Supporting Information

Soft and Hard Interactions between Polystyrene Nanoplastics and Human Serum Albumin Protein Corona

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Relevant properties

Table S1. Physical properties of HSA, PS^-_s, and PS^-_L.

Name	Formula	Molecular weight / g mol^{-1}	Density / g cm^{-3}	Isoelectric point	SLD_n / 10^{-6} $Å^{-2}$
HSA	$C_{2916}H_{4624}N_{760}O_{889}S_{41}$	66500	1.360	5.5[a]	1.84-3.14[b,1]
PS^-_s	C_8H_8 (monomer)	104	1.040	<2	1.30
PS^-_L	C_8H_8 (monomer)	104	1.040	<2	1.30
H_2O	H_2O	18	0.997	NA	-0.56
D_2O	D_2O	20	1.100	NA	6.25

[a] based on a theoretical calculation (SI Figure S9)

[b] The lower limit of the SLD_n occurs when there is no hydrogen-deuterium (H/D) exchange for exchangeable hydrogens, occurs while the upper limit assumes the 100% H/D exchange.

Circular dichroism (CD) spectroscopy

Table S2. Secondary structure of HSA resolved by spectra deconvolution of CD spectra collected at pH 7.4.

Sample	α-helix	β-sheet	Random coil
Native HSA	51% ± 2%	21% ± 2%	28% ± 1%
With PS^-_s (0.1 mg mL^{-1})	52% ± 2%	20% ± 3%	29% ± 2%
With PS^-_L (0.1 mg mL^{-1})	52% ± 1%	21% ± 2%	28% ± 2%

Table S3. Secondary structure of HSA resolved by spectra deconvolution of CD spectra collected at pH 7.4.

Sample	α-helix	β-sheet	Random coil
Native HSA	51% ± 2%	20% ± 1%	29% ± 2%
With PS^-_s (0.1 mg mL^{-1})	46% ± 2%	12% ± 2%	42% ± 3%
With PS^-_L (0.1 mg mL^{-1})	52% ± 1%	19% ± 3%	29% ± 2%

Zeta potential values

Table S4. Zeta potential values of PS particles, HSA, and PS/HSA complexes at pH 7.4 and 5.0.

Sample	pH 7.4	pH 5.0
HSA	-12.2 ± 3.2 mV	-4.4 ± 4.5 mV
PS^-_S	-50.2 ± 12.0 mV	-45.3 ± 6.8 mV
PS^-_L	-58.2 ± 7.0 mV	-46.8 ± 11.6 mV
HSA/ PS^-_S	-15.5 ± 3.0 mV	-15.8 ± 9.8 mV
HSA/ PS^-_L	-43.4 ± 7.1 mV	-9.0 ± 6.8 mV

Small angle neutron scattering (SANS)

Polystyrene nanoparticles

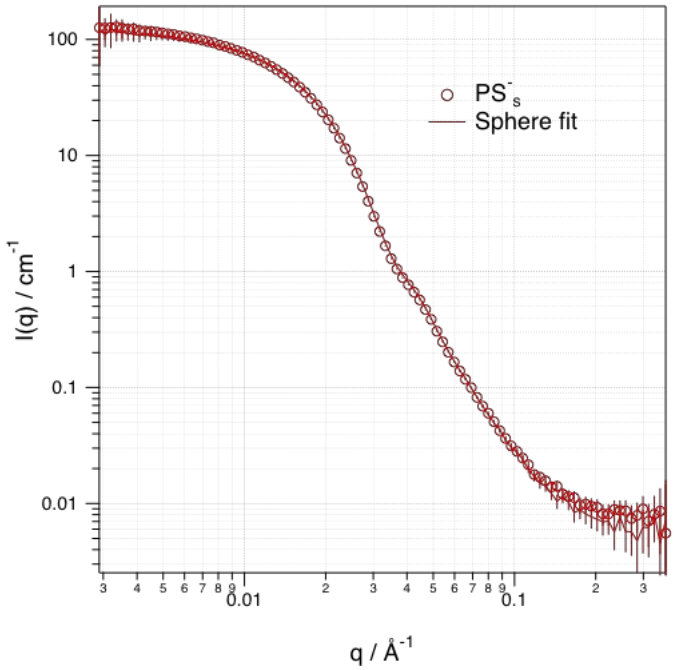

Figure S1. SANS curve of PS^-_s at 9.0 mg mL^{-1} in 100% d-buffer, fitted with sphere model

Table S5. Fitting parameters for SANS curve shown in Figure S1

Scale	Background / cm^{-1}	SLD_n / 10^{-6} $Å^{-2}$	SLD_n solvent / 10^{-6} $Å^{-2}$	Radius / Å
0.00681 ± 0.000150	0.00683 ± 0.000300	1.34	6.35	115 ± 0.100

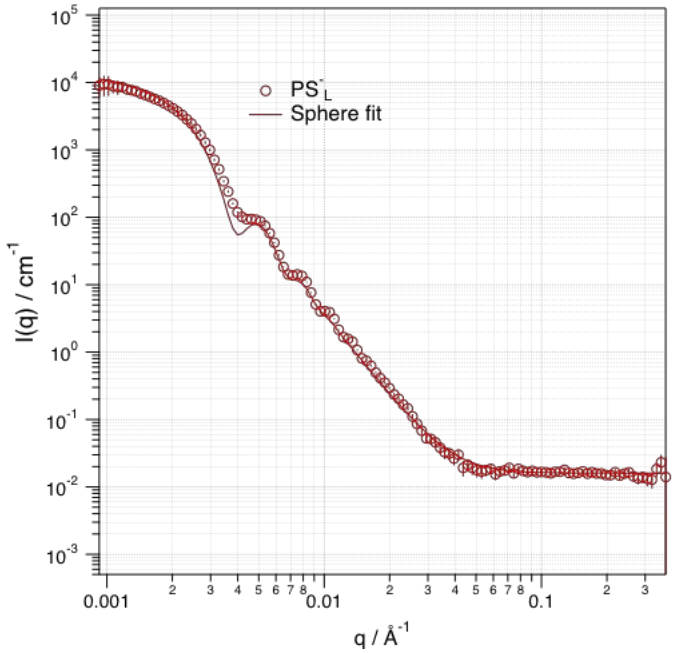

Figure S2. SANS curve for PS^-_L at 9.0 mg mL^{-1} in 100% d-buffer (pH 7.4), fitted with a sphereical model.

Table S6. Fitting parameters for SANS curve shown in Figure S2.

Scale	Background / cm^{-1}	SLD_n / 10^{-6} $Å^{-2}$	SLD_n solvent / 10^{-6} $Å^{-2}$	Radius / Å
0.000820 ± 0.00000650	0.0160 ± 0.000420	1.34	6.35	1187 ± 1.8[a]

[a] The optimised polydispersity was 2%

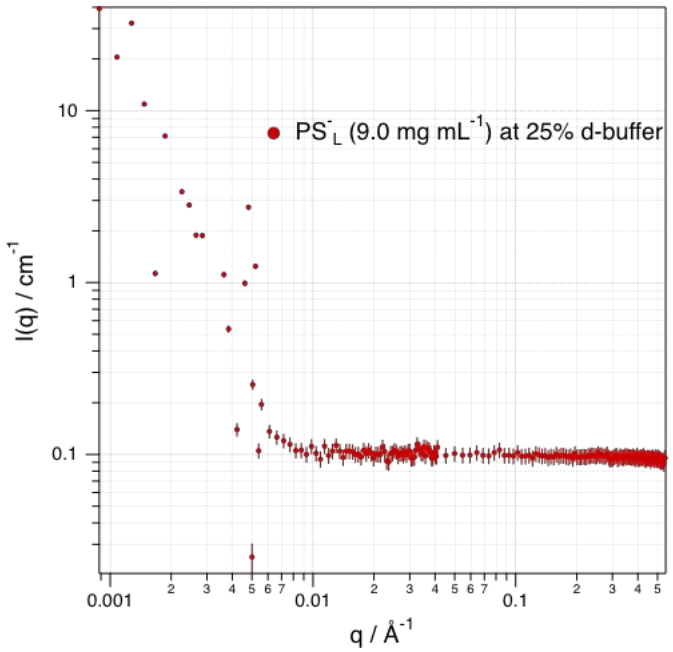

Figure S3. SANS curve of PS^-_L at 9.0 mg mL^{-1} in 25% d-buffer.

Human serum albumin (HSA)

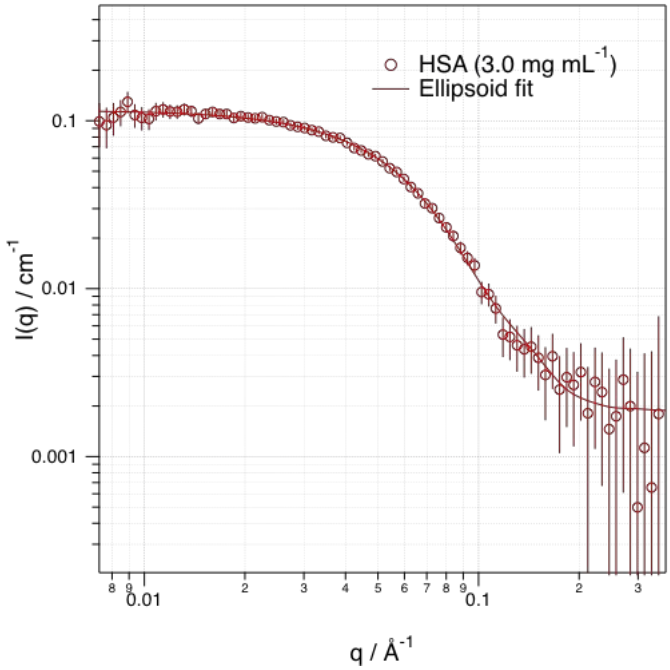

Figure S4. SANS curve for HSA at 3.0 mg mL^{-1} in 100% d-buffer (pH 7.4), fitted with elliposoidal model.

Table S7. Fitting parameters for SANS curve shown in Figure S4.

SLD_n / 10^{-6} Å^{-2}	SLD_n solvent / 10^{-6} Å^{-2}	Radius polar / Å	Radius equatorial / Å
2.40	6.35	15.6 ± 1.90	43.7 ± 0.700

PS/HSA complex

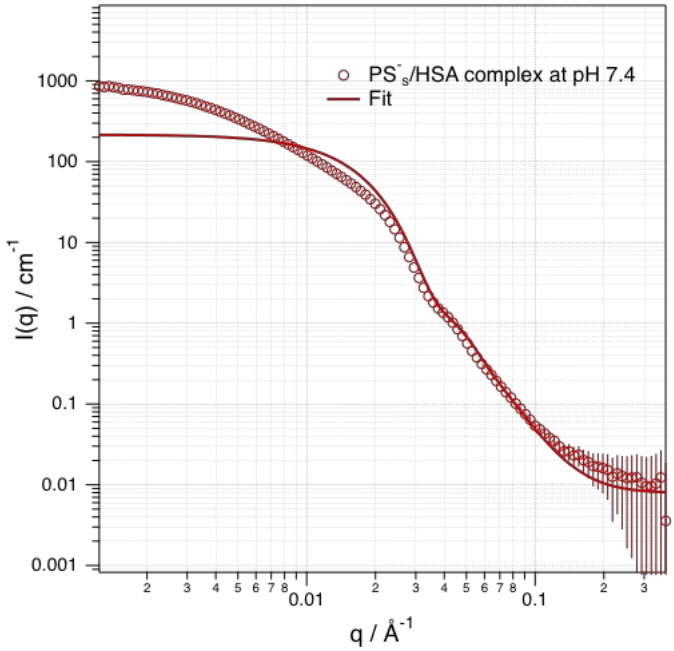

Figure S5. SANS curve for PS^-_s (9.0 mg mL^{-1})/HSA (3.0 mg mL^{-1}) complex in 100% d-buffer (pH 7.4). The scattering intensity was fitted with a combination of spherical and ellipsoidal models.

Table S8. Fitting parameters for SANS curve shown in Figure S5. The radius of PS^-_s, and dimensions of HSA were fixed with the values that were obtained in single components (Table S5 and Table S7).

Radius / Å	Radius polar / Å	Radius equatorial / Å	SLD_n solvent / 10^{-6} Å^{-2}	SLD_n PS / 10^{-6} Å^{-2}	SLD_n HSA / 10^{-6} Å^{-2}
135[a]	15.6	43.7	6.35	1.34	2.40

[a] The optimised polydispersity was 17%

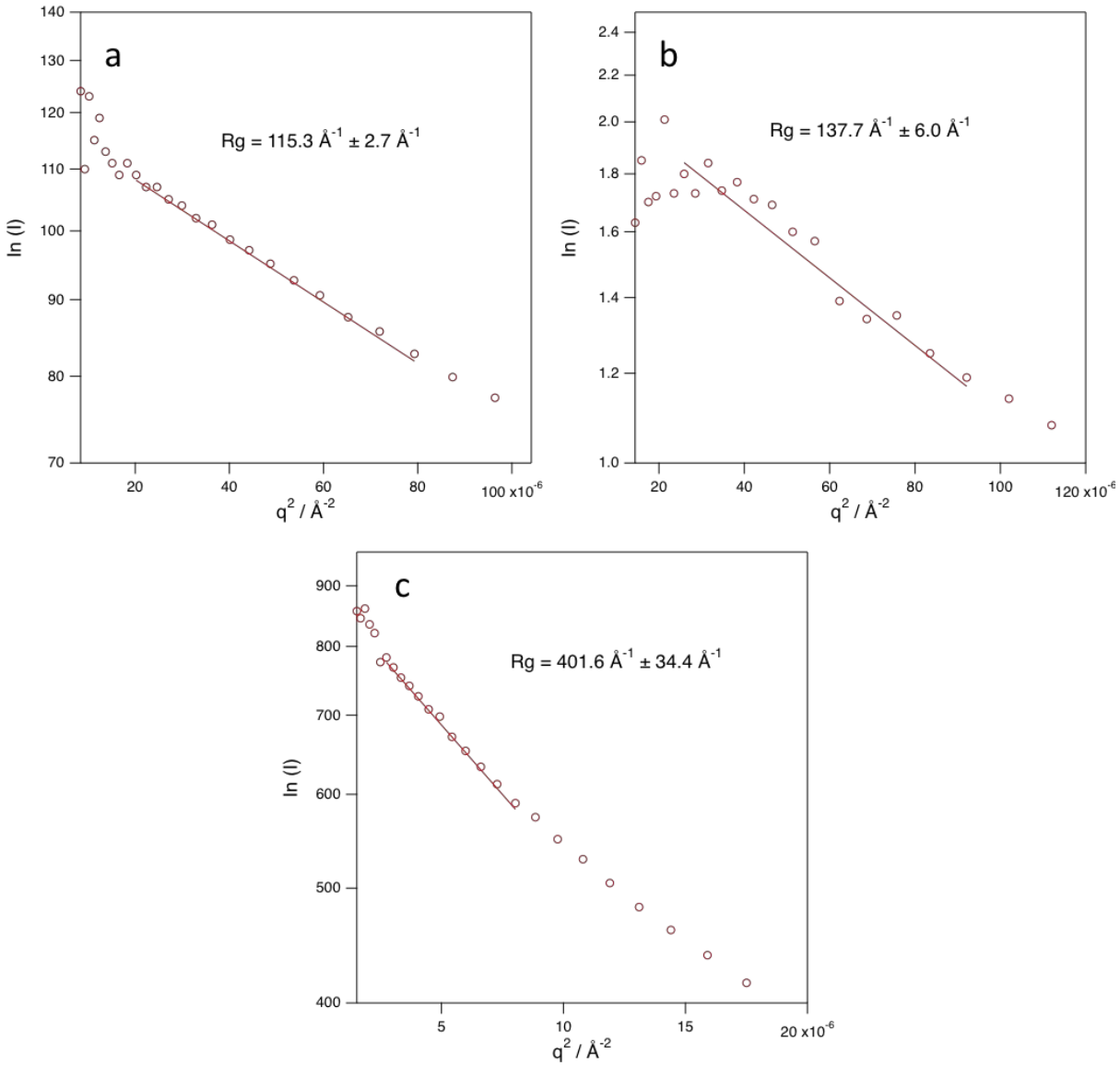

Figure S6. Guinier plots for (a) PS^{-}_{s} (9.0 mg mL^{-1}) in 100% d-buffer, (b) PS^{-}_{s}(9.0 mg mL^{-1})/HSA (3.0 mg mL^{-1}) complex in 25% d-buffer, and (c) PS^{-}_{s}(9.0 mg mL^{-1})/HSA (3.0 mg mL^{-1}) complex in 100% d-buffer.

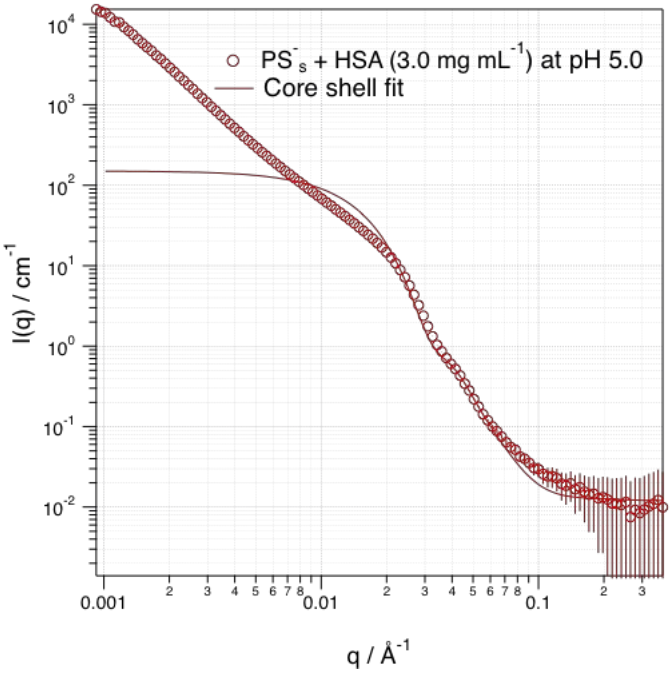

Figure S7. SANS curve for PS-s/HSA complex in 100% d-buffer (pH 5.0), fitted with core shell model.

Table S9. Fitting parameters for Figure S7

Radius / Å	Thickness / Å	SLD_n solvent / 10^{-6} Å^{-2}	SLD_n core / 10^{-6} Å^{-2}	SLD_n shell / 10^{-6} Å^{-2}
135[a]	15.6 ± 1.90	6.35	1.34	2.40

Table S10. Fitting parameters for Figure 9, PS^-_L/HSA complex at pH 7.4.

Radius / Å	Radius polar / Å	Radius equatorial / Å	SLD_n solvent / 10^{-6} Å^{-2}	SLD_n PS / 10^{-6} Å^{-2}	SLD_n HSA / 10^{-6} Å^{-2}
1187[a]	15.6	43.7	6.35	1.34	2.40

The optimised polydispersity was fitted to be 2%

Table S11. Fitting parameters for Figure 9, PS^-_L/HSA complex at pH 5.0.

Radius / Å	Radius polar / Å	Radius equatorial / Å	SLD_n solvent / 10^{-6} Å^{-2}	SLD_n PS / 10^{-6} Å^{-2}	SLD_n HSA / 10^{-6} Å^{-2}	Perturb	Stickiness
1187[a]	15.6	43.7	6.35	1.34	2.40	0.0620 $\pm$ 0.00085	0.140 $\pm$ 0.00015

The optimised polydispersity was fitted to be 2%

Representation of fractal dimension in colloidal system

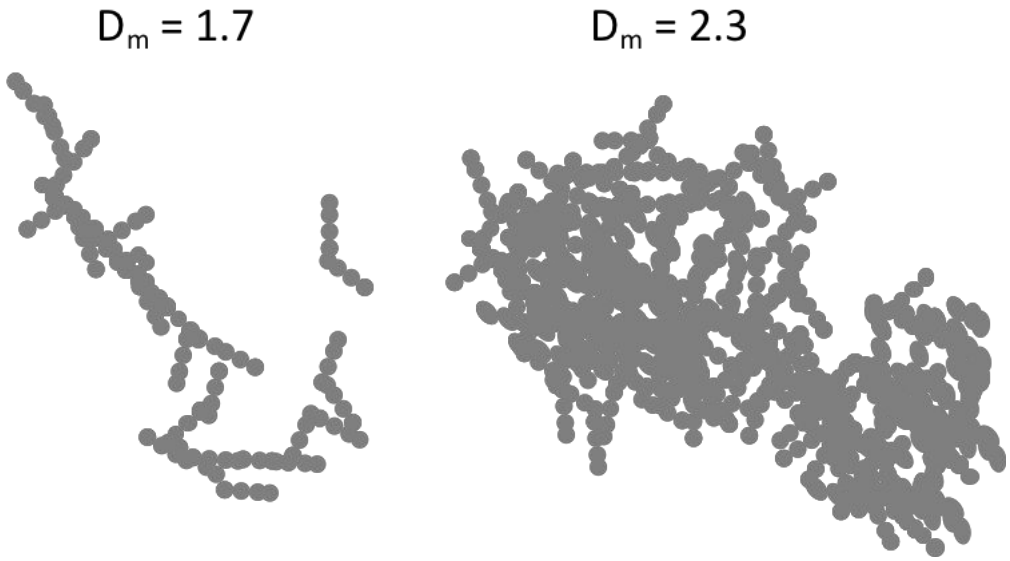

Figure S8. Graphical representation of fractals with different fractal dimensions.

Theoretical calculation of HSA net charge

The net charge of human serum albumin (HSA) was calculated with the following equation:

$$net\ charge\ (positive\ charge) = \frac{10^{-pKa}}{10^{-pKa} + 10^{-pH}} \qquad \text{Equation S1}$$

$$net\ charge\ (negative\ charge) = \frac{10^{-pH}}{10^{-pH} + 10^{-pKa}} \qquad \text{Equation S2}$$

The number and the pKa values of each amino acid used in the calculation is found in Table S11.

Table S12. Relevant properties of HSA for calculatig net charges at various pH.

Amino acid	pKa of side group[2]	Number[3]
Arginine	12.8	24
Histidine	6.0	16
Lysine	10.1	59
Aspartic acid	3.7	36
Glutamic acid	4.3	62
Cysteine*	8.2	1

*One cysteine that does not form disulfide bridge is used in this calculation.[3]

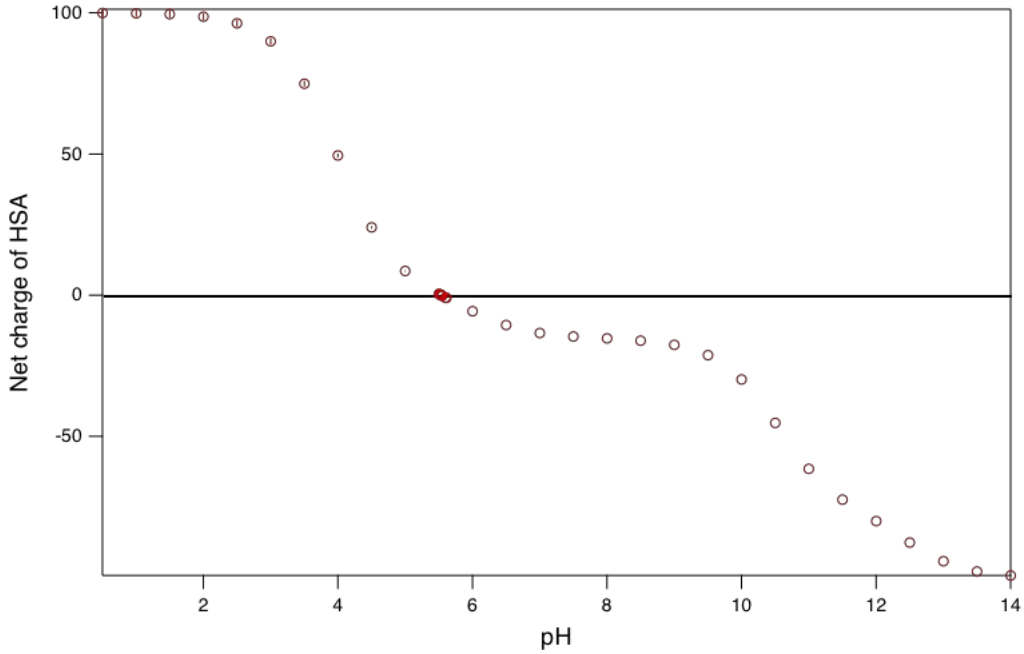

Figure S9. Theoretically calculated net charge of HSA as a function of pH.

References

1. Berts, I.; Fragneto, G.; Porcar, L.; Hellsing, M. S.; Rennie, A. R., Controlling adsorption of albumin with hyaluronan on silica surfaces and sulfonated latex particles. *J. Colloid Interface Sci.* **2017,** *504*, 315-324.

2. Lide, D., DR 1991-1992. *Handbook of chemistry and physics. 72nd edition, CRC Press, Boca Raton, FL*, 17.

3. Sugio, S.; Kashima, A.; Mochizuki, S.; Noda, M.; Kobayashi, K., Crystal structure of human serum albumin at 2.5 Å resolution. *Protein Eng.* **1999,** *12* (6), 439-446.