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Supplementary Information

Protein binding on acutely toxic and non-toxic polystyrene nanoparticles during filtration by *Daphnia magna*

Egle Kelpsiene, ^{ab} Irene Brandts, ^{cd} Katja Bernfur, ^a Mikael T. Ekvall, ^{be} Martin Lundqvist, ^{ab} Mariana Teles, ^{cd} and Tommy Cedervall *ab

^a Department of Biochemistry and Structural Biology, Lund University, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

^c Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

d Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, 08193
 Barcelona, Spain

^e Department of Aquatic Ecology Unit, Lund University, Lund, Sweden

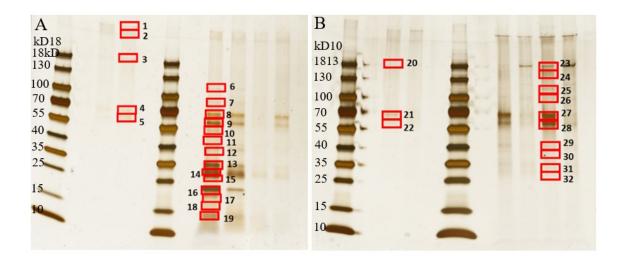
*Corresponding author. e-mail: tommy.cedervall@biochemistry.lu.se

Supplementary Table 1. The size of PS particles before and after filtration by D. magna. All measurements are at least in triplicate.

Particle	Before	filtration	After	filtration
	Diameter	Polydispersity	Diameter	Polydispersity
	(nm)	(%)	(nm)	(%)

^b NanoLund, Lund University, Box 118, SE-221 00 Lund, Sweden

53 nm PS-NH ₂	47 ± 1	10 ± 2	215 ± 12	multimodal
200 nm PS-NH ₂	179 ±	1 ± 1	180 ± 2	6 ± 2
62 nm PS-COOH	61 ± 1	14 ± 2	95 ± 6	multimodal
200 nm PS-COOH	180 ± 4	8 ± 3	191 ± 6	7 ± 2



Supplementary Figure 1. SDS gels with silver staining. The numbers represent the bands used for mass spectrometry analyses for the different conditions. (A) Control group (1-5 bands) and 53 nm PS–NH₂ incubated with *D. magna* (6-19 bands). (B) Control group (20-22 bands) and 200 nm PS–NH₂ incubated with *D. magna* (23-32 bands).

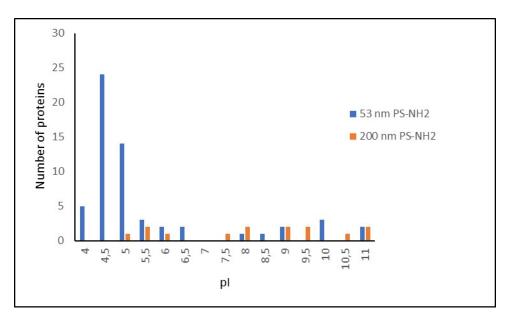
Supplementary Table 2. All identified proteins with a score number of 50 or larger and at least two identified unique peptides. ("NA" – Pi was not available as the sequence contains several consecutive undefined amino acids).

Treatment	Band	MW	Protein name	Accession number	MW	pI
53 nm PS–NH ₂	6	121	Protease m1 zinc metalloprotease	A0A0P5E8H8	109	4.59
				A0A0P6JK73	109	4.61
				A0A0N8AXC2	85	4.66
			Histone H4	A0A0N8AML8	11	11.33
	7	90	Neutral endopeptidase (Fragment)	A0A0N8A4R4	82	4.67
			Putative Neutral endopeptidase	A0A0P5QHD4	35	4.83
			Beta-galactosidase-1 protein 2	A0A0P4WLE9	63	4.53

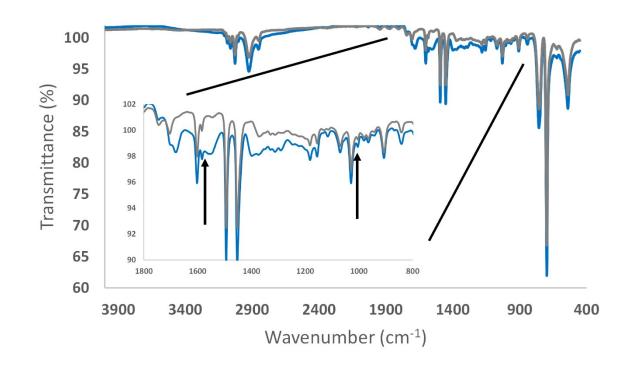
		Protease m1 zinc metalloprotease	A0A0N8B8B4	89	4.58
		Lamin-A	A0A0P6IZ96	58	5.64
8	70	Actin, alpha skeletal muscle	A0A0N8AKX4	41	5.49
			A0A0N7ZFH2	49	NA
		Tubulin beta chain	A0A0P6CCV0	49	4.78
			A0A0N7ZH16	52	4.75
		Tubulin alpha chain	A0A0P5ZYN7	50	4.94
		Fructose-bisphosphate aldolase	A0A0N8DTE5	39	8.30
		Heat shock 70 kDa protein	A0A0P6DCT5	70	5.37
		cognate Histone H2A (Fragment)	A0A0P6A1I4	14	11.23
		ATP synthase subunit beta	A0A0P6CBB6	56	5.19
		Eukaryotic initiation factor 4A-	A0A0P6CFN1	46	5.32
		II Histone H2B (Fragment)	A0A0P6A3S1	13	10.32
		`	A0A0P5WWR3	50	9.10
		Elongation factor 1-alpha			
		Armadillo segment polarity protein	A0A0P6IJT4	90	5.18
		ATP synthase subunit alpha (Fragment)	A0A0N8AAM5	51	NA
9	55	Beta-klotho	A0A0N7ZV82	48	NA
		Glucosylceramidase	A0A0N8A576	61	4.66
		Adenosine deaminase CECR1-	A0A0N7ZT57	53	4.75
		Alkaline phosphatase (Fragment)	A0A0N8B5J6	34	4.80
10	55	Lamin-A	A0A0P6IZ96	58	5.64
11	43	Actin, alpha skeletal muscle	A0A0N8AKX4	42	5.5
		Uncharacterized protein	A0A0N8E6U4	43	4.74
		Chymotrypsin elastase family member 2A	A0A0N7ZRR8	20	4.3
		Ubiquitin-40S ribosomal protein S27a	A0A0N7ZFP2	13	10.13
		Elongation factor 1-alpha	A0A0N7ZL24	49	8.81
		Calmodulin	A0A0P6C8L3	17	4.04
12	38	Mannan endo-1	A0A0N8AWI2	49	5.7
		Putative Brain chitinase and chia	A0A0N8AJS7	31	4.4
13	35	Carboxypeptidase B	A0A0P6GRN0	47	5.0
			A0A0N8A0G8	47	4.7
			A0A0N8DXD3	51	5.2
		Poly(U)-specific endoribonuclease	A0A0N8AU82	41	4.5
		Serine protease	A0A0N7ZYN7	32	4.9

			Zinc carboxypeptidase	A0A0N8ASM6	49	5.3
			Pancreatic triacylglycerol lipase	A0A0N8A9Z9	38	NA
			Uncharacterized protein	A0A0N7ZR74	50	4.56
	14	30	Serine protease	A0A0N7ZYN7	32	4.9
			Chymotrypsin elastase family member 2A	A0A0N7ZN74	33	5.4
			Neurexin IV	A0A0N8BJ04	32	4.2
	15	27	Chymotrypsin elastase family member 2A	A0A0N7ZN74	33	5.4
			Uncharacterized protein	A0A0N7ZEA0	20	4.53
			Metalloendopeptidase (Fragment)	A0A0N8BGG7	33	6.1
	16	22	Chymotrypsin elastase family member 2A	A0A0N7ZRR8	20	4.6
	17	19	Chymotrypsin elastase family member 2A	A0A0N7ZRR8	20	4.6
	19	15	Elongation factor 1-alpha	A0A0N7ZYV1	50	9.0
			Heat shock 70 kDa protein	A0A0N8AGX4	67	5.29
				A0A0N8CD53	71	5.14
			C-type lectin domain family 6 member A	A0A0N7ZMI0	17	4.9
			Actin, alpha skeletal muscle (Fragment)	A0A0P5ZI52	31	4.94
			Putative Chymotrypsin elastase family member 2A	A0A0N7ZSD8	28	NA
			Uncharacterized protein	A0A0N8AVI2	40	5.33
			ADP-ribosylation factor (Fragment)	A0A0N8BN21	19	6.4
			60S ribosomal protein L23 (Fragment)	A0A164I9D0	22	10.2
			Histone H4	A0A0N8AML8	11	11.3
			ATP synthase subunit beta	A0A0N7ZMA0	57	5.14
200 nm PS–NH ₂	23	190	Vitellogenin-1	A0A0N8ERH4	171	9.0
	24	170	Vitellogenin-1	A0A0N8ERH4	171	9.0
			Actin, alpha skeletal muscle	A0A0N7ZDS7	37	5.7
	25	120	Hemocyte protein-glutamine gamma-glutamyltransferase	A0A0N8CBE8	105	7.9
				A0A0P5CTZ7	109	NA
				A0A0P5CW37	101	8.4
				A0A0N8AI27	105	8.2
			Putative Hemocyte protein- glutamine gamma- glutamyltransferase (Fragment)	A0A0P5MNQ2	45	5.90
			Histone H4	A0A0N8AML8	11	11.33
			Elongation factor 1-alpha	A0A0P5WWR3	50	9.10
			40S ribosomal protein S3	A0A0P6A3F6	27	9.68
			Actin, alpha skeletal muscle	A0A0N8AKX4	42	5.49

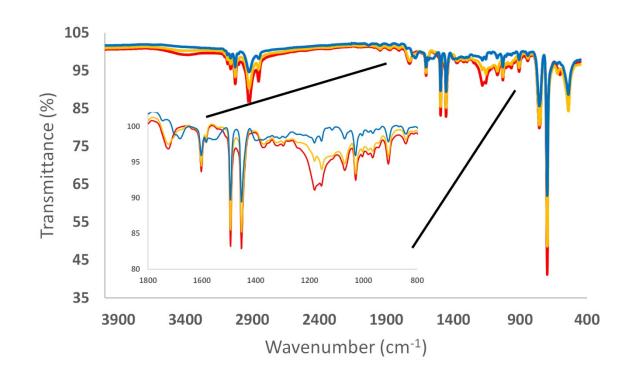
			40S ribosomal protein S1	A0A0P6CD52	18	10.99
			Putative Vitellogenin-1 (Fragment)	A0A0N8ABJ0	92	NA
			Eukaryotic initiation factor 4A- II	A0A0P6CFN1	46	5.32
			Elongation factor 1-gamma (Fragment)	A0A0P6CFP1	50	6.33
			60S ribosomal protein L8	A0A0P6CEH7	28	11.04
			40S ribosomal protein S14	A0A0N8B0M4	22	9.60
			Heat shock 70 kDa protein cognate	A0A0N7ZMQ1	71	5.25
				A0A0P6A337	72	5.10
			40S ribosomal protein S9	A0A0P6DE13	23	10.66
			40S ribosomal protein S25	A0A0P6CKS1	14	10.12
			40S ribosomal protein S26 (Fragment)	A0A0N8A385	18	10.80
	26	100	Hemocyte protein-glutamine gamma-glutamyltransferase	A0A0P5CTZ7	109	NA
				A0A0N8AI27	105	8.2
				A0A0P5C8I4	108	7.3
	27	70	Putative Hemocyte protein- glutamine gamma- glutamyltransferase	A0A0N8AYD5	68	6.6
			Double oxidase: two peroxidase domains	A0A0N8CQ82	131	NA
				A0A0N7ZFU0	54	9.2
	28	60	Hemocyte protein-glutamine gamma-glutamyltransferase	A0A0P5PVU7	93	NA
				A0A0N8AXK8	103	NA
				A0A0P5MNQ2	45	5.9
	29	45	Uncharacterized protein	A0A0N7ZTW3	59	5.98
	30	38	Uncharacterized protein	A0A0N7ZTW3	59	5.98
			Putative Hemocyte protein- glutamine gamma- glutamyltransferase	A0A0N8AYD5	68	6.6
	31	33	Hemocyte protein-glutamine gamma-glutamyltransferase	A0A0P5CTZ7	109	NA
				A0A0N8AI27	105	8.2
	32	30	Hemocyte protein-glutamine gamma-glutamyltransferase	A0A0P5CTZ7	109	NA
				A0A0N8AI27	105	8.2
			Chymotrypsin elastase family member 2A	A0A0N7ZRR8	20	4.6
Control	20	190	Vitellogenin-1	A0A0N8ERH4	171	9.0



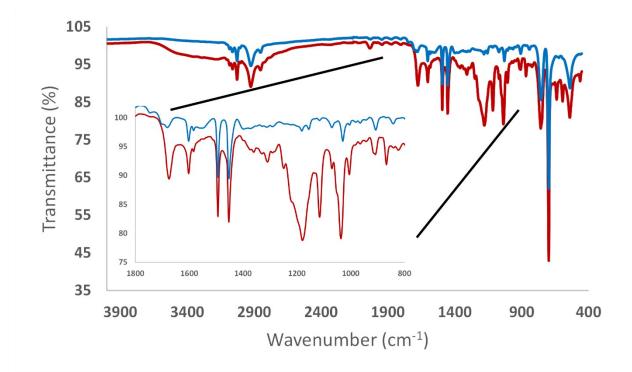
Supplementary Figure 2. The distribution pf proteins bound to 50 nm and 200 nm PS-NH₂. The proteins are grouped with pl between 4.0 to 4.5 and so on.



Supplementary Figure 3. Comparison between plain and amine polystyrene particles of the size \sim 200 nm. ATR-FTIR spectrum for PS-NH2 200 nm in blue and PS 195 nm in gray. The inset is a zoom-in on the area of wavenumber 800 -1800 cm⁻¹. The two arrows point at signals at wavenumber \sim 1570 cm⁻¹ which have been assigned to the stretching vibrations of NH₂ (Ref: 10.1021/acs.est.6b02846) and wavenumber \sim 1005 cm⁻¹ which have been assigned to stretching vibrations of C-N bonds (ref:10.1016/j.jcis.2015.03.052).



Supplementary Figure 4. Comparison between amine polystyrene particles of the size **50, 52, and 200 nm**. ATR-FTIR spectrum for PS-NH2 200 nm in blue, PS-NH2 50 nm in red, and PS-NH2 52 nm in orange. The inset is a zoom-in on the area of wavenumber 800 - 1800 cm⁻¹.



Supplementary Figure 5. Comparison between amine polystyrene particles of the size **180 and 200 nm**. ATR-FTIR spectrum for PS-NH2 200 nm in blue and PS-NH2 180 nm in dark red. The inset is a zoom-in on the area of wavenumber 800 -1800 cm⁻¹.

Supplementary Table 3. Proteins were identified in the content of the pellets after nanoparticles were filtrated by the digestive system of *D. magna* for 4 h in comparison with a control group. All proteins with a score number of 50 or larger and at least two identified unique peptides were taken into consideration.

Treatment	Accession	Score	Num. of	emPAI	Description
			significant		
			sequences		
53 nm PS-	A0A0N8ERH4	1371	33	1.74	Vitellogenin-1
NH ₂					
	A0A0P5XJT6	227	5	1.56	Uncharacterized protein (Fragment)
	A0A164FM53	148	3	10.53	Uncharacterized protein (Fragment)
	A0A0P5UGM5	115	3	0.99	Uncharacterized protein (Fragment)
	A0A0N8B1S8	1006	16	1.23	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0N8AYD5	555	9	0.86	Putative Hemocyte protein-glutamine
					gamma-glutamyltransferase
	A0A0P5E182	416	9	0.74	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0N7ZTW3	470	9	0.91	Uncharacterized protein (Fragment)
	A0A0N7ZU01	372	5	0.74	Di-domain hemoglobin
	A0A0P4XQ89	336	4	0.56	Di-domain hemoglobin
	A0A0P5F3Z1	190	4	0.68	Di-domain hemoglobin
	A0A0P5D088	247	4	0.83	Chymotrypsin elastase family member
					2A (Fragment)
	A0A0N8BSM2	211	2	0.3	Uncharacterized protein

	A0A0N8AW39	194	2	0.3	Uncharacterized protein
	A0A0P5EK64	164	2	0.28	Uncharacterized protein (Fragment)
	A0A0N7ZE53	182	2	0.18	Uncharacterized protein
	A0A0N8BCY1	177	2	0.23	Chymotrypsin BI
	A0A0P5CJN7	159	2	1.25	Mucin peritrophin
	A0A0N7ZDU7	155	4	0.61	Actin, alpha skeletal muscle
					(Fragment)
	A0A0N7ZYN7	149	3	0.48	Serine protease
	J9R260	134	3	0.61	Trypsin 152 (Fragment)
	A0A0N7ZFA7	131	3	0.59	Serine protease (Fragment)
	A0A0N7ZMQ5	134	2	0.33	Putative Serine protease P76
					(Fragment)
	A0A0P5HL93	123	5	0.23	Uncharacterized protein
	A0A0N7ZUL9	118	2	0.23	Uncharacterized protein (Fragment)
	A0A0P4Z5S1	117	2	0.5	Putative Secreted ferritin g subunit
200 nm PS-	A0A0N8ERH4	591	19	0.64	Vitellogenin-1
NH_2					
	A0A0P6B5M1	551	12	0.33	Vitellogenin-1
	A0A0P6CX53	537	13	0.44	Vitellogenin-1
	A0A0N8A166	294	3	0.27	Vitellogenin-1-like protein
	A0A0P5D5M6	269	4	0.4	Vitellogenin-1-like protein
	A0A0N8B626	169	4	0.1	Vitellogenin-1
	А0А0Р6НЈВ7	290	6	0.88	Actin, alpha skeletal muscle
	A0A0N8A3C7	268	6	0.82	Actin, alpha skeletal muscle
	A0A0N7ZEH2	194	3	0.13	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0N7ZYN7	188	4	0.68	Serine protease
	A0A0N7ZSD8	162	3	0.56	Putative Chymotrypsin elastase family
					member 2A

	A0A0N7ZES6	154	2	0.38	Obstructor-A
	A0A0A7CK57	149	4	0.52	Arginine kinase
	A0A0N7ZEE0	113	2	0.33	Gly d 3
	A0A0N7ZUW0	111	3	0.03	Mucin 5AC, oligomeric mucus/gel-
					forming
	A0A0N8AJS9	108	2	0.08	Uncharacterized protein (Fragment)
62 nm PS-	A0A0N8ERH4	1160	29	1.29	Vitellogenin-1
СООН					
	A0A0N8ACD3	328	5	0.3	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0P5CTZ7	137	3	0.13	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0P5PVU7	130	4	0.21	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0N7ZEH2	86	4	0.18	Hemocyte protein-glutamine gamma-
					glutamyltransferase
	A0A0N7ZUW0	309	7	0.06	Mucin 5AC, oligomeric mucus/gel-
					forming
	A0A0N8A626	285	8	0.79	Uncharacterized protein
	A0A0N7ZYN7	210	3	0.5	Serine protease
	A0A0P5LBK9	204	3	0.13	Uncharacterized protein
	A0A0N8AGI9	201	3	0.12	Tissue factor pathway inhibitor
	A0A0N8AJD1	131	3	0.14	Tissue factor pathway inhibitor
	A0A0N8A945	197	4	0.52	Actin, alpha skeletal muscle
	A0A0N7ZTW3	136	3	0.25	Uncharacterized protein (Fragment)
200 nm PS-	A0A0N8ERH4	875	22	0.9	Vitellogenin-1
СООН					
	A0A0P5UGM5	200	3	0.99	Uncharacterized protein (Fragment)
	A0A0P5M5E3	175	4	0.67	Vitellogenin-1-like protein (Fragment)

	A0A0P5XJT6	160	4	1.12	Uncharacterized protein (Fragment)
	A0A164FM53	149	3	5.26	Uncharacterized protein (Fragment)
	A0A164G126	138	3	5.26	Uncharacterized protein (Fragment)
	A0A0N8A945	220	5	0.64	Actin, alpha skeletal muscle
	A0A0N8AUD4	212	3	0.11	Tissue factor pathway inhibitor
	A0A0N8AGI9	161	3	0.11	Tissue factor pathway inhibitor
	A0A0N7ZYN7	180	4	0.68	Serine protease
	A0A0N7ZSD8	154	3	0.56	Putative Chymotrypsin elastase family
					member 2A
	A0A0N7ZTW3	141	2	0.15	Uncharacterized protein (Fragment)
	A0A0N7ZUW0	112	4	0.03	Mucin 5AC, oligomeric mucus/gel-
					forming
Control	A0A0N7ZYN7	242	4	0.68	Serine protease
	A0A0N7ZSD8	200	3	0.56	Putative Chymotrypsin elastase family
					member 2A
	A0A0P6H0J1	148	3	0.44	Uncharacterized protein (Fragment)
	A0A0P5CAU9	125	2	0.59	Uncharacterized protein (Fragment)
	A0A0N7ZE19	117	2	0.52	Uncharacterized protein (Fragment)
	A0A0N8BCY1	148	2	0.23	Chymotrypsin BI
	A0A0N8AZL9	130	2	0.3	Trypsin serine protease
	A0A0N7ZUL9	129	2	4.69	Uncharacterized protein (Fragment)
	A0A0P4WVW2	102	2	7.58	Uncharacterized protein
	A0A0N7ZMQ5	101	2	5.92	Putative Serine protease P76
					(Fragment)