SUPPLEMENTARY INFORMATION

Linking Protein Corona Composition to Ecotoxicological Outcomes: A comparative Study of different Engineered Nanoparticles in Mediterranean Sea Urchin *Paracentrotus lividus*

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Table SI1. Mean hydrodynamic diameter (Z-Ave), polydispersity index (PDI), and zeta potential (Z-pot) values of PS-NH_2 NPs in Milli-Q (T0) and in CF at different time points (T0, 12 h, 24 h), are shown in Figure 1A-A2. The data refer to both hard and soft corona conditions, as well as to samples washed twice with PBS, where only the hard corona remains. Data are presented as mean ± standard deviation (SD) (n = 3, 1 biological replicate with 3 technical replicates each).

	Medium	Time	Z-Ave (d.nm)	PDI	Z-pot (mV)
PS-NH_2	Milli-Q	T0	63.22 ± 1.34	0.16 ± 0.02	47.2 ± 1.35
	CF Hard+Soft	T0	208.4 ± 3.70	0.19 ± 0.02	-11.50 ± 4.04
		12h	881.50 ± 142.9	0.28 ± 0.08	-10.40 ± 2.79
		24h	151.40 ± 2.55	0.25 ± 0.02	-8.16 ± 1.89
	CF Hard	T0	208.4 ± 3.70	0.19 ± 0.02	-11.50 ± 4.04
		12h	1073.00 ± 49.57	0.34 ± 0.07	-22.80 ± 1.8
		24h	602.00 ± 157.9	0.99 ± 0.01	-22.1 ± 0.76

Table SI2. Mean hydrodynamic diameter (Z-Ave), polydispersity index (PDI), and zeta potential (Z-pot) values PS-COOH NPs in Milli-Q (T0) and in CF at different time points (T0, 12 h, 24 h), are shown in Figure 1B-B2. The data refer to both hard and soft corona conditions, as well as to samples washed twice with PBS, where only the

hard corona remains. Data are presented as mean ± standard deviation (SD) (n = 3, 1 biological replicate with 3 technical replicates each).

	Medium	Time	Z-Ave (d.nm)	PDI	Z-pot (mV)
PS-COOH	Milli-Q	T0	81.02 ± 1.05	0.16 ± 0.04	-39.60 ± 0.91
	CF Hard+Soft	T0	213.70 ± 5.71	0.25 ± 0.01	-14.00 ± 3.74
		12h	361.20 ± 33.23	0.73 ± 0.23	-11.70 ± 3.10
		24h	146.90 ± 3.53	0.21 ± 0.03	-9.26 ± 4.49
	CF Hard	T0	213.70 ± 5.71	0.25 ± 0.01	-14.00 ± 3.74
		12h	1347.00 ± 111.50	0.72 ± 0.47	-21.40 ± 0.72
		24h	530.00 ± 8.41	0.81 ± 0.06	-25.3 ± 1.40

Table SI3. Mean hydrodynamic diameter (Z-Ave), polydispersity index (PDI), and zeta potential (Z-pot) values AgcitLcys NPs in Milli-Q (T0) and in CF at different time points (T0, 12 h, 24 h), are shown in Figure 1C-C2. The data refer to both hard and soft corona conditions, as well as to samples washed twice with PBS, where only the hard corona remains. Data are presented as mean ± standard deviation (SD) (n = 3, 1 biological replicate with 3 technical replicates each).

	Medium	Time	Z-Ave (d.nm)	PDI	Z-pot (mV)
AgcitLcys	Milli-Q	T0	56.81 ± 2.28	0.64 ± 0.05	-51.80 ± 0.75
	CF Hard+Soft	T0	307.80 ± 8.19	0.26 ± 0.02	-9.87 ± 0.76
		12h	117.10 ± 2.59	0.64 ± 0.08	-11.50 ± 3.36
		24h	149.59 ± 2.25	0.62 ± 0.04	-11.20 ± 2.27
	CF Hard	T0	307.80 ± 8.19	0.26 ± 0.02	-9.87 ± 0.76
		12h	788.20 ± 163.10	0.93 ± 0.071	-19.60 ± 0.99
		24h	484.00 ± 28.98	0.71 ± 0.05	-25.3 ± 1.40

Table SI4. Mean hydrodynamic diameter (Z-Ave), polydispersity index (PDI), and zeta potential (Z-pot) values TiO_2 NPs in Milli-Q (T0) and in CF at different time points (T0, 12 h, 24 h), are shown in Figure 1D-D2. The data refer to both hard and soft corona conditions, as well as to samples washed twice with PBS, where only the hard corona remains. Data are presented as mean ± standard deviation (SD) (n = 3, 1 biological replicate with 3 technical replicates each).

	Medium	Time	Z-Ave (d.nm)	PDI	Z-pot (mV)
TiO_2	Milli-Q	T0	395.2 ± 18.59	0.42 ± 0.09	-12.03 ± 0.25
	CF Hard+Soft	T0	385.00 ± 44.14	0.52 ± 0.09	-9.36 ± 3.33
		12h	293.90 ± 33.51	0.70 ± 0.12	-7.33 ± 5.24
		24h	237.00 ± 3.93	0.34 ± 0.05	-10.6 ± 4.24
	CF Hard	T0	385.00 ± 44.14	0.52 ± 0.09	-9.36 ± 3.33
		12h	1237.00 ± 178.4	1	-25.10 ± 0.45
		24h	1228.00 ± 469.3	0.95 ± 0.09	-26.6 ± 0.58

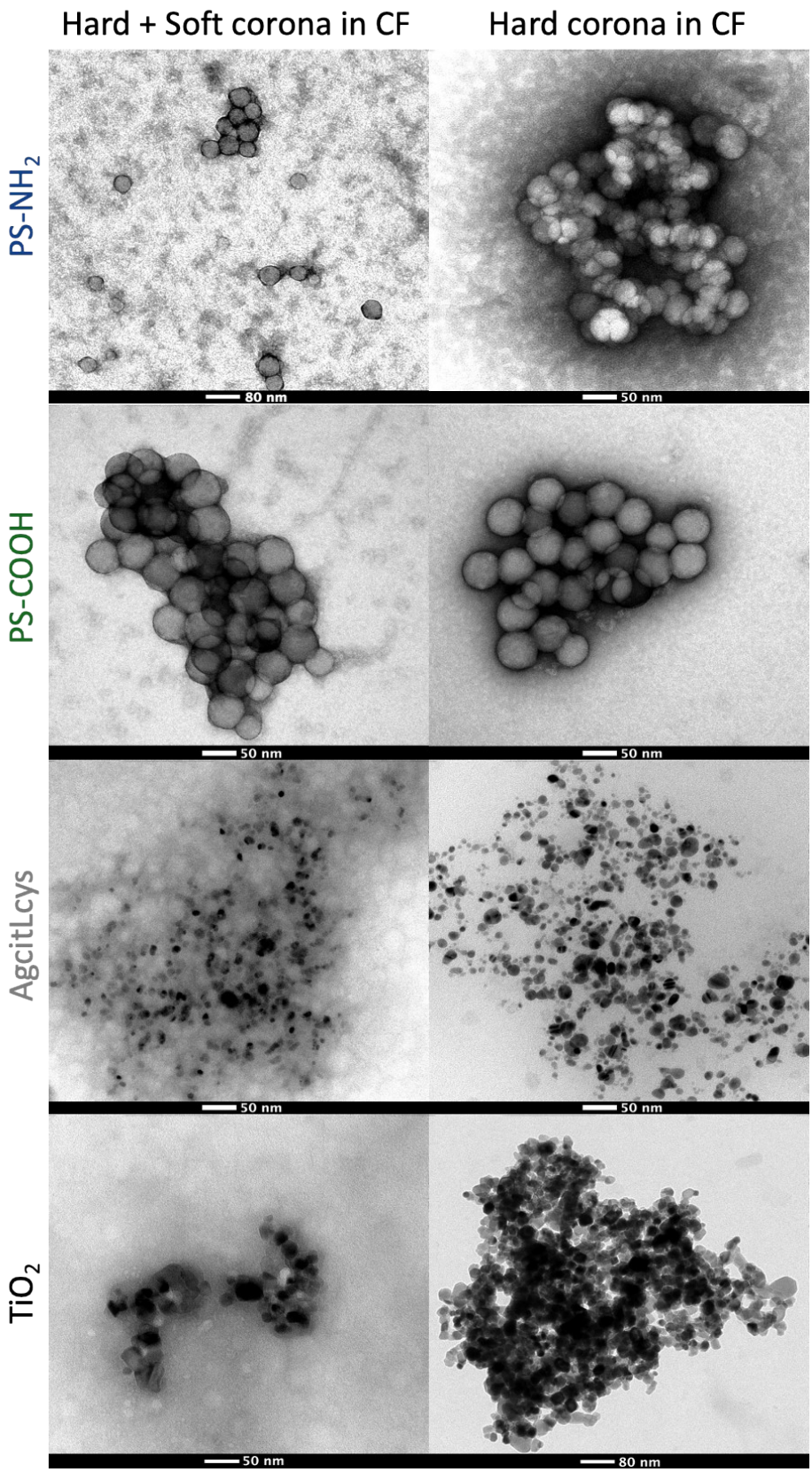

Figure SI1. Morphological and dimensional characterization of PS-NH_2, PS-COOH, AgcitLcys, and TiO_2 NPs using TEM. Panel show the NPs after 24 h of incubation in CF of *P. lividus*, highlighting the formation of the protein corona (hard + soft) and following three PBS washes, in which only the hard corona remains after removal of the loosely bound proteins.

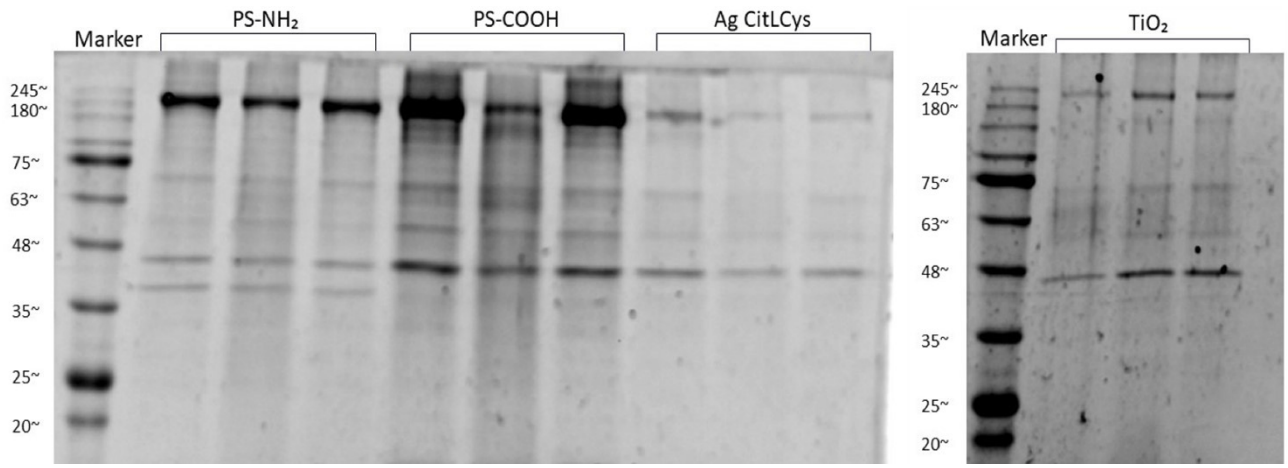

Figure SI2. SDS-PAGE (12%) showing the protein coronas formed on different NP types after 24 h incubation in CF of *P. lividus*. Lanes 2–4: PS-NH_2 NPs; lanes 5–7: PS-COOH NPs; lanes 8–10: AgcitLcys NPs; lanes 12–14: TiO_2 NPs. Each lane represents an independent replicate of the protein corona isolated from the respective NP type. Proteins were separated under reducing conditions and visualized by Coomassie Brilliant Blue staining. Triplicate analysis was performed for each NP type to evaluate the reproducibility of the corona composition. Molecular weight markers (MW) are shown on the left of each gel (in kDa).

Table SI5. Theoretical estimates of particle number for each NP type (PS-COOH, PS-NH_2, AgcitLcys and TiO_2) calculated from the nominal particle diameter at the two exposure concentrations used (25 µg/L and 25 µg/mL), using a mass-to-particle-number conversion and assuming spherical, homogeneous, non-aggregated particles.

NP	Diameter	25 µg/L	25 µg/mL
PS-COOH	62 nm	1.89×10^{11}	1.89×10^{14}
PS-NH_2	50 nm	3.64×10^{11}	3.64×10^{14}
AgNP	8.3 nm	7.96×10^{12}	7.96×10^{15}
TiO_2 P25	23 nm	1.01×10^{12}	1.01×10^{15}

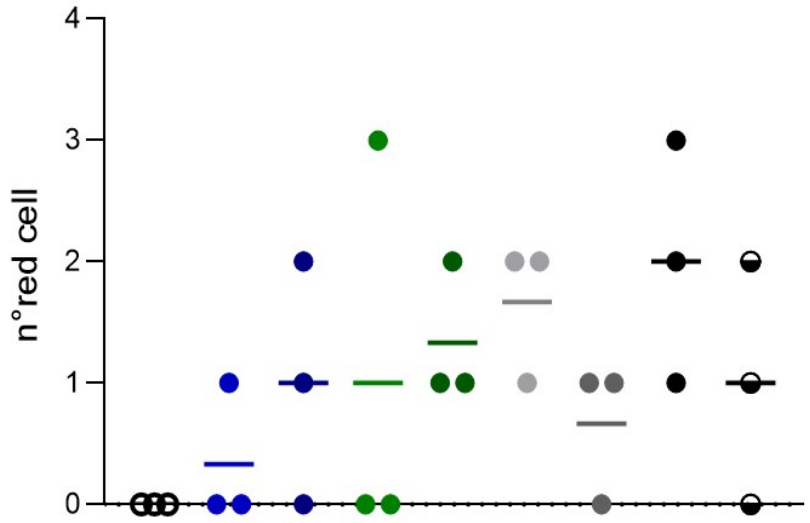

Figure SI3. Number of red cells in *P. lividus* coelomocytes following 4 h exposure to NPs in CF. Each dot represents the count of red cells per field in a biological replicate. Horizontal lines show mean values for each condition. Conditions labeled "a" correspond to 25 µg/L exposures and "b" to 25 µg/mL exposures.

Table SI6. Number of red cells in *P. lividus* coelomocytes after 4 h exposure to NPs in CF. Values represent the number of red cells per field in three biological replicates.

n° Red Cell			
Samples	**Rep 1**	**Rep 2**	**Rep 3**
CTRL	0	0	0
$PS\text{-}NH_2$ [a]	1	0	0
$PS\text{-}NH_2$ [b]	0	2	1
PS-COOH [a]	0	3	0
PS-COOH [b]	1	1	2
AgcitLcys [a]	2	2	1
AgcitLcys [b]	1	0	1
TiO_2 [a]	1	3	2
TiO_2 [b]	0	2	1

[a] exposures at 25 µg/L

[b] exposures at 25 µg/mL