Supporting information

Particle size affects cytosolic delivery of membranotropic peptide-functionalized platinum nanozymes

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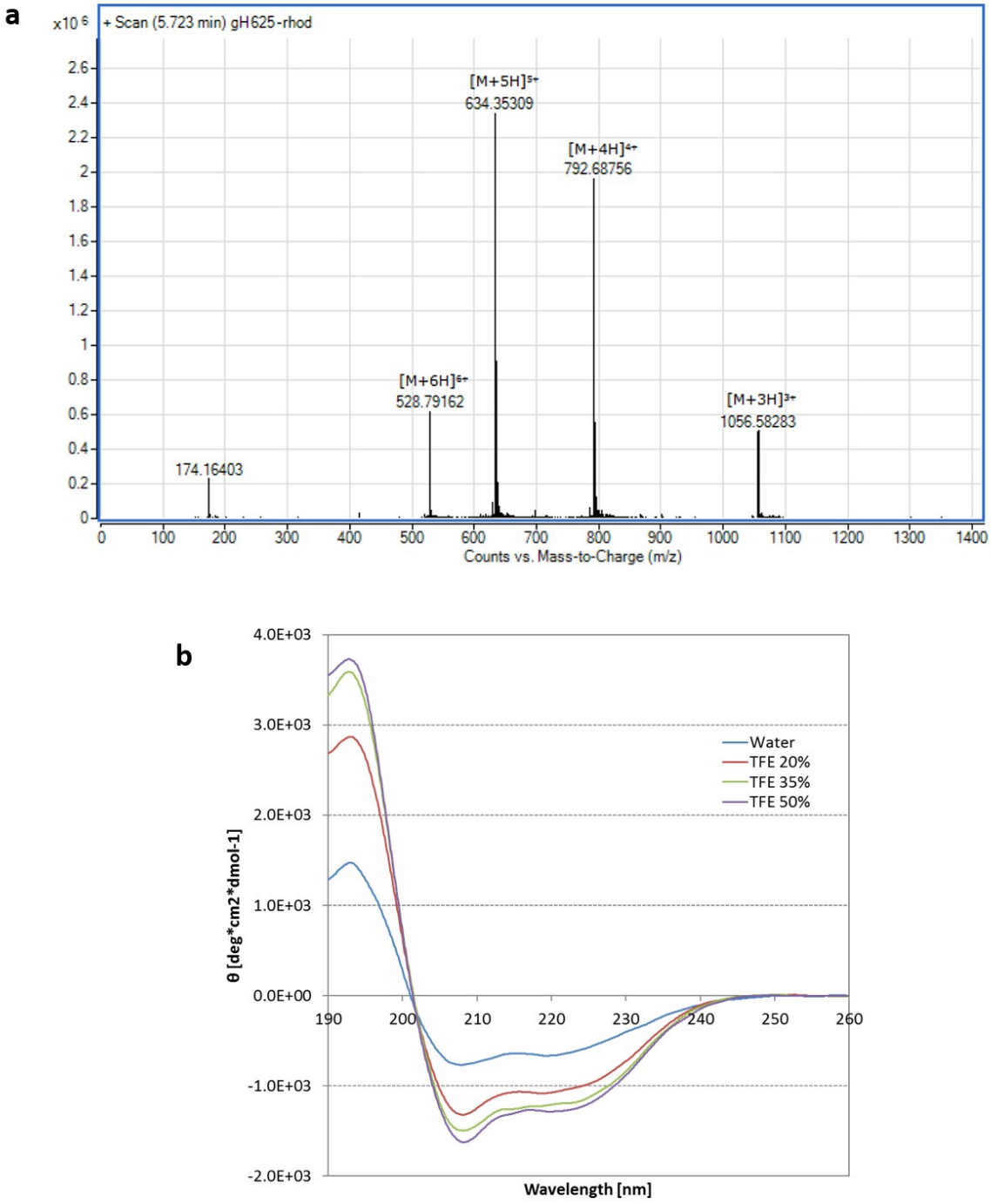

Figure S1. Characterization of rhodamine-conjugated gH625 peptide. a) Mass spectrum shows peaks of multi-charged ions at m/z = 1056.58 ($[M+3H]^{3+}$), m/z = 792.69 ($[M+4H]^{4+}$), m/z = 634.35 ($[M+5H]^{5+}$) and m/z = 528.79 ($[M+6H]^{6+}$) corresponding to 3167.73 Da molecular weight; b) Circular dichroism (CD) at increasing amounts (up to 50% v/v) of 2,2,2-trifluoroethanol (TFE), used as a structure-inducing co-solvent. The absorption peaks at 194 nm and the negative ellipticity at 208 and 220 nm rise with TFE concentration indicative of α-helix secondary structure.

Theoretical surface density (n° peptide/nm^2)	Peptide/NP ratio		
	Pt2.5	**Pt5**	**Pt20**
0.09	2	7	90
0.18	4	14	180
0.27	6	21	270
0.36	8	28	360

Table S1. Theoretical surface densities of gH625 peptide as a function of particle size and peptide/nanoparticle ratios.

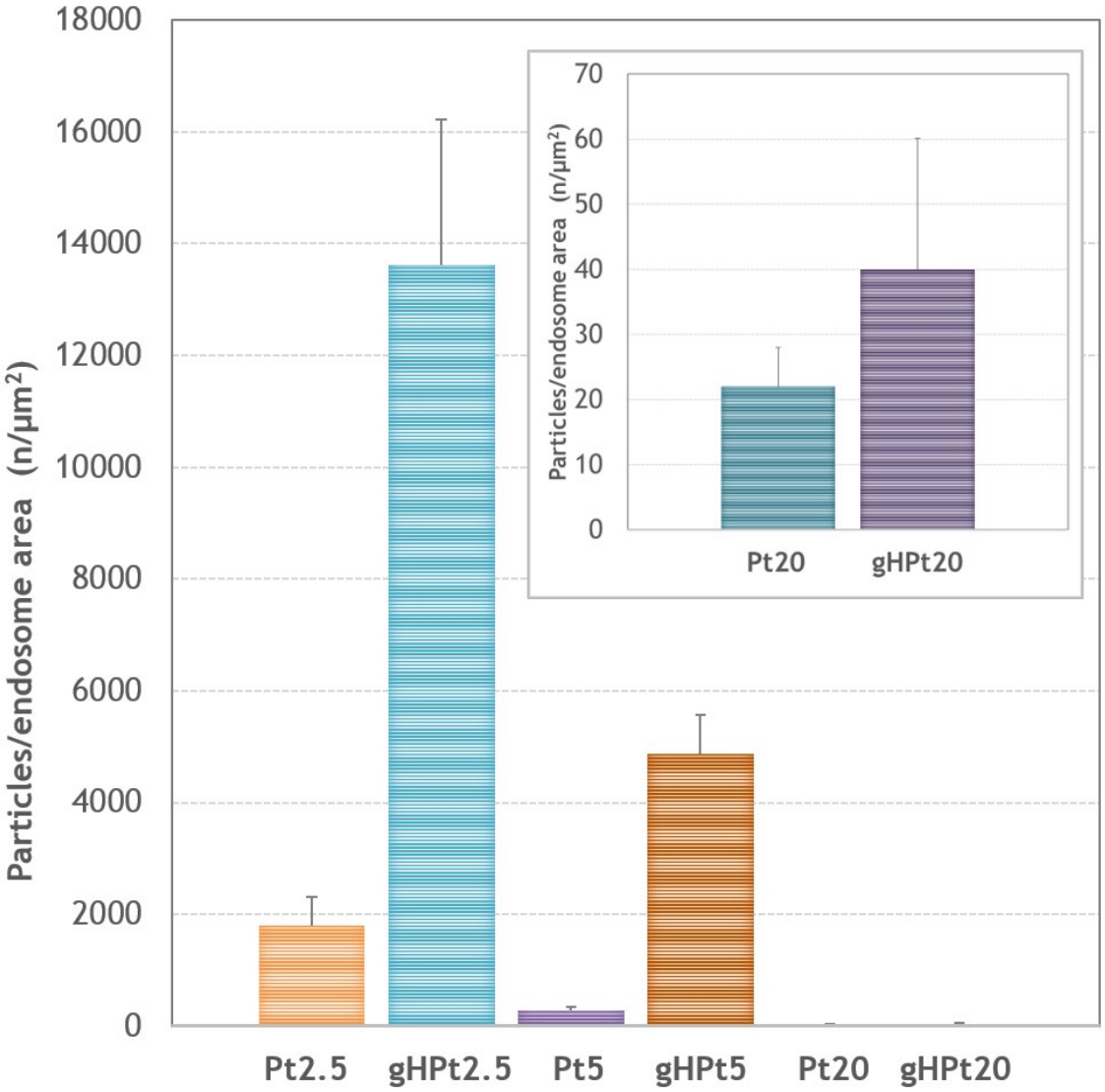

Figure S2. Semi-quantitative analysis of gH625-functionalized and bare Pt2.5, Pt5 and Pt20 NP density into endo-lysosomal compartments. Inset represents endo-lysosomal density of Pt20 and gHPt20 NPs with a lower scale of y-axis. Data are reported as mean ± s.e.m.

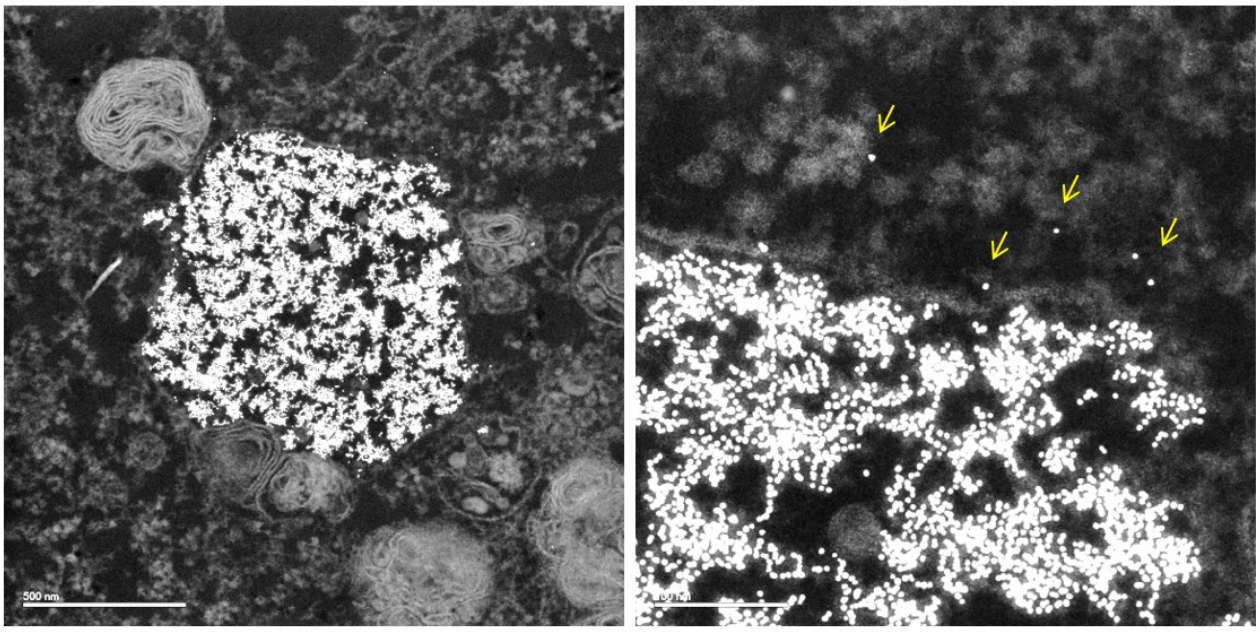

Figure S3. Additional STEM images of HeLa cells after 24 h incubation with gH625-functionalized Pt5 NPs at 50 μg/ml particle concentration showing some endo-lysosomal compartments completely full of particles and a minor fraction of NPs free in the cytoplasm. Bar 500 nm (left panel) and 200 nm (right panel).

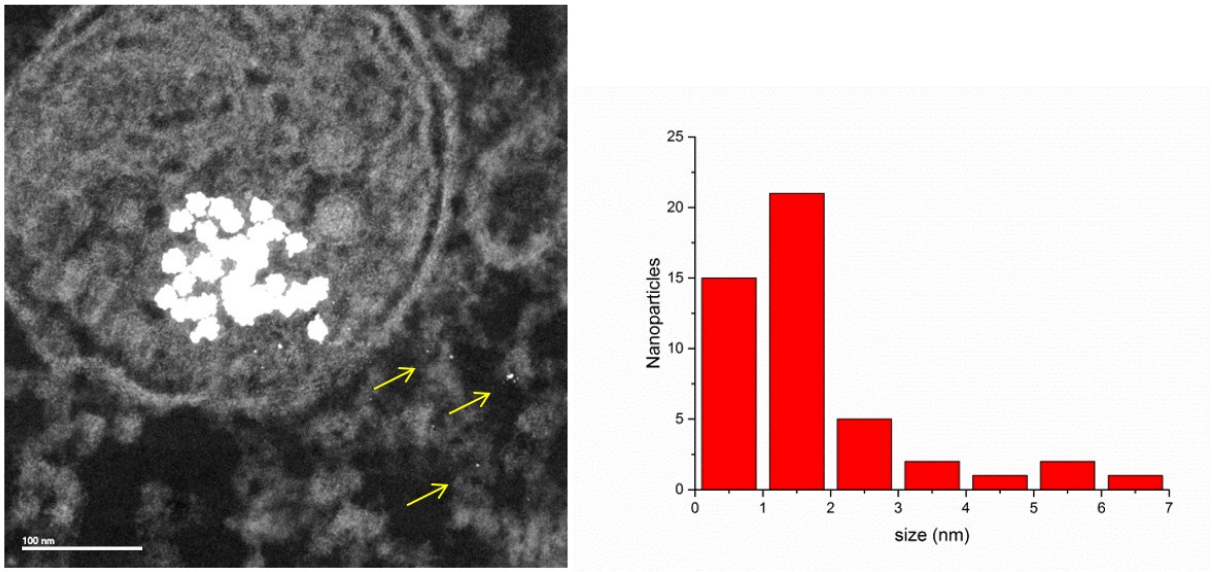

Figure S4. (Left) STEM image of HeLa cells after 24 h incubation with gH625-functionalized Pt20 NPs at 50 µg/ml particle concentration, showing some particle fragmentation inside and outside the endo-lysosomal compartment. Scale bar 100 nm. (Right) The chart indicates the size range analysis of particle fragments with a peak around 1-2 nm.

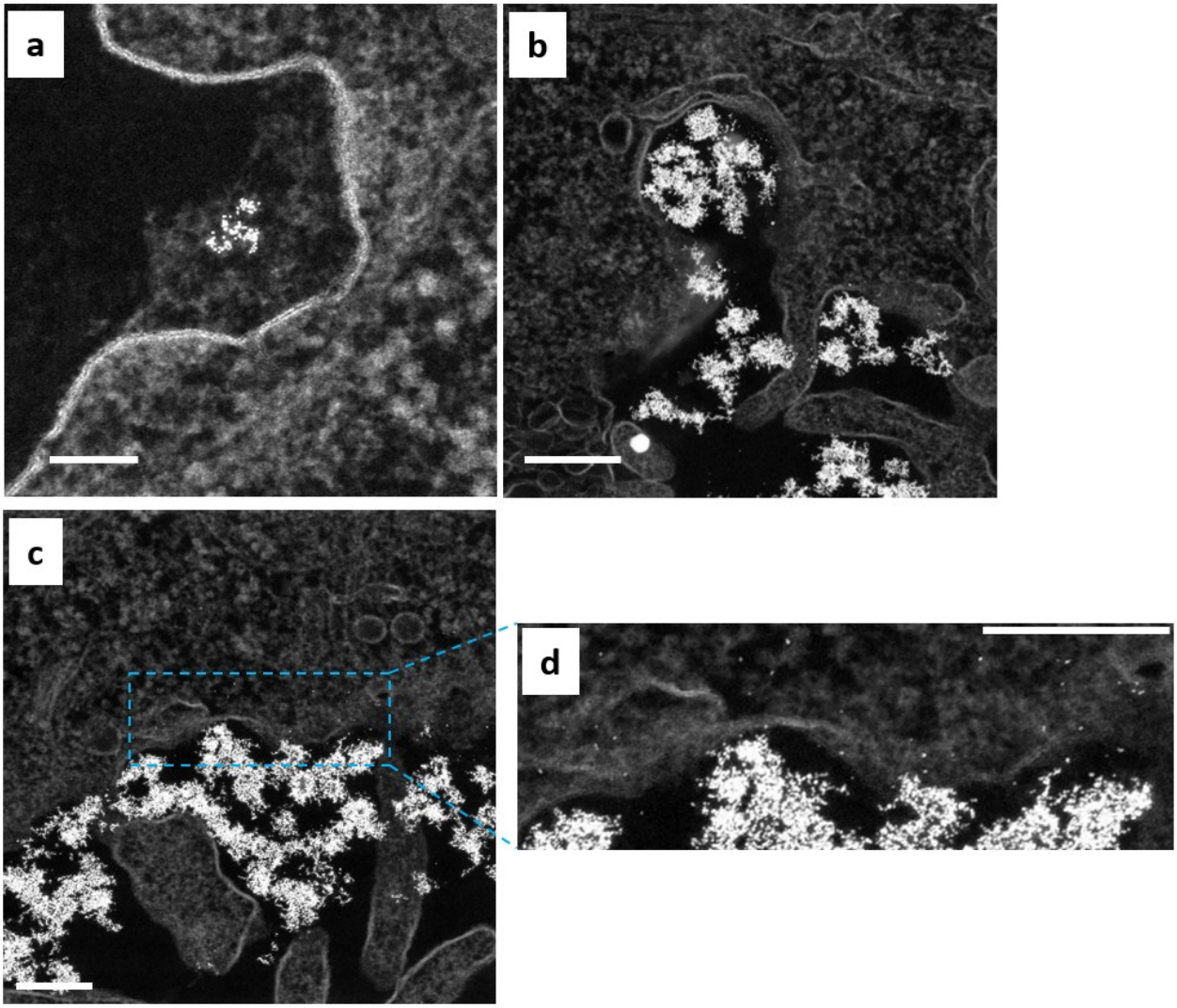

Figure S5. STEM images of gHPt2.5 NPs approaching cell membrane. a) small NP aggregate entering through a large putative clathrin-coated pit; b) large NP aggregates entering by macropinocytosis; c) single NPs penetrating cell membrane and reaching directly the cytosol; d) zoomed image of dashed squared areas in c). Scale bars: 100 nm (a) and 200 nm (b-d).

Movie S1. HAADF STEM tomogram of the 3D model showing an endosome containing gHPt2.5 nanoparticles as shown in Figure 5a.

Movie S2. HAADF STEM tomogram of the 3D model showing an endosome portion containing gHPt2.5 nanoparticles as shown in Figure 5b.