Particle
& Particle Systems Characterization

Supporting Information

Nanoparticle Tracking Analysis of Polymer Nanoparticles in Blood Plasma

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Supplementary Methods

Multicomponent Aggregate Counting.

The number of multicomponent aggregates in each NTA measurement were determined manually, where each video of flowing particles was observed. Videos were monitored for the presence of multicomponent aggregates that were identified as at least two particles moving together in the same manner without the 'wiggle' of Brownian motion. Videos were initially observed in real time to identify the presence of potential aggregates, then the sections of the videos with potential aggregates were viewed frame by frame in ImageJ to count the number of particles in the aggregates using a hand tally counter. For each group of particles determined to be an aggregate, the number of particles observed was recorded, where an individual particle was defined as "a definitive shape with precise limits and boundaries". If a figure was observed with a larger diameter but lacked the boundaries mentioned in the definition of a particle, it was only counted as a single particle.

Accounting for High Vibration Error

High vibration error represents an NTA run measuring a sample with irregular flow through the microfluidic viewing device. Specifically, if a bubble gets trapped in the device, especially when using a fluid such as blood, irregular flow can occur. NTA software is not able to properly calculate the diffusion coefficient of particles analyzed in under irregular flow, which signals the NS300 software to give a "high vibration warning" for any run affected. If this warning is seen, the run should not be included in size calculations.
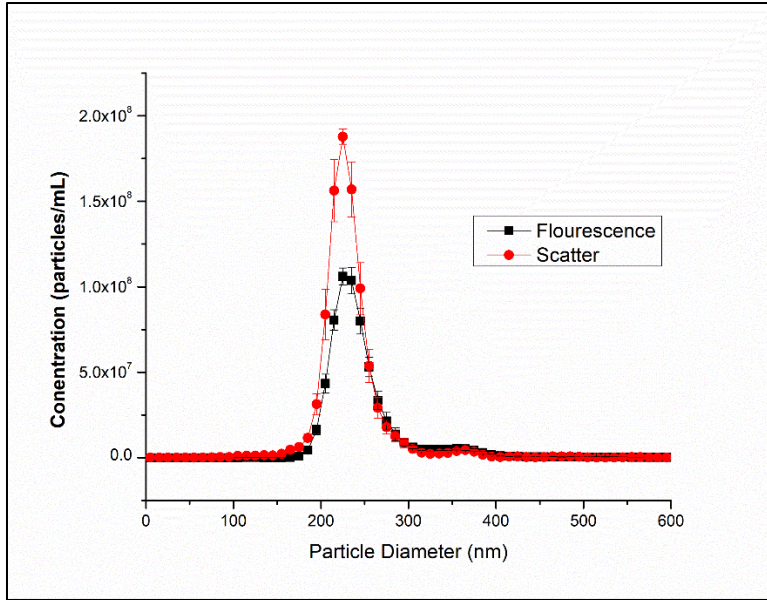
Supplementary Figures

Figure S1. Demonstrates quantitative differences between scatter and fluorescent modes using the Malvern Nanosight's NTA. Signal decreases slightly between scatter mode and fluorescent mode, resulting in a slightly lower particle concentration.
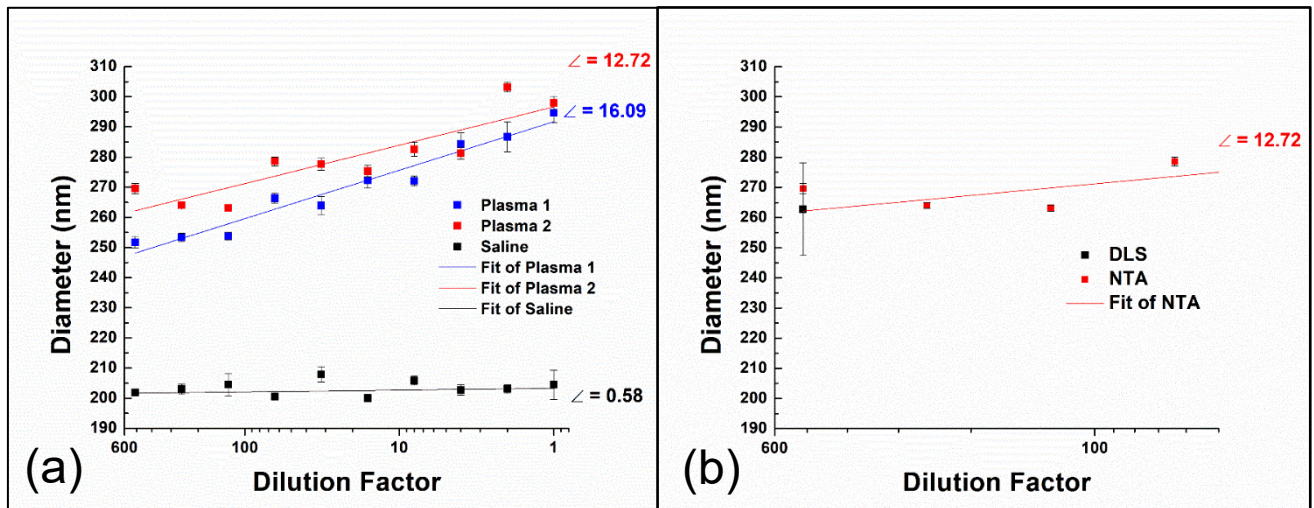

Figure S2. (a) NTA measurements of PS particles incubated in saline (black) and two batches of goat blood plasma (red, blue) and subsequently diluted. For saline measurements, the initial amount of blood plasma was replaced with saline. Displayed error bars represent standard error for each data set. (b) Comparison of results using both NTA and DLS of measurements of PS

particles incubated in blood plasma and subsequently diluted using saline. DLS measurements in solutions with dilution factors less than 512 were reported as "too poor for cumulative analysis", and were omitted from the figure. Displayed error bars represent standard error for each data set.

One-way ANOVA: Figure 2-a (Plasma 2)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
0.07%	26	269.57	8.63	(266.09, 273.05)
0.14%	23	264.01	4.84	(261.91, 266.10)
0.28%	21	263.12	4.64	(261.00, 265.23)
0.55%	25	278.48	7.68	(275.31, 281.65)
1.11%	30	277.64	11.12	(273.49, 281.79)
2.22%	20	275.32	8.57	(271.31, 279.33)
4.44%	26	282.62	11.60	(277.94, 287.31)
8.88%	23	281.25	9.61	(277.10, 285.41)
17.75%	24	303.11	7.70	(299.86, 306.36)
35.50%	22	297.98	9.63	(293.71, 302.25)

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Factor	9	92.4358	77.60	0.000

Grouping Information Using the Games-Howell Method and 95% Confidence

Factor	N	Mean	Grouping		
17.75%	24	303.11	A		
35.50%	22	297.98	A		
4.44%	26	282.62		B	
8.88%	23	281.25		B	
0.55%	25	278.48		B	
1.11%	30	277.64		B	C
2.22%	20	275.32		B	C
0.07%	26	269.57			C D
0.14%	23	264.01			D
0.28%	21	263.12			D

Means that do not share a letter are significantly different.

Figure S3. Single Factor ANOVA analysis of PS particles incubated in each dilution of Plasma 2. Equal variances were not assumed for the analysis according to the results of a multiple comparisons test for equal variance.

One-way ANOVA: Figure 2-a (Plasma 1)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
0.07%	30	251.57	9.51	(248.02, 255.12)
0.14%	30	253.45	7.61	(250.60, 256.29)
0.28%	30	253.75	7.18	(251.07, 256.44)
0.55%	49	266.35	11.30	(263.11, 269.60)
1.11%	28	263.92	15.92	(257.74, 270.09)
2.22%	25	272.23	12.76	(266.96, 277.49)
4.44%	28	272.04	8.28	(268.83, 275.25)
8.88%	35	284.34	21.41	(276.98, 291.69)
17.75%	42	286.74	32.33	(276.67, 296.81)
35.50%	42	294.73	21.78	(287.94, 301.52)

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Factor	9	128.142	35.84	0.000

Grouping Information Using the Games-Howell Method and 95% Confidence

Factor	N	Mean	Grouping				
35.50%	42	294.73	A				
17.75%	42	286.74	A	B			
8.88%	35	284.34	A	B			
2.22%	25	272.23		B	C		
4.44%	28	272.04		B	C		
0.55%	49	266.35			C		
1.11%	28	263.92			C	D	
0.28%	30	253.75				D	E
0.14%	30	253.45				D	E
0.07%	30	251.57					E

Means that do not share a letter are significantly different.

Figure S4. Single Factor ANOVA Analysis of Nanoparticles Incubated in Each Dilution of Plasma 1. Equal variances were not assumed for the analysis according to the results of a multiple comparisons test for equal variance.

One-way ANOVA: Figure 2-a (Saline)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
0.07%	30	201.917	5.329	(199.927, 203.906)
0.14%	28	203.01	8.76	(199.61, 206.41)
0.28%	27	204.51	18.96	(197.01, 212.01)
0.55%	30	200.49	5.83	(198.31, 202.66)
1.11%	25	207.86	12.34	(202.76, 212.95)
2.22%	30	200.01	6.82	(197.47, 202.56)
4.44%	30	205.79	8.02	(202.80, 208.79)
8.88%	25	202.70	8.52	(199.18, 206.21)
17.75%	30	203.04	7.59	(200.20, 205.87)
35.50%	25	204.45	23.07	(194.93, 213.97)

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Factor	9	107.551	1.94	0.054

Grouping Information Using the Games-Howell Method and 95% Confidence

Factor	N	Mean	Grouping
1.11%	25	207.86	A
4.44%	30	205.79	A
0.28%	27	204.51	A
35.50%	25	204.45	A
17.75%	30	203.04	A
0.14%	28	203.01	A
8.88%	25	202.70	A
0.07%	30	201.917	A
0.55%	30	200.49	A
2.22%	30	200.01	A

Means that do not share a letter are significantly different.

Figure S5. Single Factor ANOVA analysis of PS particles incubated in each dilution of pure saline. Equal variances were not assumed for the analysis according to the results of a multiple comparisons test for equal variance. Results show that the measured hydrodynamic diameters of the particles are not statistically different across each dilution in saline.
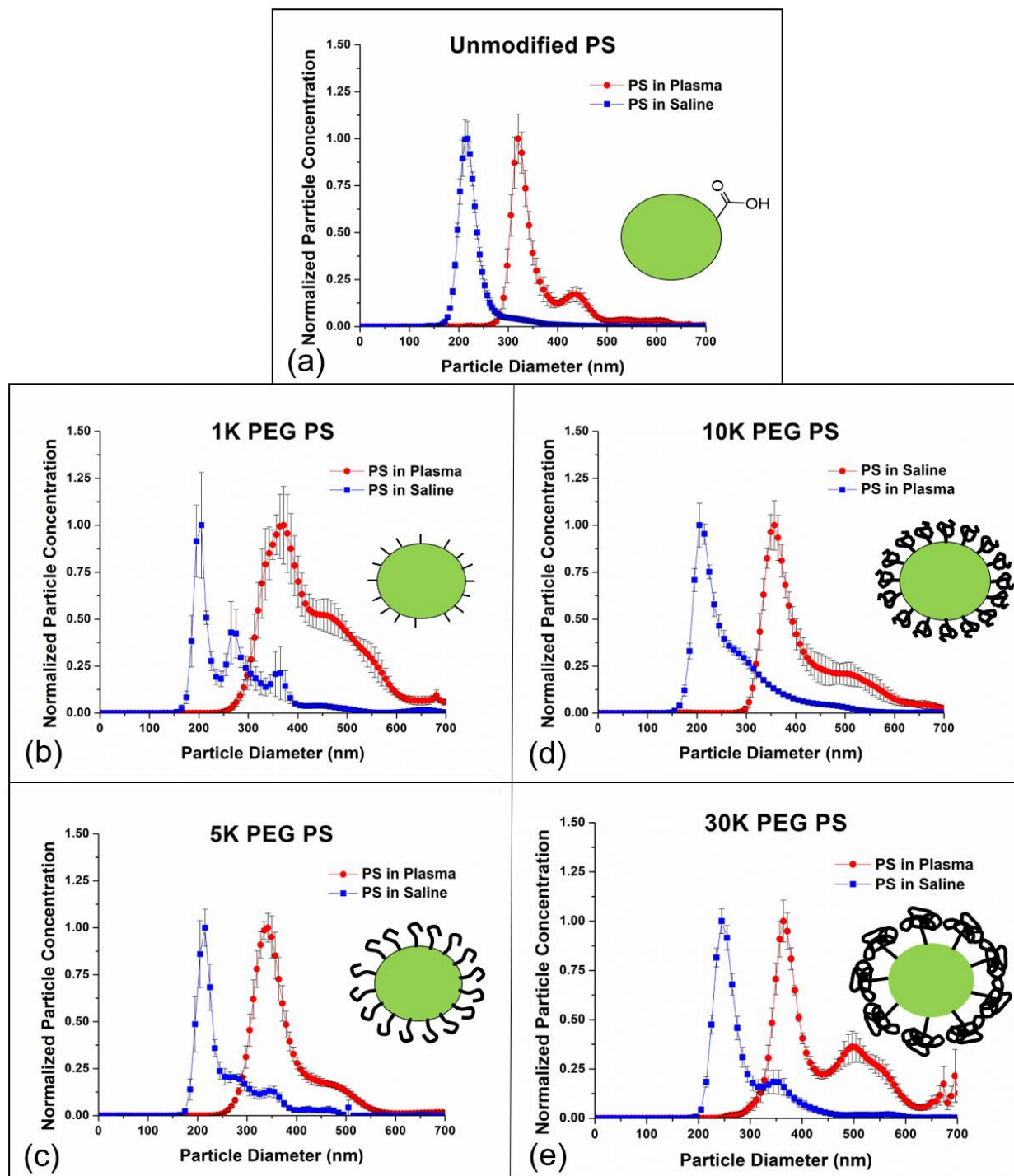
Figure S6. NTA size distribution plots and visual representations of (a) unmodified PS particles and (b) 1K, (c) 5K, (d) 10K, (e) 30K PEGylated PS particles in saline and blood plasma. Each visual representation of PEGylated particles shows the orientation of the different molecular weights of PEG (black) after reacting with carboxylates on the surface of PS particles (green). Size disparities between particles incubated in saline and plasma confirm the presence of a protein corona on all particles, regardless of the use of PEG.
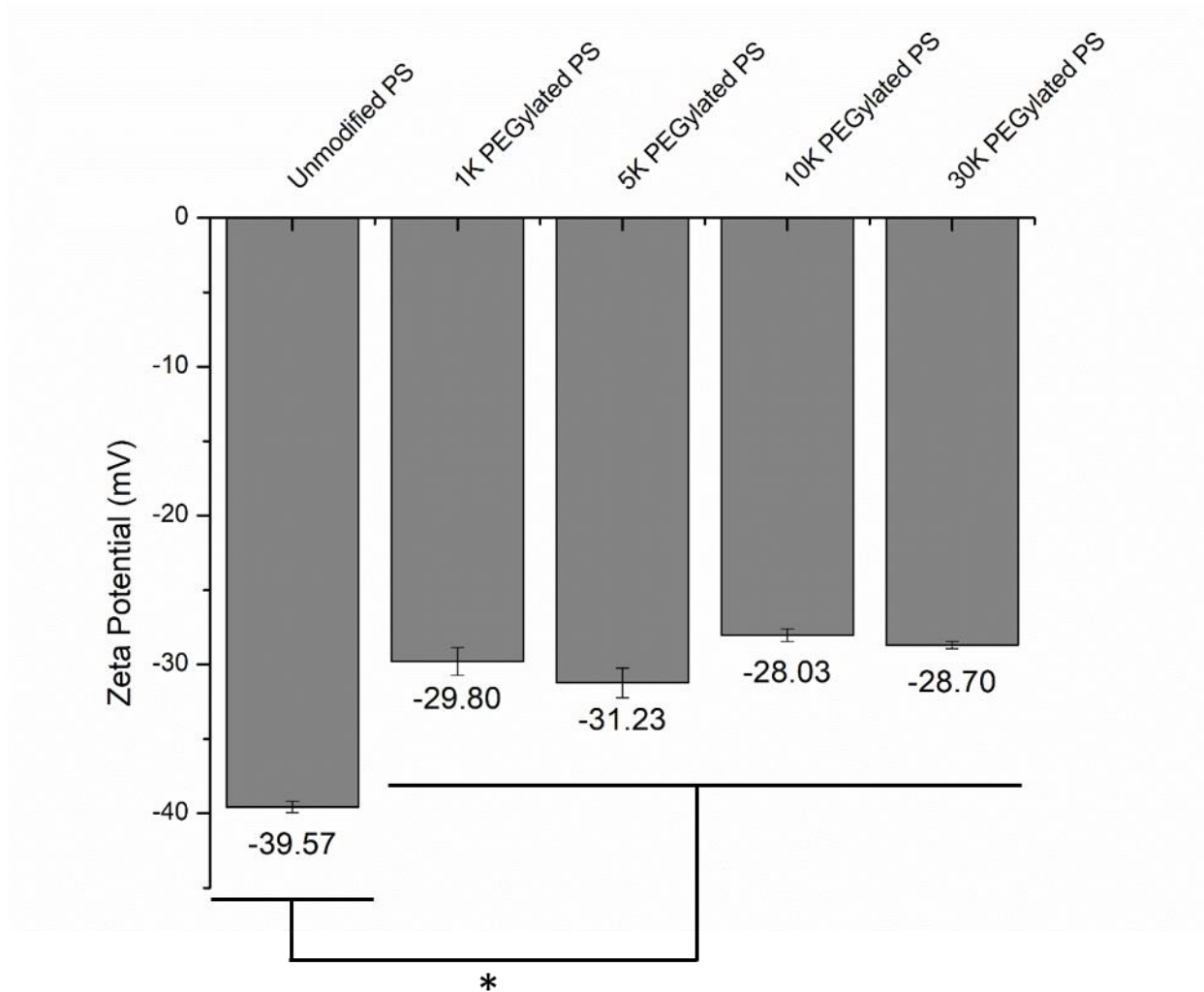
Figure S7. Zeta potential values of unmodified PS particles and PS particles PEGylated with 1K, 5K, 10K and 30K PEG. Measurements were taken with a Malvern Zetasizer.

One-way ANOVA: SI Figure 6

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
Unmodified	3	-39.567	0.814	(-41.403, -37.730)
1K	3	-29.80	2.00	(-31.64, -27.96)
5K	3	-31.23	2.11	(-33.07, -29.40)
10K	3	-28.033	0.902	(-29.870, -26.197)
30K	3	-28.700	0.529	(-30.536, -26.864)

Pooled StDev = 1.42759

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	263.65	65.913	32.34	0.000
Error	10	20.38	2.038		
Total	14	284.03			

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
10K	3	-28.033	A
30K	3	-28.700	A
1K	3	-29.80	A
5K	3	-31.23	A
Unmodified	3	-39.567	B

Means that do not share a letter are significantly different.

Figure S8. Single Factor ANOVA analysis of zeta potential values of unmodified and PEGylated polystyrene particles measured in saline. Equal variances were assumed for the analysis according to the results of a multiple comparisons test for equal variance. Shows that the zeta potential of unmodified carboxylate-PS particles is statistically different from the various particle PEGylations, which are all statistically similar to each other.
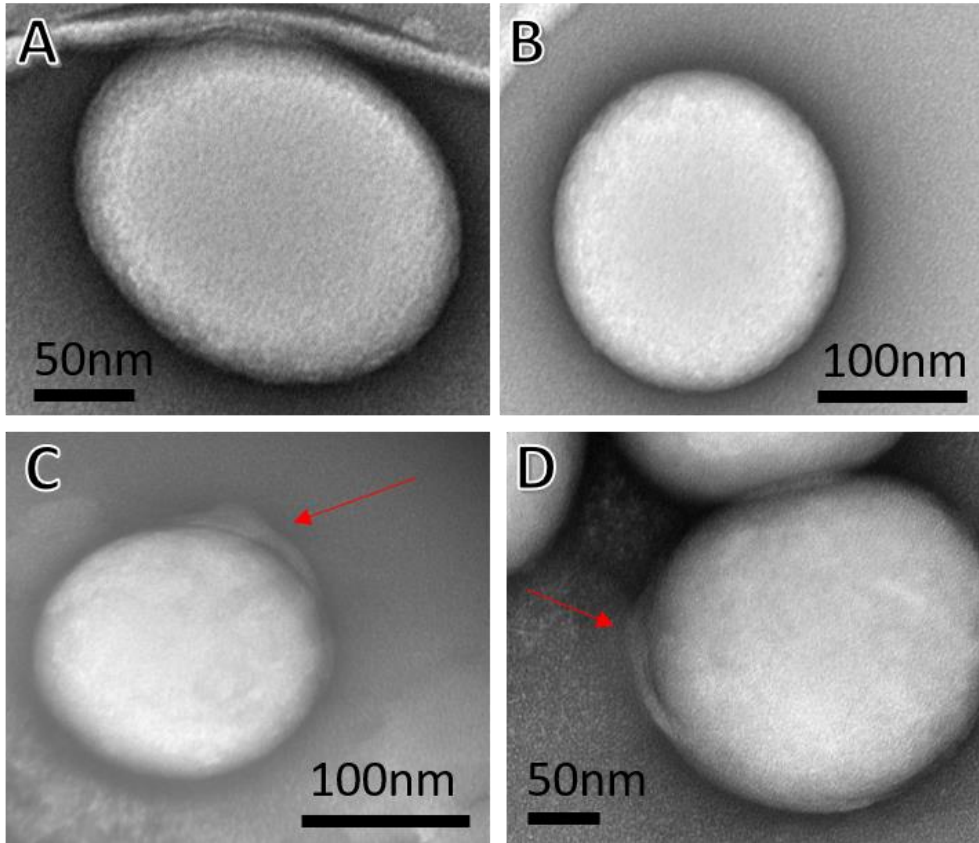
Figure S9. TEM images of PS particles A) unmodified, B) 1K PEG, C) 5K PEG, and D) 30K PEG. Red arrows point at the PEG on the surface of PS particles.

One-way ANOVA: Table 1 (Thicknesses)

Method

Null hypothesis   All means are equal
Alternative hypothesis   Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were not assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
Unmodified	7	63.07	5.33	(58.14, 68.00)
1K	5	72.39	11.39	(58.24, 86.53)
5K	5	64.80	5.69	(57.73, 71.87)
10K	5	69.33	5.46	(62.56, 76.11)
30K	7	60.79	2.91	(58.10, 63.48)

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Factor	4	10.3235	3.07	0.066

Grouping Information Using the Games-Howell Method and 95% Confidence

Factor	N	Mean	Grouping
1K	5	72.39	A
10K	5	69.33	A
5K	5	64.80	A
Unmodified	7	63.07	A
30K	7	60.79	A

Means that do not share a letter are significantly different.

Figure S10. Single Factor ANOVA analysis of calculated protein corona thickness on unmodified and various PEGylated PS particles after incubation in blood plasma. Equal variances were not assumed for the analysis according to the results of a multiple comparisons test for equal variance. Shows that the thicknesses of developed protein coronas are not statistically different across the variously functionalized PS particles.
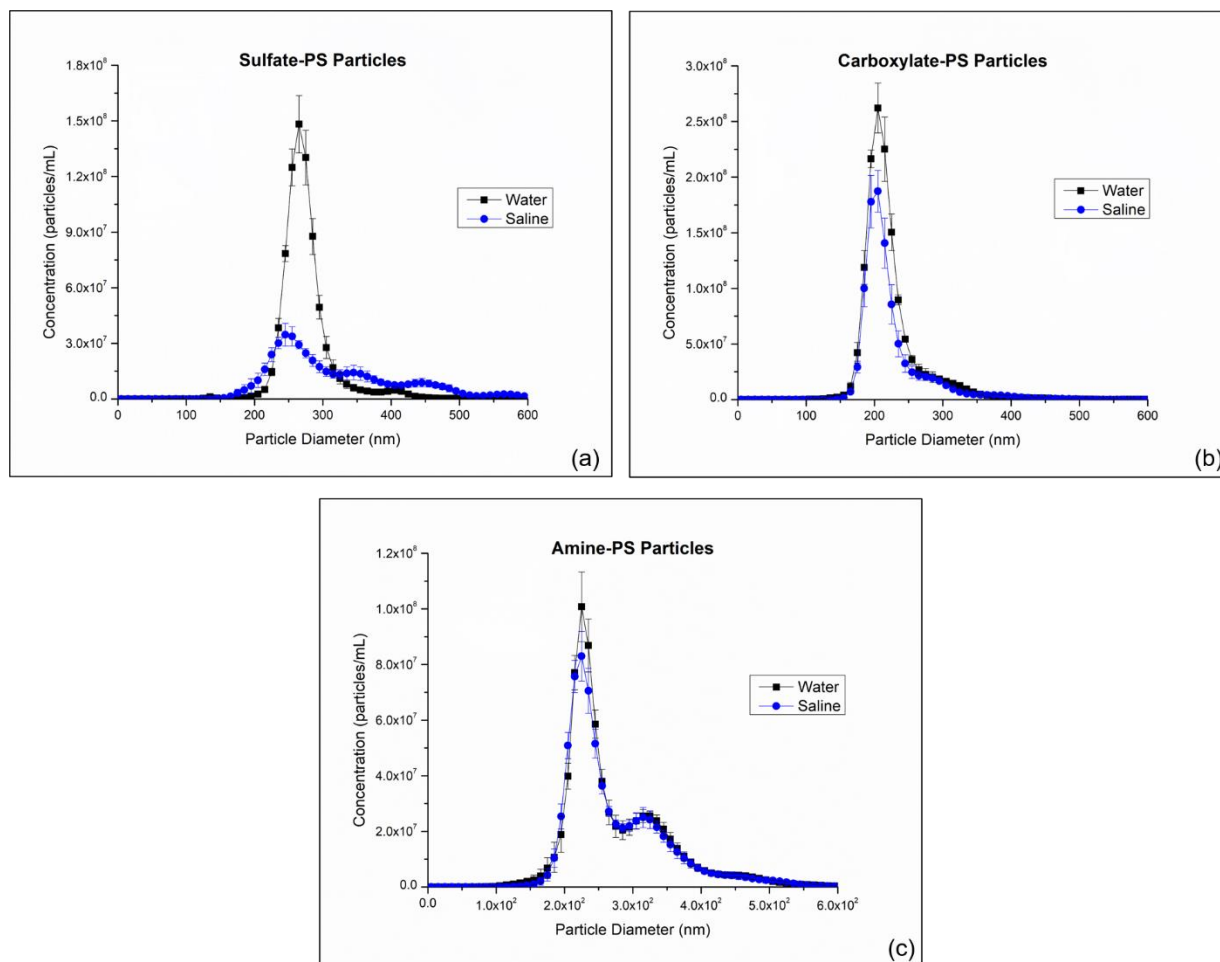
Figure S11. Aggregation behavior varies depending on surface chemistry and dispersant. NTA size distributions of PS particles with different surface modifications in saline and water. **a**) Sulfate-PS particles aggregate in saline, but not in water. **b**) Carboxylate-PS do not aggregate in saline or water. **c**) Amine-PS particles show some aggregation behavior in both saline and water.

One-way ANOVA: Figure 4-b

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were not assumed for the analysis.

Means

Factor	N	Mean	StDev	95% CI
Unmodified	9	1014	545	(595, 1433)
1K	9	201.6	103.7	(121.9, 281.4)
5K	9	236.0	238.0	(53.1, 418.9)
10K	10	262.8	222.9	(103.4, 422.2)

Welch's Test

Source	DF Num	DF Den	F-Value	P-Value
Factor	4	18.5502	4.41	0.011

Grouping Information Using the Games-Howell Method and 95% Confidence

Factor	N	Mean	Grouping	
Unmodified	9	1014	A	
30K	8	263.3		B
10K	10	262.8		B
5K	9	236.0		B
1K	9	201.6		B

Means that do not share a letter are significantly different.

Figure S12. Single Factor ANOVA analysis of PS particle aggregation behavior per 1×10^5 total particles in solution upon incubation in goat blood plasma. Equal variances were not assumed for the analysis according to the results of a multiple comparisons test for equal variance. Shows that the number of aggregates in unmodified PS particles is statistically different from the various particle PEGylations, which are all statistically similar to each other.
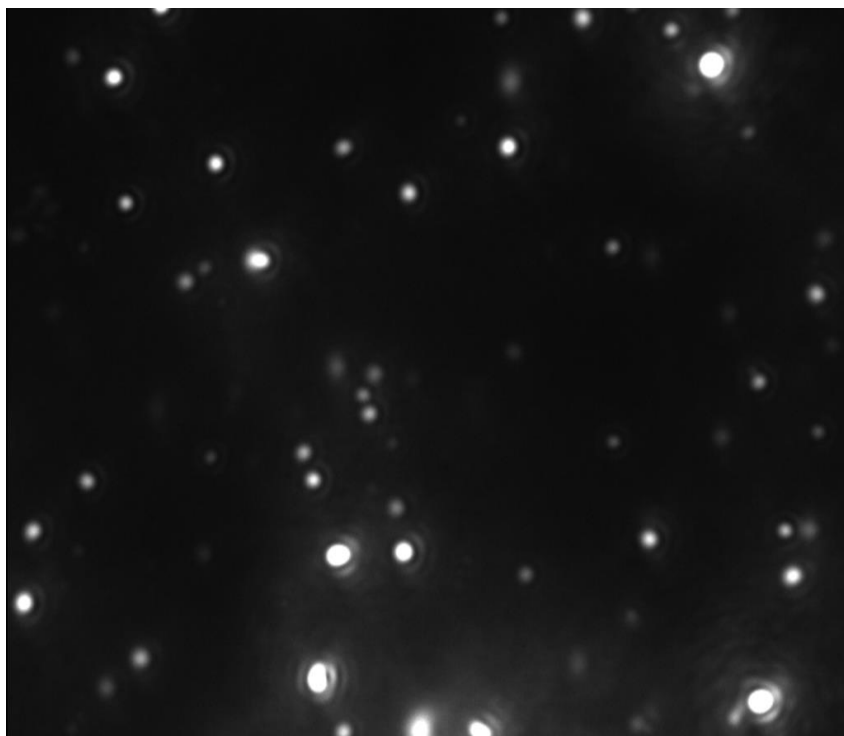
Figure S13. Still frame from a data collection of nanoparticles incubated in bovine blood plasma with sodium citrate anticoagulant under fluorescent mode, where white circles represent the nanoparticles. Using fluorescence, no other particles are seen except for the tagged nanoparticles.
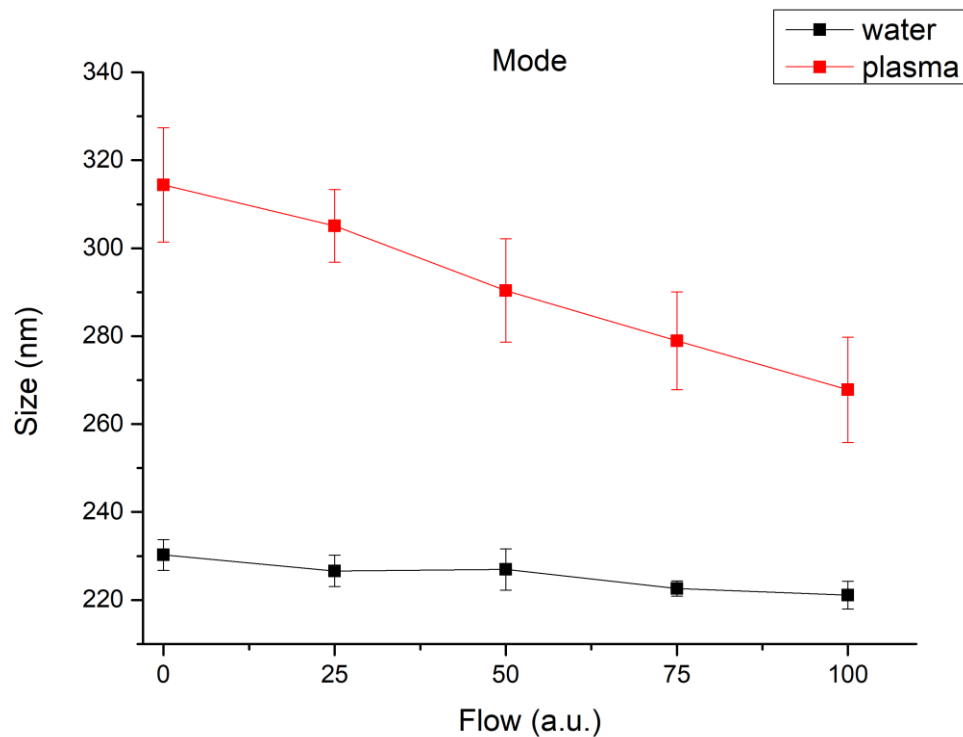
Figure S14. Demonstration of change in size by the effect of flow on PS particles when incubated with plasma. Red dots are unmodified PS particles incubated with pure plasma for 10 min and then characterized at different flow rates. Black dots are unmodified PS particles incubated with water and characterized at different flow rates. Plasma data shows a decrease in size based on the flow of the instrument while water data shows a constant size for all the different flow rates. Error bars refer to standard deviation. This experiment demonstrates a loss on the soft corona based on flow rate.

Table S1. Approximate percent plasma present for each dilution factor for goat blood with an Alsever's solution anticoagulent. For the first dilution factor, the calcuation for percent plasma was based on a 50:50 mixture of whole blood and Alsevers solution. Considering whole blood is approximately 55% plasma, this results in a baseline percent plasma of approximately 35.50%.

Dilution Factor	Percent Plasma (%)
1	35.50
2	17.75
4	8.88
8	4.44
16	2.22
32	1.11
64	0.55
128	0.28
256	0.14
512	0.07

Table S2 Kinematic Viscosity of Dilutions of Goat Blood Plasma and Saline Converted to Dynamic Viscosity Using Density

Dilution Factor	Measured Kinematic Viscosity $(\frac{m^2}{s})$	Density $(\frac{kg}{m^3})$	Converted Dynamic Viscosity $(\frac{kg}{m \times s})$	Standard Error $(\frac{kg}{m \times s})$
1	0.970	1	0.970	0.003
2	0.841	1	0.841	0.006
4	0.769	1	0.769	0.003
8	0.747	1	0.747	0.005
16	0.732	1	0.732	0.003
32	0.727	1	0.727	0.004
64	0.722	1	0.722	0.001
128	0.736	1	0.736	0.002
256	0.730	1	0.730	0.001
512	0.738	1	0.738	0.003

Table S3. Mode hydrodynamic diameters of PS particles with various PEGylations in saline and blood plasma measured by NTA. Includes calculated protein corona thickness of the particle variants in goat blood plasma. Displayed error represent standard error for each data set.

PS Particle Modification	Mode Hydrodynamic Diameter in Saline (nm)	Mode Hydrodynamic Diameter in Goat Blood Plasma (nm)	Calculated Protein Corona Thickness in Goat Blood Plasma (nm)
Unmodified	196.1 +/- 1.0	322.3+/- 2.4	63.1 +/- 2.2
1K PEGylated	209.0 +/- 3.2	353.8 +/- 4.7	72.4 +/- 4.3
5K PEGylated	208.6 +/- 1.3	338.2 +/- 2.7	64.8 +/- 2.4
10K PEGylated	216.1 +/- 2.1	354.8 +/- 4.1	69.3 +/- 3.8
30K PEGylated	244.7 +/- 1.6	366.3 +/- 2.2	60.8 +/- 2.1