Host-Guest Interactions in Polycationic Human Serum Albumin Bioconjugates

- Supporting Information -

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Keywords: cationic HSA, HSA-PEO conjugates, host-guest interactions, EPR Spectroscopy, double electron-electron resonance (DEER), fatty acids

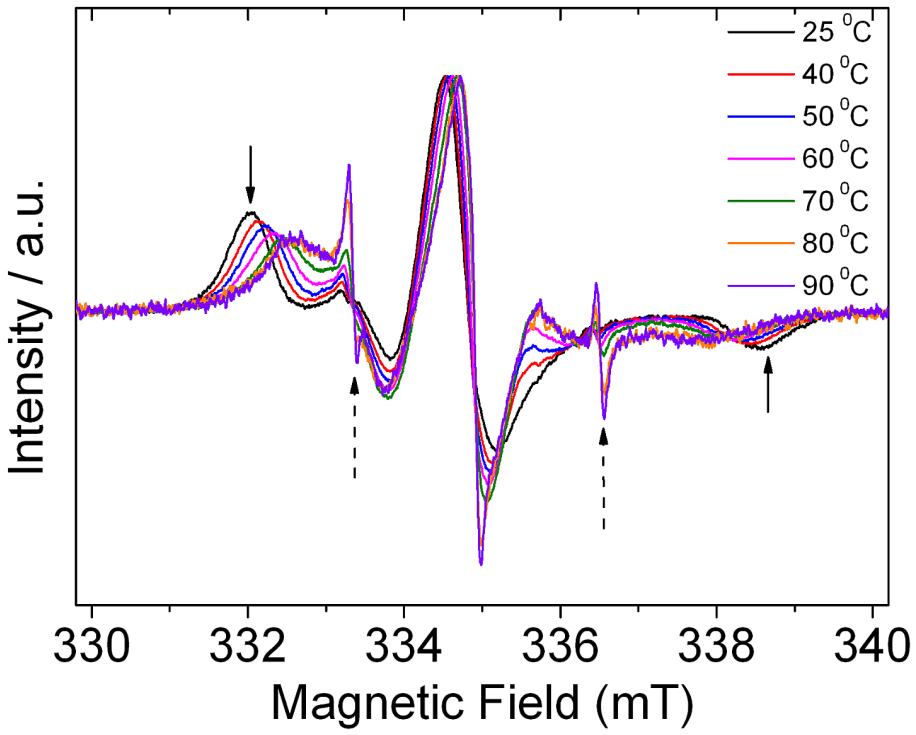

Figure S1. CW X-band EPR spectra of 5-DSA in HSA/buffer solution at an FA/HSA ratio of 4 at the temperatures of 25°C (black), 40°C (red), 50°C (blue), 60°C (magenta), 70°C (green), 80°C (orange) and 90°C (violet). The characteristic signals of 5-DSA bound to albumin are marked by solid arrows and signals of free 5-DSA are marked by dashed arrows.

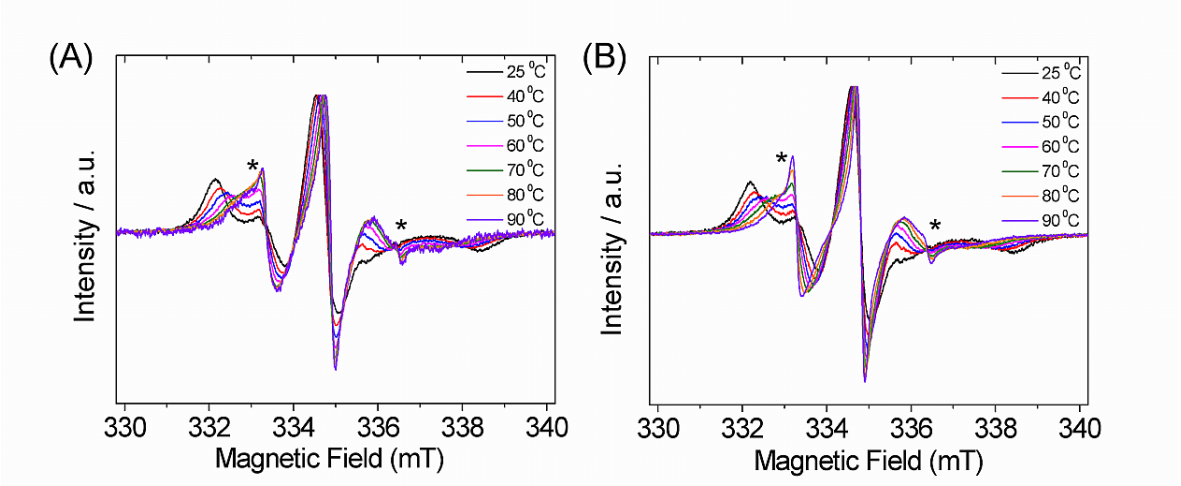

Figure S2. CW X-band EPR spectra of 5-DSA in cHSA/buffer (A) and in cHSA-PEO(2000)$_{19}$/buffer (B) solutions at an FA/HSA ratio of 4 at the temperatures of 25°C (black), 40°C (red), 50°C (blue), 60°C (magenta), 70°C (green), 80°C (orange) and 90°C (violet). Asterisks mark a new spectral component corresponding to 5-DSA bound to low affinity electrostatic binding sites of cHSA, which is not observed in HSA.

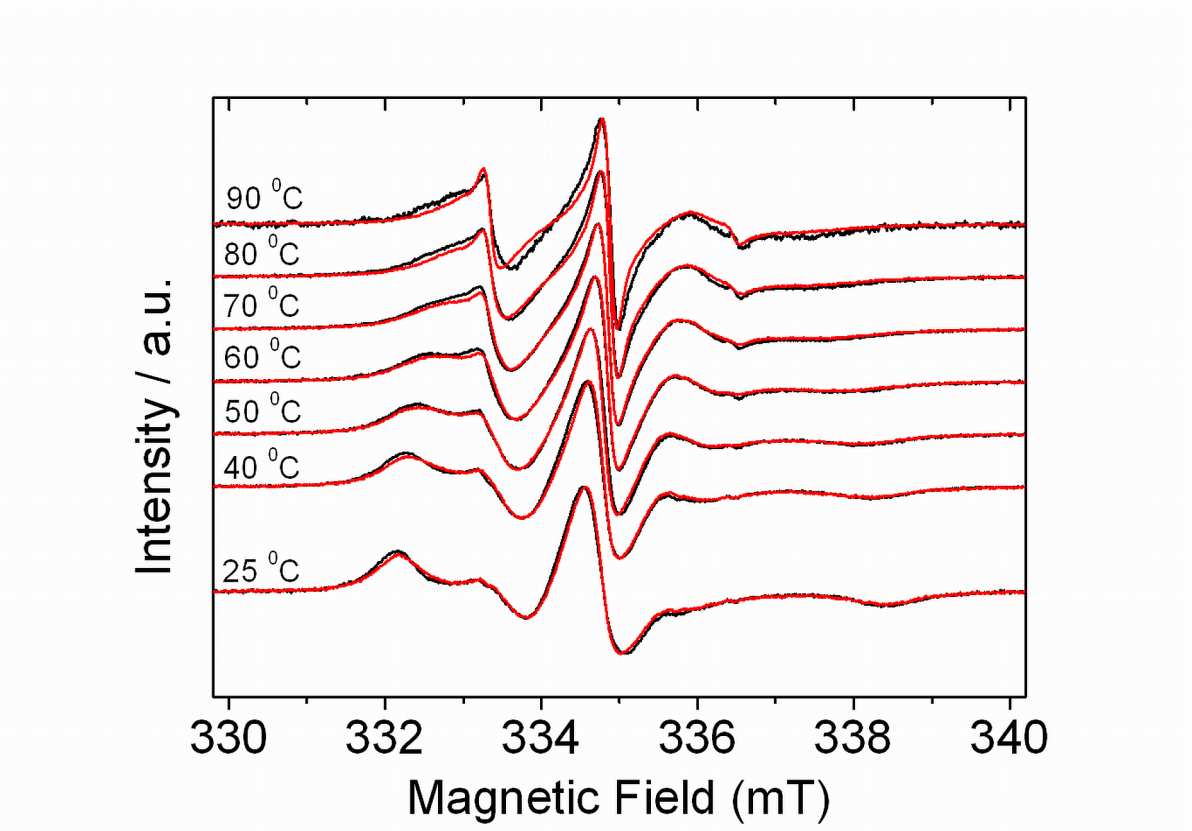

Figure S3. CW X-band EPR spectra of 5-DSA in cHSA/buffer (black) and in cHSA-PEO(2000)$_{19}$/buffer (red) solutions at a FA/HSA ratio of 4 at the temperatures of 25°C, 40°C, 50°C, 60°C, 70°C, 80°C and 90°C.

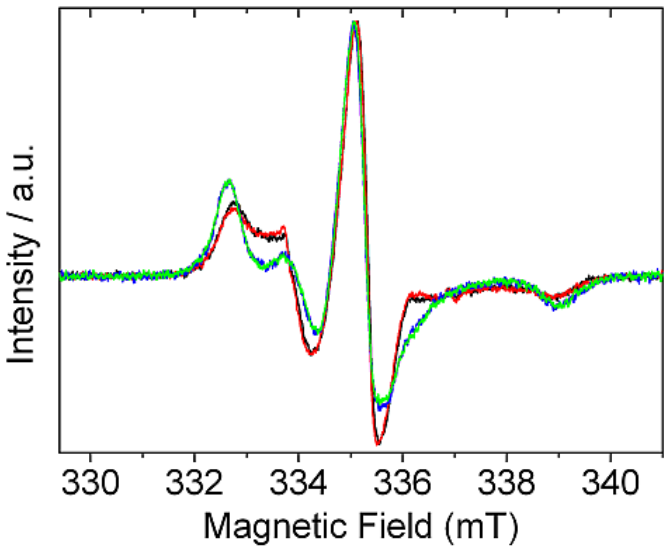

Figure S4. CW X-band EPR spectra of 5-DSA in cHSA/buffer (blue), in cHSA/PEO(750)$_{22}$/buffer (green) solutions and 16-DSA in cHSA/buffer (black), in cHSA/PEO(750)$_{22}$/buffer (red) solutions at a FA/HSA ratio of 2 at room temperature.

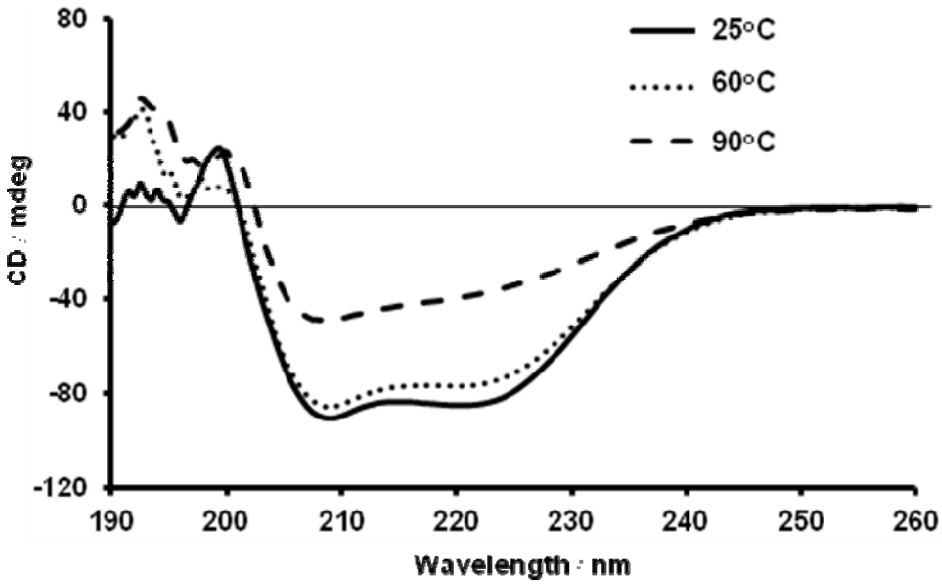

Figure S5. Circular dichroism spectra of HSA at different temperatures.

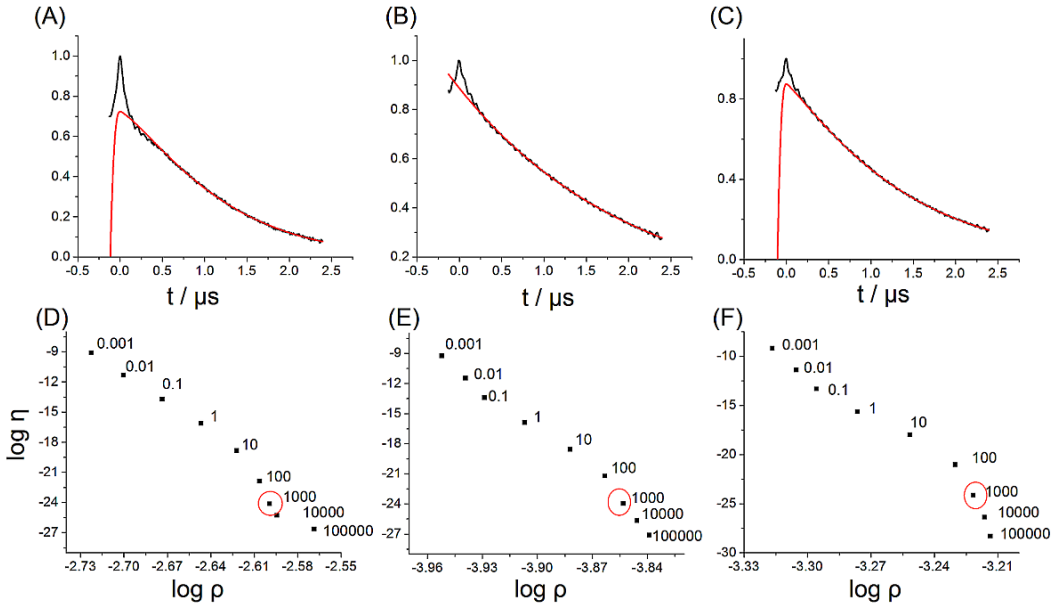

Figure S6. Time domain DEER data (black) and background fits (red) of 5-DSA in HSA (A), in cHSA (B) and in cHSA-PEO (750) (C), and the L-curves of 5-DSA in HSA (D), in cHSA (E) and in cHSA-PEO (750) (F).

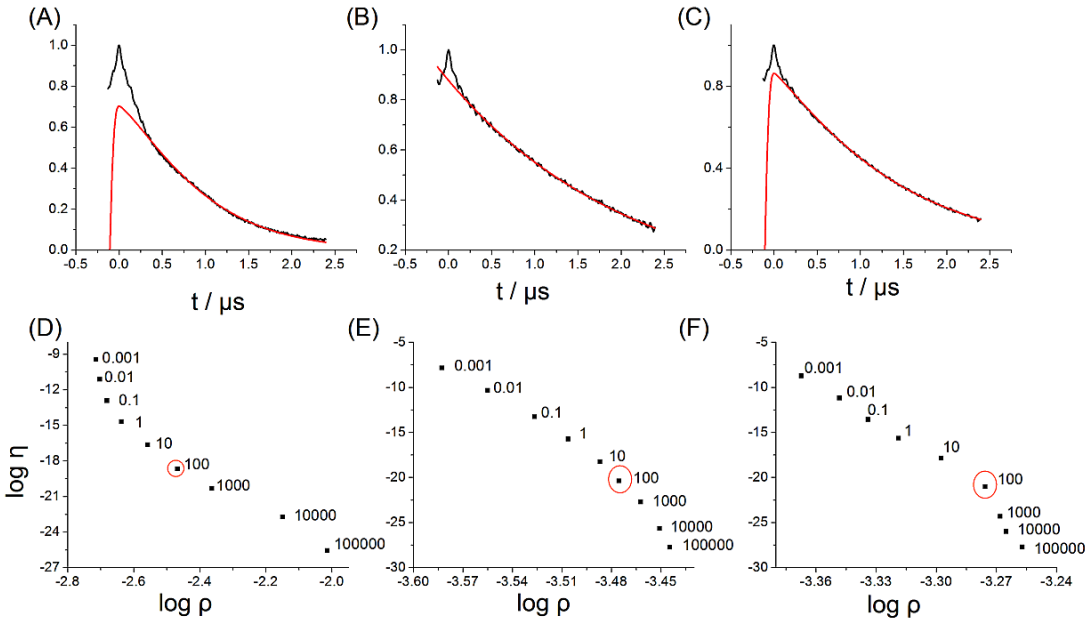

Figure S7. Time domain DEER data (black) and background fits (red) of 16-DSA in HSA (A), in cHSA (B) and in cHSA-PEO (750) (C), and the L-curves of 16-DSA in HSA (D), in cHSA (E) and in cHSA-PEO (750) (F).

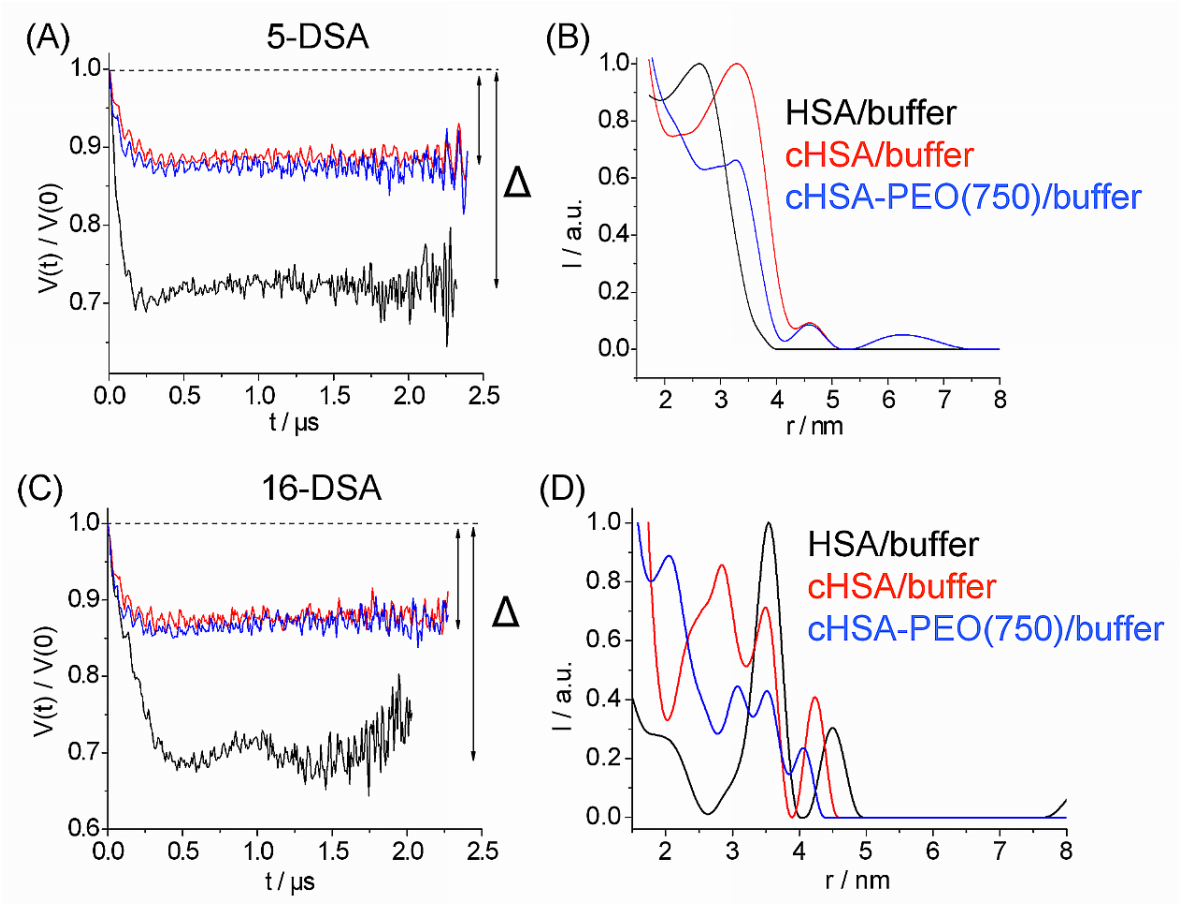

Figure S8. (A) Intramolecular part of the DEER time-domain data and the distance distribution obtained from Tikhonov regularization for 5-DSA (A, B) and 16-DSA (C, D), respectively, in HSA/buffer (black), in cHSA/buffer (red) and in cHSA-PEO(750)/buffer (blue) solutions. On average, each protein molecule is loaded with two spin-labeled FAs. The modulation depths Δ are marked. 20% (v/v) of glycerol was added to the aqueous solutions. d = 3.8 for HSA, d = 3.4 for cHSA-PEO(750)$_{22}$ and d = 3.0 for cHSA (see Fig. S6 and S7).

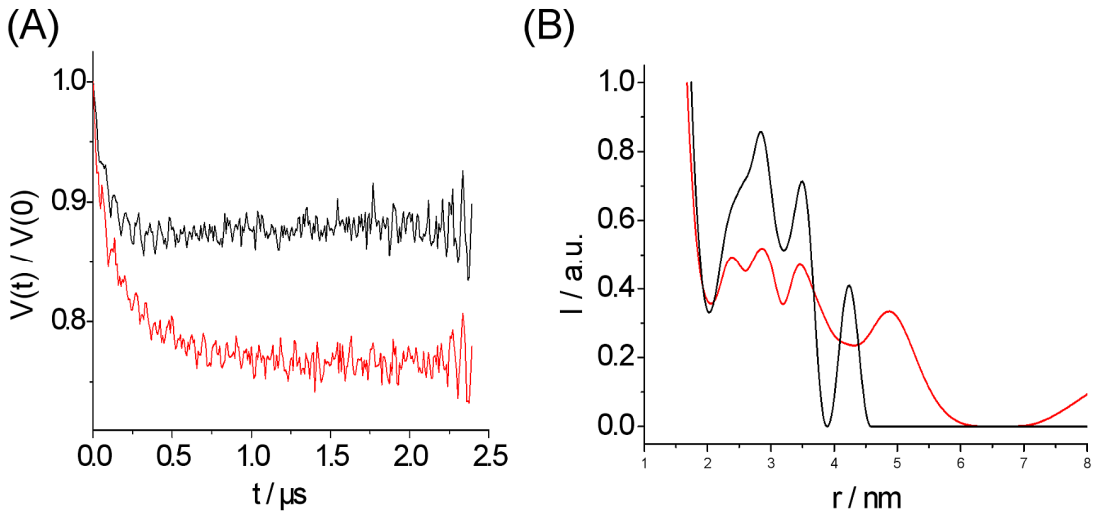

Figure S9. Intramolecular part of the DEER time-domain data (A) and extracted distance distributions by Tikhonov regularization (B) of two paramagnetic 16-DSA complexed in cHSA/buffer (black) and additional four diamagnetic analogues of 16-DSA (nitroxides reduced to hydroxylamines) in cHSA/buffer (red). 20% (v/v) of glycerol was added to the aqueous solutions.

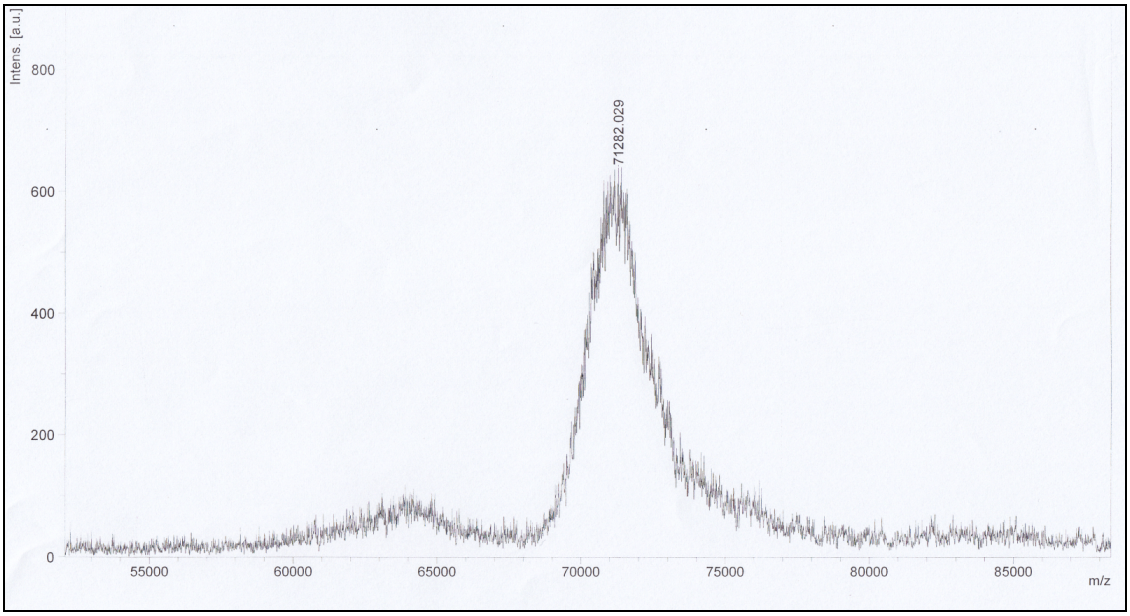

Figure S10new. MALDI-ToF spectrum of cHSA.

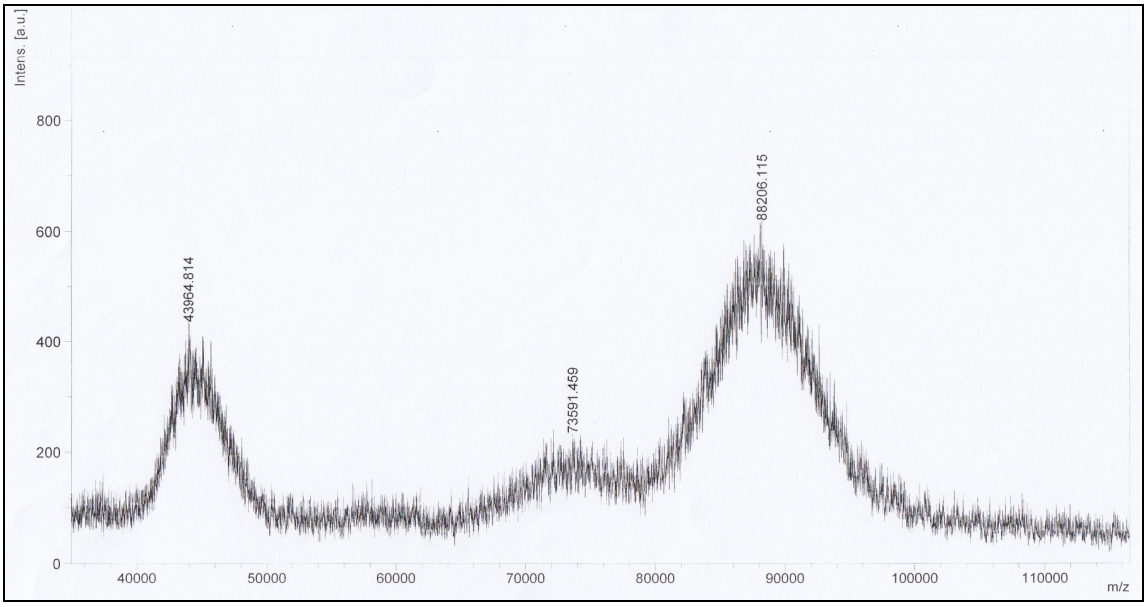

Figure S11new. MALDI-ToF spectrum of cHSA-PEO(750)$_{22}$.

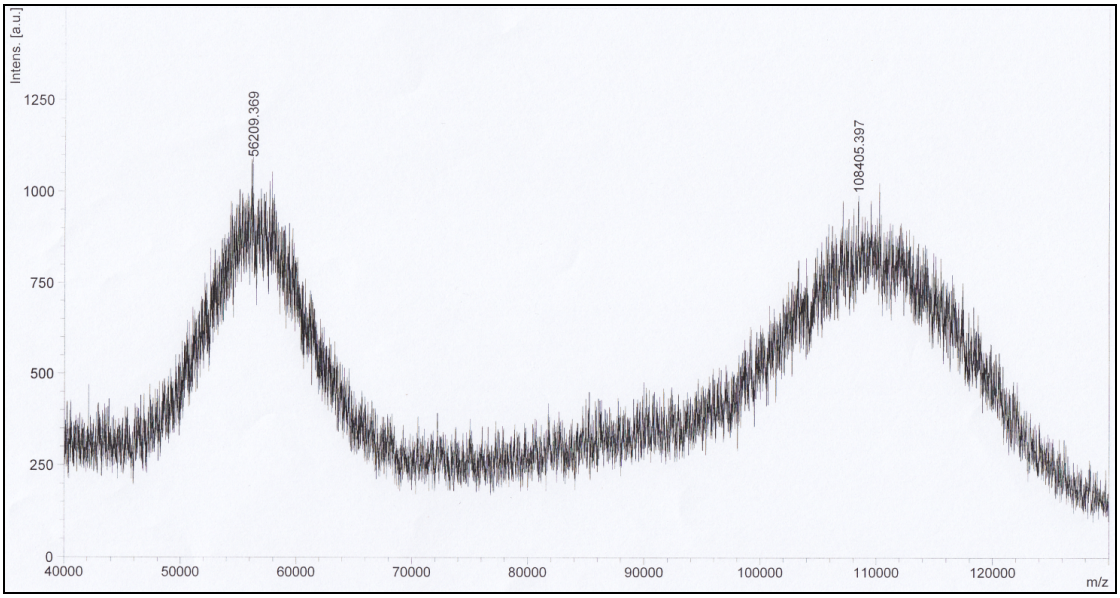

Figure S12new. MALDI-ToF spectrum of cHSA-PEO(2000)$_{19}$.