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Supporting Information

Vesicular Disruption of Lysosomal Targeting Organometallic Polyarginine Bioconjugates

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Table of Content

I. *HPLC characterization of **1**, **2**, **3** and **5***

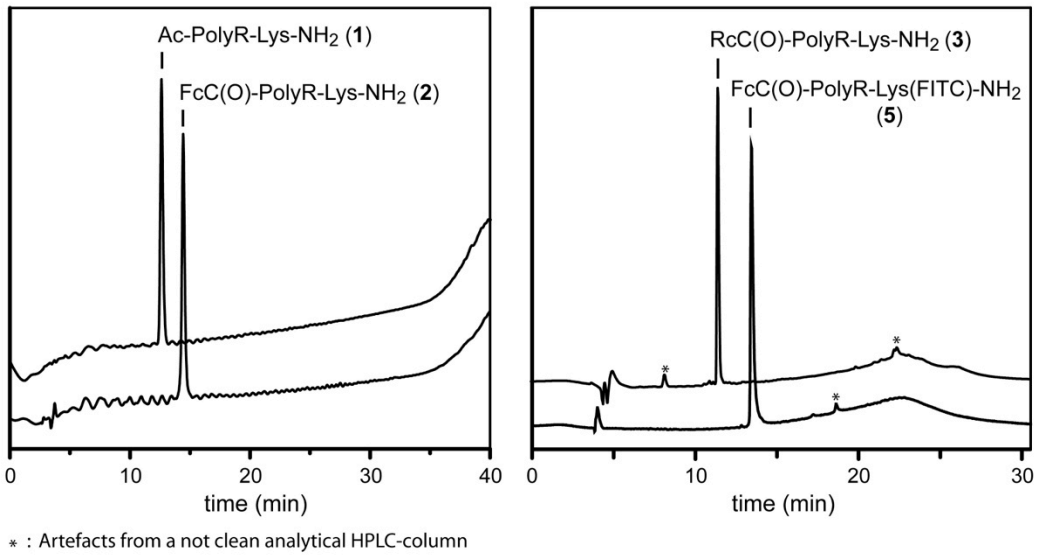

Figure S1. HPLC-chromatogram of the purified compounds **1** and **2** detected at 220 nm, run: 40 min (left); HPLC-chromatogram of the purified compounds **3** and **5** detected at 254 nm (right).

II. *Membrane Studies*

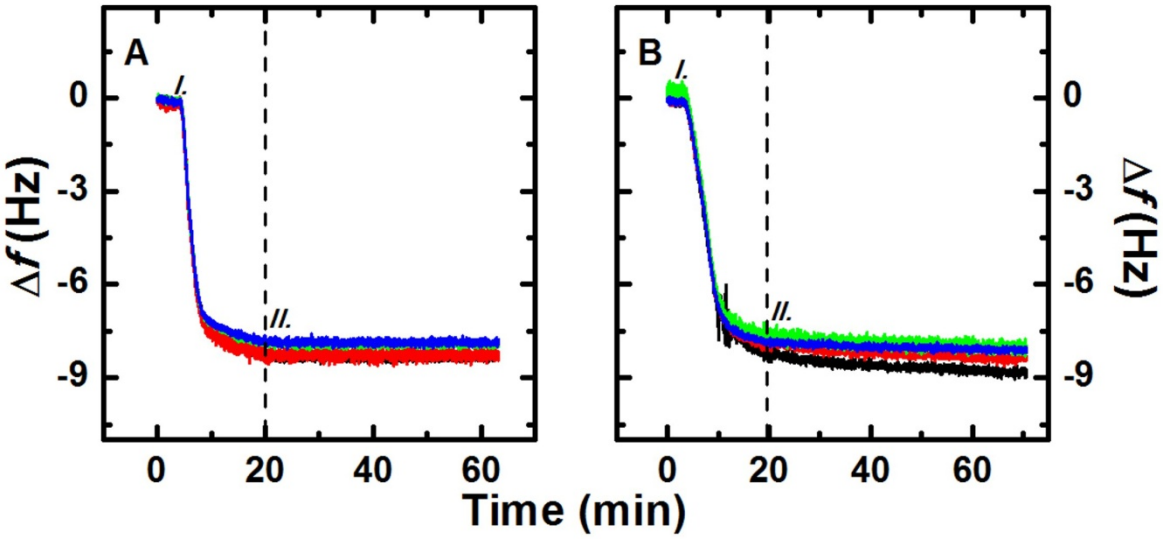

Figure S2: Δ*f*-t plots obtained for peptide **1** (**A**) and peptide **3** (**B**) interacting with a DMPC/Cholesterol membrane. The plots are for 5 μM. The harmonic represented are: 3rd (black line), 5th (red line), 7th (green line) and 9th (blue line). The vertical dash line indicates the time when the peptide flow stopped (*II.*), while *I.* corresponds to the beginning of the peptide injection into the QCM chamber.

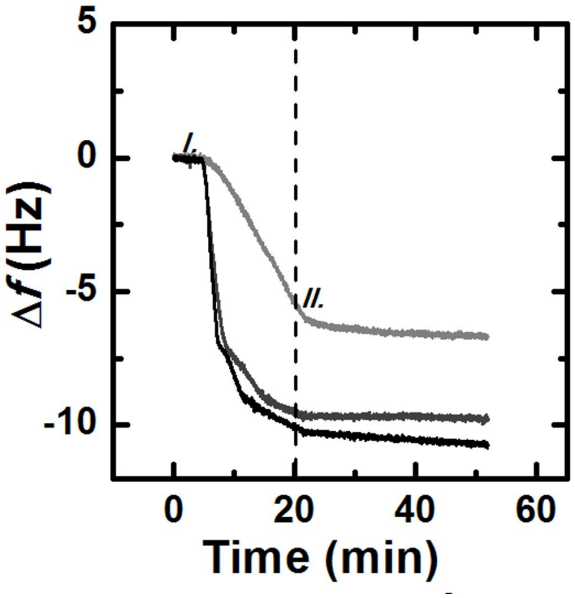

Figure S3: Δ*f*-t plots obtained for peptide **2** interacting with a DMPC/Cholesterol membrane. The concentrations used are 1, 5 and 10 μM (from the lightest to the darkest trace). The harmonic examined was the 7th. The vertical dash line indicates the time when the peptide flow stopped (*II.*), while *I.* corresponds to the beginning of the peptide injection into the QCM chamber.

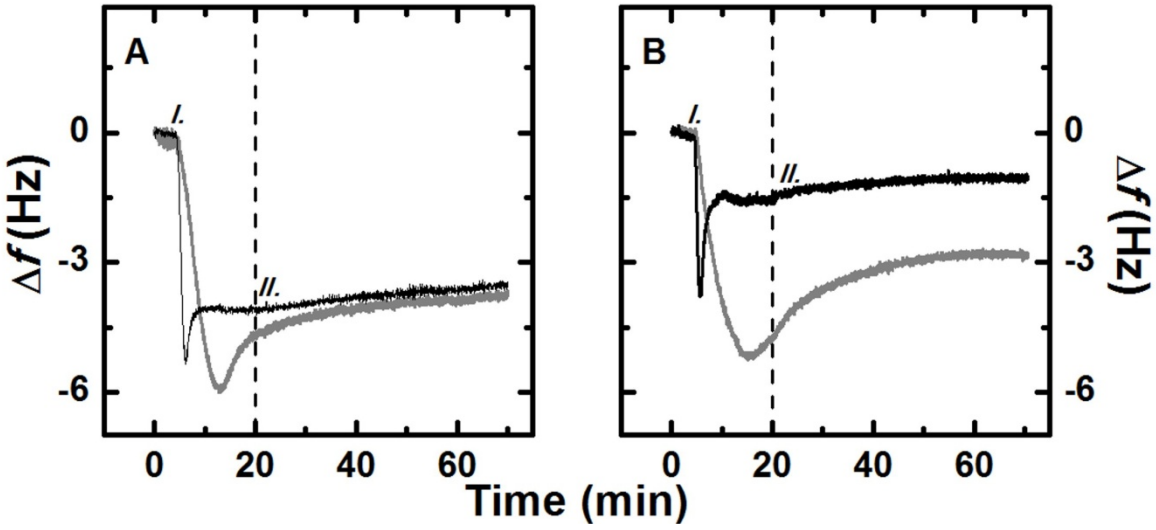

Figure S4: Δ*f*-t plots obtained for peptide **1** (**A**) and peptide **3** (**B**) interacting with a DMPC/DMPG membrane. The concentrations used are 1 μM (light grey) and 10 μM (black trace). The harmonic examined was the 7th. The vertical dash line indicates the time when the peptide flow stopped (*II.*), while *I.* corresponds to the beginning of the peptide injection into the QCM chamber.

III. *Biological Studies*

a) Uptake Studies of **4**, **5** and **6**

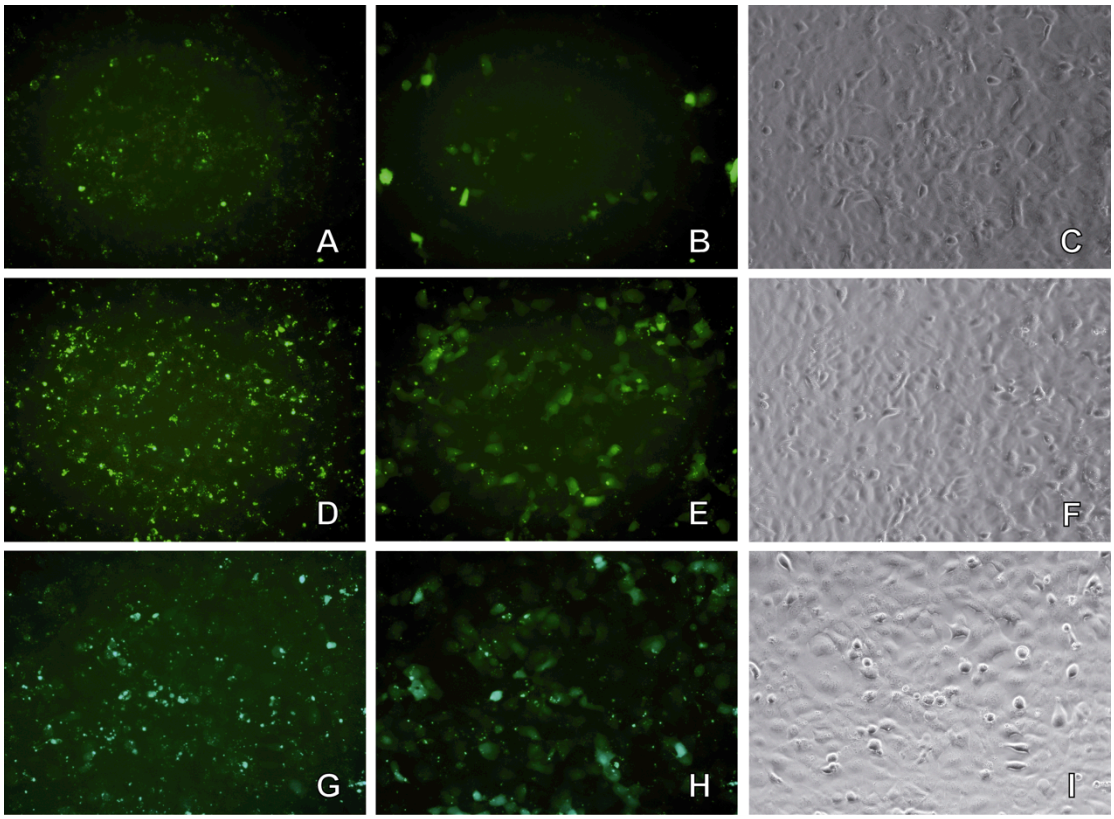

Figure S5. Cellular uptake of **4** (10 µM) in PT45 cells after 14 h incubation. A: fluorescence image, t = 0; B: fluorescence image, t = 5 min; C: phase contrast. FITC-filter, 200x magnification. Cellular uptake of **5** (10 µM) in PT45 cells after 14 h incubation. D: fluorescence image, t = 0; E: fluorescence image, t=5 min; F: phase contrast. FITC-Filter, 200x magnification. Cellular uptake of **6** (10 µM) in PT45 cells after 14 h incubation. G: fluorescence image, t = 0; H: fluorescence image, t = 5 min; I: phase contrast. FITC-Filter, 200x magnification.

b) Cytotoxicity Assays

Cytotoxicity assays:

Resazurin Assay. 1 mL of resazurin solution per plate was diluted with 9 mL medium without phenol red. 100 µL of this solution was added per well and their absorbance was measured using a Tecan plate reader at 600 nm, using a reference wavelength of 690 nm. The plates were incubated for 2 h at 37 °C and 10% CO_2 and were measured again.

Crystal Violet Assay. To perform the crystal violet assay, the resazurin solution was removed and the cells were fixed using 0.2% glutardialdehyde for 25 min. The glutardialdehyde solution was removed and exchanged for 100 µL of 0.1% triton solution. After a short incubation, all liquids were removed and the fixed cells were stained with a 0.02 M crystal violet solution for 30 min. Afterwards the wells were washed intensely with H_2O and were filled with 100 µL of 96% ethanol, followed by shaking on a softly rocking rotary shaker for 3 h. The absorption of the ethanolic solution was measured using a micro-plate reader at 570 nm.

Table S1. IC_{50} values, resazurin assay after 48 h incubation.

Compound	HeLa [µM]	PT45 [µM]	HepG2 [µM]
1	251 ± 19	290 ± 10	307 ± 38
2	62 ± 3	133 ± 18	86 ± 13
3	134 ± 15	215 ± 9	195 ± 17
FcC(O)OH, RcC(O)OH	> 1000	> 1000	> 1000
cisplatin	1.8 ± 0.1	2.3 ± 0.1	2.1 ± 0.1

c) Apoptosis Assay

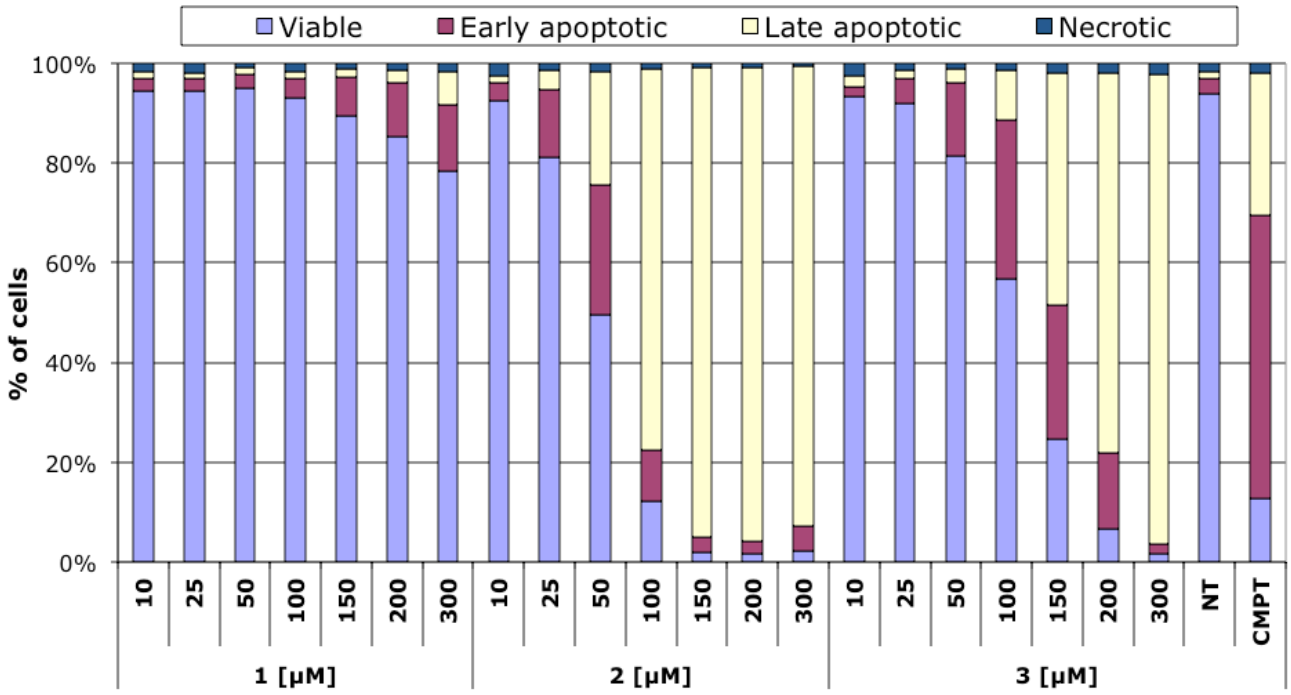

Figure S6. Apoptosis induction in HeLa cells in % of cells by **1**, **2** and **3** at the indicated concentrations after 48 h incubation.

d) Cell Cycle Studies

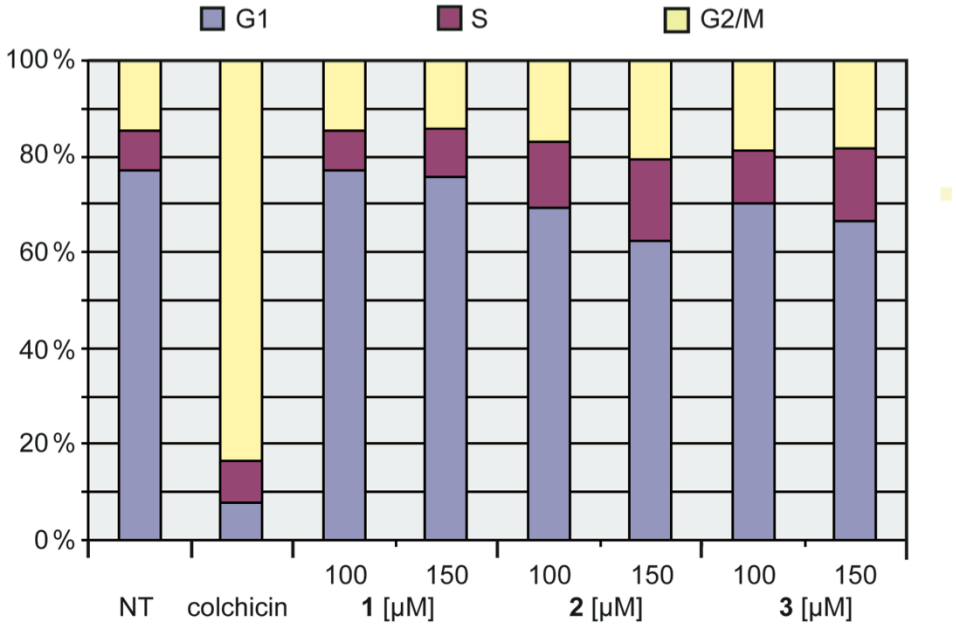

Figure S7. Cell cycle studies of **1**, **2** and **3** in HeLa cells at indicated concentrations after 24 h incubation.

IV. *Abbreviations*

AAS, atomic absorption spectrometry; AnnV, Annexin V; ATP, adenosine triphosphate; BSA, bovine serum albumin; Cp, cyclopentadiene; CPP, cell-penetrating peptide; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMF, dimethylformamide; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; DMSO, dimethyl sulfoxide; ESI, electrospray ionization; FITC, fluorescein isothiocyanate; Fmoc, 9-fluorenylmethyloxycarbonyl; HeLa, immortal cell line (cervical cancer cells); HepG2, immortal cell line (hepatocellular carcinoma); HOBt, N-hydroxybenzotriazole·H_2O; HT-29, immortal cell line (colon carcinoma); IMIM-PC2, immortal cell line (ductal pancreas carcinoma); LMP, lysosomal membrane permeabilization; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; MeCN, acetonitrile; MeOH, methanol; Mtt, 4-methyltrityl; NT, non treated cells; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl; PBS, phosphate buffered saline; Pen Strep, penicillin streptomycin solution; PI, propidium iodide; PNA, peptide nucleic acids; PT45, immortal cell line (pancreatic carcinoma cells); ROS, reactive oxygen species; SPPS, solid-phase peptide synthesis; RT, room temperature; TBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIS, triisopropylsilane.