Electronic Supplementary Material (ESI) for Soft Matter.

Electronic **S**upplementary **I**nformation

Hollow Spherical Nucleic Acid Structures Based on Polymer-Coated Phospholipid Vesicles

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Physicochemical characterization

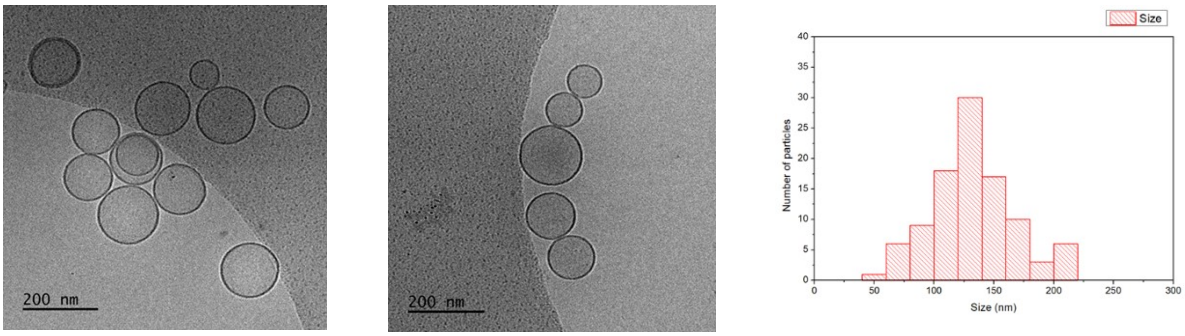

Figure S1. Cryo-TEM images and size distribution of DPPC liposomes.

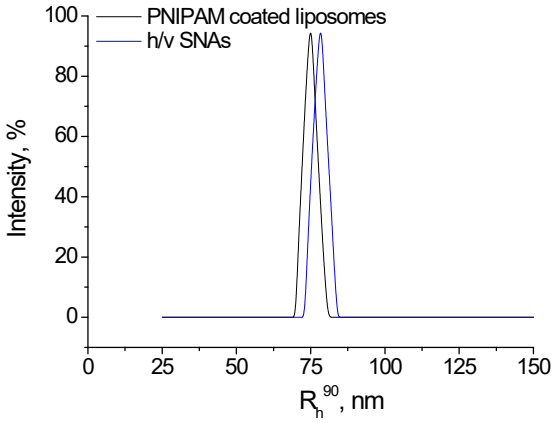

Figure S2. Size distribution curves of PNIPAM-coated liposomes (black line) and h/v SNAs (blue line). Measurements were performed at 25 °C and an angles of 90°.

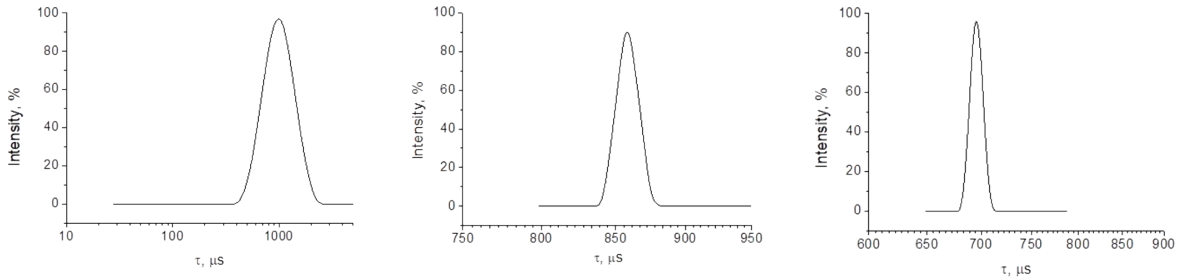

Figure S3. Relaxation time (τ) distributions from DLS for h/v SNAs measured at angles of 80°, 90°, and 110° (from left to right) and 25 °C.

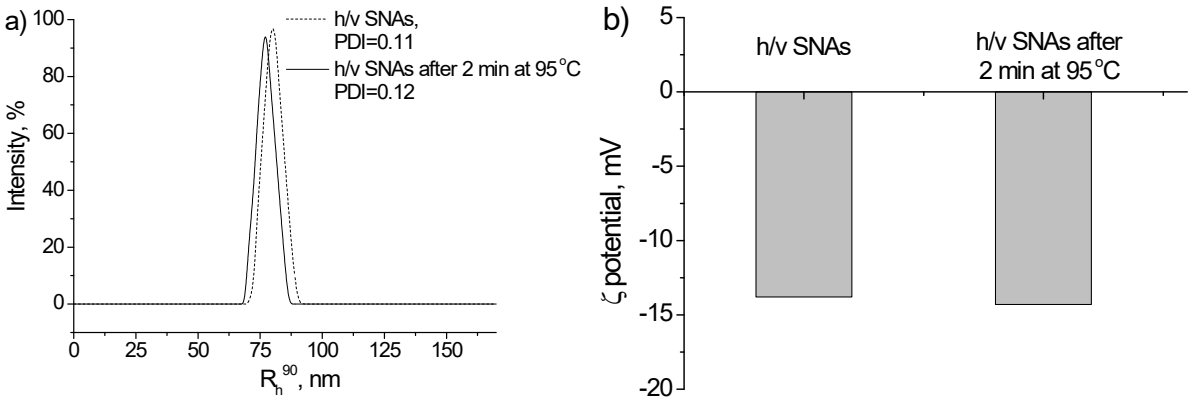

Figure S4. Particle size distribution (a) and ζ potential (b) to test the stability of the h/v SNAs at the conditions of hybridization. The initial h/v SNAs were heated to 95 °C for 2 min and then slowly cooled down to room temperature. Measurements were performed at 25 °C and angles of 90° and 15° in (a) and (b), respectively.

Calculation of the number of oligonucleotide strands per vesicle and grafting density, σ

The average number of oligonucleotide strands per vesicle was determined from the oligonucleotide content, DPPC concentration, the average size and aggregation number of the initial DPPC liposomes, and surface of the final h/v SNAs. Given the content and molar mass of the oligonucleotide of 6703 $g.mol^{-1}$ and using the Avogadro's number, the total number of oligonucleotide molecules was $2.089x10^{16}$. The total number of DPPC molecules was calculated from the concentration. From the size of the initial liposomes of 74.8 nm, assuming spherical morphology, bilayer arrangement, unilamellar structure, and area per DPPC molecule in the bilayer of 0.65 nm^{-2},[1] the aggregation number was calculated to be 220,877 DPPC molecules per liposome. Then the number of liposomes in the solution was calculated from the total number of DPPC molecules and aggregation number. The resulting value after dilution was $3.56x10^{12}$. The number of oligonucleotide strands per vesicle was calculated from the total number of strands and number of liposomes. The resulting value was 5868 strands per vesicle. Finally, the area per oligonucleotide in the vesicle and its reciprocal grafting density were calculated from the surface of a single h/v SNA vesicle and the number of strands per vesicle to be 13.12 nm^2 and 0.076 nm^{-2}, respectively.

Calculation of the Flory radius, R_F, and critical grafting density, σ_{cr}

The Flory radius is the end-to-end distance of a free polymer coil in a theta solvent, resulting from the balance between the expanding steric forces and the counteracting entropic forces from stretching of the coils. In its simplest form it is given by the following equation:[2]

$R_F = an^{3/5}$

Here *a* is the length of the monomer unit and *n* is the degree of polymerization. The methacrylamide functionalized oligonucleotide is composed of a hexaethylene glycol spacer and 21 bases. If the lengths of ethylene oxide unit and nucleotide unit are taken as 0.39 nm and 0.34 nm, respectively, and the total degree of polymerization is 27 (= 6 + 21), then for R_F we obtain:

$$R_F = \left(\frac{6}{27} \times 0.39 + \frac{21}{27} \times 0.34\right) \times (6 + 21)^{\frac{3}{5}} = 2.54\ nm$$

The oligonucleotide strands, if regarded as surface-anchored random coils,[3-6] start to interact laterally when the distance between the grafting points is equal or less than the Flory radius. Thus, the critical grafting density at the transition from an unextended (mushroom) to brush conformation, σ_{cr}, can be determined by $\sigma_{cr} = 1/R_F^2$. With R_F = 2.54 nm, for σ_{cr} a value of 0.155 nm^{-2} was obtained. The brush regime is entered when $\sigma > \sigma_{cr}$.

Biological evaluation

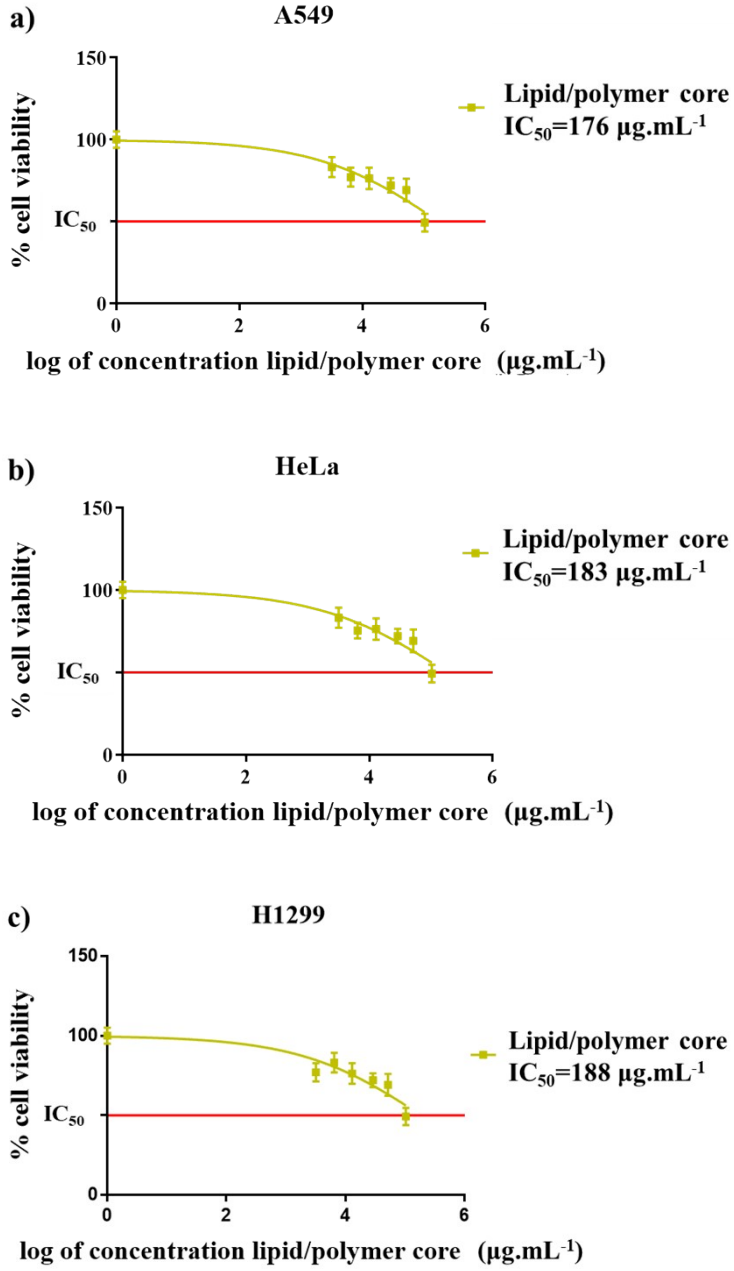

Figure S5. Cytotoxicity of PNIPAM-coated DPPC liposomes. Cell viability is presented as a percent of untreated control cells. IC_{50} values were determined by the MTT-dye reduction assay 72-hours post-continuous exposure of A549 (a), HeLa (b) and H1299 (c) human cells. Each data point represents the mean ± SD of 8 replicates in three independent experiments. IC_{50} values were calculated in GraphPad Sigma v.8 software using the "log of concentration vs normalized response (variable slope)" algorithm.

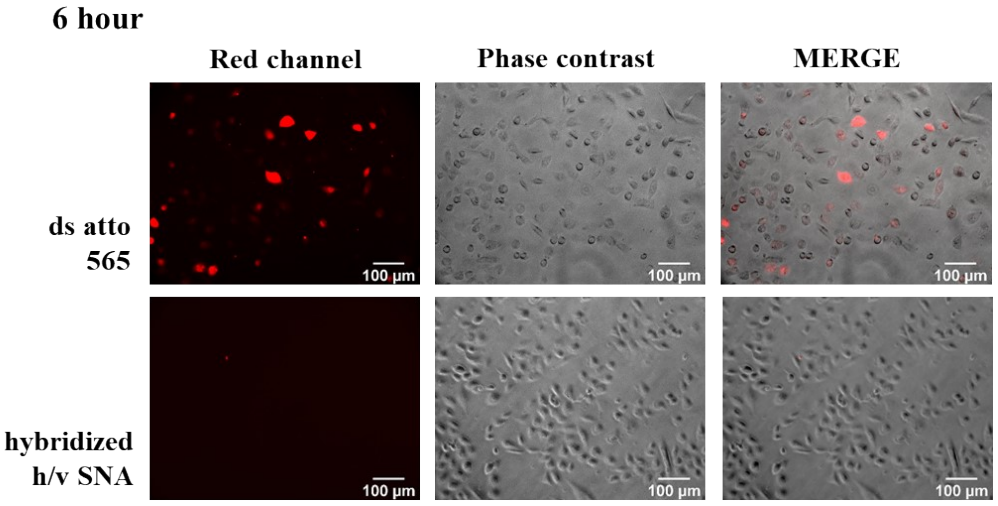

Figure S6. Fluorescent microscopy of H1299 cells after 6 h exposure to Lipofectamine2000-complexed dsAtto565 (upper row) and hybridized h/v SNAs (lower row).

References

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