Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Cell-Surface Glycosaminoglycans Regulate the Cellular Uptake of Charged Polystyrene Nanoparticles

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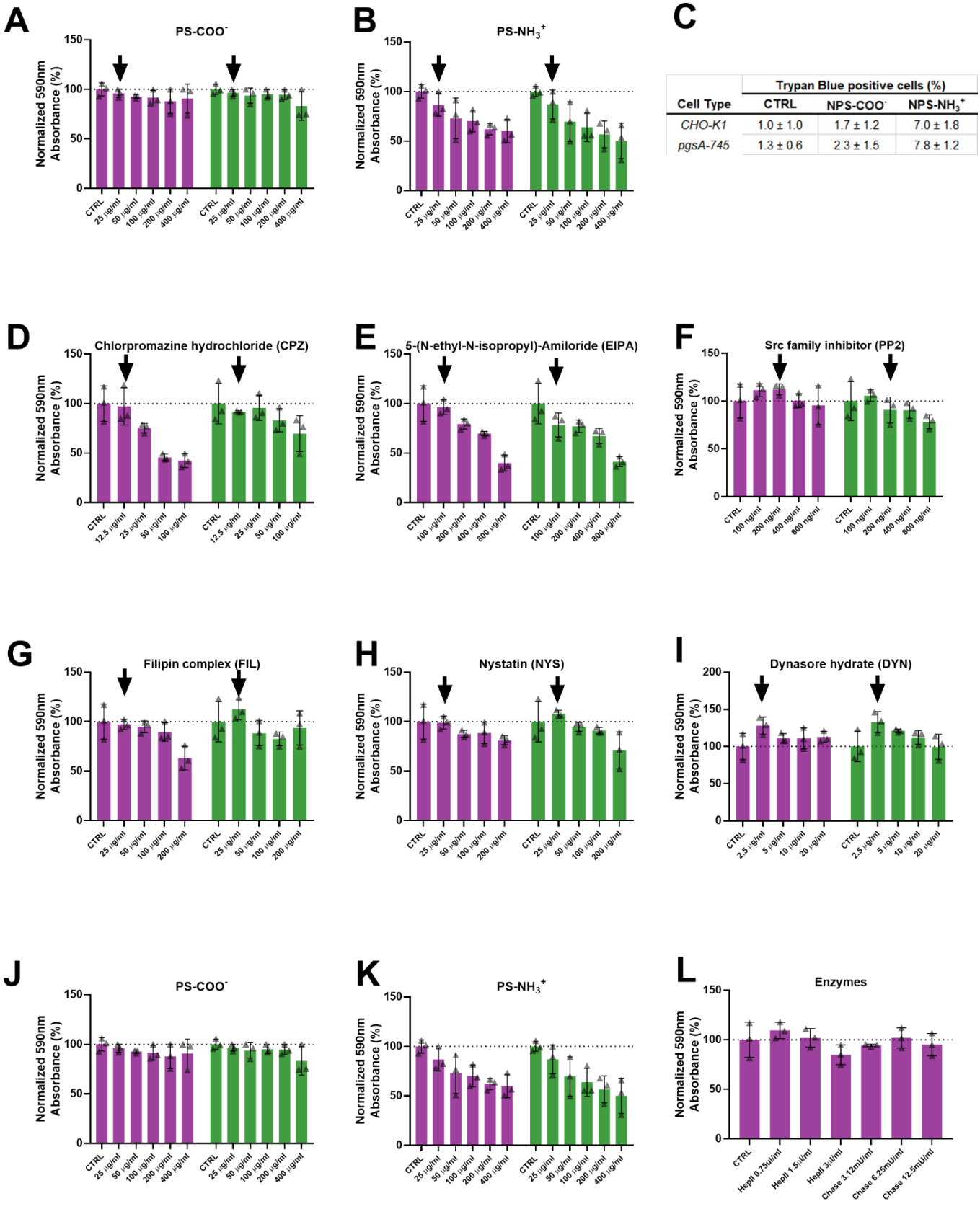
Table S1. Characterization of NP size and surface charge. Dynamic light scattering and zeta potential measurements of NPs (25 μg ml^{-1}) in Milli-Q water and F-12 cell culture medium were performed at 37°C. Similar experiments were also performed in the presence of 100 μg/mL soluble heparan sulfate (HS) or chondroitin sulfate (CS). PS-BSA corresponds do carboxyl-PS NPs coated with a bovine serum albumin corona.

Nanoparticles	Diameter (nm)		Polydispersity index		ζ potential (mV)
	Water	F-12	Water	F-12	Water
$PS\text{-}COO^-$	57.2 ± 1.8	63.1 ± 7.2	0.201 ± 0.018	0.184 ± 0.065	-58.9 ± 12.4
$PS\text{-}NH_3^+$	55.9 ± 13.4	65.4 ± 12.3	0.166 ± 0.087	0.269 ± 0.218	+35.4 ± 1.8
PS-BSA	60.9 ± 6.1	69 ± 3.7	0.091 ± 0.026	0.171 ± 0.05	-25.5 ± 0.5
$PS\text{-}COO^-$ + HS	64.3 ± 1.7	83.7 ± 10	0.134 ± 0.039	0.227 ± 0.108	-61.0 ± 1.6
$PS\text{-}NH_3^+$ + HS	222 ± 7.4	350 ± 33.5	0.389 ± 0.125	0.542 ± 0.019	-66.4 ± 1.9
$PS\text{-}COO^-$ + CS	87.5 ± 5.3	54.5 ± 4.3	0.208 ± 0.039	0.160 ± 0.042	-49.6 ± 7.6
$PS\text{-}NH_3^+$ + CS	218 ± 7.3	286 ± 25.9	0.291 ± 0.005	0.444 ± 0.059	-70.4 ± 2.2



Cell Type	Trypan Blue positive cells (%)		
	CTRL	NPS-COO⁻	NPS-NH$_3^+$
CHO-K1	1.0 ± 1.0	1.7 ± 1.2	7.0 ± 1.8
pgsA-745	1.3 ± 0.6	2.3 ± 1.5	7.8 ± 1.2

Figure S1. Cytotoxicity tests. (A ,B) Effect of NP exposure on cell viability measured by MTT assay. Arrows indicate the actual NP concentrations used in uptake studies. (C) Effect of NP exposure (25 μg ml^{-1}) on cell death measured by Trypan blue exclusion assay. (D-I) Effect of pharmacological inhibitors on cell viability measured by MTT assay. Arrows indicate the actual inhibitor concentrations used in uptake studies. (J,K) Combined effect of NP and inhibitor exposure on cell viability measured by MTT assay. NP and inhibitor concentrations are as given in A through I. (L) Effect of HepII and Chase treatment on cell viability measured by MTT assay. In all of the MTT assay results, data were normalized relative to their respective control cell group (CTRL).

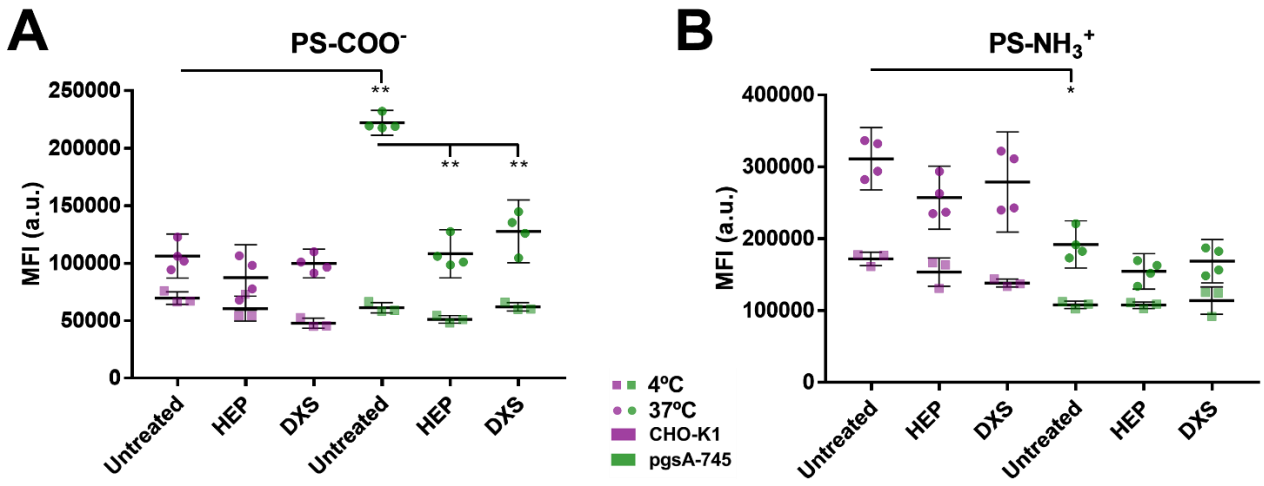


Figure S2. Influence of exogenous polyanions on NP uptake. (A, B) Median fluorescence intensities (MFI) from flow cytometry histograms of CHO cells exposed to (A) carboxyl-PS NPs and (B) amine-PS NPs in the absence (Untreated) or presence of 100 μg/mL heparin (HEP) and 100 μg/mL dextran sulfate (DXS). Net uptake can be visually discerned by considering the differences in MFI between 37 and 4°C (colored circles and squares, respectively). Shown for each tested condition are results across 3-4 biological replicates performed in technical triplicate. Error bars represent MFI ± 95% confidence interval. A two-way ANCOVA was conducted to evaluate statistically significant differences in NP uptake at 37°C, using data at 4°C as an independent covariate; (*) $p < 0.05$ and (**) $p < 0.001$.

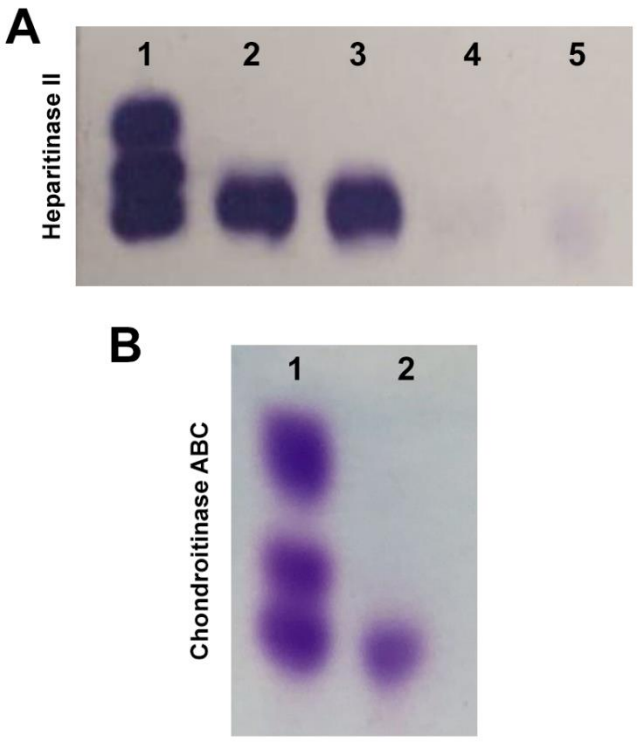


Figure S3. GAG electrophoresis for the characterization of HepII and Chase enzyme activities. GAGs were analyzed by agarose gel electrophoresis in PDA buffer. Gels were stained with 0.1% toluidine blue prepared in a solution containing 1% acetic acid and 50% ethanol, and destained with the same solution without toluidine blue. (A) Characterization of HepII activity toward HS. Lane 1: GAG mix consisting of CS, DS (dermatan sulfate) and HS (from top to bottom). Lanes 2 and 3: HS only. Lanes 4 and 5: HS treated with HepII. (B) Characterization of Chase activity toward CS. Lane 1: GAG mix consisting of CS, DS and HS (from top to bottom). Lane 2: GAG mix treated with Chase. These results confirm HepII and Chase activities toward HS and CS/DS, respectively. Note: CHO cells do not contain DS.