

Supplementary information

Hydrophobicity determines the fate of self-assembled fluorescent nanoparticles in cells

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Materials and methods

Materials

Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification. Oligomers apolar and amp1 were synthesized according to protocol¹ and oligomers amp2 and bola were kindly provided by dr. Adrien Kaeser. Alexa647 labelled antibody was kindly provided by dr.ir. Brian Janssen and preparation is described elsewhere.² Water was purified on an EMD Millipore Milli-Q integral water purification system. Fetal bovine serum (FBS) and penicillin streptomycin (Pen/Strep) were bought from Invitrogen. Phosphate Buffered Saline tablets were bought from Novagen. 8-well glass chambers for cell-seeding, electroporation and confocal microscopy were bought from LabTek. Dulbecco's Modified Eagle Medium (DMEM + phenolred + glutamine + glucose + HEPES – pyruvate), HeLa cells and Lysotraker red dnd-99 in DMSO were bought from ThermoFisher. Trypsin was bought from Sigma Aldrich.

Instrumentation

UV-spectra were recorded on a Perkin Elmer Lambda 950 or Perkin Elmer Lambda 900. Dynamic light Scattering (DLS) measurements were performed on a Malvern Zetasizer ZMV2000. Fluorescent spectroscopy was performed on a Varian Cary Eclipse fluorescence spectrometer with temperature controlled multi-cell holder. Electroporation was performed using a cellaxess CX1 system from Cellectricon. Confocal microscopy was performed using a Leica TCS SP5 AOBS equipped with a HCX PL APO CS x63/1.2 NA and a 40x water immersion lens and temperature controlled incubator. SMNPs were excited by a Chameleon Multiphoton laser.

Methods

SMNP preparation was performed according to a previously published protocol.¹ Briefly, solid oligomer was dissolved in tetrahydrofuran (THF) at 1 mM and typically 15 μ L of this concentrated solution was injected in 5 mL water or PBS resulting in a SMNP solution at 3 μ M. Synthesis and particle characterization are according to literature.^{1,3}

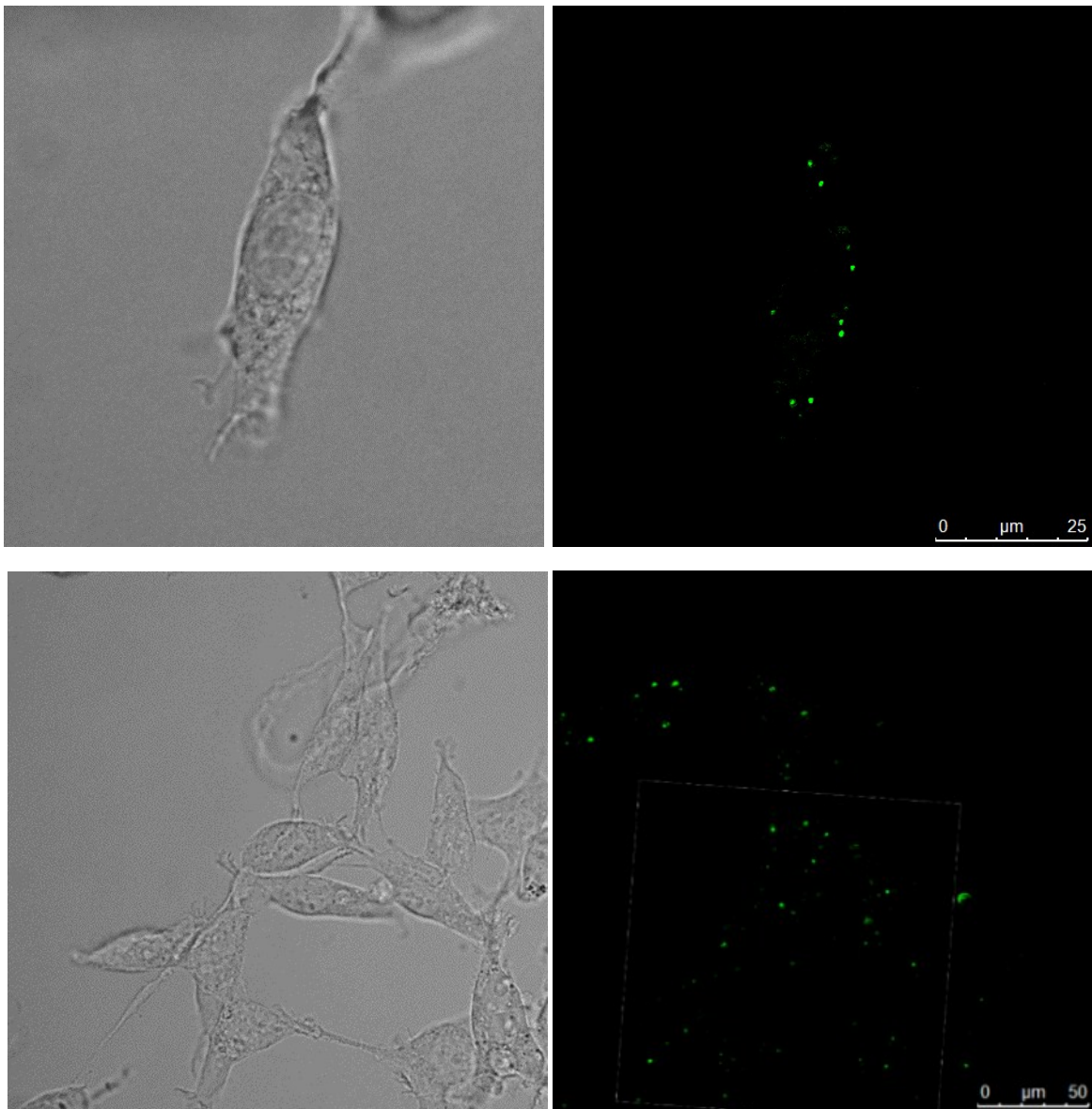
HeLa cells were cultured in cell media (DMEM + 10 % (v/v) filtered FBS + 1 % (v/v) Penstrep) and in an 8-well chamber 15 * 10⁴ cells/well were seeded the day prior to electroporation. About 1 hour prior to electroporation, cells were washed with fresh cell media. Electroporation was performed at 70 – 80 % cell confluency by using a SMNP dispense volume of 30 μ L, a dispense rate of 30 μ L/min at 120 V with 25 pulses, pulse length of 25 ms starting at 50 seconds. After electroporation, cells were kept in incubator and washed with fresh cell media prior to imaging.

In case of labelling with antibody, the cells were washed with PBS prior to fixation. Fixation was performed by washing cells 3 x with PBS and subsequently incubating the cells with 4% formaldehyde / PBS solution for 10 min. Cells were again washed 3 x with PBS and stored in fridge.

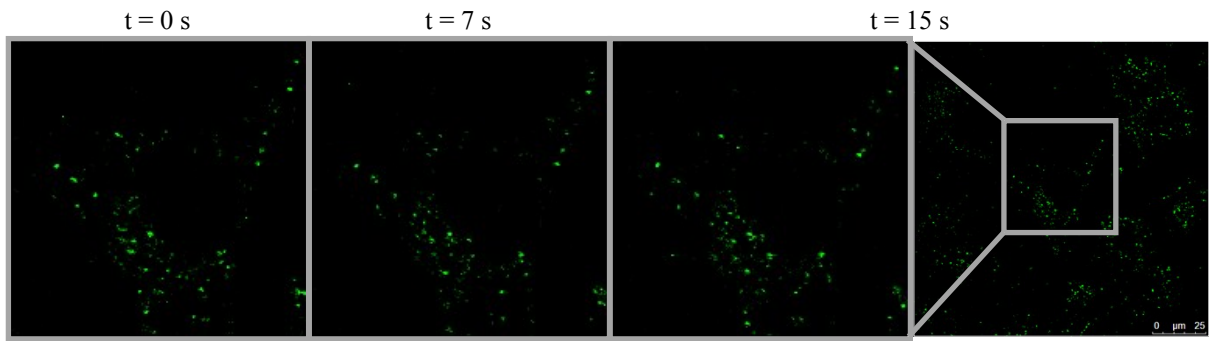
SMNP visualization by two-photon microscopy on live cells was performed by exciting at 725 nm and detecting emission between 480 and 680 nm using a fully opened pinhole (600 μm). Lyso-tracker® was visualized using white light laser with pinhole at 1 airy unit, exciting at 577 nm and emission was detected between 590 and 623 nm.

Cetuximab-647 was imaged on fixed cells by white light laser exciting at 648 nm and detecting the emission between 650 and 690 nm. Cetuximab-647 incubation was performed for 15 minutes in PBS on fixed cells followed by one PBS wash step.

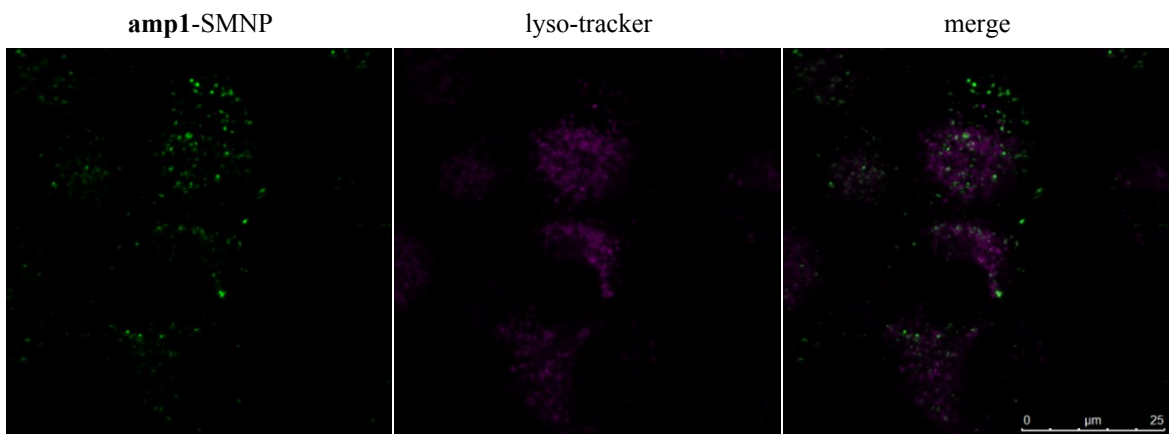
Supporting microscopy images



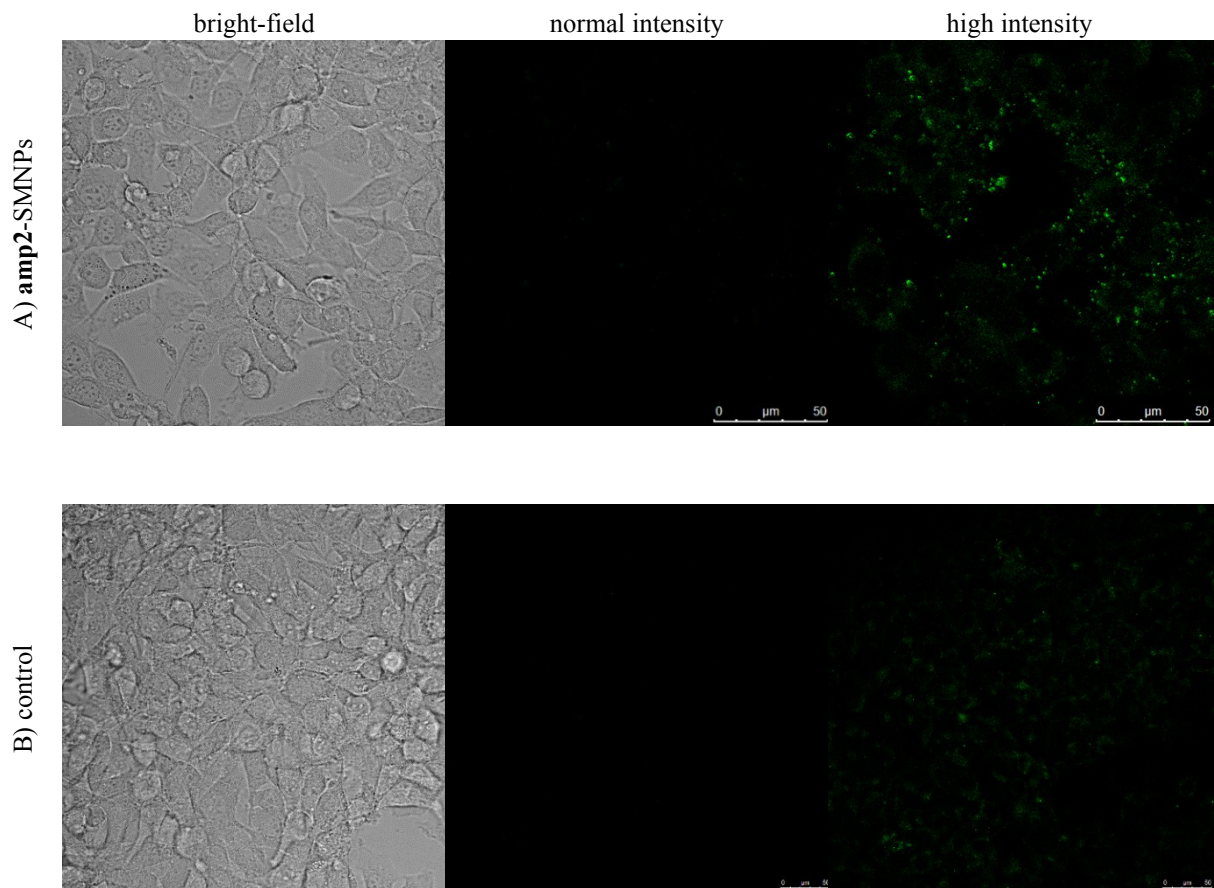
SI 1: Single and multiple cell microscopy pictures of *apolar*-SMNP containing HeLa cell. Bright-field (left), two-photon excitation fluorescence emission channel (right).



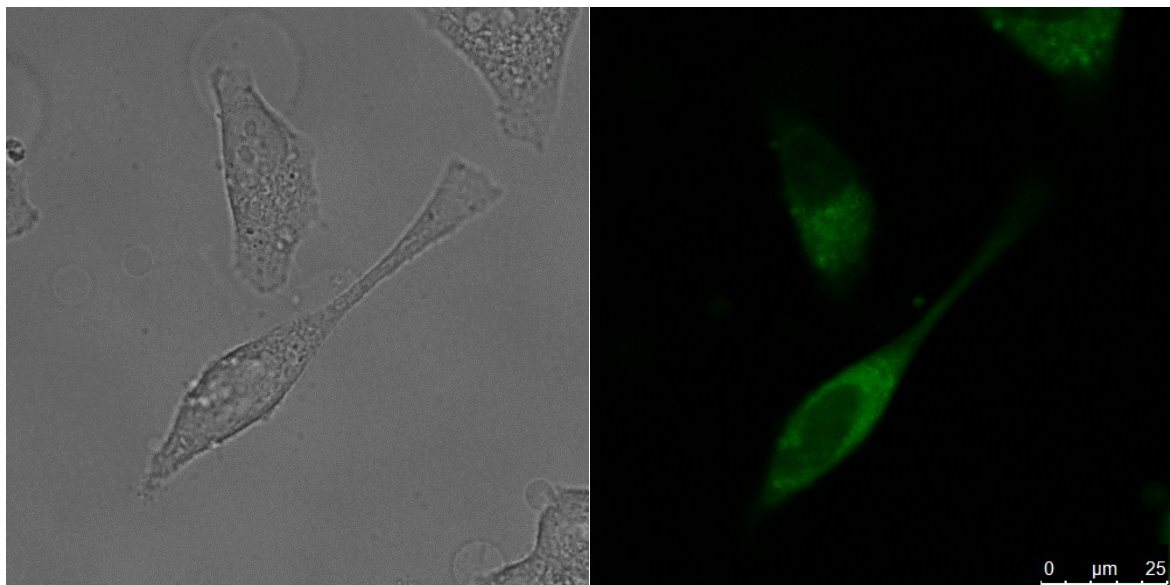
SI 2: *Timelapse of **amp1**-SMNPs in living HeLa cells after electroporation via two-photon excitation fluorescence microscopy. The insert in the picture on the right was magnified for particle tracking in a single cell for 15 s.*



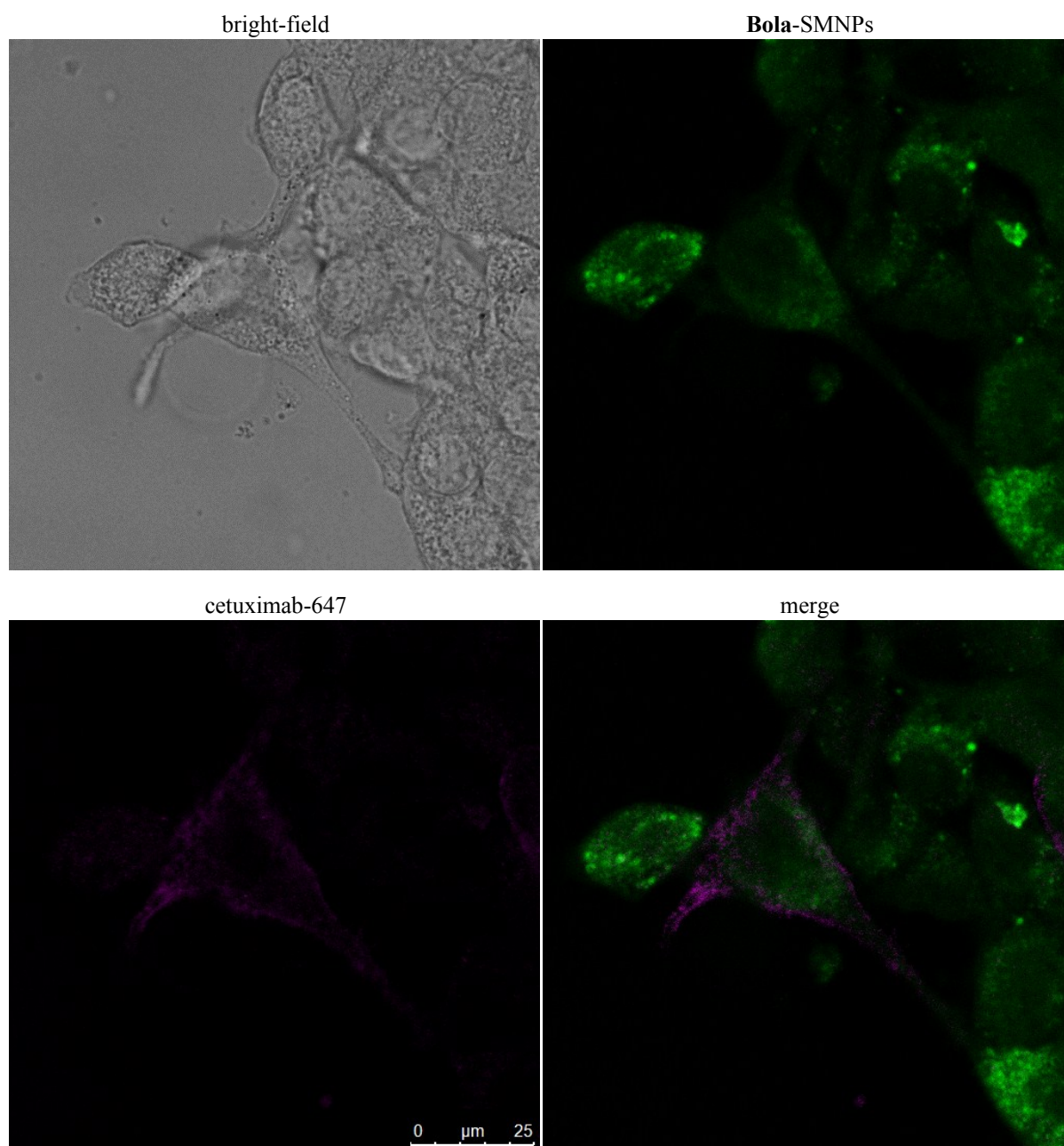
SI 3: *Co-localization of **amp1**-SMNPs electroporated in HeLa-cells and Lyso-tracker via two-photon (SMNPs) and single photon (Lyso-tracker) excitation fluorescence microscopy.*



SI 4: A) *Amp2*-SMNPs electroporated in HeLa-cells and B) PBS electroporated in HeLa-cells which served as control. Bright-field (left), two-photon excitation fluorescence emission channel (right).



SI 5: Single cell microscopy pictures of *bola*-SMNP in HeLa cells. Bright-field (left), two-photon excitation fluorescence emission channel (right).



SI 6: *Bola-SMNPs* microscopy pictures electroporated in HeLa-cells and incubated with cetuximab-647 after fixation.

References

1. Kaeser, A. *et al.* Side Chains Control Dynamics and Self-Sorting in Fluorescent Organic Nanoparticles. *ACS Nano* **7**, 408–416 (2013).
2. Brian Janssen. DNA-based control of protein activity. (Eindhoven University of Technology, 2015).
3. Abbel, R. *et al.* Multicolour Self-Assembled Fluorene Co-Oligomers: From Molecules to the Solid State via White-Light-Emitting Organogels. *Chem. – Eur. J.* **15**, 9737–9746 (2009).