Supporting Information

Voigt et al. 10.1073/pnas.1322356111

SI Materials and Methods

MTT Assay. To test the nanoparticle (NP) toxicity, an 3-(4,5-Dimethylthiazol-2yl)2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay was executed after the incubation with NPs.

Cells were seeded in a 96-well plate 1 d prior to the experiment. The medium was aspirated, and NPs suspended in medium were added and incubated for 5 h. Cell were washed with PBS and 100 μL of a solution of MTT (0.25 mg/mL in RPMI without phenol red) was added and incubated for 3 h. The MTT solution was removed, 100 μL DMSO was added to each well, and the absorbance at 550 nm was measured using a Synergy HT plate reader (BioTek).

Inhibition of Caveolae-Mediated Uptake on NPs in HeLa Cells. Cells were seeded on 24-well plates and grown to 80% confluence. Medium was aspirated, and cells were washed with PBS and then incubated at 37 °C in the presence of 0.0075 mg/mL of Filipin III in RPMI without serum. After 15 min of incubation, the inhibitor solution was aspirated and NPs (5 μL/mL in RPMI, without serum) were added, and the cells were incubated for 30 min at 37 °C. Following this, the medium was aspirated, and cells were washed twice with PBS and detached with trypsin for FACS analysis. Cells that were not treated with Filipin served as controls.

Immunofluorescence. Cells were seeded in eight-well chamber slides. After incubation with NPs for 2 h, cells were washed twice with PBS and fixed with paraformaldehyde [4% (vol/vol)] for 15 min. After blocking with BSA [1% (wt/vol) in PBS] for 30 min, cells were incubated with the primary antibody (caveolin-1, CAV1; Cell Signaling; source, rabbit, #3238) overnight at 4 °C. Slides were washed with PBS and incubated with the secondary antibody [Alexa Fluor 488 goat, anti-rabbit, lgG(H+L) Invitrogen, A11008] for 1 h at room temperature. After 3 times washing with PBS, the slides were mounted onto coverslips with Vectashield mounting medium.

Western Blot. For immunoblotting, cells were lysed in laemmli buffer, and protein concentration was determined using BCA protein assay kit (Pierce). We loaded 20 μg of protein per sample and resolved it on a 10% (wt/vol) polyacrylamide–SDS gel and transferred it onto nitrocellulose membranes, which were immediately blocked with 3% (wt/vol) BSA for 1 h. Membranes were then probed with an antibody against CAV1 (Cell Signaling; source, rabbit, #3238). GAPDH (Santa Cruz Biotechnology, SC-25778) was used as a loading control. Blots were developed using peroxidase-conjugated secondary antibodies and chemiluminescence system (Thermo Scientific).

Quantitative Real-Time PCR. Total RNA was extracted using RNeasy Mini Kit (Qiagen). Reverse transcription of mRNA to cDNA was done using QuantiTect reverse transcription kit (Qiagen). Quantitative real-time PCR was performed using Rotor-Gene SYBR-Green PCR assay (Qiagen, Germany) on a Rotor-Gene Q machine (Qiagen, Germany), with the following thermal cycling conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. All biological samples were measured in triplicates, and the resulting data were normalized to RRS18N.

Statistics. Statistical analysis was done with a Student *t* test in excel (two-tailed, homoscedastic). Error bars represent standard deviations.

Octanol-Water Partition Coefficient. The octanol-water partition coefficient (LogP) values were calculated using the Molinspiration Cheminformatics open source module for calculating Molecular Properties and Bioactivity Score. For the calculation of LogP, the monomer repeat unit structure was input into the software and the values calculated using inbuilt tools.

Transmission Electron Microscopy. One drop of the NP suspension was applied to a Quantifoil grid. The thickness of the aqueous film was reduced with filter paper, and the grid was plunged immediately into liquid ethane as cryogen. After cryotransfer, the sample was measured at –170 °C using a Gatan Cryo-TEM holder.

a

Surface electrolyte	Size [nm] (б)	Polydispersity index (б)	Zeta potential [mV] (б)
PSS	183.5 (12.5)	0.199 (0.006)	-31.3 (3.1)
PAA	185.7 (9.1)	0.188 (0.022)	-55.4 (26.0)
PVS	180.3 (15.6)	0.194 (0.033)	-46.3 (10.3)
PSM	176.7 (14.5)	0.199 (0.026)	-37.2 (3.9)
PAS	181.4 (22.5)	0.194 (0.014)	-41.5 (6.1)

b

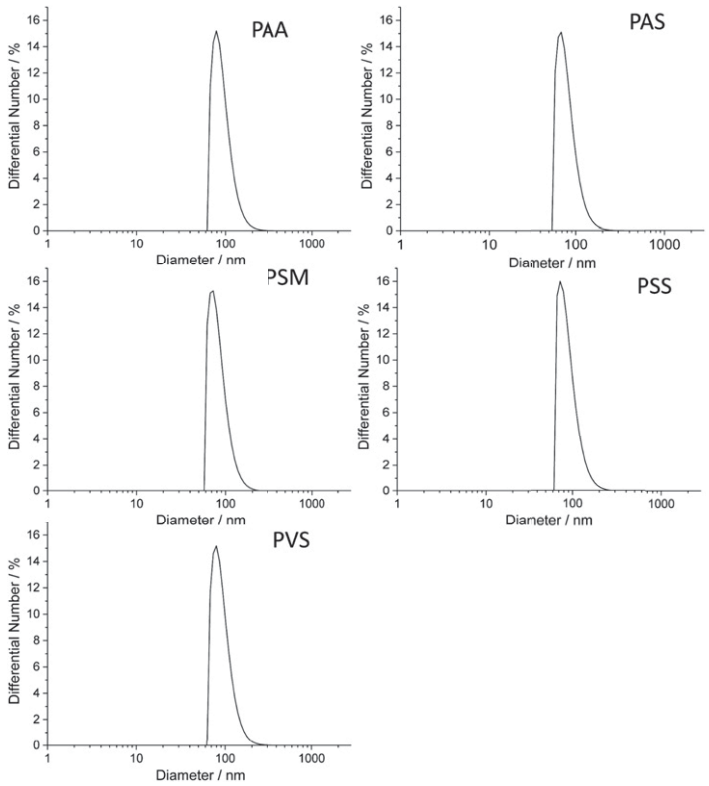

Fig. S1. NP characteristics determined by dynamic light scattering (six sets of NPs measured in triplicate) (*A*) and representative number distribution graphs (*B*).

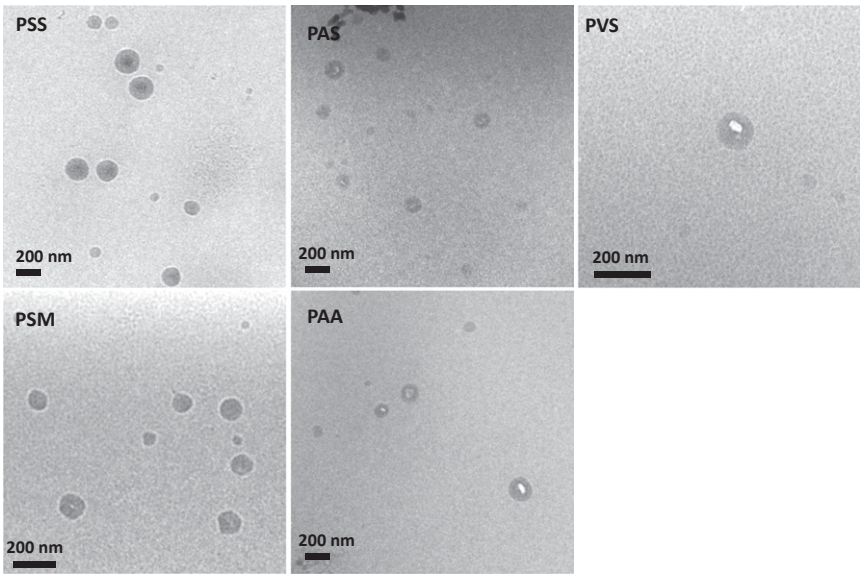

Fig. S2. Cryo-transmission electron micrographs of lipid NPs.

surface electrolyte	Δsize [nm] (б)	Δpolydispersity index (б)	zeta potential [mV] (б)
PSS	6.2(10.8)	0.010(0.037)	-39.2(2.3)
PAA	-6.7(14.3)	-0.019(0.055)	-39.2(1.6)
PVS	0.6(23.1)	-0.019(0.036)	-34.7(2.3)
PSM	37.1(34.0)	0.054(0.033)	-37.7(1.4)
PAS	-2.1(11.6)	-0.01(0.022)	-36.9(4.1)

Fig. S3. NP characteristics after 1 h incubation with medium at 37 °C determined by light scattering. Δ values are ($value_{water}$ – $value_{medium}$).

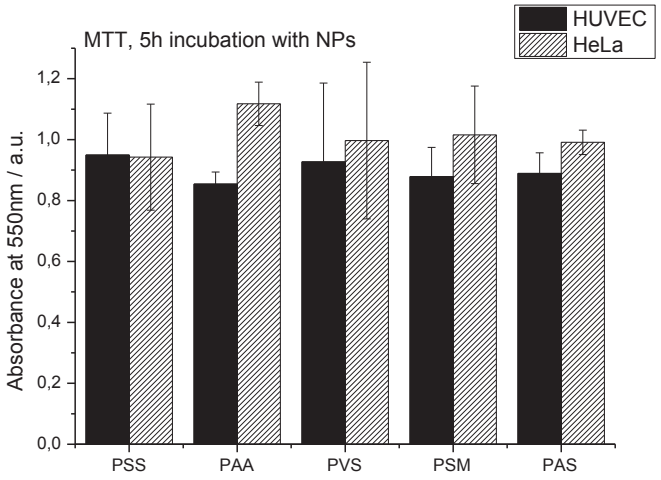

Fig. S4. Viability of HeLa cells and human umbilical vein endothelial cells (HUVECs) after exposure to NPs for 5 h as determined by MTT assay.

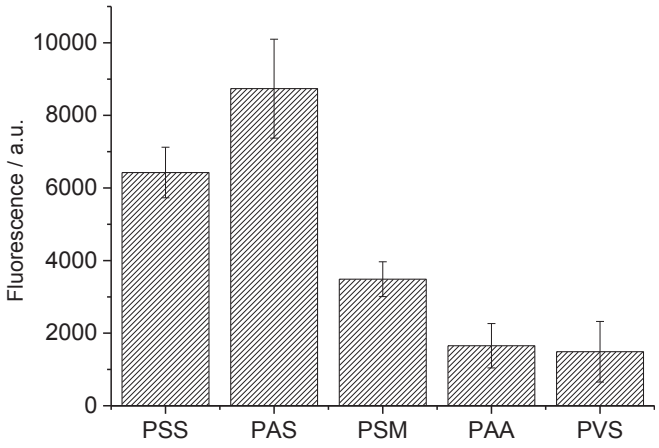

Fig. S5. Uptake of lipid NPs in HUVECs in serum-free medium determined with FACS.

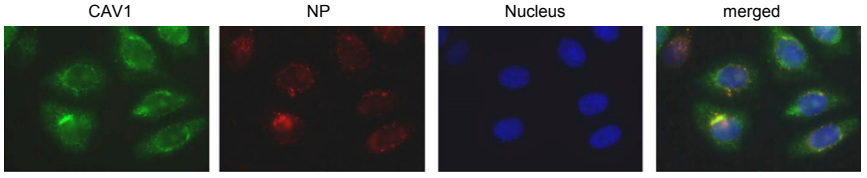

Fig. S6. Colocalization of poly(styrene sulfonate) (PSS) lipid NPs with caveolin-1 in HeLa cells. Because the caveolin expression is very low in HeLa cells, the exposure times had to be significantly increased in comparison with HUVEC images to make the caveolae visible for comparison.

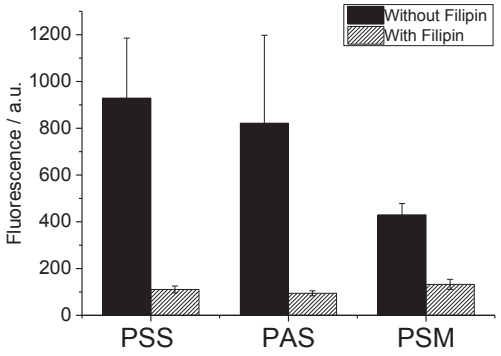

Fig. S7. Inhibition of caveolae-mediated uptake of PSS, poly(anetholesulfonic acid), and poly(4-styrenesulfonic acid–comaleic acid) NPs in HeLa cells.

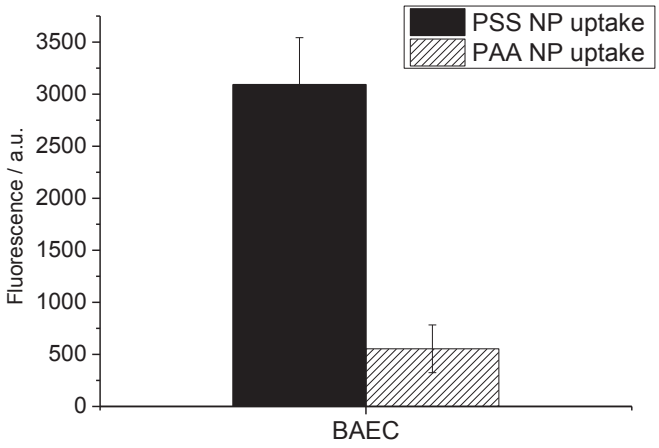

Fig. S8. FACS data of bovine aortic endothelial cells incubated with PSS and PAA lipid NPs.

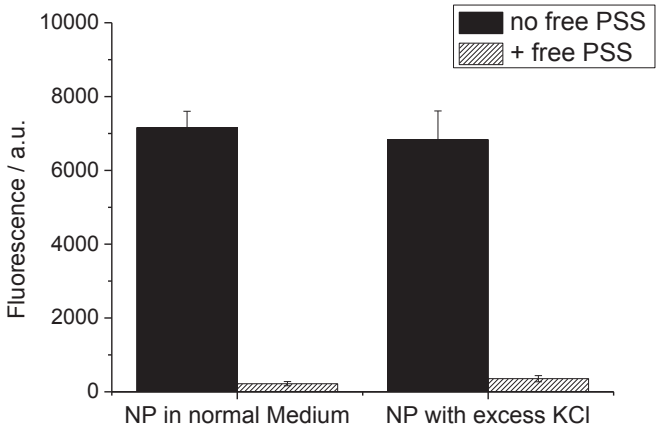

Fig. S9. NP uptake is also inhibited in the presence of excess K^+ ions showing that the K^+ binding ability of PSS has no influence on the observed effect.

CAV1_F:	GCACTTGCAACCGTCTGTTA
CAV1_R:	CTCCTCCCCCATCTTCTTTC
CAV2_F:	GCCTAATGGTTCTGCCTTCA
CAV2_R:	CGTCCTACGCTCGTACACAA
RRS18N_F:	CGGCTACCACATCCAAGGAA
RRS18N_R:	GCTGGAATTACCGCGGCT

Fig. S10. List of primers used for quantitative real-time PCR.