Supporting information for: Interaction of Human Serum Albumin with uremic toxins: A thermodynamic study

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Table S1: Thermodynamic parameters obtained with different fitting models for the different experimental conditions in Section 3.1.

I (mM)	T ($^{\circ}C$)	Model	N_{total}	ΔH_1 (kJ/mol)	$K_{b1} \cdot 10^3$ (M^{-1})	ΔH_2 (kJ/mol)	$K_{b2} \cdot 10^3$ (M^{-1})	χ^{2*}
20	25	One Site	1	-23.0±0.2	17.3±0.4	-	-	80
			2	-11.0±0.1	21.1±0.8	-	-	144
		Sequential Binding	2	-20.1±0.6	21.8±1	-8±1	0.95±0.3	50
20	30	One Site	1	-24.9±0.3	12.9±0.4	-	-	109
		Sequential Binding	2	-20.7±0.7	17.4±0.9	-12±1	0.9±0.2	50
20	37	One Site	1	-29.4±0.6	10.3±0.2	-	-	37
		Sequential Binding	2	-26.8±0.6	11.5±0.5	-0.7±0.3	0.84±0.3	27
150	37	One Site	1	-12.6±0.2	5.6±0.1	-	-	8
		Sequential Binding	2	-12.7±0.3	5.5±0.2	5±3	0.14±0.05	8

χ^2 is the error of the fit obtained by the implemented ITC Data analysis software by MicroCal.

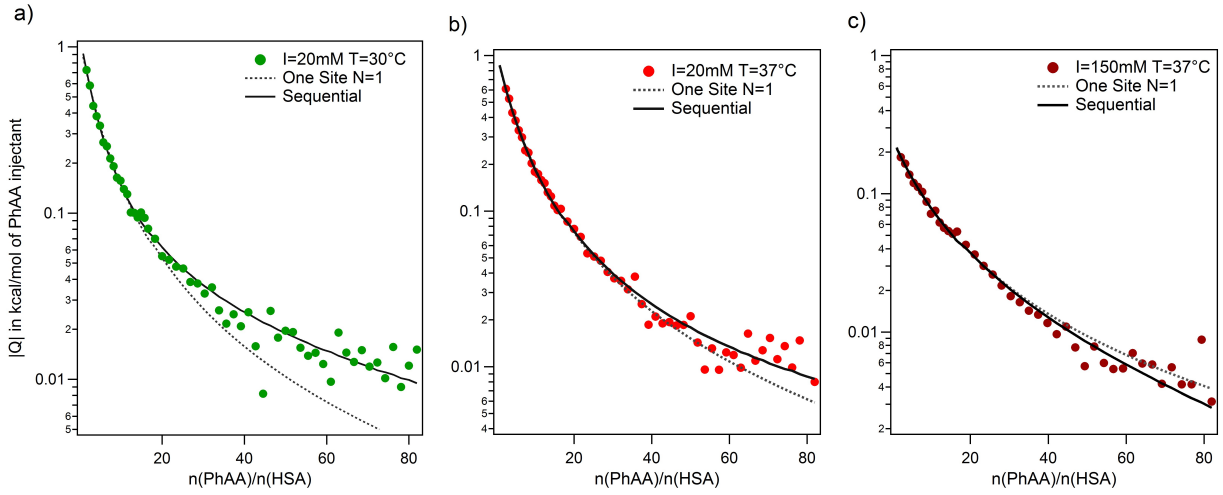

Figure S1: Binding model analysis of PhAA adsorption to native HSA. Logarithmic plots as shown before to discriminate the quality of two binding models SSIS (solid line) and SBS (dotted line). Three representative binding cases are shown for I=20 mM at (a) 30°C and (b) 37°C and for (c) I=150 mM and 37°C.

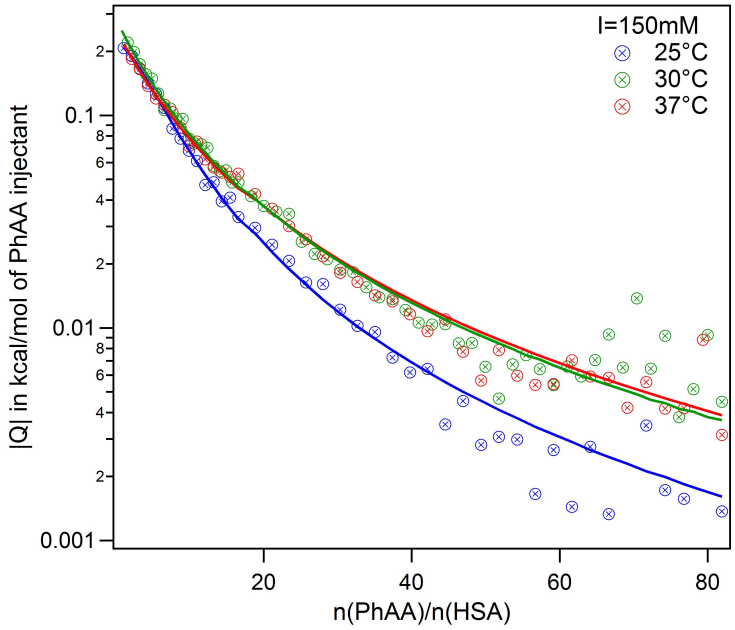

Figure S2: PhAA adsorption to native HSA. Absolute heats |Q| are shown for ionic strength I=150 mM at different temperatures and respective fits are shown.

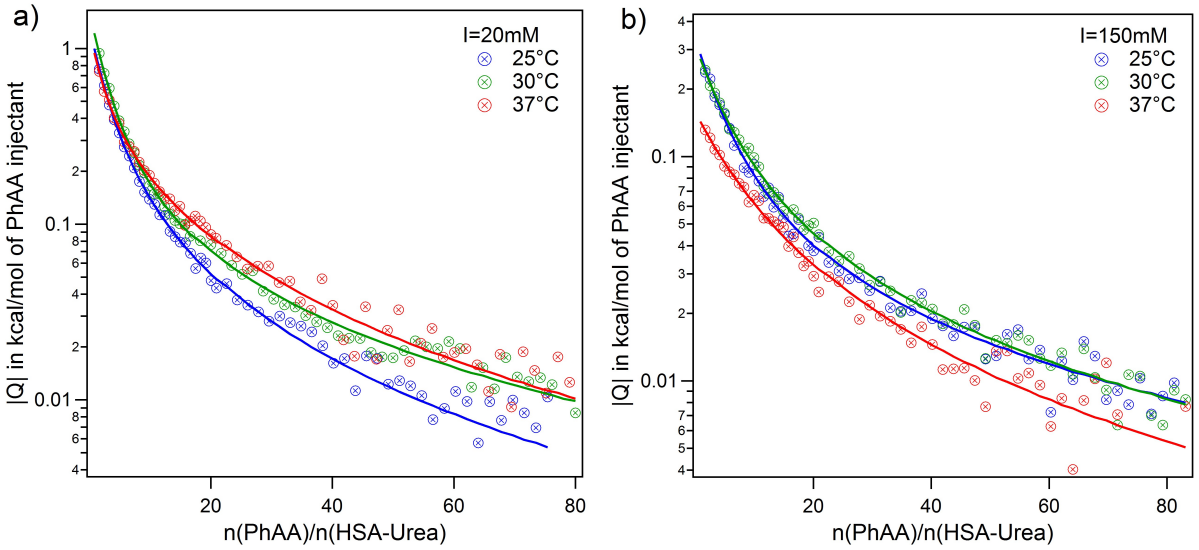

Figure S3: Effect of HSA urea modification on PhAA adsorption. Isotherms for adsorption of PhAA upon urea modified HSA for (a) I=20mM and (b) I=150mM at different temperature is shown.

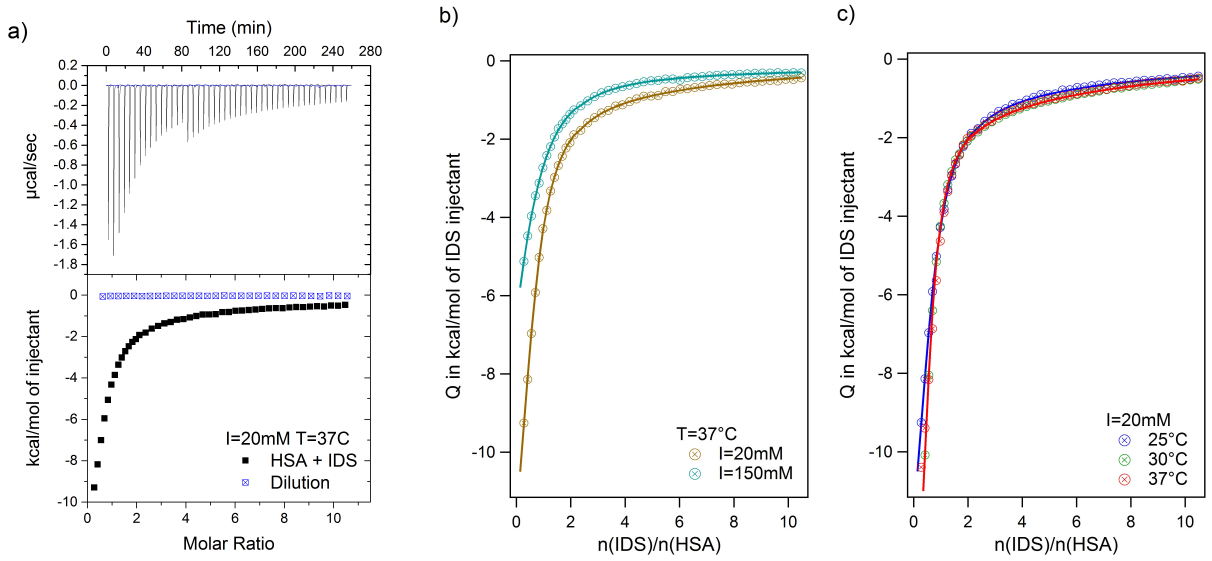

Figure S4: Interaction of IDS with native HSA. (a) Titration peaks and integrated heats for I=20 mM at 37°C. (b) Dilution corrected isotherms with according fits at 37°C and 20 mM and 150 mM. (c) Temperature series of adsorption for I=20 mM.

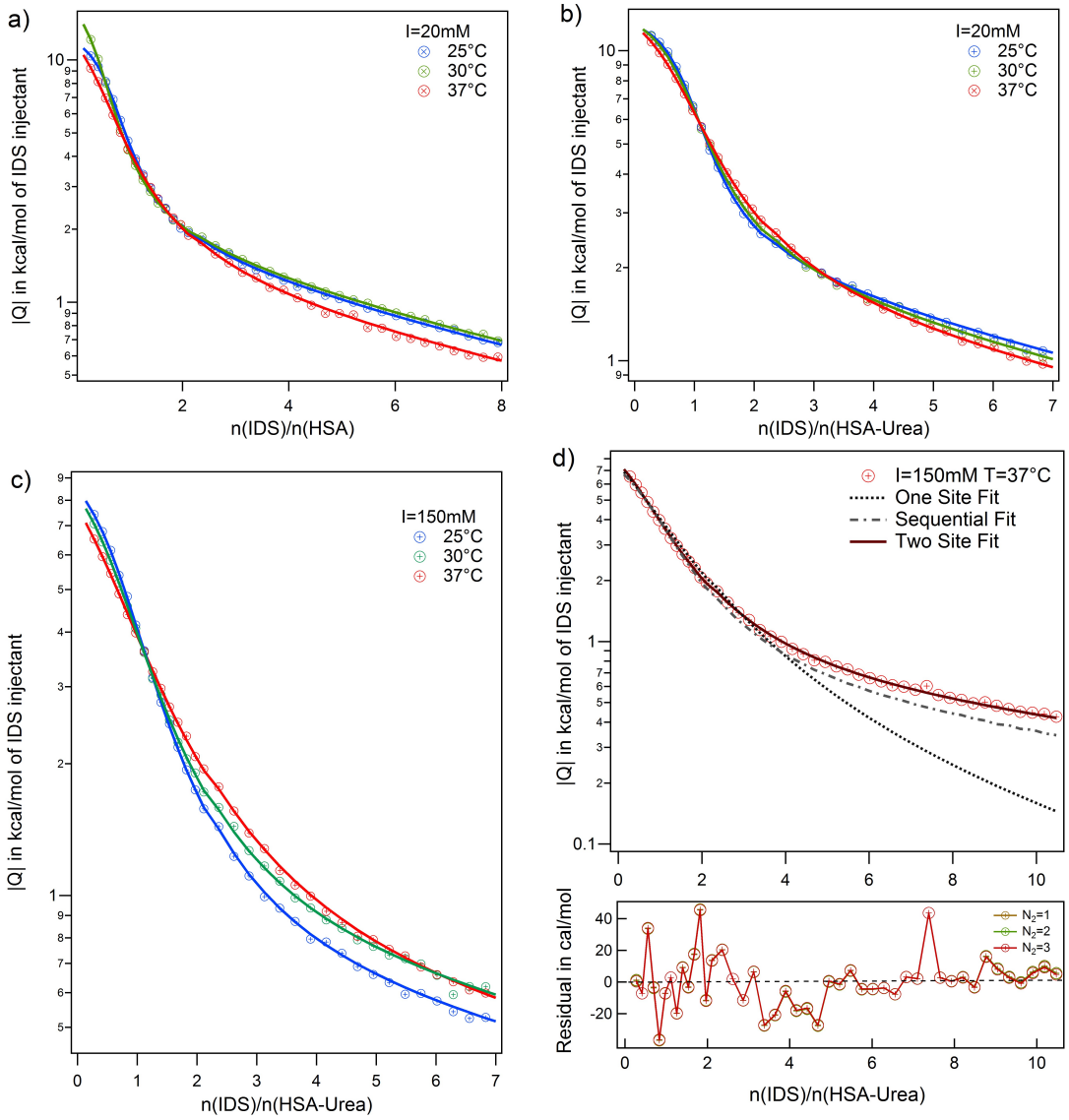

Figure S5: Adsorption of IDS to native and modified HSA. Temperature series and according fits using the TSIS model are shown for (a) native HSA with I=20mM and urea modified HSA with (b) I=20 mM and (c) 150 mM. (d) Quality of different fit models and parameters are demonstrated by comparing three models SSIS (dotted line), SBS (dashed line) and TSIS (solid line) for IDS interaction to modified HSA with I=150 mM and 37°C. Beneath the graph is depicted the residual errors for TSIS fits with different fixed N_2 values.

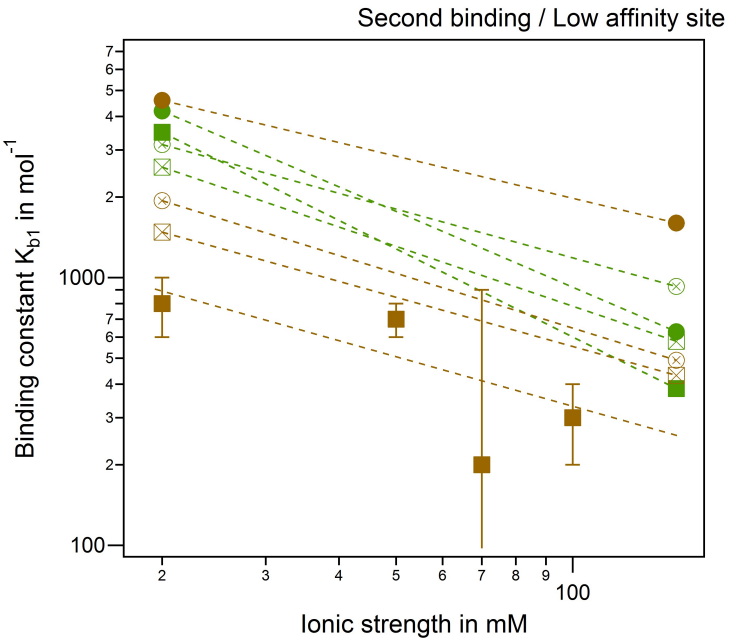

Figure S6: Ionic strength dependence of the second binding process and low affinity site. Adsorption of of PhAA (brown) and IDS (green) to native (full symbols) and modified HSA (empty symbols) are shown. Spheres and rectangles represent 25°C and 37°C.

Ionic strength (mM)	T (°C)	ΔH_1^{ITC} (kJ/mol)	$K_{b1} \cdot 10^3$ (mol^{-1})	ΔG_{b1} (in k_BT) (kJ/mol)	ΔH_{b1} (kJ/mol)	ΔS_{b1} (J/mol/K)	ΔH_2^{ITC} (kJ/mol)	$K_{b2} \cdot 10^3$ (mol^{-1})	ΔG_{b2} (in k_BT) (kJ/mol)	ΔH_{b1} (kJ/mol)	ΔS_{b2} (J/mol/K)
Native HSA											
	25	-20.3±0.6	22±1	-24.7±0.1			-8±1	0.9±0.3	-16.9±0.8		
20	30	-20.7±0.7	17.4±0.9	-24.6±0.1	-37±4	-46±14	-12±1	0.9±0.2	-17.1±0.7	-9±22	27±78
	37	-26.5±0.6	11.9±0.5	-24.2±0.1			-6±1	0.8±0.2	-17.2±0.4		
50	37	-19.2±0.4	11.2±0.4	-24.0±0.1	-	-	-3±1	0.7±0.1	-17.1±0.4	-	-
70	37	-18.9±0.4	9.9±0.3	-23.7±0.1	-	-	-15±4	0.2±0.7	-14±8	-	-
100	37	-15.5±0.5	8.8±0.4	-23.4±0.1	-	-	-15±4	0.3±0.1	-14.6±0.9	-	-
	25	-6±1	12±1	-23.2±0.5			-3±1	4.6±0.7	-20.9±0.4		
150	30	-9.3±0.8	8.6±0.9	-22.8±0.3	-47±8	-79±27	-3±1	1.6±0.4	-18.7±0.6	-	-
	37*	-11.6±0.2	5.6±0.1	-22.3±0.1			-	-	-		
Urea modified HSA											
	25	-19.2±0.6	22±1	-24.8±0.1			-9.2±0.5	1.9±0.3	-18.8±0.4		
20	30	-24.4±0.5	21.0±0.7	-25.1±0.1	-16±4	29±14	-15.9±0.6	1.1±0.2	-17.5±0.4	-22±16	-14±52
	37	-20±1	17±2	-25.2±0.2			-18±1	1.5±0.3	-18.8±0.5		
	25	-10.6±0.6	9.3±0.7	-22.63±0.02			-11±2	0.5±0.2	-15.4±0.9		
150	30	-11.9±0.4	7.6±0.4	-22.50±0.01	-42±7	-65±24	-9±1	0.6±0.1	-16.0±0.4	-10±15	20±53
	37	-9.3±0.7	4.8±0.4	-21.84±0.02			-4±1	0.4±0.2	-15.6±1.3		

Table S2: Thermodynamic parameters for the binding of PhAA to native and urea modified HSA at temperatures 25°C, 30°C and 37°C and ionic strengths from 20mM to 150mM.

* Data is fitted with SSIS as explained in section *Data analysis* (see also Table S1 from the ESI).

I (mM)	T (°C)	N_1	ΔH_1^{ITC} (kJ/mol)	$k_{b1} \cdot 10^3$ (mol^{-1})	ΔG_{b1} (kJ/mol)	ΔH_{b1} (kJ/mol)	ΔS_{b1} (J/mol/K)	ΔH_2^{ITC} (kJ/mol)	$k_{b2} \cdot 10^3$ (mol^{-1})	ΔG_{b2} (kJ/mol)	ΔH_{b2} (kJ/mol)	ΔS_{b2} (J/mol/K)
Native HSA												
	25	0.68±0.01	-56.6±0.5	343±6	-31.59±0.06			-82.3±0.8	4.2±0.08	-20.68±0.05		
20	30	0.48±0.01	-82.2±0.5	279±3	-31.60±0.05	-50±2	-61±7	-42.0±0.6	4.1±0.2	-20.98±0.09	-10±3	35±10
	37	0.57±0.01	-71±1	136±5	-30.49±0.09			-34.2±0.8	3.5±0.2	-21.0±0.1		
	25	0.59±0.01	-48±1	122±2	-29.04±0.05			-68±54	0.63±0.09	-16.0±0.3		
150	30	0.60±0.01	-52±2	83±4	-28.6±0.1	-51±3	-75±10	-78±16	0.5±0.1	-15.6±0.6	-35±27	-63±90
	37	0.55±0.02	-59±2	55±2	-28.1±0.1			-82±39	0.4±0.2	-15.4±1		
Urea modified HSA												
	25	0.91±0.01	-55.7±0.3	349±8	-31.64±0.06			-60±1	3.1±0.1	-19.96±0.08		
20	30	0.89±0.01	-59.3±0.5	237±8	-31.20±0.08	-62±2	-100±8	-56±2	3.0±0.1	-20.2±0.1	-12±4	28±13
	37	0.89±0.01	-65.4±0.6	134±4	-30.44±0.07			-56±2	2.6±0.1	-20.3±0.1		
	25	0.87±0.01	-46.5±0.5	130±4	-29.19±0.08			-60±9	0.9±0.1	-16.9±0.3		
150	30	0.84±0.01	-48.3±0.8	103±5	-29.1±0.1	-49±4	-68±13	-61±10	1.0±0.1	-17.4±0.3	-4±19	42±63
	37	0.83±0.01	-55.6±2	59±3	-28.3±0.1			-81±18	0.6±0.2	-16±1		

Table S3: Thermodynamic parameters for the binding of IDS to native and urea modified HSA at 25°C, 30°C and 37°C and ionic strengths 20mM and 150mM. An average N_2 of 2 is assumed for all binding processes.