Electronic Supplementary Information

Probing Cell Internalisation Mechanics with Polymer Capsules

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Experimental Section

Materials. Silica particles with 519 nm diameter were purchased from Microparticles (GmbH). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC), sodium acetate (NaOAc), phosphate buffered saline (PBS), (1,8-bismaleimido)diethyleneglycol (BM(PEG)$_2$), hydrofluoric acid (HF), poly(*N*-vinylpyrrolidone) (PVPON, M_w 10 kDa), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), dithiothreitol (DTT), 3-(*N*-morpholino)propanesulfonic acid (MOPS), 2-(*N*-morpholino)ethanesulfonic acid (MES), poly(sodium 4-styrenesulfonate) (PSS, M_w 1 MDa), and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma-Aldrich and used as received. Poly(methacrylic acid) sodium salt (PMA, M_w 15 kDa, 15 wt% in water) was obtained from Polysciences (USA). Pyridine dithioethylamine hydrochloride (PDA-HCl) was purchased from Shanghai Speed Chemical Co. Ltd., China. Alexa Fluor 633 hydrazide (AF633) and Alexa Fluor 488 (AF488) goat antimouse IgG were from Invitrogen. Roswell Park Memorial Institute medium (RPMI), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), 4% paraformaldehyde (PFA), Dulbecco's phosphate buffered saline (DPBS), Hoechst 33342, Alexa Fluor 488 wheat germ agglutinin (AF488 WGA) and SnakeSkin™ dialysis tubing (Molecular weight cut off: 3.5 kDa) were obtained from Life Technologies. Mouse antihuman

LAMP-1 antibody (CD107a) was purchased from BD Pharmingen. Highly purified water with resistivity greater than 18.0 MΩ·cm obtained from a Millipore Milli-Q purification system was used.

Preparation of Thiolated PMA (PMA_{SH}). Poly(methacrylic acid) with 12% thiol groups was synthesised as reported elsewhere.[1] Briefly, a PMA solution (187.2 mg of 30 wt% solution) was diluted with 3 mL of phosphate buffer (50 mM, pH 7.4). The resulting solution was pre-mixed with 43.0 mg of EDC and the mixture was stirred at ambient temperature for 30 min. Subsequently, 28.4 mg of PDA–HCl was added to the mixture and the reaction was allowed to proceed for 24 h. The resulting solution was purified via dialysis for 3 days against Milli-Q water and subsequently freeze dried for 48 h to obtain PMA–PDA as a powder. The content of thiol groups on PMA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific). To expose thiol groups, PMA–PDA was dissolved in 0.5 M DTT solution in MOPS buffer (20 mM, pH 8) at a concentration of 100 g L^{-1}, and was shaken (800 rpm) for 15 min at 37 ˚C. The stock solution was then diluted with NaOAc buffer (50 mM, pH 4) to the desired concentration prior to layer-by-layer (LbL) assembly.

Fabrication of PMA Capsules. SiO_2 particles (5 mg) were pre-washed and redispersed in NaOAc buffer (50 mM, pH 4) three times by centrifuging at 1000 *g* for 2 min and vortexing for 1 min The particles were then resuspended in 50 μL of NaOAc buffer by vortexing and sonication for 5 min. 50 μL of PVPON solution (4 g L^{-1} in 50 mM NaOAc buffer) was then added to the SiO_2 particles, which was then incubated 10 min with mixing to allow polymer adsorption. After incubation, the PVPON-coated particles were washed and redispersed in NaOAc buffer three times and then resuspended in 50 μL of NaOAc buffer. 50 μL of PMA_{SH} solution (4 g L^{-1} in 50 mM NaOAc buffer) was then added and incubated for 10 min. Following this, the particles were washed and redispersed in NaOAc buffer three times and finally resuspended in 50 μL of NaOAc buffer. The adsorption of PVPON and PMA_{SH} constituted the assembly of a single bilayer. The layering process was repeated until four, five, six, seven, eight, nine, or 10 bilayers were deposited. The polymer multilayers were crosslinked via the formation of covalent bonds between the thiol groups of PMA_{SH} and the maleimides of $BM(PEG)_2$. PVPON/PMA_{SH} core-shell particles were incubated in 2.8 mM $BM(PEG)_2$ in MES buffer (50 mM, pH 6) with gentle vortexing overnight. The stabilised core-shell particles were collected by washing and redispersing twice in MES buffer and subsequently twice in NaOAc buffer. Capsules were obtained by dissolving the silica

templates using 5 M HF for 5 min *(Caution! HF is highly dangerous and extreme care must be taken when handling!)*, followed by three washing and redispersion cycles with PBS buffer. The capsules were then fluorescently labelled by mixing the capsules with 5 μL of AF633 (5 mg mL^{-1}) and 1 mg of DMTMM in 200 μL of PBS for 12 h. Fluorescently labelled capsules were collected via centrifugation at 3500 *g* for 5 min. After washing with PBS four times, the capsules were resuspended in cell culture medium.

Characterisation of PMA Capsules. Fluorescently labelled PMA capsules were imaged using a structured illumination microscope equipped with a 60× 1.24 NA oil objective (DeltaVision OMX Blaze 3D-SIM from Applied Precision) under a standard FITC/CY5 filter set. Shell thicknesses of PMA capsules were measured by atomic force microscopy using an MFP-3D atomic force microscope (Asylum Research). Imaging was performed in AC mode in air using ultrasharp SiN gold-coated cantilevers (NT-MDT). Capsule counting was performed using an Apogee A50-Micro flow cytometer (Apogee Flow Systems Ltd.) with a laser excitation wavelength of 638 nm.

Osmotic Pressure of PSS Solutions. The osmolarity (mosmoles kg^{-1}) of PSS solution was measured by a vapour pressure osmometer (Vapro® model 5600). The osmolarity was then converted to osmotic pressure (MPa) by using the following formula: Osmotic pressure (MPa) = -Osmolarity×2.58×10^{-3} according to the Van't Hoff equation. Thus, the osmotic pressure for a certain PSS concentration could be read from the calibration curve.

Deformation of PMA Capsules in PSS Solution. 1×10^8 of AF633-labelled capsules was mixed with 100 μL of PSS solution of different concentrations. Fluorescence images of PMA capsules suspended in PSS solution were then collected using SIM equipped with a 60× 1.24 NA oil objective under a standard CY5 filter. Images were obtained by processing with *Imaris 6.3.1* (Bitplane) using the maximum intensity projection. The percentage of extracellular deformation of capsules in PSS solution was quantified by analysing at least 500 capsules.

Cell Culture. Macrophage-like THP-1 (dTHP-1) cells were differentiated from THP-1 cells by the incubation with 200 nM TPA for 48 h in complete RPMI medium (RPMI containing 10% FBS, termed as cRPMI) at 37 °C in a 5% CO_2 humidified atmosphere.

Intracellular Deformation of PMA Capsules. dTHP-1 cells were plated into 8-well Lab-Tek I chambered coverglass slides (Thermo Fisher Scientific, Rochester) at a density of 5×10^5 cells per well. The cells were then treated with PMA capsules at a capsule-to-cell ratio of 100:1 for 24 h at 37 °C in 5% CO_2. After treatment, the cells were fixed with 4% PFA for 10 min at room temperature (25 °C) and the cell nuclei were stained with 200 μL of Hoechst 33342 (1 μg mL^{-1}) for 15 min at room temperature (25 °C) followed by washing with DPBS three times. The cell membrane was stained with AF488-WGA (0.5 μg mL^{-1}) for 15 min at room temperature (25 °C), followed by washing with DPBS three times. Fluorescence images and optical sections were collected using a SIM equipped with a 60× 1.24 NA oil objective under a FITC/CY5 filter set. Images were processed with *Imaris 6.3.1* (Bitplane). The deformation percentage was defined as the number of deformed capsules divided by the total number of capsules. The intracellular deformation was quantified by analysing at least 500 internalised capsules.

Cellular Uptake of PMA Capsules. dTHP-1 cells were plated in 24-well plates at a density of 1×10^5 cells per well. Cells were then incubated with PMA capsules at a capsule-to-cell ratio of 100:1 for a 12 h period at 37 °C in 5% CO_2. After treatment, the cells were washed with DPBS three times and then harvested by trypsinization at 37 °C. At least 10,000 cells were analysed by flow cytometry.

Intracellular Fate. 5×10^4 cells dTHP-1 cells were plated in 8-well Lab-Tek I chambered coverglass slides. Then, dTHP-1 cells were incubated with AF633-labelled PMA capsules at a capsule-to-cell ratio of 100:1 at 37 °C in 5% CO_2. After 24 h incubation, cells were fixed with 4% PFA for 30 min at room temperature (25 °C). To permeabilise the cells for immunostaining, 500 μL of Triton X-100 (0.1% in PBS buffer) was allowed to incubate with fixed cells for 5 min at ambient temperature. Late endosomes and lysosomes were immunostained with 200 μL of mouse anti-human antibody (2.5 μg mL^{-1}) and 200 μL of AF488 goat anti-mouse IgG (2 μg mL^{-1}) for 45 min at ambient temperature, respectively. Fluorescence images and optical sections were collected using a SIM equipped with a 60× 1.24 NA oil objective under a standard FITC/CY5 filter set. Images were processed with *Imaris 6.3.1* (Bitplane).

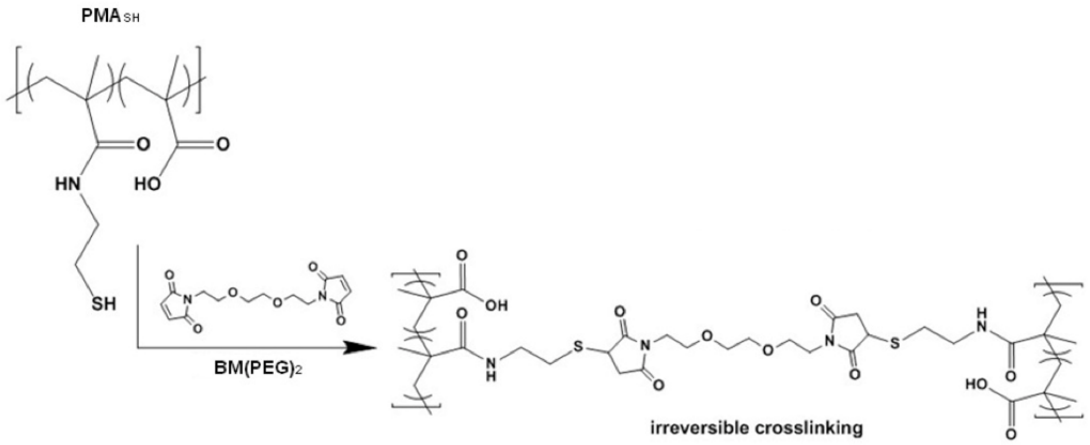

Fig. S1 Cross-linking of thiol groups on PMA into non-cleavable thioether bonds using $BM(PEG)_2$.

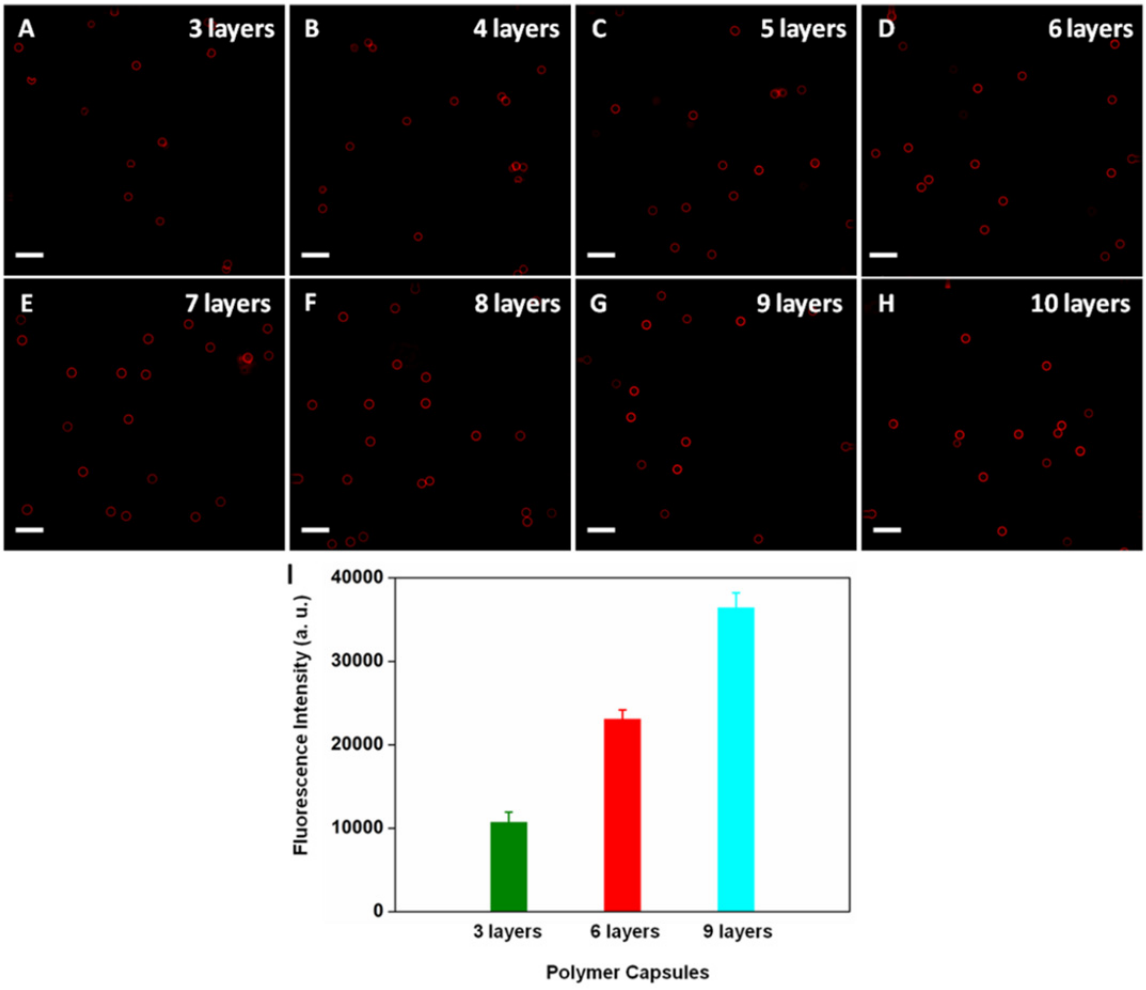

Fig. S2 (A-H) SIM images of PMA capsules with different polymer layers suspended in a cell culture cRPMI medium. Images represent the maximum intensity projection. Scale bars are 2 μm. (I) Fluorescence intensity evolution of PMA capsules with different polymer layers (three, six, or nine layers). The data were obtained from SIM images. At least 50 capsules were analysed for each sample.

Table S1 Size of PMA capsules composed of different polymer layers analysed using SIM images. The sizes are expressed as the mean and standard deviation obtained from at least 50 capsules

Number of polymer layers	4	6	8	10
Capsule diameter (μm)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1

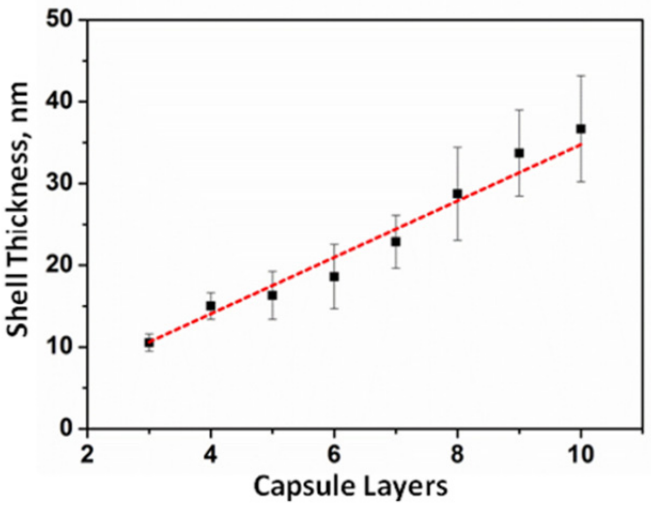

Fig. S3 Film thickness as a function of the number of polymer layers. Data are expressed as the mean ± standard error, obtained from measuring at least 30 capsules for each sample.

Table S2 The values of experimental and regressive shell thickness of PMA capsules with different polymer layers

Number of polymer layers	3	4	5	6	7	8	9	10
Experimental shell thickness (nm)	10.5	15.0	16.3	18.6	22.9	28.7	33.7	36.7
Linear regression of shell thickness (nm)	10.5	14.2	17.9	21.6	25.3	29.0	32.7	36.4

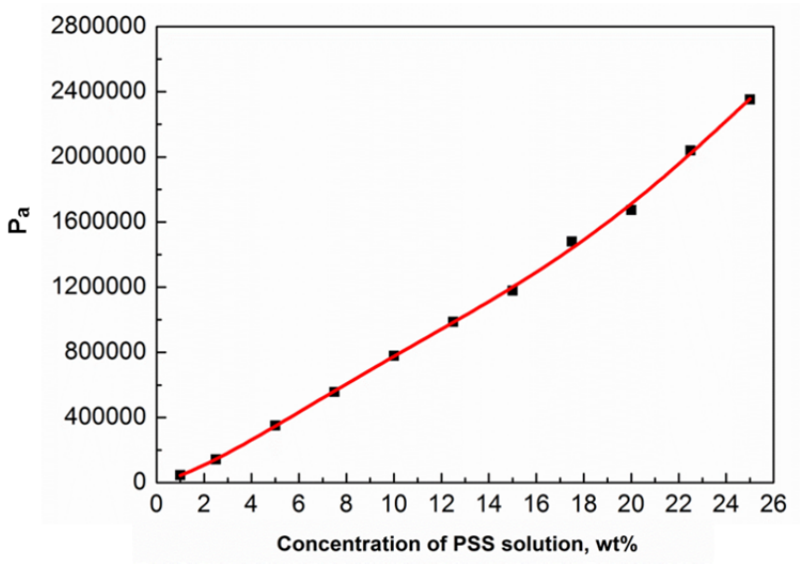

Fig. S4 Osmotic pressure calibration curve of PSS solution.

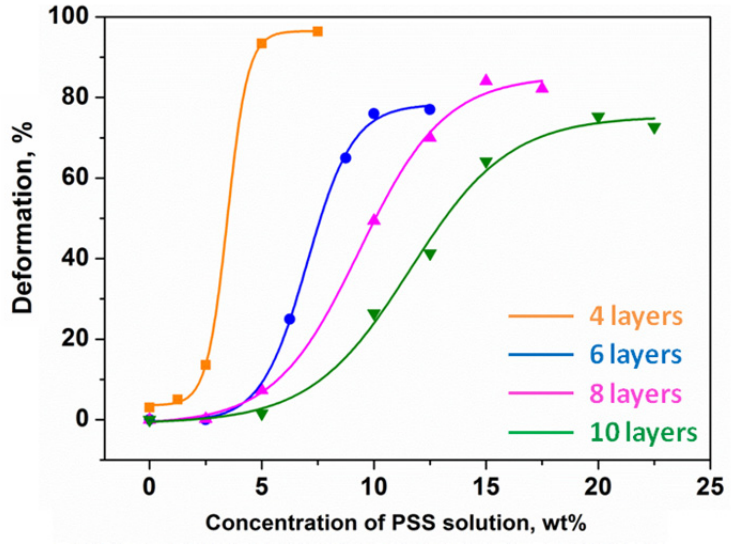

Fig. S5 Percentage of deformed capsules with four, six, eight, and ten layers as a function of the PSS concentration. Each data point is obtained by analysing at least 500 capsules.

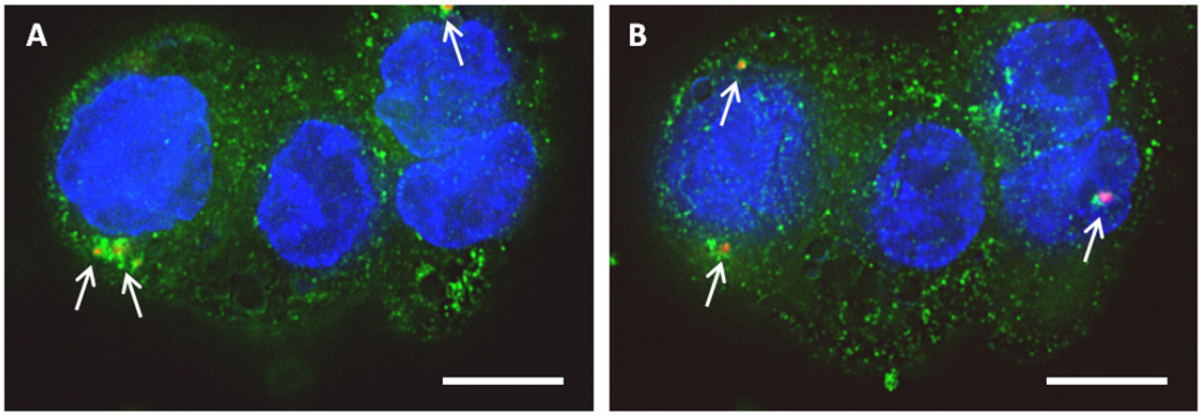

Fig. S6 SIM images of co-localization of AF633-labeled PMA capsules (red, as marked by white arrows) with lysosomes after internalization in dTHP-1 cells (A: three-layer capsules; B: six-layer capsules). After 12 h incubation, the cells were stained with an anti-LAMP 1 antibody (green) that binds to lysosomes. Cell nuclei were stained with Hoechst 33342 (blue). The images were taken at a single focal plane. Scale bars are 10 μm.

REFERENCES

1. A. Fery and R. Weinkamer, *Polymer*, 2007, **48**, 7221-7235.