Electronic Supplementary Material (ESI) for RSC Advances.

Pre-coating with Protein fractions inhibits nano-carrier aggregation in human blood plasma.

Laura K. Müller[1], Johanna Simon[1], Susanne Schöttler[1,2],Katharina Landfester[1], Volker Mailänder[1,2,], Kristin Mohr[1,*]*

[1]Max Planck-Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

[2]III. Medical Clinic (Hematology, Oncology and Pulmonology), University Medical Center Mainz, Langenbeckstraße 1, 55131 Mainz, Germany

Corresponding and Senior Authors:

Dr. Kristin Mohr, Max-Planck-Institute for Polymer Research, mohr@*mpip*-mainz.mpg.de

PD Dr. Volker Mailänder, University Medicine Mainz and Max-Planck-Institute for Polymer Research, volker.mailaender@unimedizin-mainz.de

SUPPLEMENTARY INFORMATION

CONTENT:

1. LC-MS analysis of different protein fractions

Table S1. LC-MS analysis of different protein fractions. (A detailed overview can be found in a separate excel file).

	IgG	IgG depleted plasma	HSA depleted plasma	HSA (AffiBlue only)	HSA (columns in series)
Sum of all Immunoglobulins	94,20%	5,87%	27,70%	34,60%	3,58%
Albumin	0,56%	56,92%	4,08%	47,39%	84,14%
Others	5,23%	37,22%	68,22%	18,01%	12,28%

2. Series-connection of Protein A and AffiGel Blue columns

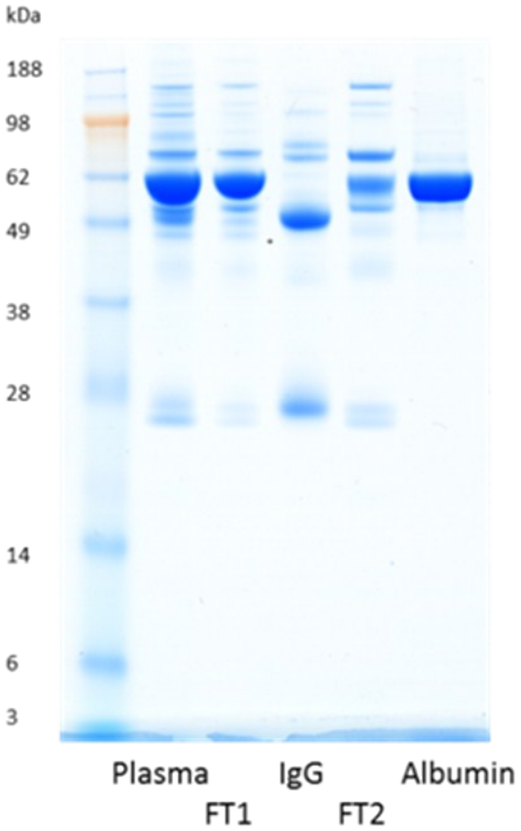
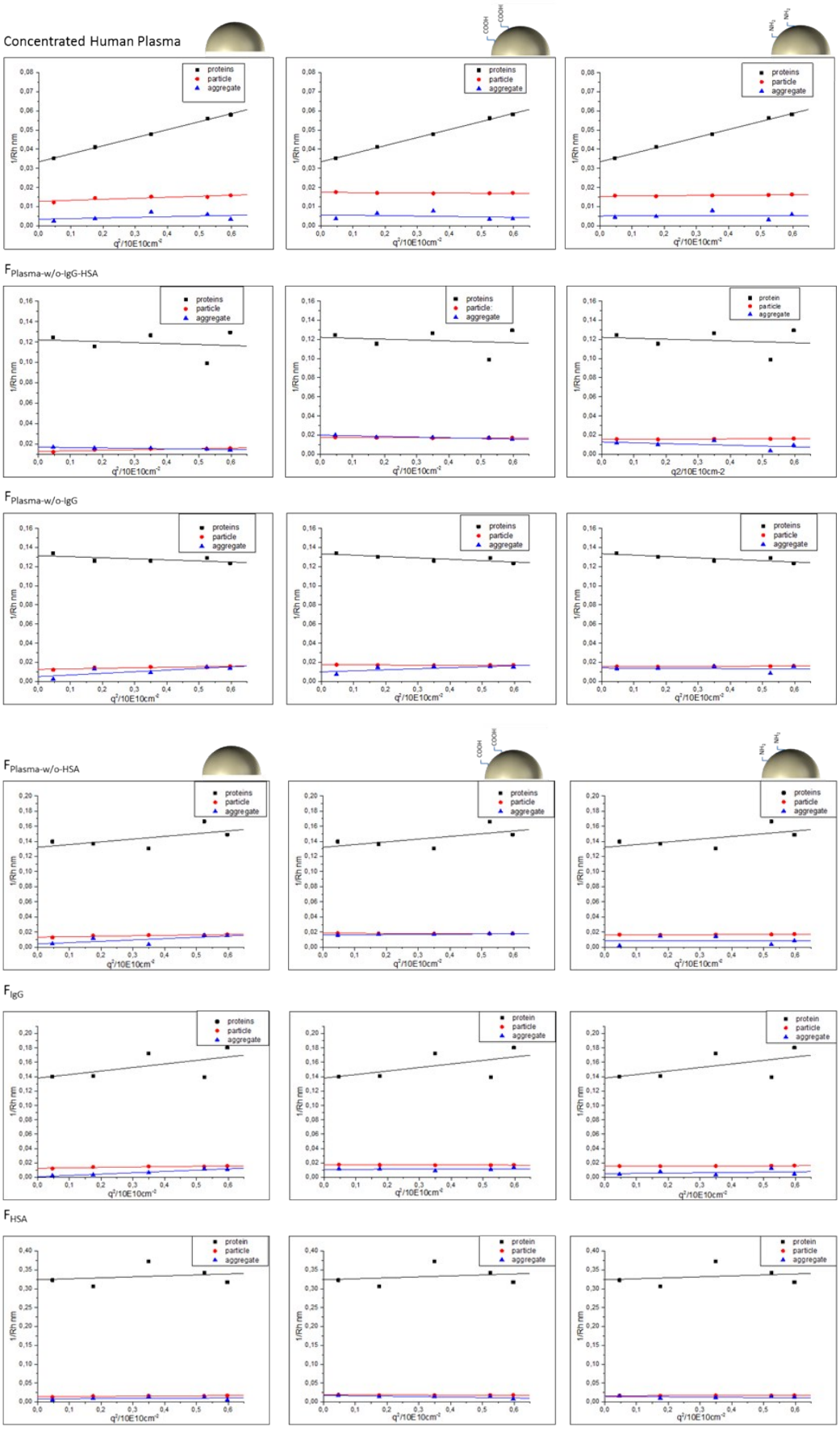


Figure S1. SDS-Page of AffiBlue affinity chromatography with prior IgG-depletion. (FT = flow through)

3. **Detailed overview of DLS data**



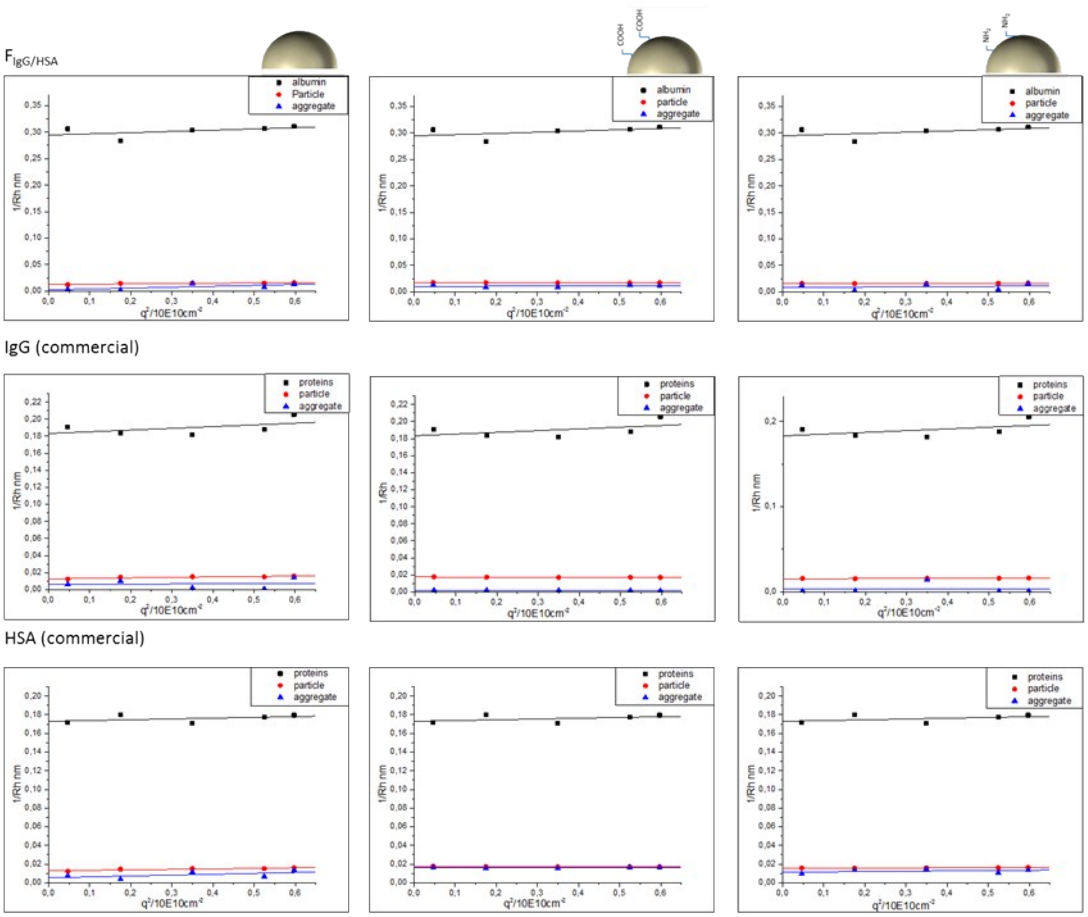
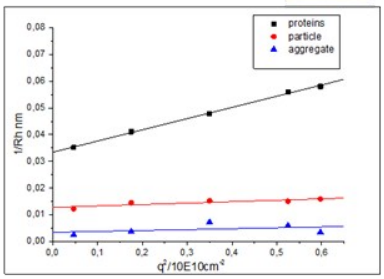
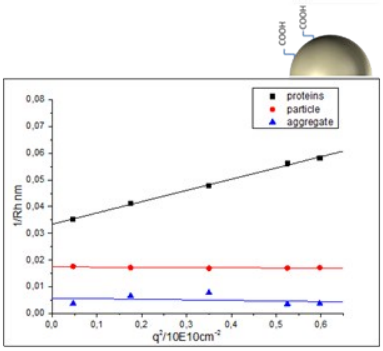
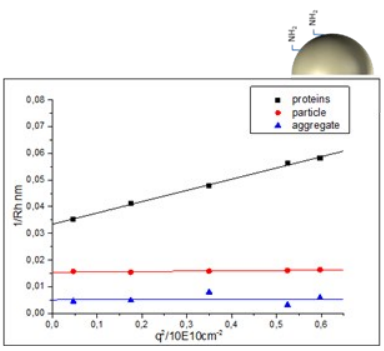


Figure S2. Angular dependency of the hydrodynamic radius of the measured samples.

uncoated

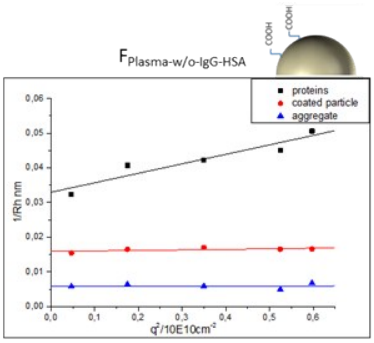






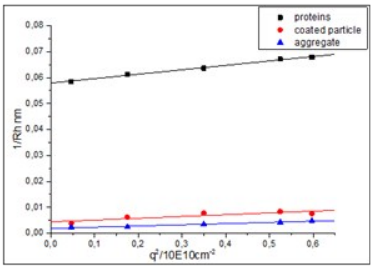
NO AGGREGATION

$F_{Plasma\text{-}w/o\text{-}IgG\text{-}HSA}$

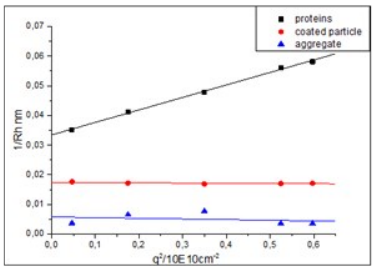


NO AGGREGATION

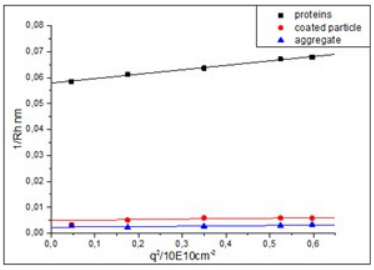
$F_{Plasma\text{-}w/o\text{-}HSA}$



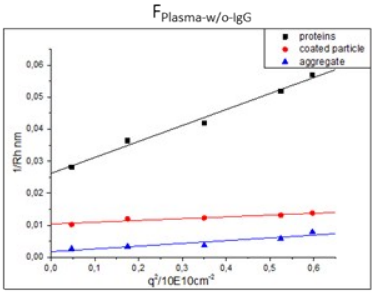
$F_{Plasma\text{-}w/o\text{-}HSA}$



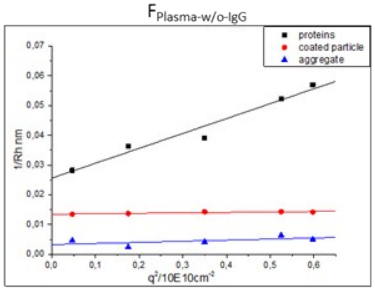
$F_{Plasma\text{-}w/o\text{-}HSA}$



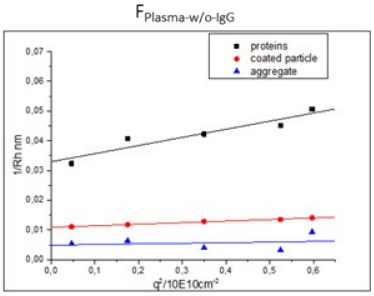
$F_{Plasma\text{-}w/o\text{-}IgG}$



$F_{Plasma\text{-}w/o\text{-}IgG}$



$F_{Plasma\text{-}w/o\text{-}IgG}$



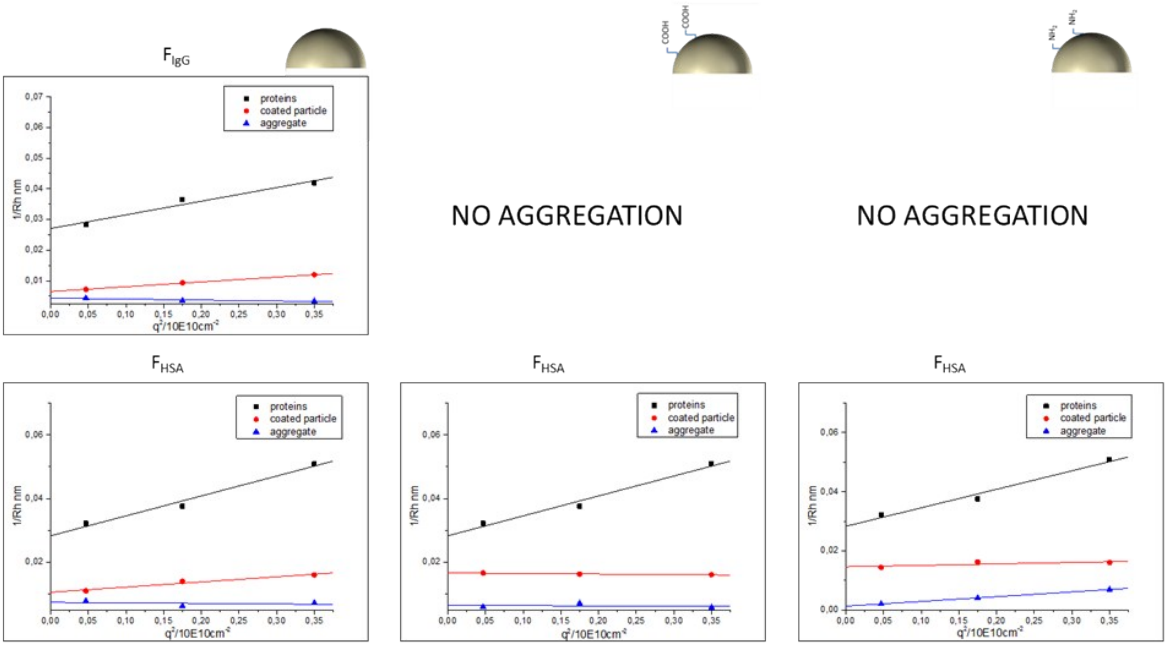


Figure S3. Angular dependency of (coated) particles in concentrated Human Plasma

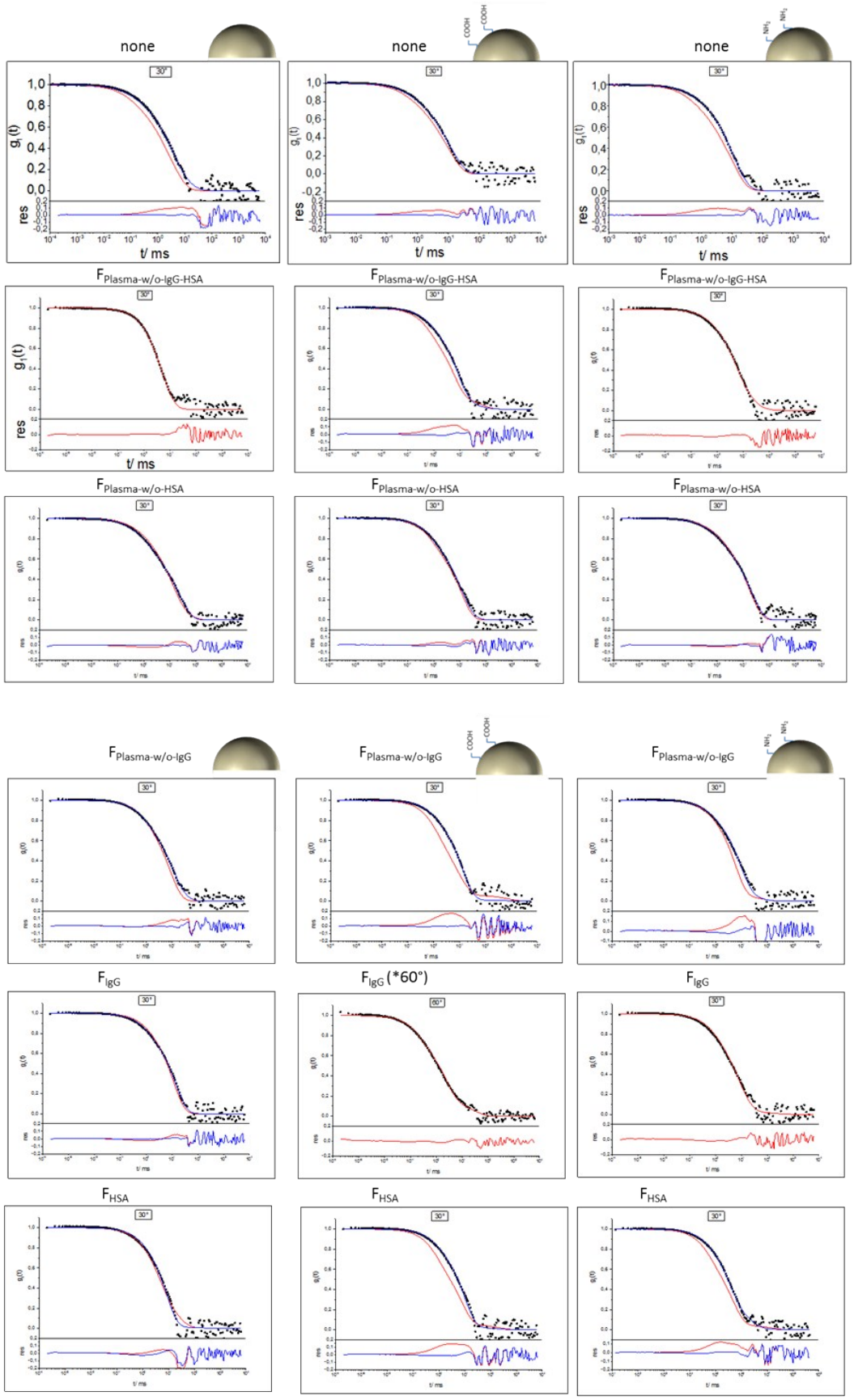
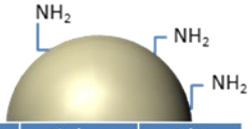


Figure S4. Autocorrelation functions of uncoated as well as coated particles in concentrated human plasma at a scattering angle of 30°.

Table S2. DLS results with duplicates of all particles without pre-coating.



	Rh / nm extrapolated		Rh / nm 30°	I / % 30°	Rh/ nm extrapolated		Rh / nm 30°	I / % 30°	Rh / nm extrapolated		Rh / nm 30°	I / % 30°
naked	69				58				65			
Plasma diluted	93	87.5	126	34	80	95	68	95	74	71	110	21
	82		115	93	110		166	33	68		-	0
IgG	191	224	423	24	99	95.5	137	34	285	206.5	229	37
	257		470	30	92		84	32	128		206	21
Albumin-IgG-mixture (affi_only)	241	180	335	22	95	90.5	76	100	121	137.5	95	65
	119		184	9	86		77	100	154		80	58
Albumin (row)	125	121.5	309	15	57	61.5	57	100	67	72	70	92
	118		381	28	66		71	42	77		64	100
IgG-depleted Plasma	198	146	407	13	67	83.5	62	100	71	70	75	98
	94		765	15	100		193	19	69		84	95
Albumin-depleted Plasma	102	137	477	15	54	57.5	53	100	88	102	104	72
	172		219	16	61		62	58	116		491	18
Albumin- and IgG - depleted Plasma	59	61	59	100	57	53.5	24	9	72	77.5	-	0
	63		46	100	50		50	100	83		-	0
IgG com in running buffer	226	194.5	790	13	503	642	507	96	408	345	651	13
	163		163	45	781		854	81	282		894	12
HSA com in running buffer	152	169.5	109	97	62	64	60	100	88	80.5	105	55
	187		132	43	66		65	54	73		91	73
IgG com in PBS	210	183	269	19	101	90.5	72	99	224	174.5	4169	4
	156		333	22	80		70	100	125		2234	2
Albumin com in PBS	212	194	647	11	72	70.5	57	100	67	67	207	13
	176		325	7	69		63	100	67		80	98
Fibrinogen com In PBS	238	206.5	509	24	189	158	219	35	182	152.5	228	46
	175		196	54	127		184	45	123		141	81

4. **Zetapotential of particles in the different protein fractions**

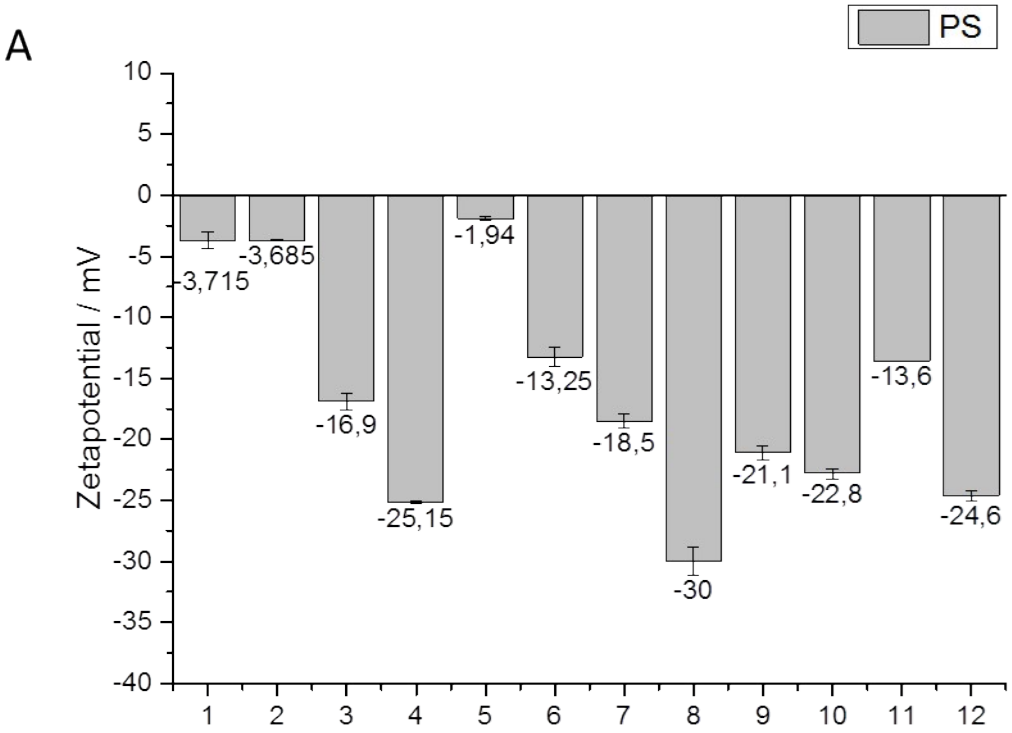


Figure S5. Zetapotential of PS in the different protein fractions with and without separation of unbound protein, representing hard and soft corona (see table below).

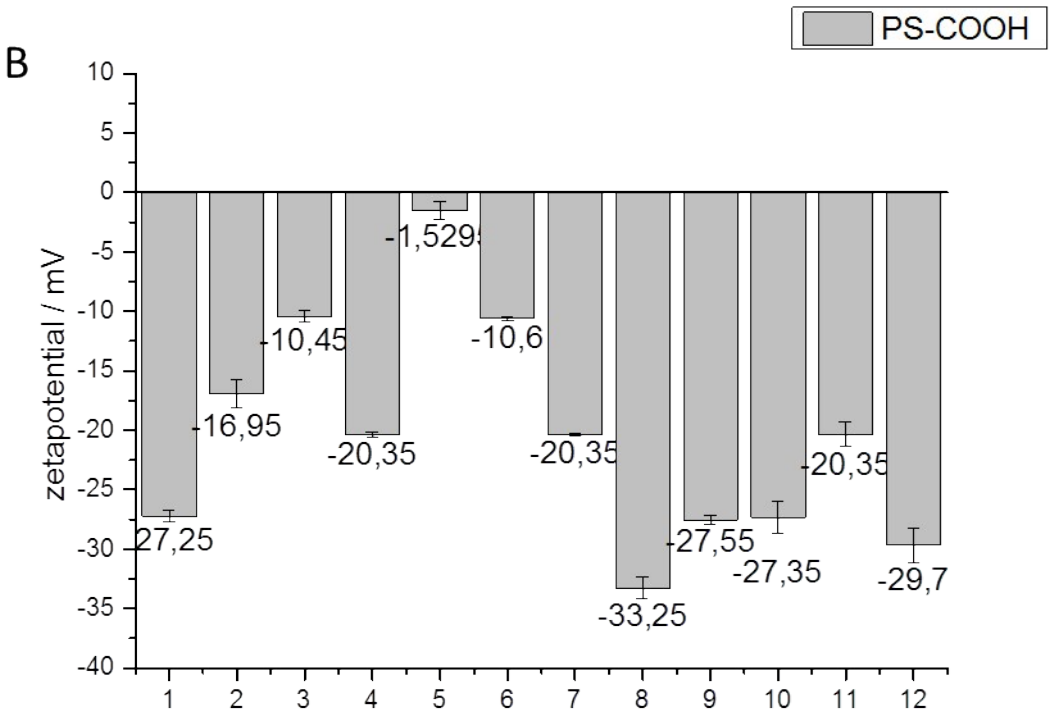


Figure S6. Zetapotential of PS-COOH in the different protein fractions with and without separation of unbound proteins, representing hard and soft protein corona (see table below).

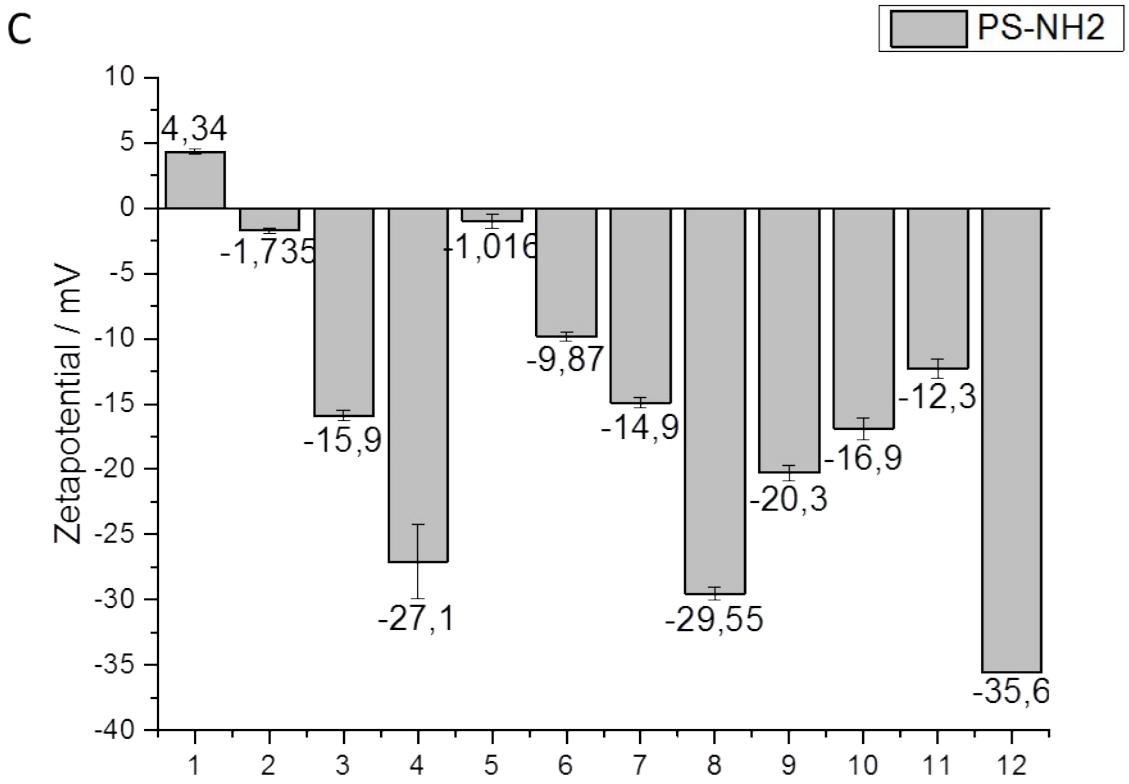
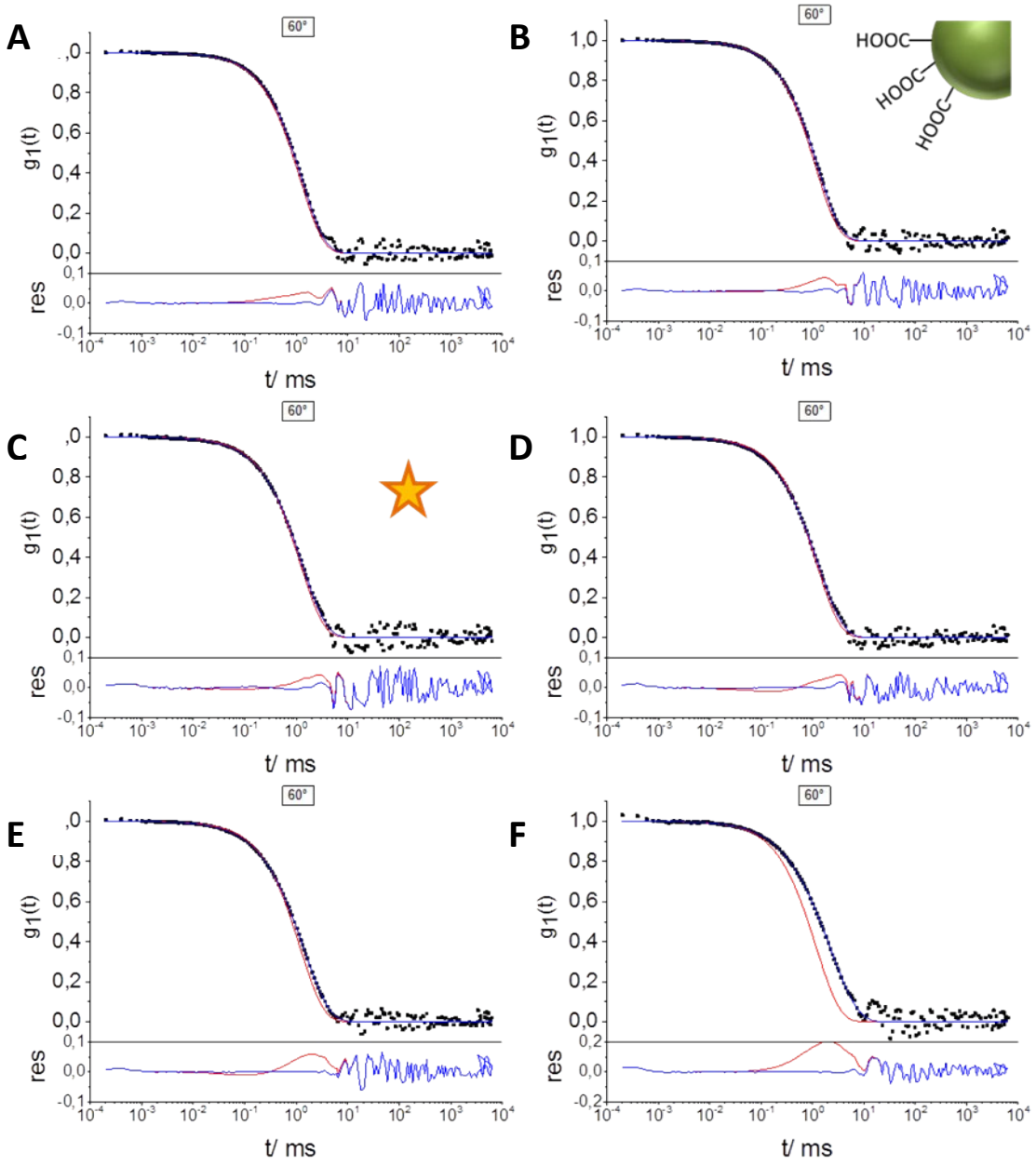


Figure S7. Zetapotential of PS-NH_2 in the different protein fractions with and without separation of unbound proteins, representing hard and soft protein corona (see table below).

Table S3. Index of Figure S5-S7.

1	Particle
2	Particle centrifugated
3	Particle / $F_{Plasma\text{-}IgG}$ mixture
4	3 centrifugated
5	Particle / F_{IgG} mixture
6	5 centrifugated
7	Particle / $F_{Plasma\text{-}IgG\text{-}HSA}$ mixture
8	7 centrifugated
9	Particle / HSA mixture
10	9 centrifugated
11	Particle / $_{FPlasma\text{-}HSA}$ mixture
12	11 entrifugated

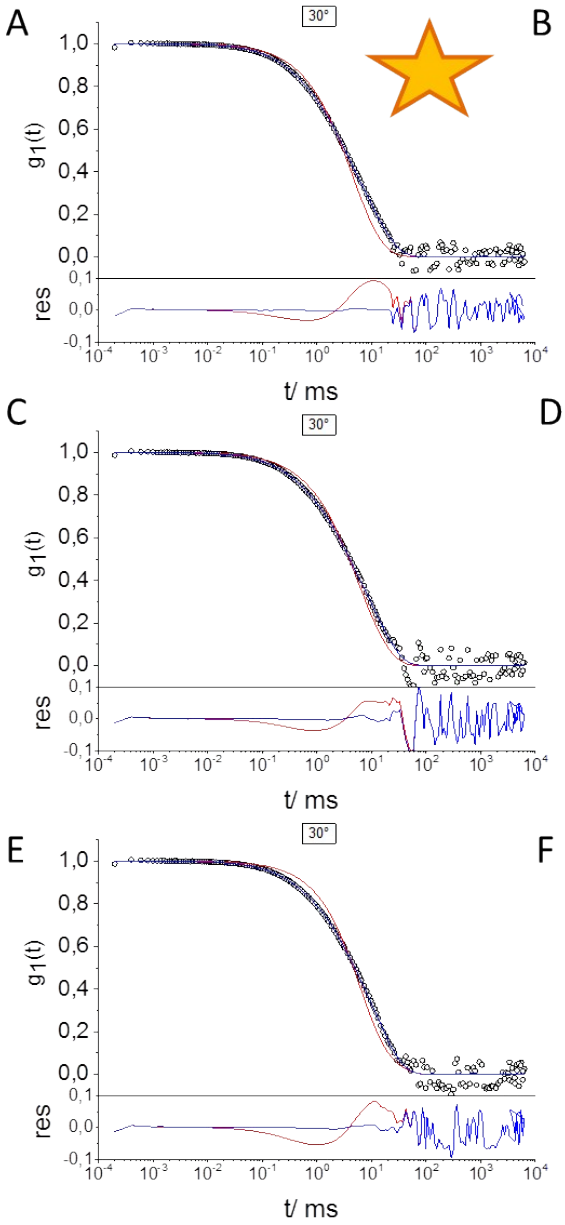
5. Dependency of NP stability on protein concentration



Sample	A	B	C	D	E	F
5x10^{-4} m^2 surface /	0.25 mg	0.5 mg	1 mg	2 mg	3 mg	4 mg

Figure S8. Autocorrelation function at Θ = 60° of PS-COOH in $F_{Plasma\text{-}w/o\text{-}IgG}$ including data points (•), force fit (red) and fit with aggregate formation (blue) From A to D, the protein amount per given surface area was increased by a factor of 16.

6. Influence of protein amount used for pre-coating on particle stability against aggregation in concentrated human plasma



F

sample	mg protein	$\langle 1/R_h \rangle_z^{-1}$ / nm
A	0	182
B	1 eq	216
C	2 eq	222
D	3 eq	207
E	4 eq	214

Figure S9. ACF at Θ = 30° including data points, force fit and fit with aggregation in concentrated human plasma of A) PS-COOH, B) PS-COOH coated with $F_{Plasma\text{-}w/o\text{-}IgG}$ as described in the manuscript, C) PS-COOH coated with twice the amount of proteins, D) PS-COOH coated with thrice the amount of proteins and D) PS-COOH coated with four times the amount of proteins.