Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information for:

Effect of protein corona on nanoparticle-plasma membrane and nanoparticle-biomimetic membrane interactions

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Table S1. Representative proteins identified in the protein corona formed on uncharged, carboxyl-, and amine-polystyrene nanoparticles by LC-MS-MS.

SPNP**		CPNP**		APNP**	
Acc. no.*	**Protein identity**	**Acc. no.***	**Protein identity**	**Acc. no.***	**Protein identity**
P06727	Apolipoprotein A-IV	**P02647**	Apolipoprotein A-I	**P02647**	Apolipoprotein A-I
P02768	Serum albumin	**P02649**	Apolipoprotein E	**P02649**	Apolipoprotein E
P02647	Apolipoprotein A-I	**P06727**	Apolipoprotein A-IV	**P04004**	Vitronectin
P02649	Apolipoprotein E	**P01024**	Complement C3	**P02652**	Apolipoprotein A-II
P01024	Complement C3	**P01008**	Antithrombin-III	**P10909-5**	Isoform 5 of Clusterin
P04114	Apolipoprotein B-100	**P10909-4**	Isoform 4 of Clusterin		
P10909-4	Isoform 4 of Clusterin	**P02768**	Serum albumin		
P02652	Apolipoprotein A-II	**P04004**	Vitronectin		
P01009	Alpha-1-antitrypsin	**P00734**	Prothrombin		
P04004	Vitronectin	**P04196**	Histidine-rich glycoprotein		
P02654	Apolipoprotein C-I	**P02652**	Apolipoprotein A-II		
P01008	Antithrombin-III	**P02654**	Apolipoprotein C-I		
P02774-3	Isoform 3 of Vitamin D-binding protein	**P04114**	Apolipoprotein B-100		
P02655	Apolipoprotein C-II	**P55056**	Apolipoprotein C-IV		
P00338-4	Isoform 4 of L-lactate dehydrogenase A chain	**Q03591**	Complement factor H-related protein 1		
P05546	Heparin cofactor 2	**P05546**	Heparin cofactor 2		
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	**P19823**	Inter-alpha-trypsin inhibitor heavy chain H2		
P30101	Protein disulfide-isomerase A3	**O14791-3**	Isoform 3 of Apolipoprotein L1		
P00738	Haptoglobin				
P31146	Coronin-1A				
P06703	Protein S100-A6				
P17661	Desmin				
P19823	Inter-alpha-trypsin inhibitor heavy chain H2				
P05090	Apolipoprotein D				
P16402	Histone H1.3				
P07195	L-lactate dehydrogenase B chain				
P00734	Prothrombin				

* Uniprot accession number.

** SPNP: 100 nm sulfate-functionalized polystyrene nanoparticles; CPNP: 100 nm carboxyl-functionalized polystyrene nanoparticles; APNP: 100 nm amine-functionalized polystyrene nanoparticles.

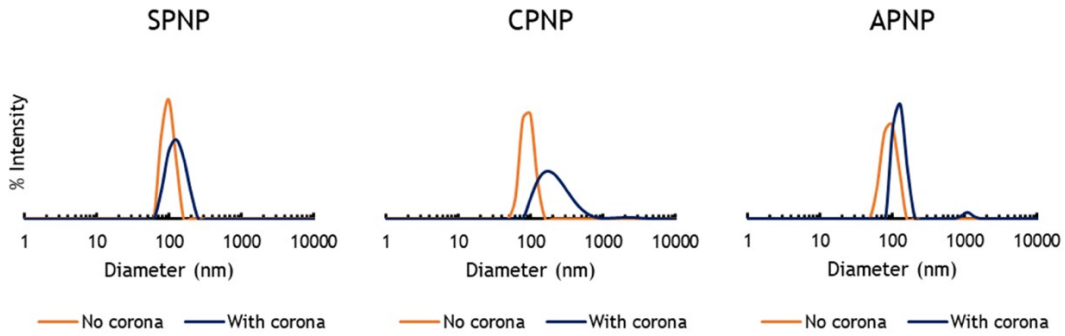

Figure S1. Size distribution of polystyrene nanoparticles with and without the presence of a protein corona. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP.

Table S2. Hydrodynamic diameters and Zeta potential of PNPs in FBS-absent cell culture media DMEM and complete media cDMEM.

		In DMEM		In cDMEM	
		Without corona	With corona	Without corona	With corona
Hydrodynamic Diameter (nm)	Sulfate-PNP	85.86 ± 4.15	114.63 ± 12.78	104.85 ± 10.00	106.14 ± 20.58
	Carboxyl-PNP	95.62 ± 10.22	136.04 ± 28.79	101.64 ± 12.72	124.03 ± 35.92
	Amine-PNP	645.96 ± 107.95	135.13 ± 18.53	123.53 ± 31.81	128.26 ± 35.42
Zeta potential (mV)	Sulfate-PNP	-30.39 ± 1.43	-12.14 ± 2.56	-7.00 ± 4.12	-6.65 ± 5.53
	Carboxyl-PNP	-25.70 ± 1.64	-8.78 ± 6.11	-11.45 ± 5.05	-8.97 ± 4.98
	Amine-PNP	-5.30 ± 12.79	-10.45 ± 3.65	-10.78 ± 0.76	-12.39 ± 4.38

Table S3. Unpaired t-test result of viability MTT assay.

PNP	Time	Level 1	Level 2	Mean 1	Mean 2	Mean Difference	P-value	P value summary
SPNP	4h	-FBS/-corona	-FBS/+corona	109.177	86.282	22.895	0.0571	ns
		-FBS/-corona	+FBS/-corona	109.177	85.978	23.199	0.0133	*
		-FBS/-corona	+FBS/+corona	109.177	90.923	18.254	0.0252	*
		-FBS/+corona	+FBS/-corona	86.282	85.978	0.304	0.9747	ns
		-FBS/+corona	+FBS/+corona	86.282	90.923	-4.641	0.6118	ns
		+FBS/-corona	+FBS/+corona	85.978	90.923	-4.945	0.3933	ns
	15h	-FBS/-corona	-FBS/+corona	128.520	102.928	25.592	0.0195	*
		-FBS/-corona	+FBS/-corona	128.520	101.062	27.458	0.0123	*
		-FBS/-corona	+FBS/+corona	128.520	94.193	34.327	0.0065	**
		-FBS/+corona	+FBS/-corona	102.928	101.062	1.866	0.6374	ns
		-FBS/+corona	+FBS/+corona	102.928	94.193	8.736	0.0311	*
		+FBS/-corona	+FBS/+corona	101.062	94.193	6.870	0.1405	ns
CPNP	4h	-FBS/-corona	-FBS/+corona	108.325	84.957	23.368	0.0768	ns
		-FBS/-corona	+FBS/-corona	108.325	92.251	16.075	0.0564	ns
		-FBS/-corona	+FBS/+corona	108.325	97.417	10.908	0.1441	ns
		-FBS/+corona	+FBS/-corona	84.957	92.251	-7.293	0.4797	ns
		-FBS/+corona	+FBS/+corona	84.957	97.417	-12.460	0.2591	ns
		+FBS/-corona	+FBS/+corona	92.251	97.417	-5.166	0.14	ns
	15h	-FBS/-corona	-FBS/+corona	130.606	112.074	18.532	0.0579	ns
		-FBS/-corona	+FBS/-corona	130.606	103.329	27.277	0.0014	**
		-FBS/-corona	+FBS/+corona	130.606	86.544	44.062	0.0092	**
		-FBS/+corona	+FBS/-corona	112.074	103.329	8.745	0.2733	ns
		-FBS/+corona	+FBS/+corona	112.074	86.544	25.530	0.0567	ns
		+FBS/-corona	+FBS/+corona	103.329	86.544	16.785	0.1406	ns
APNP	4h	-FBS/-corona	-FBS/+corona	79.281	95.553	-16.272	0.0307	*
		-FBS/-corona	+FBS/-corona	79.281	89.520	-10.239	0.1827	ns
		-FBS/-corona	+FBS/+corona	79.281	95.867	-16.586	0.0071	**
		-FBS/+corona	+FBS/-corona	95.553	89.520	6.033	0.443	ns
		-FBS/+corona	+FBS/+corona	95.553	95.867	-0.314	0.9537	ns
		+FBS/-corona	+FBS/+corona	89.520	95.867	-6.347	0.3616	ns
	15h	-FBS/-corona	-FBS/+corona	7.461	82.070	-74.609	0.0026	**
		-FBS/-corona	+FBS/-corona	7.461	79.603	-72.143	0	***
		-FBS/-corona	+FBS/+corona	7.461	77.620	-70.160	0	***
		-FBS/+corona	+FBS/-corona	82.070	79.603	2.466	0.7977	ns
		-FBS/+corona	+FBS/+corona	82.070	77.620	4.449	0.659	ns
		+FBS/-corona	+FBS/+corona	79.603	77.620	1.983	0.6364	ns

Table S4. Unpaired t-test result of viability LDH assay.

PNP	Time	Level 1	Level 2	Mean 1	Mean 2	Mean Difference	P-value	P value summary
SPNP	4h	-FBS/-corona	-FBS/+corona	2.027	4.817	-2.790	0.1436	ns
		-FBS/-corona	+FBS/-corona	2.027	2.761	-0.734	0.5381	ns
		-FBS/-corona	+FBS/+corona	2.027	4.936	-2.909	0.0609	ns
		-FBS/+corona	+FBS/-corona	4.817	2.761	2.056	0.2105	ns
		-FBS/+corona	+FBS/+corona	4.817	4.936	-0.120	0.9330	ns
		+FBS/-corona	+FBS/+corona	2.761	4.936	-2.175	0.0038	**
	15h	-FBS/-corona	-FBS/+corona	1.886	3.119	-1.233	0.3405	ns
		-FBS/-corona	+FBS/-corona	1.886	2.933	-1.047	0.3891	ns
		-FBS/-corona	+FBS/+corona	1.886	1.539	0.347	0.7606	ns
		-FBS/+corona	+FBS/-corona	3.119	2.933	0.186	0.7614	ns
		-FBS/+corona	+FBS/+corona	3.119	1.539	1.580	0.0512	ns
		+FBS/-corona	+FBS/+corona	2.933	1.539	1.394	0.0042	**
CPNP	4h	-FBS/-corona	-FBS/+corona	1.172	4.333	-3.162	0.0497	*
		-FBS/-corona	+FBS/-corona	1.172	1.991	-0.820	0.2184	ns
		-FBS/-corona	+FBS/+corona	1.172	4.970	-3.798	0.0038	**
		-FBS/+corona	+FBS/-corona	4.333	1.991	2.342	0.1089	ns
		-FBS/+corona	+FBS/+corona	4.333	4.970	-0.637	0.6276	ns
		+FBS/-corona	+FBS/+corona	1.991	4.970	-2.979	0.0110	*
	15h	-FBS/-corona	-FBS/+corona	2.067	3.083	-1.015	0.0900	ns
		-FBS/-corona	+FBS/-corona	2.067	1.365	0.702	0.0318	*
		-FBS/-corona	+FBS/+corona	2.067	1.568	0.499	0.1529	ns
		-FBS/+corona	+FBS/-corona	3.083	1.365	1.718	0.0202	*
		-FBS/+corona	+FBS/+corona	3.083	1.568	1.514	0.0278	*
		+FBS/-corona	+FBS/+corona	1.365	1.568	-0.203	0.5045	ns
APNP	4h	-FBS/-corona	-FBS/+corona	10.954	5.114	5.840	0.0035	**
		-FBS/-corona	+FBS/-corona	10.954	5.070	5.884	0.0001	***
		-FBS/-corona	+FBS/+corona	10.954	5.539	5.415	0.0011	**
		-FBS/+corona	+FBS/-corona	5.114	5.070	0.044	0.9679	ns
		-FBS/+corona	+FBS/+corona	5.114	5.539	-0.424	0.7267	ns
		+FBS/-corona	+FBS/+corona	5.070	5.539	-0.469	0.5980	ns
	15h	-FBS/-corona	-FBS/+corona	44.606	5.766	38.840	0.0001	***
		-FBS/-corona	+FBS/-corona	44.606	4.588	40.017	0.0007	***
		-FBS/-corona	+FBS/+corona	44.606	4.472	40.133	0.0008	***
		-FBS/+corona	+FBS/-corona	5.766	4.588	1.178	0.4884	ns
		-FBS/+corona	+FBS/+corona	5.766	4.472	1.294	0.4465	ns
		+FBS/-corona	+FBS/+corona	4.588	4.472	0.116	0.8071	ns

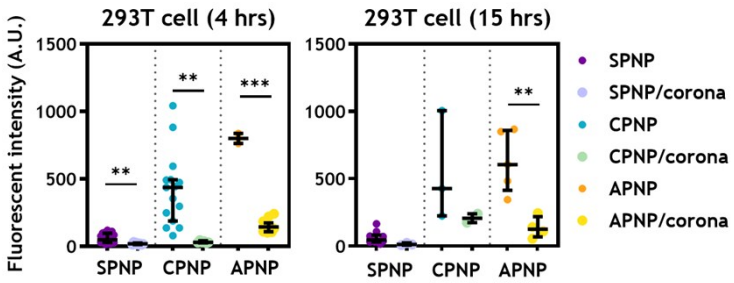

Figure S2. Fluorescent intensity of adsorbed nanoparticles on 293T cells after 4 h and 15 h incubation. Medians and interquartile ranges of calibrated fluorescence intensity were demonstrated along with individual values in graphs. There is no significant increase of fluorescent intensity between 4 h and 15 h. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP. (Unpaired t-test, * significant at $p < .05$, ** significant at $p < .01$, *** significant at $p < .001$).

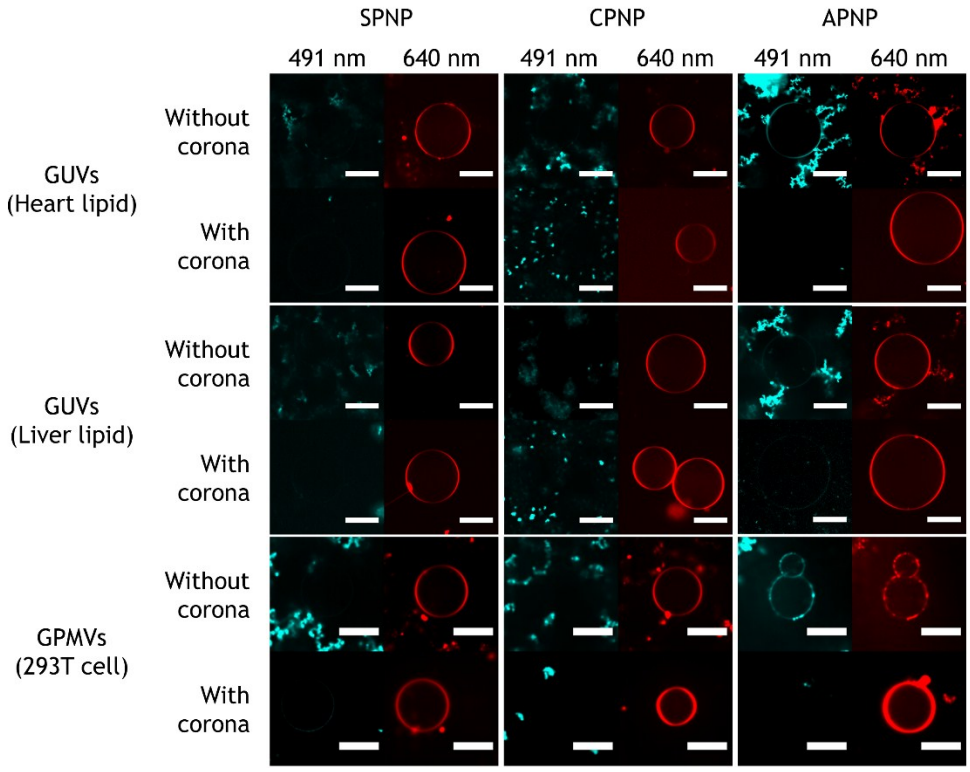

Figure S3. Confocal microscopy images of DiD-stained GUVs and GPMVs with green fluorescent PNPs after 4 h incubation. Scale bar in GUVs panels: 30 µm, in GPMVs panels: 10 µm. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP.

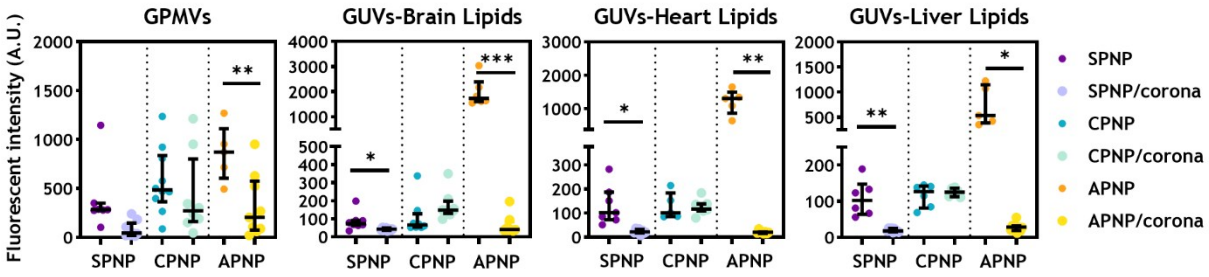

Figure S4. Fluorescent intensity of adsorbed nanoparticles on lipid membranes of GPMVs and GUVs after 15 h. Medians and interquartile ranges of calibrated fluorescence intensity were demonstrated along with individual values in graphs. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP. (Unpaired t-test, * significant at $p < .05$, ** significant at $p < .01$, *** significant at $p < .001$).

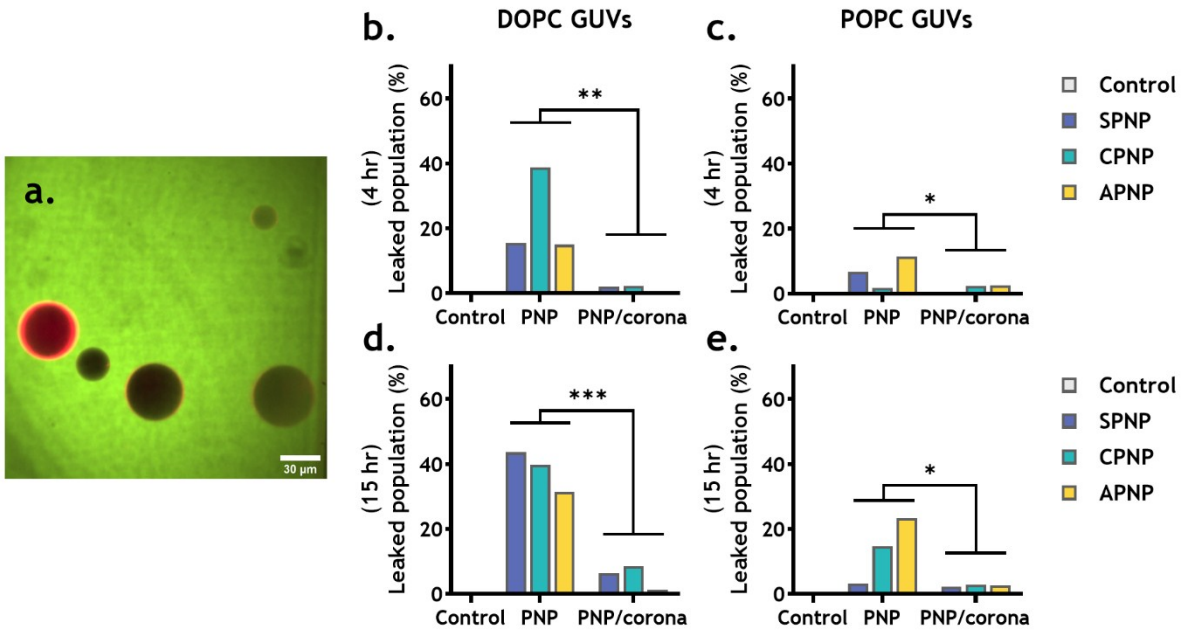

Figure S5. Effect of PNPs and protein corona on membrane integrity of pure lipid GUVs. DOPC GUVs and POPC GUVs were incubated with PNPs in 0.5 mg/mL calcein buffer for 4 h and 15 h. Inflow of calcein was observed after incubation with PNPs, percentages of leaked vesicles are presented in graphs companied with control groups where PNPs were absent. (a) Confocal microscopy image of DOPC GUVs (DiD stained, red fluorescence) in 0.5 mg/mL calcein (green fluorescence) buffer for 15 h in the absence of PNPs. (scale bar: 30 µm). (b-e) Percentages of leaked DOPC GUVs after 4 h (b) and 15 h (d) incubation with PNPs as well as relative leaked population of POPC GUVs after 4 h (c) and 15 h (e) incubation with PNPs. The control groups in all the leakage assays showed 0% leaked population. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP (Unpaired t-test, * significant at $p < .05$, ** significant at $p < .01$, *** significant at $p < .001$)

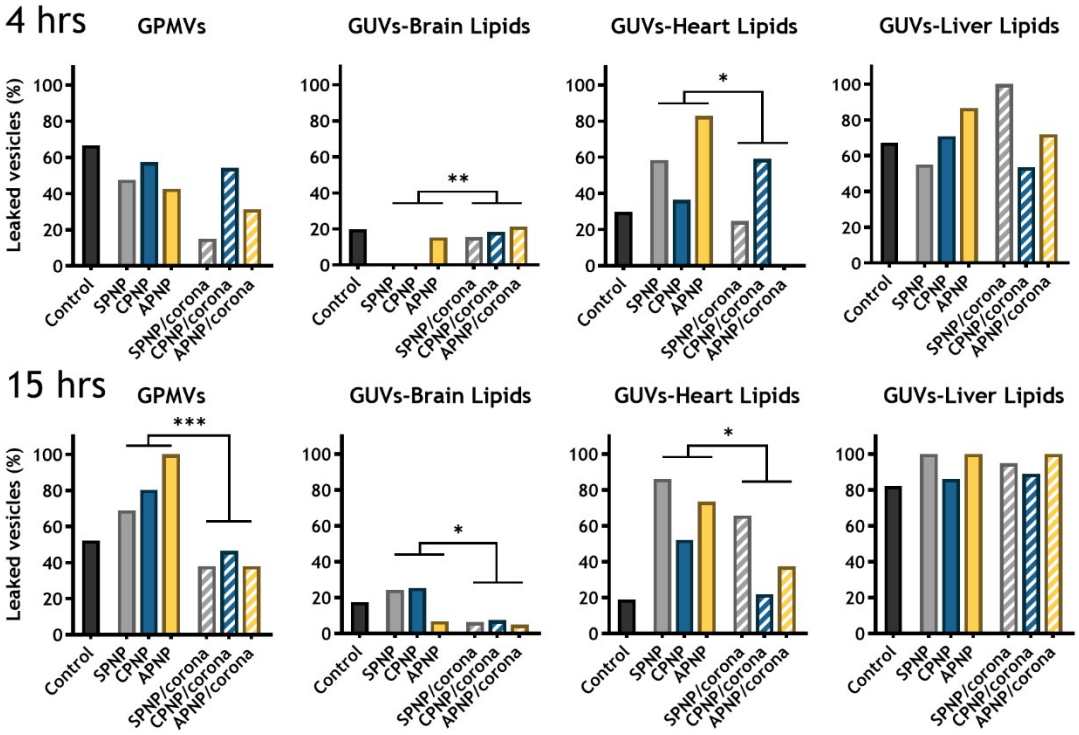

Figure S6. Effect of PNPs and protein corona on model membrane integrity. GPMVs and GUVs were incubated with PNPs in 0.5 mg/mL 10 kDa rhodamine-dextran buffer for 4 h and 15 h. Inflow of dextran was observed after incubation with PNPs, percentages of leaked vesicles are presented in graphs companied with control groups where PNPs were absent. Sulfate-PNPs, carboxyl-PNPs, and amine-PNPs are denoted as SPNP, CPNP and APNP. (Unpaired t-test, * significant at $p < .05$, ** significant at $p < .01$, *** significant at $p < .001$)