbed by (*S*)-FBPMe, while 83% is absorbed by the protein.

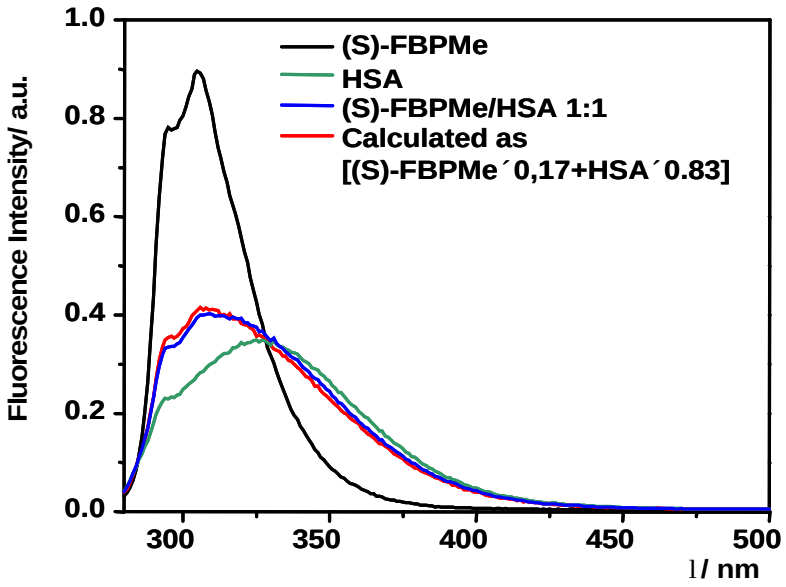


Figure S15. Emission spectra (λ_{exc} = 266 nm) of isoabsorptive solutions (A = 0.2) of (*S*)-FBPMe, HSA and 1:1 (*S*)-FBPMe/HSA in PBS. Emission of the complex shows a good matching with the calculations from the independent emissions of the two components, taking into account the relative absorbance.