Supporting Information

Responsive Nucleic Acid-Based Nanoparticles

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A. Instrumentation

Attenuated total reflection Fourier transform infrared (ATR-FTIR) transmission spectra were measured using a Shimadzu IRAffinity-1 spectrometer. The spectra were recorded by drop-casting solutions or, in the case of organosilica particles, placing the powders onto the sample holder of the instrument. ATR-FTIR spectra of the particle samples were recorded at a spectral resolution of Δ=4 cm^{-1}, with 64 scans accumulated and the ATR-FTIR spectra were ATR-corrected (penetration depth mode).

For DNAs, mass spectrometry (MS) experiments were performed on a Bruker Daltonics microToF spectrometer (Bruker Daltonik GmgH, Bremen, Germany) with the electrospray ionization (ESI) interface operating in a negative mode. For PNAs, UPLC-MS analyses were performed on a Waters Acquity Ultra Performance LC system equipped with a Waters Acquity SQ Detector, an ESI interface operating in positive mode, and a Waters Acquity UPLC BEH 300 column (C18, 1.7 μm, 300 Å, 2.1 x 50 mm).

Absorption spectra were measured on a Shimadzu UV-3600 spectrophotometer double-beam UV–VIS–NIR spectrometer or on a Thermo Scientific Evolution 260 BIO spectrophotometer and baseline corrected. Quartz cuvettes with a path length of 10.0 mm were used for the recording of UV-Vis absorption spectra.

Emission spectra were measured on a Horiba Jobin−Yvon IBH FL-322 Fluorolog 3 spectrometer equipped with a 450 W xenon arc lamp, double grating excitation, and emission monochromators (2.1 nm mm^{-1} of dispersion; 1200 grooves mm^{-1}) and a TBX-04 single photocounting detector was used. Quartz cuvettes with a path length of 10 mm were used for all recorded spectra. Relative FRET efficiencies were recorded on a BMG Labtech (CLARIOstar Plus), running Kaleido 3.0 and MARS 4.01 R2 as software. To this end, black opaque OptiPlateTM-96 polystyrene microwell plates with transparent well from Perkin Elmer were used. The instruments were equipped with monochromatic fluorescence intensity detection (top- and bottom-reading) and filter- and monochromator-based absorbance detection and temperature control.

Scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) images were recorded on a FEI Quanta FEG 250 instrument (FEI corporate, Hillsboro, S3 Oregon, USA) with an acceleration voltage of 20 kV. The SEM sample was prepared by drop-casting the aqueous dispersion of particles diluted 1 : 9 in EtOH onto a glass coverslip and, subsequently, sputter coated with Au (Emitech K575X Peltier cooled) for 60 s at 60 mA prior to a fixation on an Al support.

Transmission electron microscopy (TEM) samples were analyzed on an FEI/PHILIPS CM120 system operating at 100 kV. Samples were prepared by drop casting aqueous dispersions of particles diluted 1 : 9 in EtOH (0.015 $mg \cdot mL^{-1}$) onto Formvar-coated Cu grids (400 mesh) and allowed to dry overnight prior to visualization.

ζ-potential measurements were conducted on a DelsaNano C Particle Analyzer (Beckman Coulter, operative wavelength 655 nm). Measurements were performed on the dispersions of the particles in water.

DLS measurements were performed using a Malvern ZetaSizer nano-device. The intensity of the scattered light was measured at a fixed angle (173°), while the laser light wavelength for the scattering experiments was 633 nm. Data analysis was performed according to standard procedures using Malvern software. Briefly, the decay rates were determined by the following relationship, where η is the viscosity of the medium (water), D_h is the hydrodynamic diameter, and θ is the scattering angle.

$$\Gamma = D_h \cdot \left(\frac{4\pi\eta}{\lambda} \cdot \sin\left(\frac{\theta}{2}\right)\right)^2$$

To fit the autocorrelation function, the non-negatively constrained least squares (NNLS) algorithm built into the software was used, which allows the determination of the diffusion coefficient (*d*), from which the hydrodynamic diameter of the particles is calculated using the Stokes-Einstein equation (see below), where k_b is the Boltzmann constant, T is the temperature, and η is the viscosity of the medium (water).

$$D_h = \frac{k_b \cdot T}{6 \cdot \pi \cdot \eta \cdot d}$$

The resulting intensity distribution was in some cases recalculated as a volume distribution (or number-weighted distribution) by the algorithm built into the software, which is based on the Mie theory.

Small-angle X-ray scattering (SAXS) set-up comprised the SAXSess mc2 instrument from Anton Paar GmbH, containing a slit collimation system, and the PW3830 laboratory X-ray generator (40 kV, 50 mA) with a long-fine focus sealed X-ray tube (CuKα wavelength of λ = 0.1542 nm) from PANalytical. Detection was performed with the 2D imaging-plate reader Cyclone® by Perkin Elmer. The measurement was performed on a powder sample for 5 min and the data collected up to a scattering vector q value of 7 nm^{-1}, where $q = (4\pi\sin\vartheta)/\lambda$ and 2ϑ is the scattering angle. The 2D data were converted to 1D data and background-corrected by using SAXSQuant software (Anton Paar GmbH, Austria).

X-ray photoelectron spectroscopy (XPS) analysis was performed using a K-Alpha™+ X-ray Photoelectron Spectrometer System (Thermo Scientific). Monochromatic Al K alpha X-rays were used (15 keV, 72 W, 200 mm spot diameter). Spectra were measured using pass energy of 200 eV for survey spectra and 50 eV for core-level spectra. The samples were analyzed as powders. The Advantage software was used for background subtraction (smart mode), and deconvolution of peaks.

The oligonucleotides were synthesized on a solid support using a BioAutomation Mer Made MM6 DNA synthesizer using a standard phosphoramidite protocol.

Reversed-phase HPLC purification of amino-modified DNA strands was carried out on an Agilent Infinity 1260 instrument. Column used: Hamilton PRP 4.6 x 250 mm. Reversed-phase HPLC purification of the PNA strands was performed on an Agilent 1260 Series LC system. Column used: Jupiter C18, 5 μm, 300 Å, 10 x 250 mm by Phenomenex.

Section I. Synthesis and characterization of DNA and PNA oligonucleotides and their alkoxysilane derivatives

I.I DNA oligomers

General information. 1 μmol Universal 1000 Å LCAACPG supports, and standard reagents used for automated DNA synthesis were purchased from Bioautomation. Besides the serinol amino modifier, cyanine 3-, and cyanine 5-phosphoramidites, which were purchased from GlenTech, all other phosphoramidites were purchased from ChemGenes Corporated. 5-(ethylthio)tetrazole was purchased from Merck. TEAA buffer was prepared with triethylammonium acetate (50mM) and adjusted to pH 8 with acetic acid. All organic solvents were purchased from Merck and used as received. DMSO (analytical reagent grade) was purchased from Fisher Chemical and dried over Na_2SO_4 before use. 3-(triethoxysilyl)propyl isocyanate (ICPTES, 95 %), and triethylamine (TEA, >99 %), were purchased from Merck.

Solid phase DNA synthesis. Oligonucleotides were synthesized on a controlled pore glass (CPG) solid support with a BioAutomation Mer Made MM6 DNA synthesizer using a standard phosphoramidite

protocol for regular monomers. Amino functionalities were introduced with serinol amino modifier (Am, GlenTech) and fluorescent markers with cyanine-3-phosphoramidite (Cy3) and cyanine-5-phosphoramidite (Cy5). These monomers were introduced as follows: 3 % dichloroacetic acid in dichloromethane was used to remove the DMT-protecting group from sequence growing on the CPG, then the modifier phosphoramidite was dissolved in anhydrous CH_3CN at a final concentration of 0.1 M, and coupling was performed for 10 min in a glove box under inert atmosphere, after activation with 5-(ethylthio)tetrazole (0.25 M). After completion of the synthesis, the CPG was treated with 28 % aqueous ammonium hydroxide solution for 16 – 18 h at 60 °C. The crude mixture was then concentrated under reduced pressure at 60 °C, dispersed in MilliQ water, filtered through a 0.22 µm centrifugal filter, and purified by RP-HPLC. Pure Cy3-labelled, diamino ssDNA (H_2N-CAATGTAGCT-Cy3-NH_2) was obtained as a pink solid (0.2 µmol, yield 20 % compared to the original CPG loading).

HPLC purification. Solvent system: A: 50 mM triethylamine-acetic acid buffer (TEAA buffer), pH 8; B: Acetonitrile. TEAA buffer (50 mM triethylammonium acetate, pH = 8.0) was filtered through a 0.22 µm cellulose membrane before use. All DNA strands with amino-modified monomer were purified by RP-HPLC. Elution gradient used: 3 – 50% acetonitrile over 30 minutes at 60 °C. Column used: Hamilton PRP 15 µm 4.6 x 250 mm. Approximately 0.5 – 0.75 OD_{260} of crude DNA was injected as a 20 – 50µL solution in Millipore water and then detected using a diode array detector monitoring absorbance at 260 nm.

DNA silanization: Bis-alkoxysilane derivatives Si-ssDNA-Si and Si-4mer-DNA-Si. To a dispersion of H_2N-CAATGTAGCT-Cy3-NH_2 or H_2N-GCGC-NH_2 (2 or 0.8 mg, 0.5 µmol) in dry DMSO (50 µL), ICPTES (0.27 µL of a 4.04 M ICPTES solution in DMSO, 1.1 µmol, 1.1 eq) and TEA (0.5 µL) were added. The reaction mixture was stirred for 1 h at room temperature and used for the preparation of organosilica particles without further purification. Prior to use for the preparation of organosilica particles, an aliquot of the crude material was taken and subjected to MS analysis to confirm the formation of the silanization product. Despite a general tendency to undergo early oligomerization leading to the formation of precipitates in solvents used for ESI analysis (*i.e.* H_2O, acetonitrile, MeOH), Si-ssDNA-Si was soluble enough to record a representative spectrum (see below).

Cy3-ssDNA. H_2N-CAATGTAGCT-Cy3-NH_2, ε (260 nm): 146600 $M^{-1}cm^{-1}$, 20 % yield. HPLC-MS (ESI-TOF, negative mode, water): r.t. = 3.6 – 3.8 min, m/z calcd 3979.9 [M], found 1989.6 $[M-2H]^{2-}$, 1326.1 $[M-3H]^{3-}$, 994.3 $[M-4H]^{4-}$, 795.2 $[M-5H]^{5-}$, 662.5 $[M-6H]^{6-}$ (Figure S13). The UV-Vis spectrum is shown in Figure S14.

Cy5-ssDNA. Cy5-AGCTACATTG, ε (260 nm): 158000 $M^{-1}cm^{-1}$, 20 % yield. HPLC-MS (ESI-TOF, negative mode, water): r.t. = 17.8 – 18.1 min, m/z calcd 3560.6[M], found 3563.0 (Figure S15). The UV-Vis spectrum is shown in Figure S16.

4mer-DNA. H_2N-GCGC-NH_2, ε (260 nm): 93300 $M^{-1}cm^{-1}$, 70 % yield. HPLC-MS (ESI-TOF, negative mode, water): r.t. = 1.2 – 1.3 min, m/z calcd 1623.1 [M], found 1622.4 $[M-1H]^{1-}$, 810.2 $[M-2H]^{2-}$, 539.8 $[M-3H]^{3-}$; m/z calcd 3242.2 [2M], found 1080.9 $[2M-3H]^{3-}$(Figure S17).

Si-ssDNA-Si. (ICPTES)HN-CAATGTAGCT-Cy3-NH(ICPTES). MS (ESI-TOF, negative mode, water), m/z calcd 4309.3 for fully hydrolyzed product [M − 6 Ethyl], found 860.4 $[M-6H]^{6-}$, 717.0 $[M-7H]^{7-}$, 614.3 $[M-8H]^{8-}$; m/z calcd 4146.1 for monosubstituted, fully hydrolyzed intermediate [M − 3 Ethyl], found 827.8 $[M-5H]^{5-}$, 689.7 $[M-6H]^{6-}$, 591.1 $[M-7H]^{7-}$ (Figure S18).

I.II PNA oligomers

General information. Fmoc-PNA(Bhoc)-OH monomers for PNA synthesis were purchased from LGC link or PolyOrg Inc, while 5(6)-Carboxytetramethylrhodamine (TAMRA), Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Dde)-OH and all the other commercially available reagents and solvents were bought from Sigma-Aldrich and used without further purification. Dry solvents were stored over 4 Å molecular sieves and DMF (peptide synthesis grade) was purged with nitrogen before use to avoid the presence of dimethylamine.

Solid phase PNA synthesis. All PNA were synthesized manually on Rink Amide-ChemMatrix® resin (Sigma-Aldrich) loaded with Fmoc-Gly-OH (0.17 mmol/g) or Fmoc-Lys(Boc)-OH (0.16 mmol/g) in 10 μmol scale. The following solutions were used: a) Fmoc deprotection: 20 % piperidine in DMF; b) Capping: acetic anhydride/DIPEA/ dry DMF 5/6/89; c) DIPEA wash: 5 % DIPEA in DMF; d) Cleavage: TFA/*m*-cresol 9 : 1.

All the PNA monomers and amino acids residues were coupled according to standard Fmoc/Bhoc protocols for solid phase synthesis,[1] consisting of a) Fmoc deprotection, 2 x 8 min; b) DCM wash; c) DMF wash; d) Kaiser test (1 min, positive);[2] e) Coupling, 30 min (activation for 2 min; Activation solution: 5 eq of PNA monomer or amino acid residue, 4.9 eq of HBTU, 10 eq of DIPEA in dry DMF); f) DMF wash; g) Kaiser test (1 min, negative); h) capping, 2 x 1 min; i) DMF wash; j) DIPEA wash, 2 x 2 min; k) DMF wash. All the fluorescent dyes were attached as the last monomer according to the modified procedures that are reported below. The outcome of the various syntheses was checked in the middle and at the end of the PNA sequence by UPLC-MS analysis (see below). Treatment of the dried resins with the cleavage solution (2 x 1 h) resulted in the liberation of the PNA oligomers, and the deprotection of their nucleobases and lysine side chains. The crude samples were precipitated with 50 volumes of Et_2O at −20 °C and then purified by reversed-phase HPLC (see below). The purity of the resulting PNAs was checked by UPLC-MS analysis (see below), while their concentration was extracted from the UV absorbance of diluted solutions at λ = 260 nm, assuming an additive contribution of all nucleobases and fluorophores to the total ε (260 nm) of the oligomers. Accordingly, the molar extinction coefficients were calculated by considering the following values for each UV-absorbing unit: T = 8600 $M^{-1}cm^{-1}$, C = 6600 $M^{-1}cm^{-1}$, A = 13700 $M^{-1}cm^{-1}$, G = 11700 $M^{-1}cm^{-1}$, TAMRA = 31980 $M^{-1}cm^{-1}$.

Conjugation with TAMRA. TAMRA was linked at the N-term of ssPNA according to the following procedure: a) Fmoc deprotection, 2 x 8 min; b) DCM wash; c) DMF wash; d) Kaiser test (1 min, positive); e) Coupling, 3 h (activation for 10 min; Activation solution: 5 eq of TAMRA, 4.9 eq of HBTU, 10 eq of DIPEA in dry DMF); f) DMF wash; g) Kaiser test (1 min, negative); h) DCM wash.

HPLC purification. Solvent system: A: MilliQ H_2O + 0.1 % TFA, B: Acetonitrile + 0.1 % TFA. Elution gradient used: 5 minutes in H_2O + 0.1 % TFA, then a linear gradient from H_2O + 0.1 % TFA to 50 % MeCN + 0.1 % TFA in 30 minutes at a flow rate of 4.0 ml/min, 40 °C. Column used: Jupiter C18.5 μm, 300 Å, 10 x 250 mm by Phenomenex. Approximately 0.5 – 1 μmol of crude PNA was injected as a 50 – 100 μL solution in MilliQ water and then detected monitoring their absorbance at 260 nm.

UPLC-MS analyses. Solvent system: A: Milli Q H_2O + 0.2 % formic acid, B: Acetonitrile + 0.2 % formic acid. Elution gradient used: 0.90 minutes in H_2O + 0.2 % formic acid, then linear gradient to 50 % MeCN + 0.2 % formic acid in 5.70 minutes at a flow rate of 0.25 mL/min, 35 °C. Column used: Waters Acquity UPLC BEH 300 C18, 1.7 μm, 300 Å, 2.1 x 50 mm. Approximately 10 nmol of crude PNA was injected as a 10 μL solution in MilliQ water and then detected using a Waters Acquity SQ Detector in positive mode.

PNA silanization: Bis-alkoxysilane derivative Si-ssPNA-Si. To a solution of ssPNA (0.5 mg, 0.15 μmol) in DMSO (30 μL), ICPTES (1.54 μL of a 0.2 M solution in DMSO, 0.315 μmol), and TEA (0.2 μL, 1.4 μmol) were

added, and the reaction mixture was stirred for 1 h at room temperature. The resulting crude was used for the preparation of organosilica particles without further purification.

ssPNA: TAMRA-Lys-CAATGTAGCT-Lys, ε (260 nm): 135480 $cm^{-1}M^{-1}$, 18.9 % yield. UPLC-MS (ESI+, H_2O): r.t. = 3.18 min and 3.56 min, m/z calcd 3395.5 [M], found 1133.0 $[M+3H]^{3+}$, 849.9 $[M+4H]^{4+}$, 680.2 $[M+5H]^{5+}$, 567.0 $[M+6H]^{6+}$, 486.2 $[M+7H]^{7+}$, 425.5 $[M+8H]^{8+}$ (Figure S19).

Section II. Preparation and characterization of DNA and PNA-bridged organosilicas

II.I Preparation and characterization of model mesoporous silica particles

General information. Hexadecyltrimethylammonium bromide (CTAB, Acros Organics, 99+ %), ammonia solution (VWR, 28 %), tetraethyl orthosilicate (TEOS, Sigma-Aldrich, ≥ 99 % GC), 5-(ethylthio)tetrazole (TEOS, Sigma–Aldrich, 95 %).

Preparation of model MSPs. In a glass vial, CTAB (25 mg, 0.068 mmol) was dissolved in an H_2O/EtOH mixture (0.739 : 0.26 v/v; 12.3 mL) at room temperature under stirring. Subsequently, NH_3 (28 wt% in H_2O, 5 µL) was added. Finally, TEOS (62.4 µL, 0.279 mmol) was added and the reaction mixture was stirred overnight at room temperature. The particles were collected by centrifugation and purified by means of re-dispersion and centrifugation in H_2O (1 ×) and EtOH (2 ×). The organic template was removed by extracting the particles in refluxing EtOH for 24 h, and the particles were collected by centrifugation and dried under reduced pressure.

II.II Preparation and characterization of ssDNA-OSPs

General information. Hexadecyltrimethylammonium bromide (CTAB, Acros Organics, 99+ %), ammonia solution (VWR, 28 %), tetraethyl orthosilicate (TEOS, Sigma-Aldrich, ≥ 99 % GC), 5-(ethylthio)tetrazole (TEOS, Sigma–Aldrich, 95 %).

Preparation of ssDNA-OSPs. In a 2 ml Eppendorf tube equipped with a small magnetic stirrer, CTAB (6.25 mg, 17.15 µmol) was dissolved in an EtOH/H_2O mixture (0.26 : 0.739 *v*/*v*, 3.075 mL) and $NH_{3(aq)}$ (1.25 µL, 28 wt%) was added. Subsequently, TEOS (69.4 µmol, 15.5 µL) was added to the crude reaction mixture of Si-ssDNA-Si (1 mol% in respect to TEOS), which in turn was added to the stirring surfactant mixture. The reaction was stirred for a further 12 h at room temperature in the dark. The precipitate was collected by centrifugation and washed with water (1×) and EtOH (1 ×). The surfactant was extracted by dispersing the particles in 1 mL of fresh EtOH and refluxing the particle dispersion for 12 h. The extraction process was repeated one more time before finally collecting the particles by centrifugation. The collected precipitate was further washed with EtOH (1 ×) by means of dispersion and centrifugation and finally dried under reduced pressure.

Preparation 4mer DNA-bridged organosilica particles. In a 2 ml Eppendorf tube equipped with a small magnetic stirrer, CTAB (6.25 mg, 17.15 µmol) was dissolved in an EtOH/H_2O mixture (0.26 : 0.739 *v*/*v*, 3.075 mL) and $NH_{3(aq)}$ (1.25 µL, 28 wt%) was added. Subsequently, TEOS (69.4 µmol, 15.5 µL) was added to the crude reaction mixture of the 4mer bis-alkoxysilane ssDNA, rapidly mixed, and added to the stirring surfactant mixture and the reaction was stirred for further 12 h at room temperature. The precipitate was collected by centrifugation and washed with water (1 x) and EtOH (1 ×). The surfactant was extracted by dispersing the particles in 1 mL of fresh EtOH and refluxing the particle dispersion for 12 h. The extraction process was repeated one more time before finally collecting the particles by centrifugation. The collected precipitate was further washed with EtOH (1 ×) by means of dispersion and centrifugation and finally dried under reduced pressure.

Quantification of DNA in ssDNA-OSPs.
UV-vis absorption spectroscopy was used to determine the amount of DNA present in ssDNA-OSP(Cy3).

For this purpose, a calibration function was first created with Cy3-ssDNA and used to recalculate the concentration based on the absorbance value of a dispersion of ssDNA-OSP(Cy3) (Fig. S4).

Calibration function:

$$\text{abs} = 0.05378 \cdot [\text{ssDNACy3}] + 0.03271$$

Using the absorbance value of a dispersion of ssDNA-OSPs(Cy3), abs = 0.064 (the concentration of the particle dispersion was 0.02 mg·mL^{-1}), the concentration of DNA present in the particles can be calculated, which is as follows:

$$[\text{ssDNACy3}] = \frac{0.064 - 0.03271}{0.05378} = 0.582\ \mu M$$

The amount of substance of Cy3-labeled ssDNA can be calculated from the measured total volume of particle dispersion, which was 0.15 mL:

$$c_{ssDNACy3} = \frac{n_{ssDNACy3}}{V} \Rightarrow n_{ssDNACy3} = c_{ssDNACy3} \cdot V = (0.582\ \mu M) \cdot (0.00015\ L) = 0.0000873\ \mu mol$$

The mass of nanoparticles present in the dispersion can be recalculated considering the total measured volume of the particle dispersion, which was 0.15 mL:

$$c_{particles} = \frac{m}{V} \Rightarrow m = c_{particles} \cdot V = (0.02\ mg \cdot mL^{-1}) \cdot 0.15\ mL = 0.003\ mg$$

Taking into account the amounts of particles and DNA calculated above and the fact that the total mass of ssDNA-OSPs obtained from one synthesis is 23.4 mg:

$$n_{total\ ssDNA} = \frac{0.0000873\ \mu mol \cdot 23.4\ mg}{0.003\ mg} = 0.68\ \mu mol$$

The initial molar amount of ssDNA used to prepare the NPs was 0.70 µmol, so the final yield of DNA incorporation into the particles can be calculated:

$$yield = \frac{100 \cdot 0.68}{0.70} = 97\ \%$$

From the molar amount of Cy3-labeled ssDNA in 23.4 mg particles, the amount of DNA per milligram of particles can be calculated back to 0.029 µmol·mg^{-1}, which corresponds to 11.6 % (w/w).

II.III Enzyme-triggered degradation of ssDNA-OSPs

ssDNA-OSPs (0.02 mg) were dispersed in Tris-buffer (0.5 mL see below the composition of the buffer) and DNase I solution (10 µL of a solution with 9563 units/mL from Thermo Scientific) was added. The mixture was stirred at room temperature for 1 hour.

Tris buffer composition: Prepared from distilled water. Contains: Tris(hydroxymethyl)aminomethane (10 mM), calcium chloride (0.5 mM), magnesium chloride (2.5 mM). The pH of the buffer solution was 7.6.

II.IV FRET-based study of ssDNA hybridization by ssDNA-OSPs

FRET experiments with ssDNA-OSPs and Cy5-ssDNA. The FRET fluorescence spectra were recorded on the Horiba Jobin-Yvon IBH FL-322 Fluorolog 3 using quartz cells (2.5 mL total volume; [ssDNA-OSPs] = 0.06 mg·mL^{-1}; [Cy5-ssDNA] = 0 – 3.52 µM). For the determination of FRET efficiencies and FRET single point fluorescence intensities, measurements were performed using a BMG Labtech plate reader (CLARIOstar Plus; total well volume 150 µL). After mixing the particle dispersion (0.12 mg·mL^{-1}) with the Cy5-ssDNA solution (0 − 5 µM), the system was stirred at room temperature for 30 minutes. Then, the emission spectra of the sample solution were recorded upon light excitation at λ_{ex} = 520 nm. For single point measurements, the emission intensities were recorded at $\lambda_{em,Cy3}$ = 570 nm and $\lambda_{em,Cy5}$ = 665 nm. All FRET-related emission spectra and single-point fluorescence intensity measurements were corrected for the background emission of Cy5 for each concentration. All experiments were performed in Tris-HCl buffer (10 mM containing 3 mM $MgCl_2$, pH = 7.4).

The relative FRET efficiency was then calculated given the equation below:

$$rel.FRET\ efficiency\ = \frac{I_{Cy5}}{I_{Cy5} + I_{Cy3}}$$

Reference FRET efficiencies for the hybridization of free Cy3-ssDNA and Cy5-ssDNA were recorded according to the same protocol.

II.V Preparation of ssPNA-OSPs

Preparation of ssPNA-OSPs. In a 2 ml Eppendorf tube equipped with a small magnetic stirrer, CTAB (1.56 mg, 4.28 µmol) was dissolved in an EtOH/H_2O (0.26:0.739 *v*/*v*, 0.768 mL) mixture and aq. NH_{3aq} (0.3 µL, 28 wt%) was added. Subsequently, TEOS (17.33 µmol, 3.87 µL) was added to the crude reaction mixture of Si-ssPNA-Si, rapidly mixed, and added to the stirring surfactant mixture. The reaction was stirred for further 12 h at room temperature. The precipitate was collected via centrifugation and washed with water (1 ×) and EtOH (1 ×). The surfactant was extracted by dispersing the particles in 1 mL of fresh EtOH and refluxing the particle dispersion for 12 h. The extraction process was repeated one more time before finally collecting the particles by centrifugation. The collected precipitate was further washed with EtOH (1 ×) by dispersion and centrifugation and finally dried under reduced pressure.

II.VI FRET-based study in ssDNA hybridization by ssPNA-OSPs

FRET experiments ssPNA-OSPs and Cy5-ssDNA. In three different cuvettes, three samples were prepared by dissolving both ssPNA-OSPs and Cy5-ssDNA in 10 mM Tris buffer containing 3 mM $MgCl_2$, pH = 7.4. The final concentrations of the Cy5-Strand were 0, 1.76, 3.52, 5.28, 7.04, 8.8, 10.56 and 12.32 µM, respectively, whereas the final concentration of ssPNA-OSPs was in all three samples 0.96 µg·mL^{-1}. After mixing the particle dispersion with the Cy5-Strand solution, the system was mixed for 30 min at room temperature. Subsequently, the emission spectra of the sample solution were recorded upon light excitation at λ_{ex} = 500 nm.

Section IV: Supplementary figures

TEM images and size distribution of model MSP.

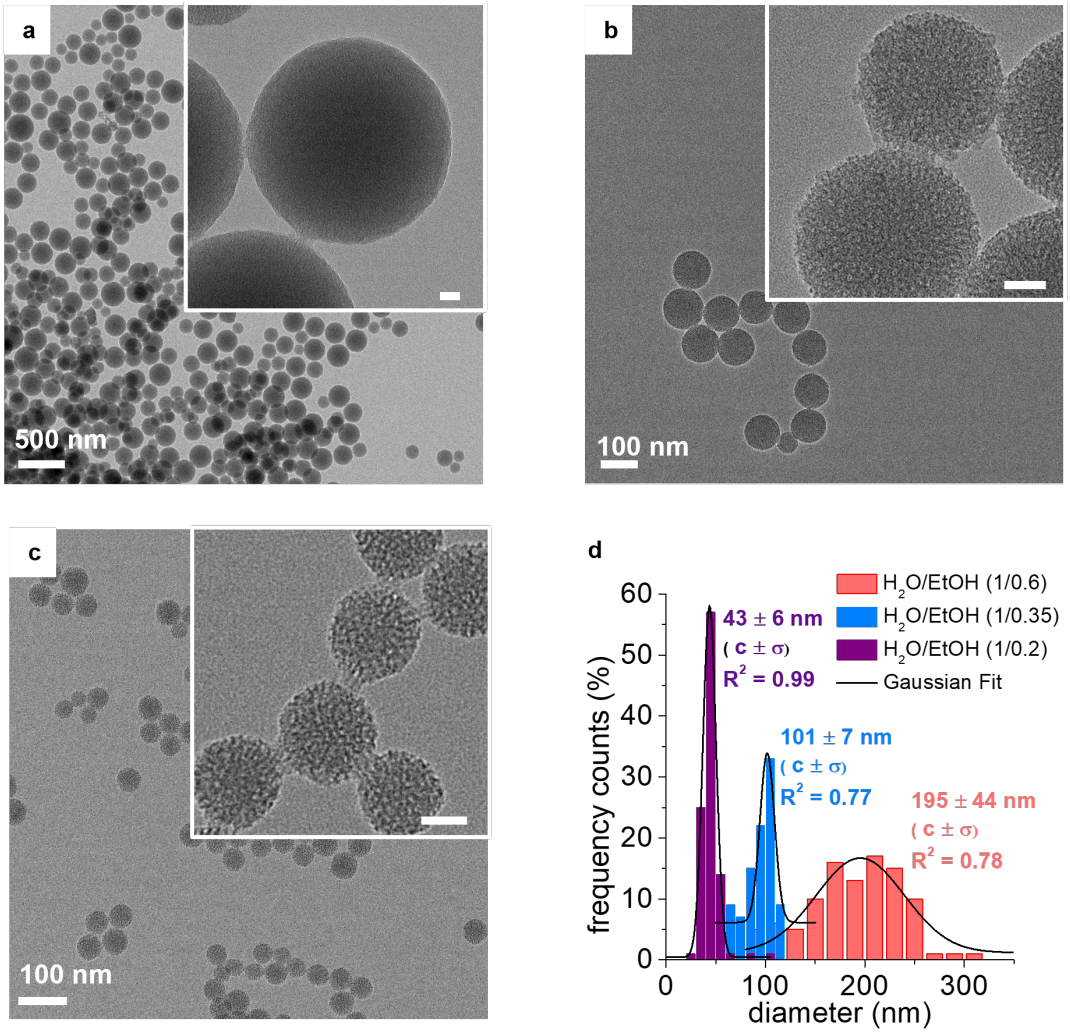


Figure S1. **a**–**c**, TEM images of model MSPs. **d**, the size distribution of model MSPs calculated from TEM images**.** Inset scale bar = 20 nm.

Electron microscopy analysis of ssDNA-OSPs.

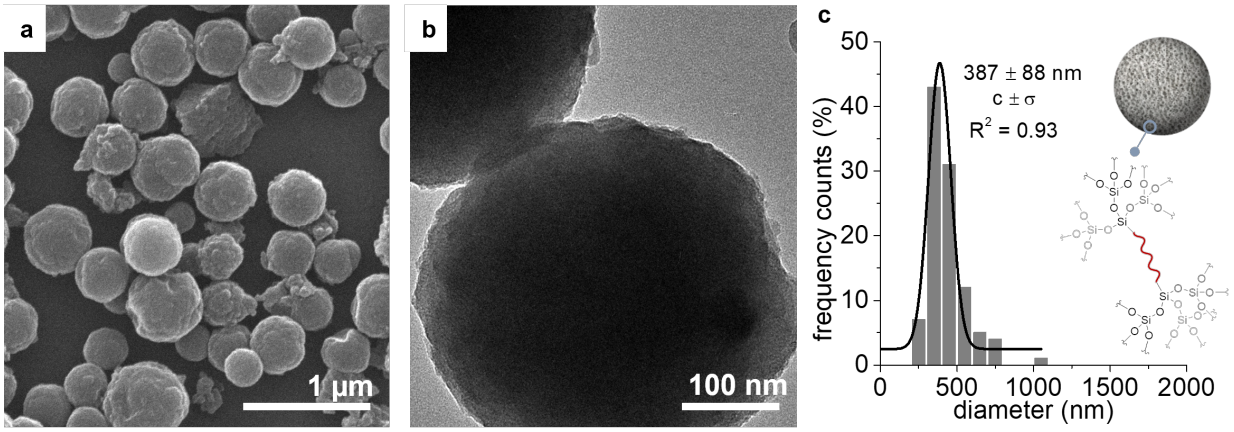


Figure S2. a, SEM images recorded on ssDNA-OSPs. **b**, TEM images at higher magnification of ssDNA-OSPs. **c**, the size distribution of ssDNA-OSPs calculated from TEM images.

Characterization of ssDNA-OSPs.

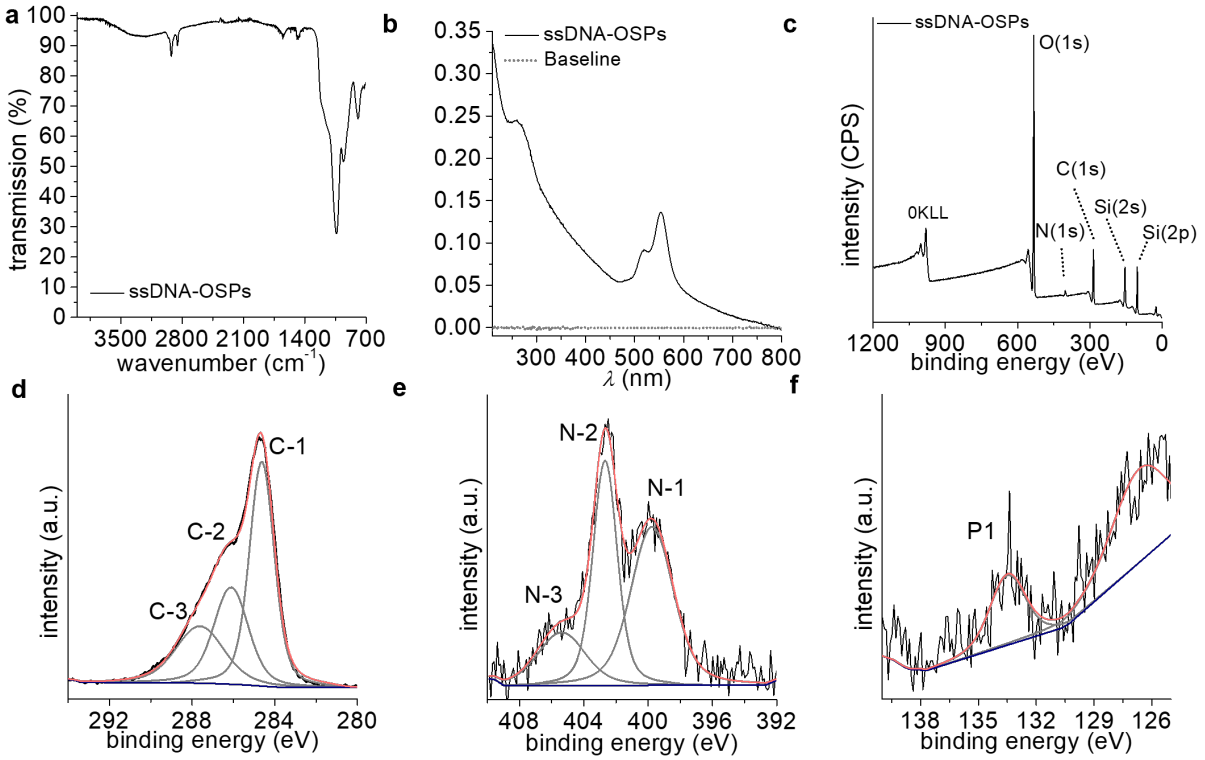


Figure S3. **a**, ATR-FTIR spectra recorded on a powder of ssDNA-OSPs show the presence of the characteristic transmission bands at 1474 cm^{-1} and 1649 cm^{-1}, which are both due to the presence of DNA.[3] The transmission bands occurring from 2848 – 2928 cm^{-1} can be attributed to C_{sp2}–H and Cs_{p3}–H stretching vibrations of ssDNA and residual CTAB within ssDNA-OSPs. **b**, the absorption spectra recorded on a dispersion of ssDNA-OSPs (c = 0.02 mg·mL^{-1}) confirmed the presence of the Cy3-labeled DNA thanks to the characteristic absorption band maxima centered at λ = 260 nm and λ = 550 nm, which can be attributed to purine and pyrimidine DNA nucleobases and the Cy3 moiety, respectively. **c**, the survey XPS spectra recorded on ssDNA-OSPs indicated the presence of C(1s) and N(1s) signals at 285 and 403 eV, respectively, confirming the presence of the organic functional group within ssDNA-OSPs. **d**, the deconvoluted HR scan for C (1s) peak shows the presence of three components C-1 (284.4 eV), C-2 (286.1 eV), and C-3 (287.5 eV) corresponding to C–C, C–O, and C=O carbon species, respectively. **e**, the deconvoluted HR scan for the N(1s) peak shows the presence of three components N-1 at 399.7 eV, N-2 at 402.7 eV, and N-3 at 405.9 eV. The component N-1 can be attributed to the nucleobase C–NH_2 and C=N-C species,[4] whereas the N-2 component can be attributed to residual CTAB (quaternary ammonium) within the particles.[5] **f**, The deconvoluted HR scan for the P(2p) peak shows the presence one component centered at 133.4 eV (P1), which can be attributed to the presence of phosphate groups within ssDNA-OSPs.[6]

Determination of Cy3-ssDNA in ssDNA-OPSs(Cy3).

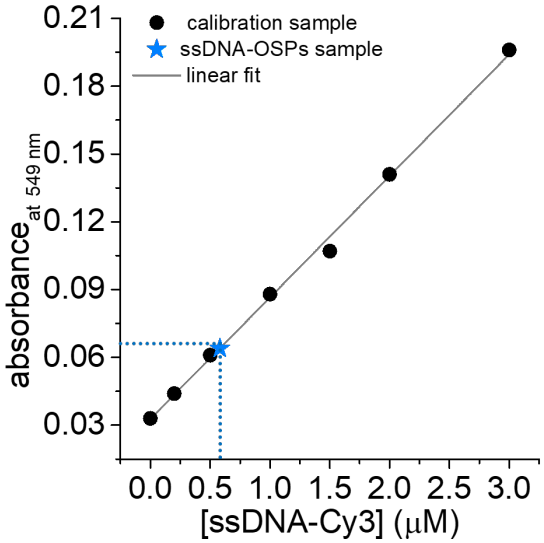
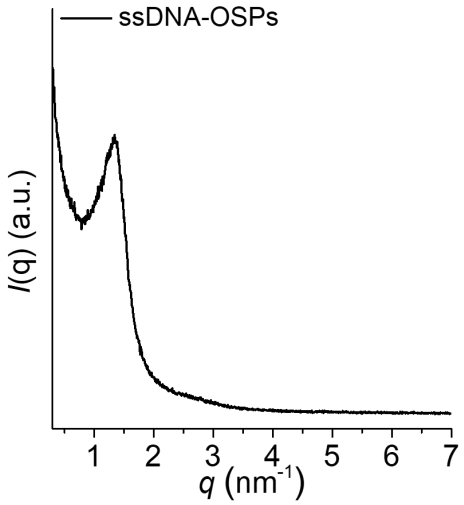


Figure S4. UV-Vis absorbance of a ssDNA-OSPs(Cy3) particle dispersion (water, λ_{ab} = 549 nm) and the calibration function used to determine the amount of incorporated Cy3-ssDNA.

SAXS analysis of ssDNA-OSPs.



Assuming that an ordered hexagonally arranged structure composed of small silica particles exists within ssDNA-OSPs and that the observed SAXS pattern centered at q=1.3 nm^{-1} corresponds to the (100) diffraction plane of a hexagonally arranged phase, then the mean size of the scatterers can be calculated as follows: $d = \frac{2\pi}{q} = 4.8\ nm$

Figure S5. SAXS analysis was recorded on a powder of ssDNA-OSPs.

TEM images of organosilica particles prepared with the 4mer-bis-alkoxysilane ssDNA.

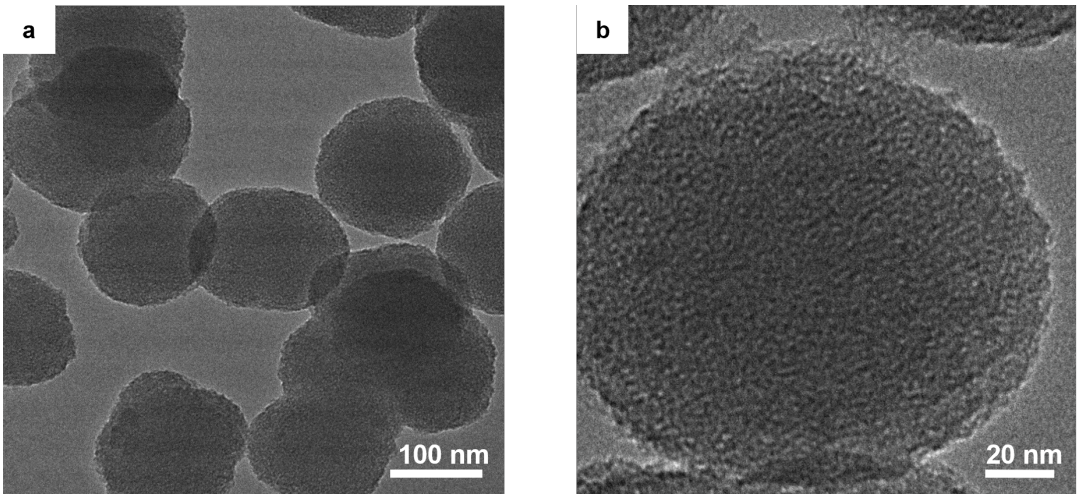


Figure S6. a, low and **b**, high magnification TEM images of ssDNA-OSPs prepared with the 4mer-bis-alkoxysilane-ssDNA. At contrary to what observed with 10mer-bis-alkoxysilane-ssDNA (**Figure S2b**), the particles show pronounced porosity, as indicated by the frequent high-low contrast regions within the particles.

Degradation study via SEM of ssDNA-OSPs in the presence or absence of DNase I.

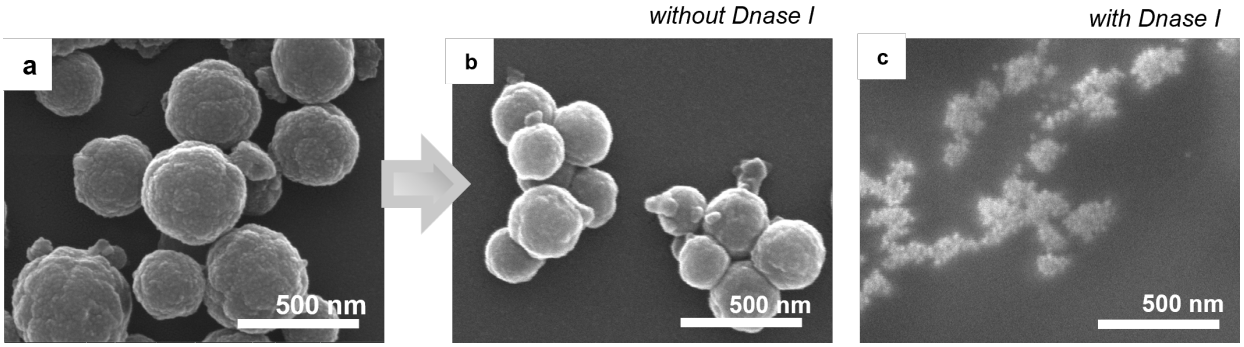


Figure S7. a, SEM image of ssDNA-OSPs at t_0. **b**, SEM images of ssDNA-OSPs after 1 h in the absence of DNaseI. **c**, SEM images of ssDNA-OSPs after being mixed for 1 h with DNaseI.

DLS intensity size distributions of ssDNA-OSPs.

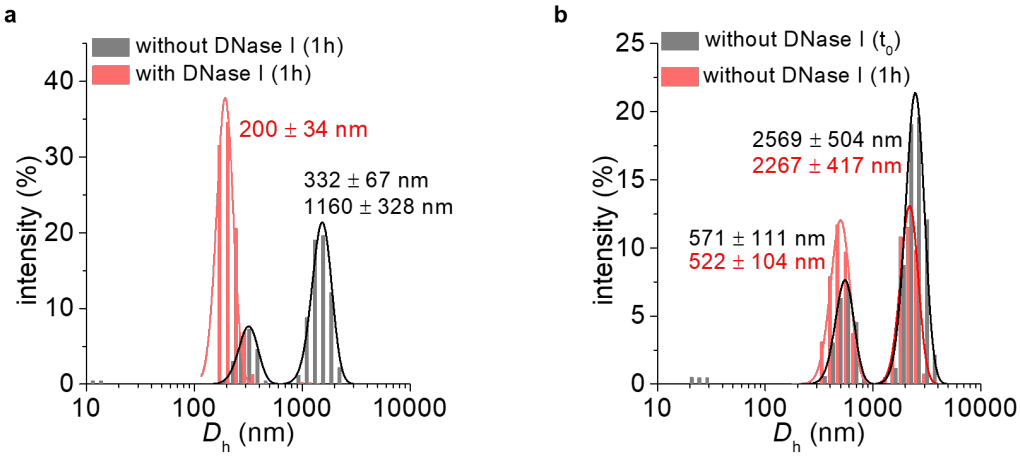


Figure S8. **a**, DLS intensity size distributions of ssDNA-OSPs that was stirred for 1 h with and without DNase I (Tris-HCl buffer 10 mM, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH = 7.6). **b**, DLS intensity size distributions of ssDNA-OSPs at t_0 and that were stirred for 1 h in the absence of DNase I.

Normalized absorption and emission spectra of Cy3-ssDNA and Cy5-ssDNA.

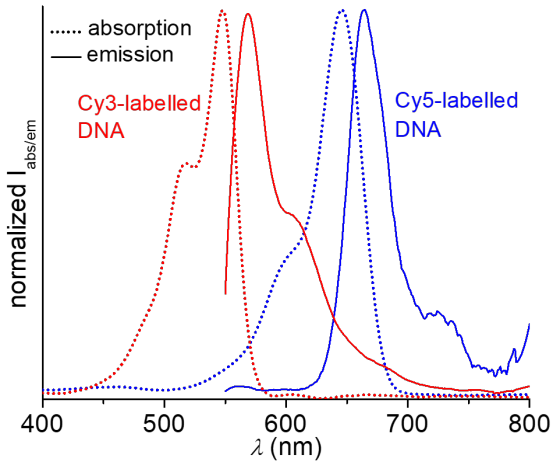


Figure S9. Normalized absorption and emission spectra of Cy3-ssDNA (λ_{ex} = 520 nm) and Cy5-ssDNA (λ_{ex} = 650 nm). The spectra were recorded in PBS (10 mM, pH = 7.4).

Time-dependent relative FRET efficiency.

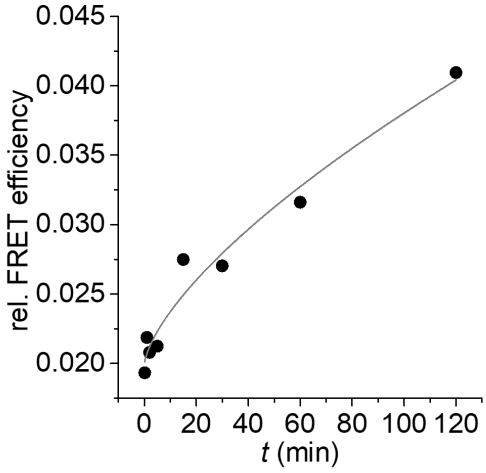


Figure S10. Time-dependent relative FRET efficiency from a ssDNA-OSPs(Cy3) dispersion (1.0 µg-mL^{-1} ≙ 0.072 nM Cy3-labeled DNA) to which Cy5-ssDNA (1.5 µM) was added. The emission intensities were recorded at $\lambda_{em,Cy3}$ = 570 nm and $\lambda_{em,Cy5}$ = 665 nm ($\lambda_{ex,Cy3}$ = 520 nm) in Tris-HCl buffer (10 mM containing 3 mM $MgCl_2$, pH = 7.4).

PNA-bridged organosilica particles.

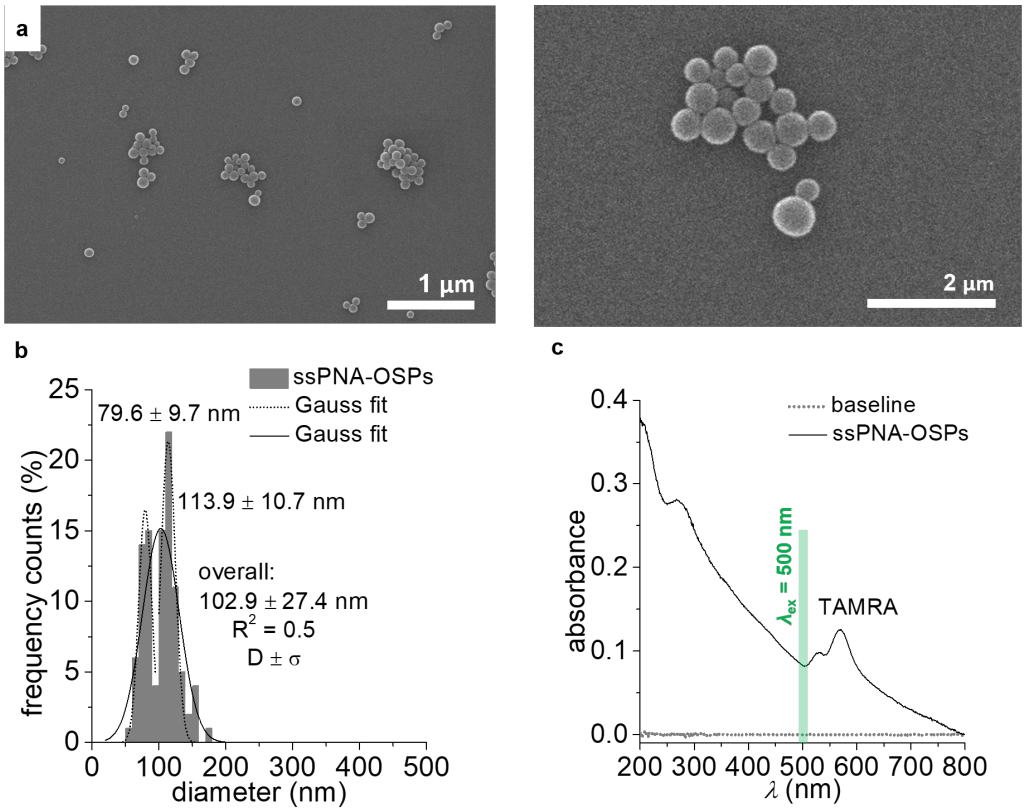


Figure S11. **a**, SEM images recorded on ssPNA-OSPs. **b**, the size distribution of ssPNA-OSPs calculated from SEM images. **c**, absorption spectrum of a dispersion of ssPNA-OSPs(TAMRA), in which the nucleobase and the fluorophore absorption maxima at λ = 260 nm and λ = 583 nm, respectively, confirmed the presence of the TAMRA-labelled PNA.

DLS analysis of ssPNA-OSPs treated with DNAse I.

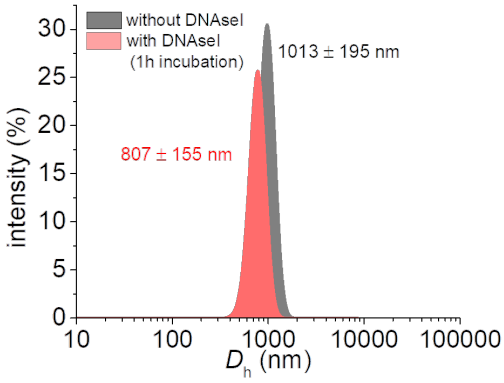


Figure S12. The hydrodynamic diameter of ssPNA-OSPs with and without DNAseI in Tris-HCl buffer (10 mM, 3 mM $MgCl_2$, pH = 7.5).

Additional chromatograms and mass or UV spectra.

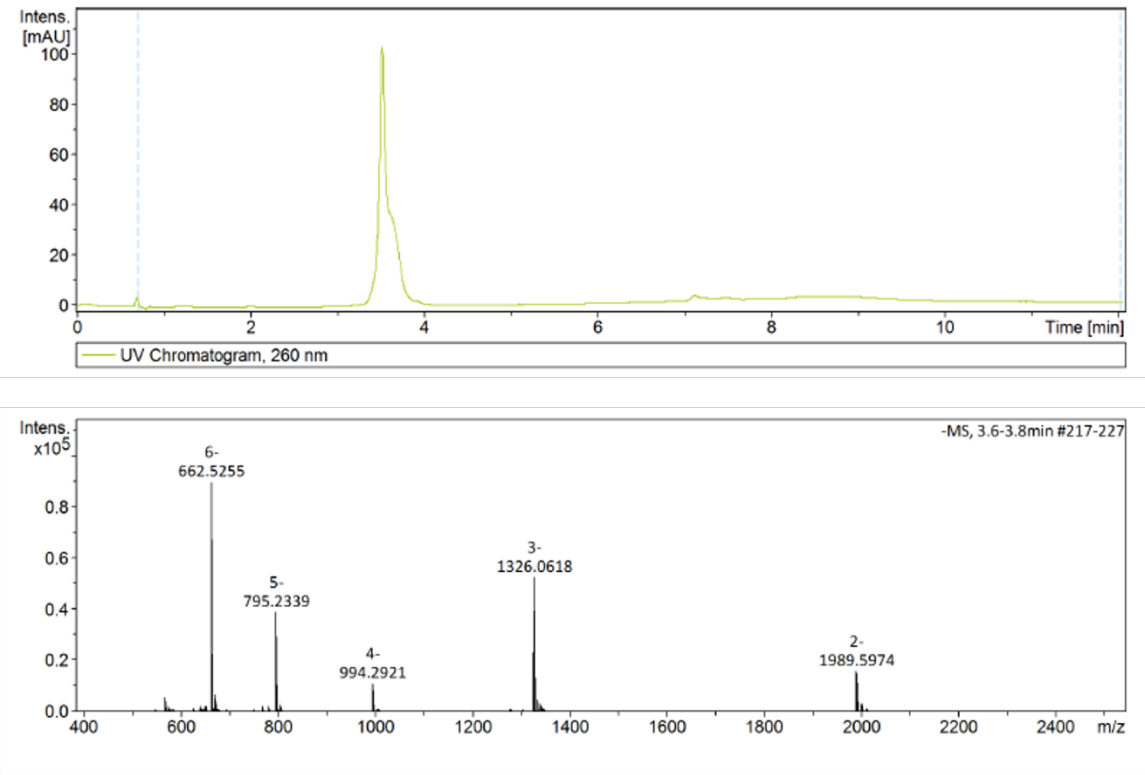


Figure S13. UPLC-MS chromatogram (ESI-TOF, negative mode, water) of pure Cy3-ssDNA (top) and ESI-MS spectrum for the peak at 3.6 – 3.8 min (bottom).

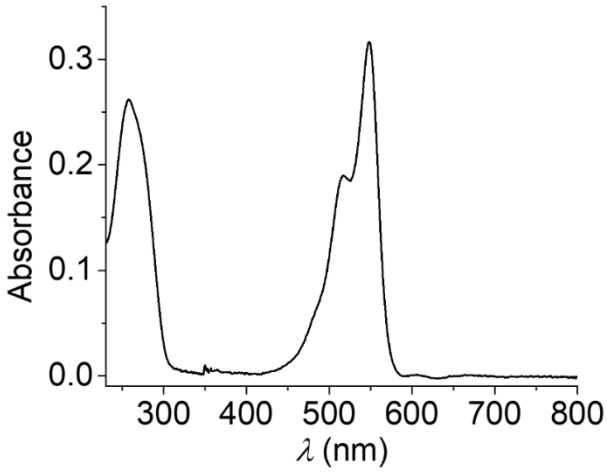


Figure S14. Normalized UV-VIS spectrum of Cy3-ssDNA in water.

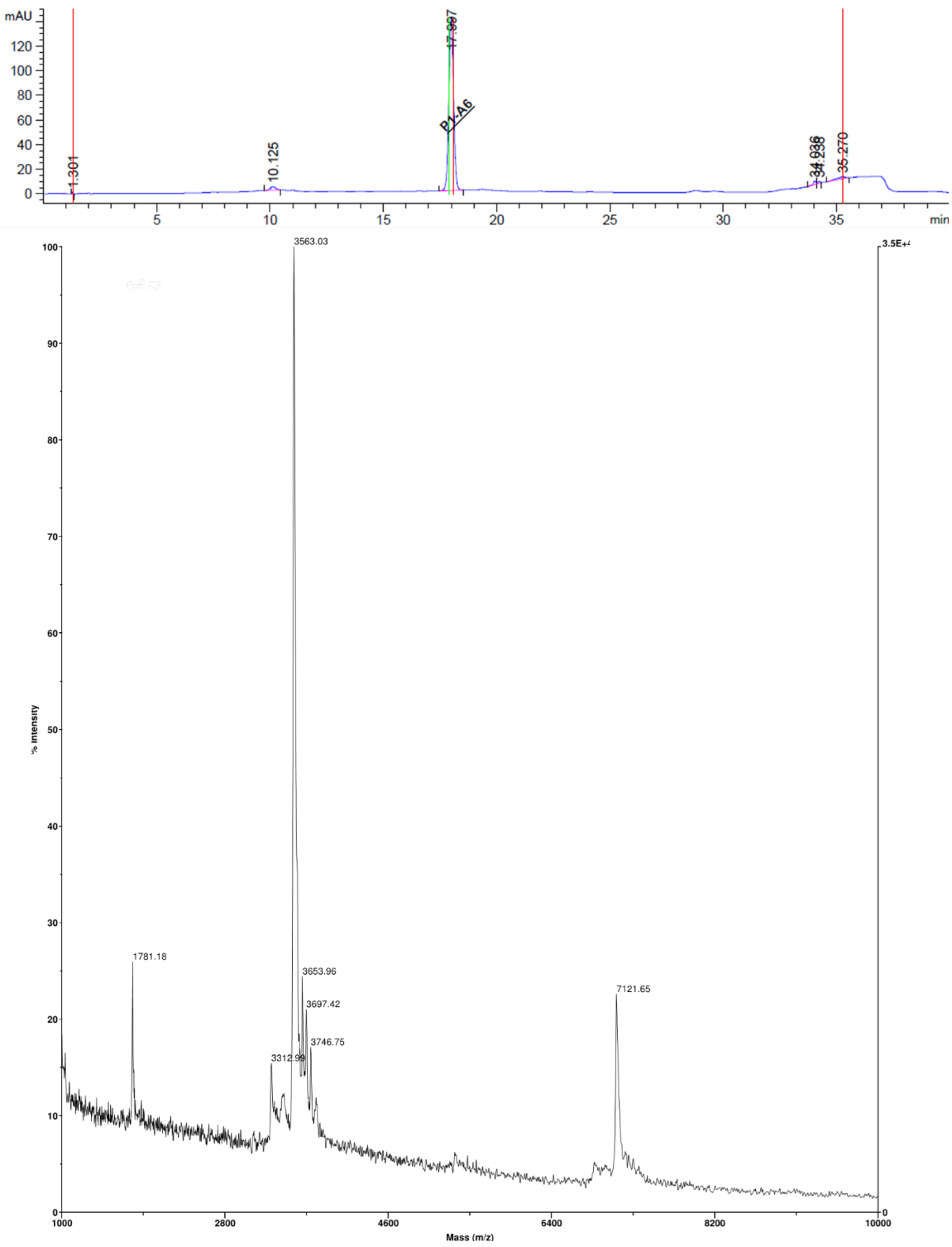


Figure S15. HPLC chromatogram of pure Cy5-ssDNA (top) and deconvoluted ESI-MS spectrum (ESI-TOF, negative mode, water) for the peak at 18.0 min (bottom).

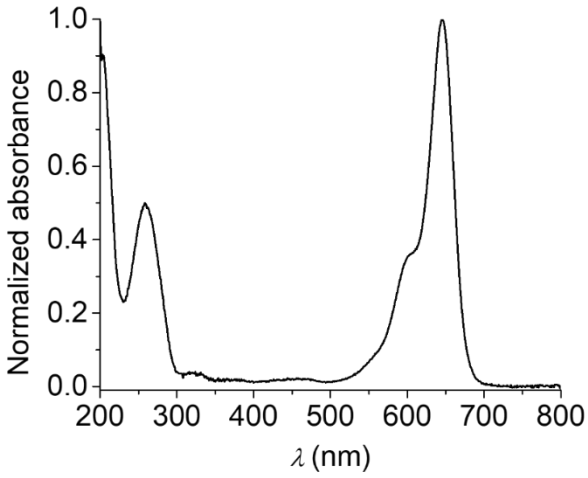


Figure S16. Normalized UV-VIS spectrum of Cy5-ssDNA in water.

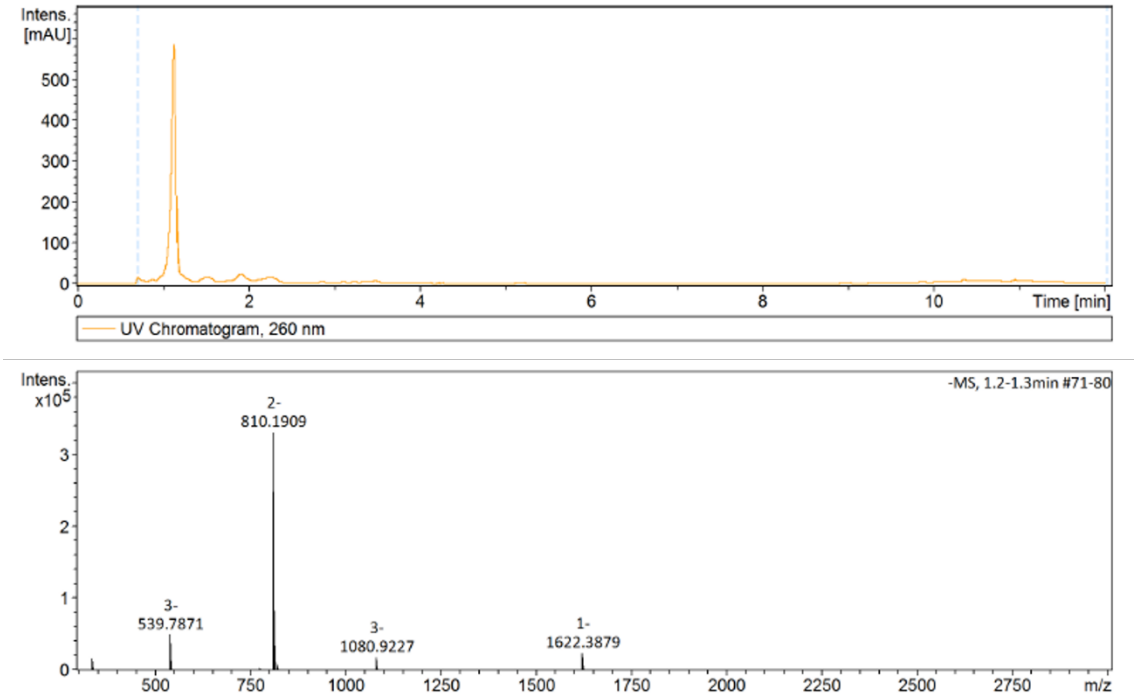


Figure S17. UPLC-MS chromatogram (ESI-TOF, negative mode, water) of pure 4mer-ssDNA (top) and ESI-MS spectrum for the peak at 1.2 – 1.3 min (bottom).

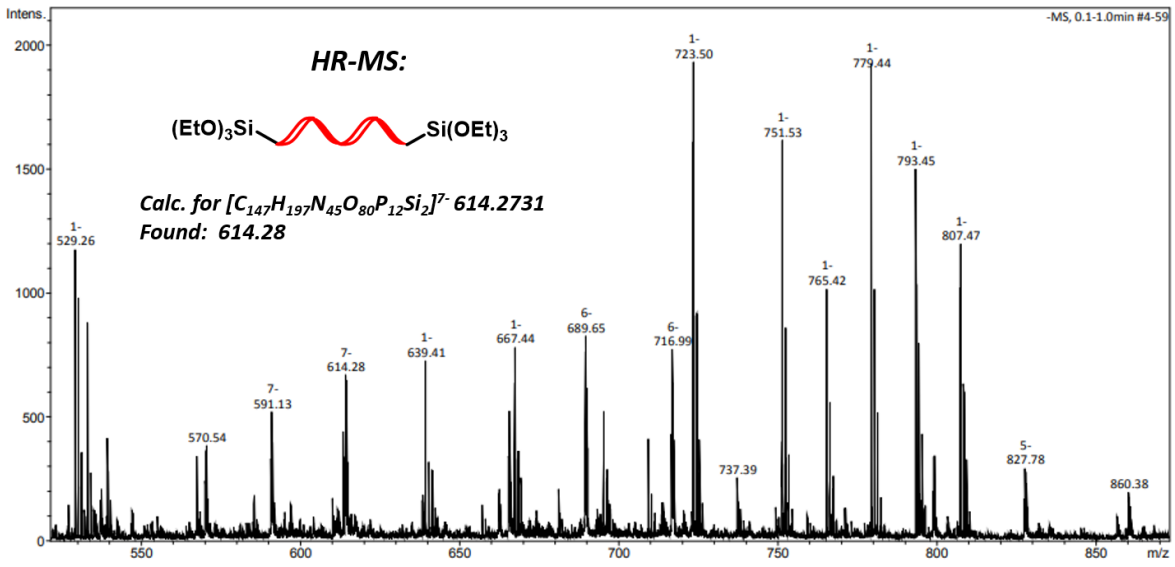


Figure S18. MS spectrum (ESI-TOF, negative mode, water) of an aliquot of crude Si-ssDNA-Si.

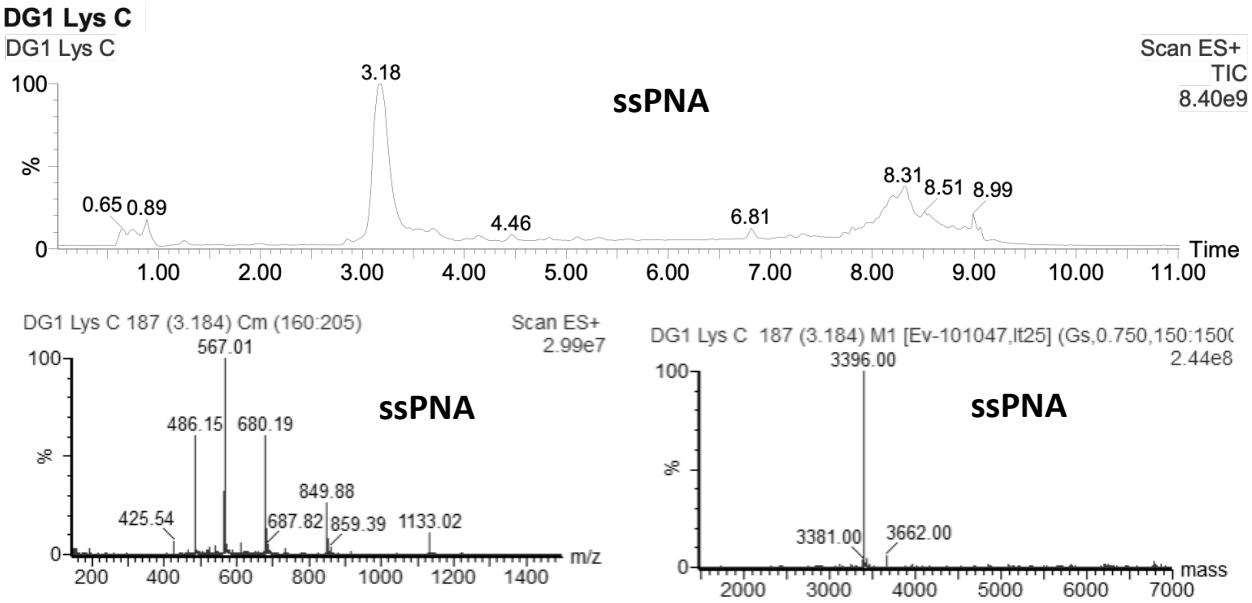


Figure S19. UPLC-MS (ESI-MS, positive mode, water) chromatogram of pure ssPNA (top) and ESI-MS spectrum for the peak at 3.18 min with the corresponding mathematic deconvolution of the multicharged signals (bottom).

B. References

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