

Fluorinated Human Serum Albumin as Potential ¹⁹F Magnetic Resonance Imaging Probe

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Figure S1. Structures of nonfluorinated and S-fluorinated 2,5-diketopiperazine and tripeptide derivatives. 2

Table S1. The ingredients and reaction conditions of the reaction between HTL and perfluoroarenes for mechanism studies.....4

HTL pKa determinations 6

Figure S2. SDS–PAGE of PFT-HSA (line 1), PFX-HSA (line 2), HSA (line 3), and Hcy-HSA (line 4) conjugates under Laemmli condition without addition of DTT with subsequent Coomassie blue staining. Bands corresponding to monomeric protein (MW = 66.5 kDa) and its dimer (MW ~ 130 kDa) were observed in samples. 6

Figure S3. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Number. 6

Figure S4. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Volume.....7

Figure S5. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Intensity. 7

Figure S6. DLS size distribution of HSA by Intensity. 7

Figure S7. Cellular uptake of fluorescently labeled albumin conjugates. 7

Figure S8. Typical albumin proteolysis SDS-PAGE data. Susceptibility of HSA and its conjugates to tryptic proteolysis (enzyme/substrate ratio 1:100). The bands in the gel lower 66.5 kDa (HSA MW) were marked as digested albumin. Quantitative data were obtained by digitizing the gel (SDS-PAGE) using GelPro Analyzer software (Media Cybernetics). SDS-PAGE (10%), pt. 6 h. The gel was stained with Coomassie Brilliant Blue. Line 1, MW markers (Sigma S8445); line 2, PFX-HSA; line 3, PFT-HSA; line 4, HSA; line 5, Hcy-HSA. 8

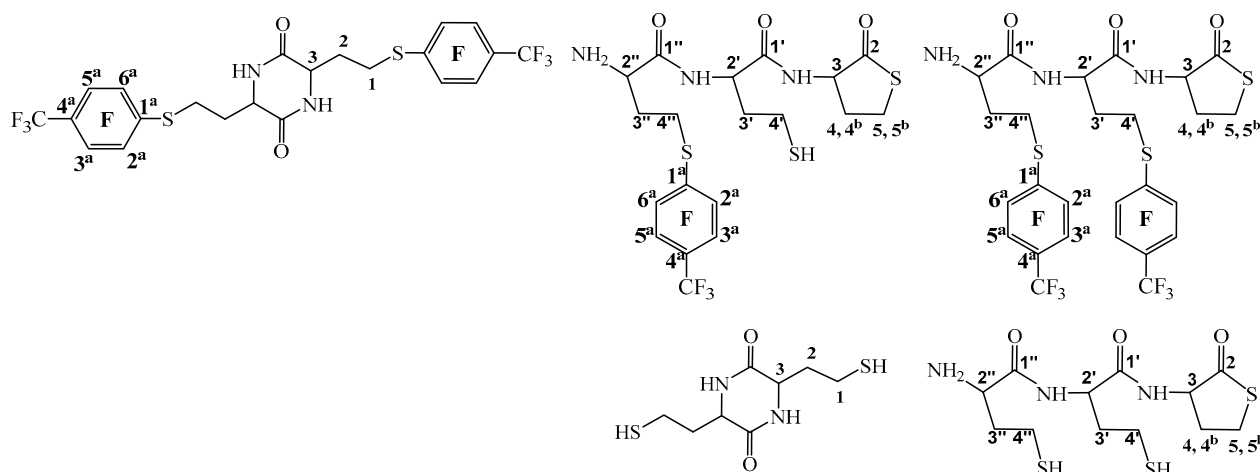


Figure S1. Structures of nonfluorinated and S-fluorinated 2,5-diketopiperazine and tripeptide derivatives.

3,6-Bis(2-(2,3,5,6-tetrafluoro-(trifluoromethyl)phenylthio)ethyl) piperazine-2,5-dione

UV-vis (CH₃CN): $\lambda_{\max}$ 279 nm ($\epsilon = (1.9 \pm 0.1) \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$). ¹⁹F NMR (CD₃CN): δ 107.12 (t, 6F, CF₃), 30.12 (m, 4F, F-2^a, F-6^a), 20.90 (m, 4F, F-3^a, F-5^a), $J_{\text{CF}_3, 3a \text{ and } 5a} = 21.4$. ¹H NMR (CD₃CN): δ 6.68 (br.s, 2H, NH), 3.95 (t, 2H, H-3), 3.10 (m, 4H, H-1), 1.92 (m, 4H, H-2), $J_{1,2} = 7.5$, $J_{2,3} = 6.0$. ¹³C NMR (CD₃CN): δ 168.6 (CO), 149.4 (C-2^a, C-6^a), 147.0 (C-3^a, C-5^a), 120.7 (C-1^a), 113.2 (C-4^a), 109.0 (CF₃), 54.2 (C-3), 34.8 (C-2), 30.5 (C-1). ESI MS negative mode (m/z) calculated for C₂₂H₁₂N₂O₂S₂F₁₄ [M]: 666.012, found 665.891; [M+K-2H]: 702.960, found 702.891.

S-(*p*-perfluorotolyl)-homocysteinyll-*S*-(*p*-perfluorotolyl) homocysteinyll-homocysteine thiolactone

UV-vis (CH₃CN): $\lambda_{\max}$ 240 nm, 279 nm. ¹⁹F NMR (DMSO-*d*₆): δ 107.08 (t, $J_{\text{CF}_3, 3a \text{ and } 5a} = 21.8$, 6F, CF₃), 30.30 (m, 4F, F-2^a, F-6^a), 20.97 (m, 4F, F-3^a, F-5^a). ¹H NMR (DMSO-*d*₆): δ 4.54 (dd, 1H, $J_4 = 12.8$, $J_{4b} = 7.0$, H-3), 4.40 (m, 1H, H-2'), 3.34 (m, 1H, H-2''), 3.33 (m, 1H, H-5), 3.25 (m, 1H, H-5^b), 3.21 (m, 4H, H-4', H-4''), 2.37 (m, 1H, H-4^b), 2.05 (m, 4H, H-3', H-3''), 1.85 (m, 1H, H-4). ¹³C NMR (CD₃CN): δ 204.6 (C-2), 178.2 (C-1', C-1''), 149.3 (C-2^a, C-6^a), 146.9 (C-3^a, C-5^a), 120.7 (C-1^a), 113.2 (C-4^a), 109.0 (CF₃), 64.2 (C-3), 54.4 (C-2'), 54.0 (C-2''), 33.9 (C-3', C-3''), 32.3 (C-4', C-4''), 30.3 (C-5), 27.6 (C-4). ESI MS positive mode (m/z) calculated for C₂₆H₂₀N₃O₃S₃F₁₄ [M+H]: 784.044, found 783.790; [M+Na]: 806.026, found 805.789.

S-(*p*-perfluorotolyl)homocysteinyll-homocysteinyll-homocysteine thiolactone

UV-vis (CH₃CN): $\lambda_{\max}$ 240 nm, 279 nm. ¹⁹F NMR (DMSO-*d*₆) δ 107.10 (t, $J_{\text{CF}_3, 3c} = 21.2$, 3F, CF₃), 30.31 (m, 2F, F-2^a, F-6^a), 20.98 (m, 2F, F-3^a, F-5^a). ¹H NMR (DMSO-*d*₆) δ 4.48 (dd, 1H, $J_4 = 12.8$, $J_{4b} = 7.0$, H-3), 4.34 (m, 1H, H-2'), 3.34 (m, 1H, H-2''), 3.28 (m, 1H, H-5), 3.25 (m, 1H, H-5^b), 3.21 (m, 2H, H-4''), 2.50 (m, 2H, H-4'), 2.37 (m, 1H, H-4^b), 2.05 (m, 2H, H-3''), 1.87 (m, 2H, H-3'), 1.83 (m, 1H, H-4). ¹³C NMR (CD₃CN) δ 204.6 (C-2), 178.2 (C-1', C-1''), 149.3 (C-2^a, C-6^a), 146.9 (C-3^a, C-5^a), 120.7 (C-1^a), 113.2 (C-4^a), 109.0 (CF₃), 64.2 (C-3), 54.2 (C-2'), 54.0 (C-2''), 33.9 (C-3''), 32.3 (C-4''), 31.0 (C-3'), 30.3 (C-5), 27.6 (C-4),

21.0 (C-4'). ESI MS negative mode (m/z) calculated for C₁₉H₁₉N₃O₃S₃F₇ [M-H]: 566.048, found 565.893; [M+Na-H]: 589.037, found 588.887.

Homocysteine 2,5-diketopiperazine

¹H NMR (DMSO-d₆): δ 8.20 (br. s, 2H, NH), 3.90 (t, 2H, H-3), 2.40 (t, 2H, H-1), 1.95 (m, 4H, H-2), *J*_{1,2}=7.5, *J*_{2,3}=6.1. ¹³C NMR (DMSO-d₆): δ 168.5 (CO), 53.0 (C-3), 36.4 (C-2), 19.0 (C-1). ESI MS (m/z) calculated for C₈H₁₃N₂O₂S₂ [M-H]: 233.042, found 233.041.

Homocysteinyll homocysteinyll-homocysteine thiolactone

¹H NMR (DMSO-d₆): δ 4.50 (dd, 1H, *J*₄= 12.7, *J*_{4b}=7.1, H-3), 4.40 (m, 1H, H-2'), 3.33 (m, 1H, H-2''), 3.30 (m, 1H, H-5), 3.25 (m, 1H, H-5^b), 2.40 (m, 4H, H-4', H-4''), 2.36 (m, 1H, H-4^b), 1.95 (m, 4H, H-3', H-3''), 1.85 (m, 1H, H-4). ¹³C NMR (CD₃CN): δ 203.0 (C-2), 178.0 (C-1', C-1''), 64.6 (C-3), 55.4 (C-2'), 54.0 (C-2''), 33.8 (C-3', C-3''), 30.3 (C-5), 27.6 (C-4), 20.0 (C-4', C-4'').

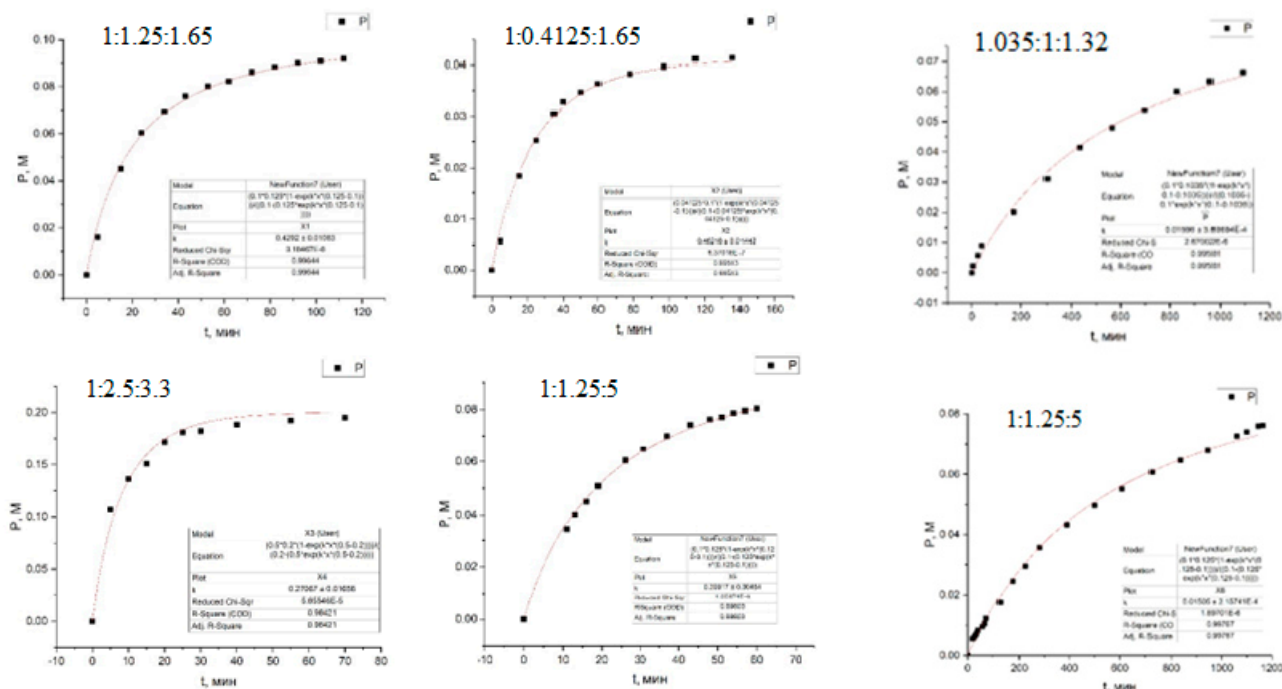
Table S1. The ingredients and reaction conditions of the reaction between HTL and perfluoroarenes for mechanism studies.

Entry	C(PFX), M	C(HTL), M	C(NEt ₃), M	Ratio (PFX:HTL:NEt ₃)	k_{eff} , M ⁻¹ s ⁻¹
1	0.2	0.5	0.66	1:2.5:3.3	See below
2	0.1	0.125	0.165	1:1.25:1.65	0.43 ± 0.01
3	0.1	0.125	0.5	1:1.25:5	0.399 ± 0.005
4	0.1	0.04125	0.165	1:0.4125:1.65	0.46 ± 0.02
	C(PFT), M	C(HTL), M	C(NEt ₃), M	Ratio (PFT:HTL:NEt ₃)	
5	0.1	0.125	0.5	1:1.25:5	0.0151 ± 0.0002
6	0.1035	0.1	0.132	1.035:1:1.32	0.0160 ± 0.0004

Mechanism (1)

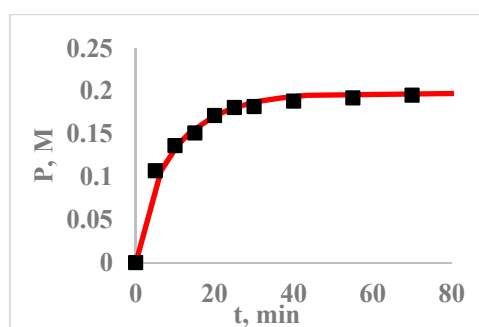
PFX experiments data

PFT experiments data



The experiment with PFX:HTL:NEt₃ concentration ratio of 1:2.5:3.3 is not realized by mechanism (1). Such reaction behavior may be calculated by mechanism (2) (Figure 4).

Mechanism (2) PFX:HTL:NEt₃ = 1:2.5:3.3



Mechanism (1) Figure 4. Derivation of the equation P(t) by mechanism (1):

$$\begin{cases} \frac{dA}{dt} = \frac{dB}{dt} = k_{-1}C - k_1AB \\ \frac{dC}{dt} = k_1AB - (k_{-1} + k_2)C \approx 0 \Rightarrow C = \frac{k_1}{k_{-1} + k_2} AB \\ \frac{dP}{dt} = k_2C \end{cases}$$

$$\frac{dA}{dt} = \left(k_{-1} - \frac{k_1}{k_{-1} + k_2} \right) AB = -k_{eff} \cdot AB \quad (1)$$

$$\frac{dA}{dt} = -\frac{dP}{dt} = -k_{eff} \cdot (A_0 - P)(B_0 - P) \quad (2)$$

$$\frac{1}{A_0 - B_0} \ln \left[\frac{B_0 (A_0 - P)}{A_0 (B_0 - P)} \right] = k_{eff} \cdot t \quad (3)$$

$$P(t) = \frac{A_0 \left(\exp[(A_0 - B_0)k_{eff} \cdot t] - 1 \right)}{\frac{A_0}{B_0} \exp[(A_0 - B_0)k_{eff} \cdot t] - 1} \quad (4)$$

Mechanism 2 Figure 4. Derivation of the equation P(t) by mechanism (2):

$$\frac{dC}{dt} = k_1AB - (k_{-1} + k_2B)C \cong 0 \Rightarrow C = \frac{k_1}{k_{-1} + k_2B} AB \quad (10)$$

$$\frac{dP}{dt} = k_2CB = \frac{k_1k_2}{k_{-1} + k_2B} AB^2 \quad (11)$$

$$\left[\frac{k_{-1}}{(A_0 - P)(B_0 - P)^2} + \frac{k_2}{(A_0 - P)(B_0 - P)} \right] dP = k_1k_2 \cdot dt \quad (12)$$

$$k_{-1} \int_0^{P(t)} \left(\frac{11,11}{0,2 - P} - \frac{11,11}{0,5 - P} - \frac{3,33}{(0,5 - P)^2} \right) + k_2 \cdot 3,33 \left(\frac{1}{A_0 - P} - \frac{1}{B_0 - P} \right) dP = k_1k_2 \int_0^t dt \quad (13)$$

$$\begin{aligned} t &= \left(\frac{11,11 \cdot k_{-1}}{k_1k_2} + \frac{3,33}{k_1} \right) \left[\ln \frac{0,2}{0,2 - P(t)} - \ln \frac{0,5}{0,5 - P(t)} \right] - \frac{3,33 \cdot k_{-1}}{k_1k_2} \left(\frac{1}{0,5 - P(t)} + 2 \right) = \\ &= a \left[\ln \frac{0,2}{0,2 - P(t)} - \ln \frac{0,5}{0,5 - P(t)} \right] - b \left(\frac{1}{0,5 - P(t)} + 2 \right) \end{aligned} \quad (14)$$

The closest approximation occurs at a = 13,709 and b = 0,175.

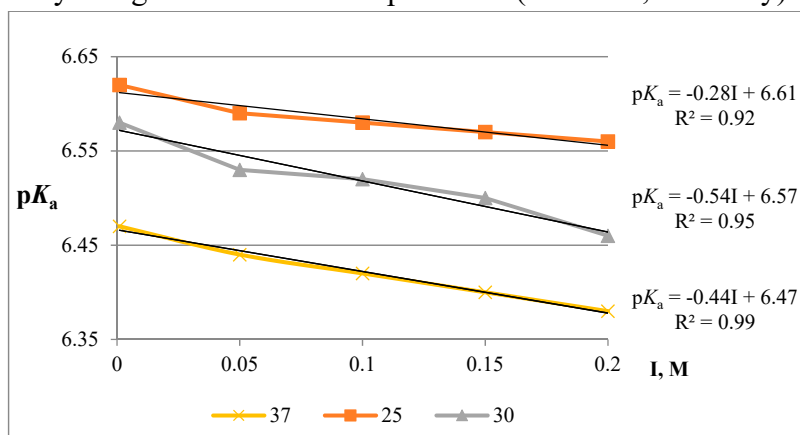
$$\begin{cases} a = 13,709 = \frac{11,11 \cdot k_{-1}}{k_1k_2} + \frac{3,33}{k_1} \\ b = 0,175 = \frac{3,33 \cdot k_{-1}}{k_1k_2} \end{cases} \Rightarrow 0,584 + \frac{3,33}{k_1} = 13,709 \Rightarrow k_1 = 0,2537 \text{ M}^{-1}\text{s}^{-1}$$

$$\frac{k_{-1}}{k_1 k_2} = \frac{k_{-1}}{0,2537 \cdot k_2} = \frac{0,175}{3,33} = 0,05255 \Rightarrow \frac{k_2}{k_{-1}} = 75$$

$$k_1 = 0,2537 \text{ M}^{-1}\text{s}^{-1} \text{ and } k_2/k_{-1} = 75.$$

HTL pKa determinations

The pKa of HTL was determined at 25 °C, 30 °C, and 37 °C by potentiometric titration of the hydrochloride salt of HTL (1 mM) with NaOH (8 mM). For ionic strength adaptation, sodium chloride was used. pH was monitored by using a Sartorius PP-15 pH meter (Sartorius, Germany).



I = 0.167 M (for PBS buffer). pKa (25 °C) = 6.56, pKa (30 °C) = 6.48, pKa (37 °C) = 6.40,

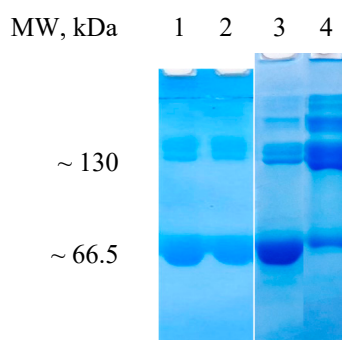


Figure S2. SDS–PAGE of PFT-HSA (line 1), PFX-HSA (line 2), HSA (line 3), and Hcy-HSA (line 4) conjugates under Laemmli condition without addition of DTT with subsequent Coomassie blue staining. Bands corresponding to monomeric protein (MW = 66.5 kDa) and its dimer (MW ~ 130 kDa) were observed in samples.

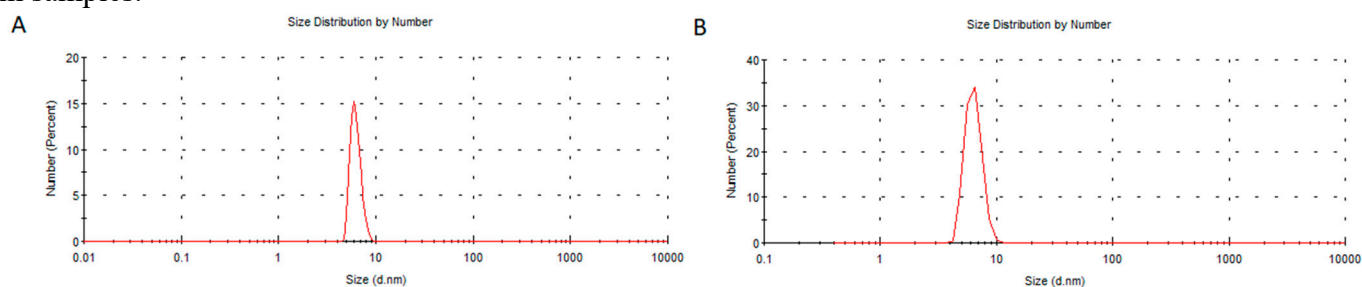


Figure S3. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Number.

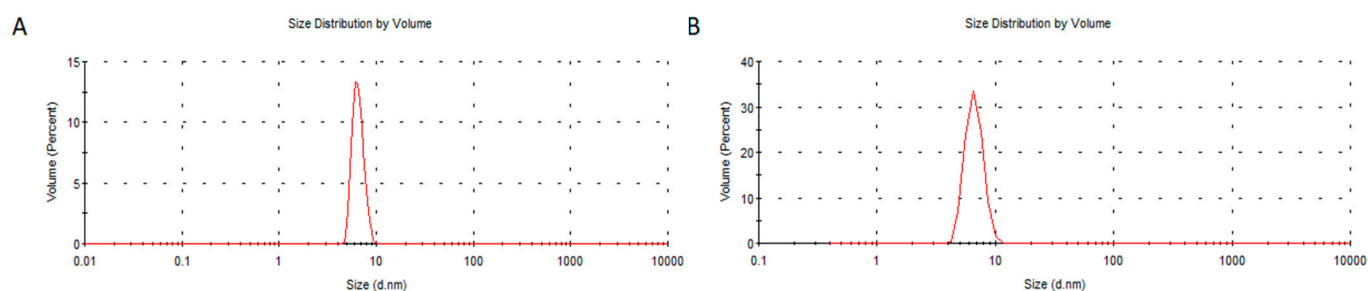


Figure S4. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Volume.

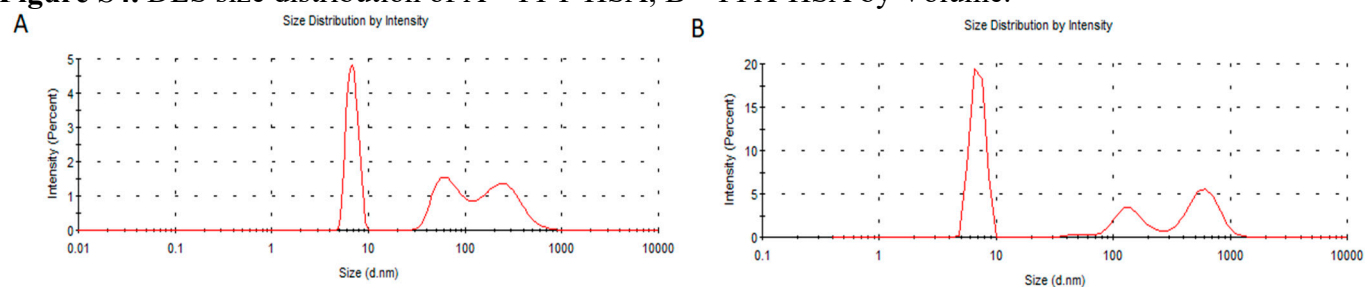


Figure S5. DLS size distribution of A – PFT-HSA; B – PFX-HSA by Intensity.

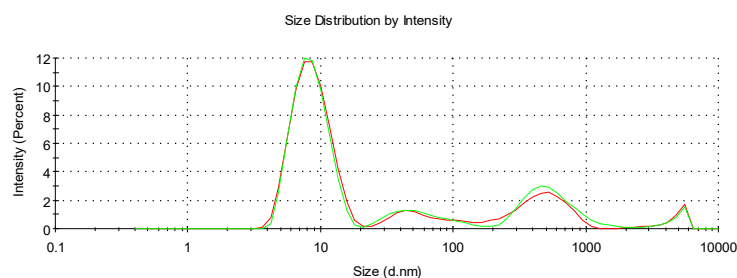


Figure S6. DLS size distribution of HSA by Intensity.

Flow cytometry. Cellular uptake of Cy5-labeled albumin conjugates was investigated by flow cytometry. A 2×10^5 T98G cells per well were seeded in 12-well plates in complete medium, treated with albumin conjugates, and incubated for 2 h. Afterward, the cells were washed with PBS three times, trypsinized using 0.25 % Trypsin-EDTA, and centrifuged at 1000 rpm for 5 min. The cells were fixed with 2% formaldehyde in PBS (20 min, 25 °C). The samples were measured in relative fluorescence units (RFU) on a FACS-Canto II flow cytometer (Becton Dickinson) using FACS Diva Software (BD Biosciences). Intact (non-stained) cells were used as a control.

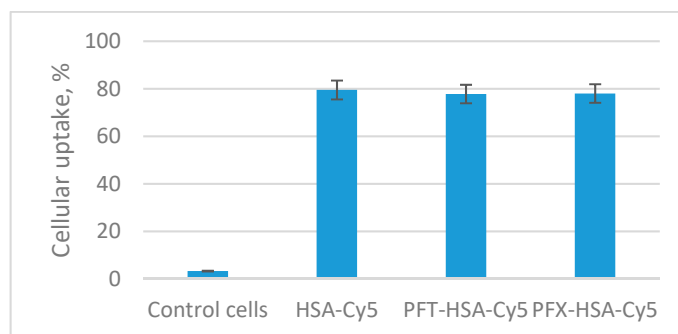


Figure S7. Cellular uptake of fluorescently labeled albumin conjugates.

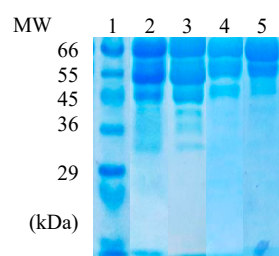


Figure S8. Typical albumin proteolysis SDS-PAGE data. Susceptibility of HSA and its conjugates to tryptic proteolysis (enzyme/substrate ratio 1:100). The bands in the gel lower 66.5 kDa (HSA MW) were marked as digested albumin. Quantitative data were obtained by digitizing the gel (SDS-PAGE) using GelPro Analyzer software (Media Cybernetics). SDS-PAGE (10%), pt. 6 h. The gel was stained with Coomassie Brilliant Blue. Line 1, MW markers (Sigma S8445); line 2, PFX-HSA; line 3, PFT-HSA; line 4, HSA; line 5, Hcy-HSA.