Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Tetraphenylethylene-DNA Conjugates: Influence of Sticky Ends and DNA Sequence Length on the Supramolecular Assembly of AIE-Active Vesicles

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1. General methods

All reagents and solvents were purchased from commercial suppliers and used without further purification. The synthesis of (*E*)-4-(4-(2-(4-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)but-1-yn-1-yl)phenyl)-1,2-diphenylvinyl) phenyl)but-3-yn-1-yl (2-cyanoethyl) diisopropylphosphoramidite, required for the solid-phase synthesis of the tetraphenylethylene (TPE)-modified oligonucleotides, followed published procedures.[1] Unmodified DNA single strands were purchased from Microsynth (Switzerland). Water was used from a Milli-Q system. Mass spectra were obtained from the Analytical Research and Services (ARS) of the University of Bern, Switzerland, on a Thermo Fisher LTQ Orbitrap XL using Nano Electrospray Ionization (NSI). All mass spectra were measured in negative ion mode in mixtures of acetonitrile/water/triethylamine. UV-Vis spectra were recorded on an Agilent Cary 100 spectrophotometer using quartz cuvettes with an optical path of 1 cm. Fluorescence spectra were collected on a Cary Eclipse fluorescence spectrophotometer using an excitation slit of 2.5 nm and an emission slit of 5 nm. Spectroscopic data were measured from at least five minutes thermally equilibrated samples at the corresponding temperature. Supramolecular assembly proceeded *via* thermal disassembly and reassembly: the sample solution was heated to 75 °C, followed by a controlled cooling of 0.5 °C/min to 20 °C in a Cary Eclipse fluorescence spectrophotometer equipped with a Peltier thermostat. Atomic force microscopy (AFM) experiments were conducted on a Nanosurf FlexAFM instrument in tapping mode under ambient conditions. AFM samples were prepared on (3-aminopropyl)triethoxysilane (APTES)-modified mica sheets (Glimmer "V1", 20 mm x 20 mm, G250-7, Plano GmbH) according to published procedures, using a sample adsorption time of 7 min.[1] Dynamic light scattering (DLS) experiments were performed on a Malvern Zetasizer Nano Series instrument (λ = 633 nm) in particle size distribution (PSD) mode (number value) at 25 °C. Samples for cryo-EM were plunge frozen using the FEI Vitrobot Mark 4 at room temperature and 100% humidity.

In brief, copper lacey carbon grids were glow discharged (air – 10 mA for 20 seconds). 3 μL of the sample were pipetted on the girds and blotted for 3 seconds before plunging into liquid ethane. Sample grids were stored in liquid nitrogen. Images were acquired using a Gatan 626 cryo holder on a Falcon III equipped FEI Tecnai F20 in nanoprobe mode. Due to the nature of the sample, acquisition settings had to be adjusted for a low total electron dose (less than 20 $e^-/Å^2$) using EPU software. Distance measurements were done in Fiji[2,3] using the multi-point tool to set marks. After the read-out of the x- and y-values, the distances between the marks were calculated. The reported distances are mean values with the corresponding standard deviation.

2. Solid-phase oligomer synthesis

All TPE-DNA conjugates **ON1-ON5** (Table S1) were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer applying a standard cyanoethyl phosphoramidite coupling protocol on a 1 μmol scale. A coupling time of 30 s was employed for the DNA nucleobases (0.1 M in anhydrous acetonitrile) and 2 min for TPE phosphoramidite (0.1 M solution in anhydrous 1,2-dichloroethane). The synthesis was started with an TPE-modified long chain alkylamine controlled pore glass (LCAA-CPG) solid-support, which was prepared according to previously reported procedures.[1] After the solid-phase synthesis, **ON1-ON5** were cleaved and deprotected by treatment with aqueous NH_4OH (28-30%) at 55 °C overnight. The supernatants were collected, and the solid-support was washed three times with a solution of ethanol and Milli-Q H_2O (1:1, 3x1 mL), before the crude TPE-DNA conjugates were lyophilized.

All TPE-DNA conjugates **ON1-ON5** were purified by reversed-phase HPLC (*Shimadzu LC-20AT*, *LiChrospher 100 RP-18*, 5 μm, 250 x 4 mm) at 50 °C with a flow rate of 1 mL/min, λ: 330 nm. Solvent A: aqueous 2.1 mM triethylamine (TEA) / 25 mM 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) pH 8; solvent B: acetonitrile; applying the gradients listed in Table S1. The purified TPE-DNA conjugates **ON1** and **ON2** were dissolved in Milli-Q H_2O (1 mL), **ON3-ON5** were dissolved in a 1:1 ethanol/Milli-Q H_2O mixture (1 mL). The absorbance was measured at 260 nm to determine the concentration of the stock solutions and the yields of **ON1-ON5**. The calculation was according to the Beer-Lambert law. The following molar absorptivities (at 260 nm) in [L/mol·cm] were used for the DNA nucleobases: ε_A: 15’300; ε_T: 9’000; ε_G: 11’700; ε_C: 7’400. A molar absorptivity of ε_{TPE}: 35’975 was used for TPE. The corresponding HPLC traces and mass spectra of **ON1-ON5** are displayed in Fig. S1 and Fig. S2–Fig. S6, respectively.

Table S1 TPE-DNA oligonucleotide sequences **ON1-ON5**, HPLC gradients, calculated and found masses by NSI-MS, and yields.

Oligomer	Sequence (5'→3')	HPLC gradient B [%] (t_R [min])	calc. mass	found mass	Yield [%]
ON1	(TPE)-CTT CCT TGC ATC GGA CCT TG-(TPE)	5 (0), 40 (24)	7095.2951	7095.3432	10
ON2	$(TPE)_2$-CTT CCT TGC ATC GGA CCT TG-$(TPE)_2$	5 (0), 40 (24)	8155.6448	8155.6722	9
ON3	$(TPE)_3$-CTT CCT TGC ATC GGA CCT TG-$(TPE)_3$	5 (0), 50 (24)	9215.9945	9216.0492	25
ON4	$(TPE)_3$-CTT CCT TGG ACC TTG-$(TPE)_3$	5 (0), 50 (24)	7691.0132	7690.7916	21
ON5	$(TPE)_3$-CTT CCT TGC ACT GAA TCG GAC CTT G-$(TPE)_3$	5 (0), 50 (24)	10765.0008	10765.2660	7

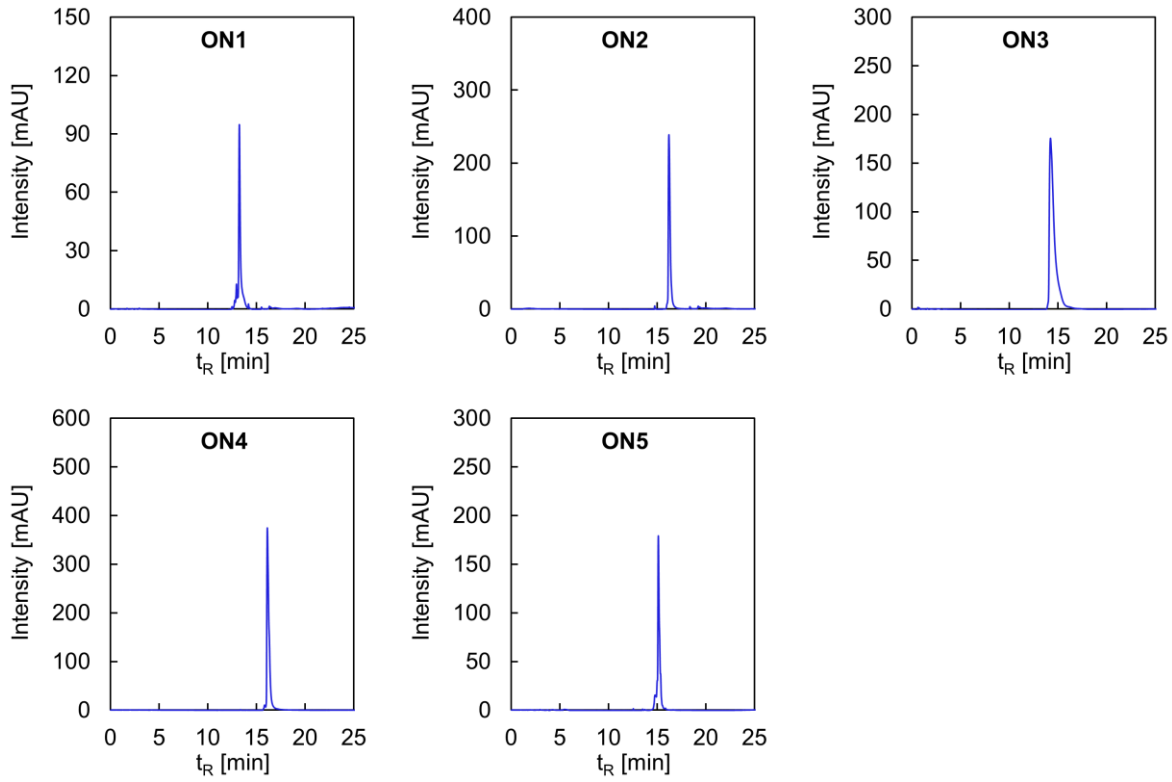

Fig. S1 HPLC traces of **ON1-ON5**.

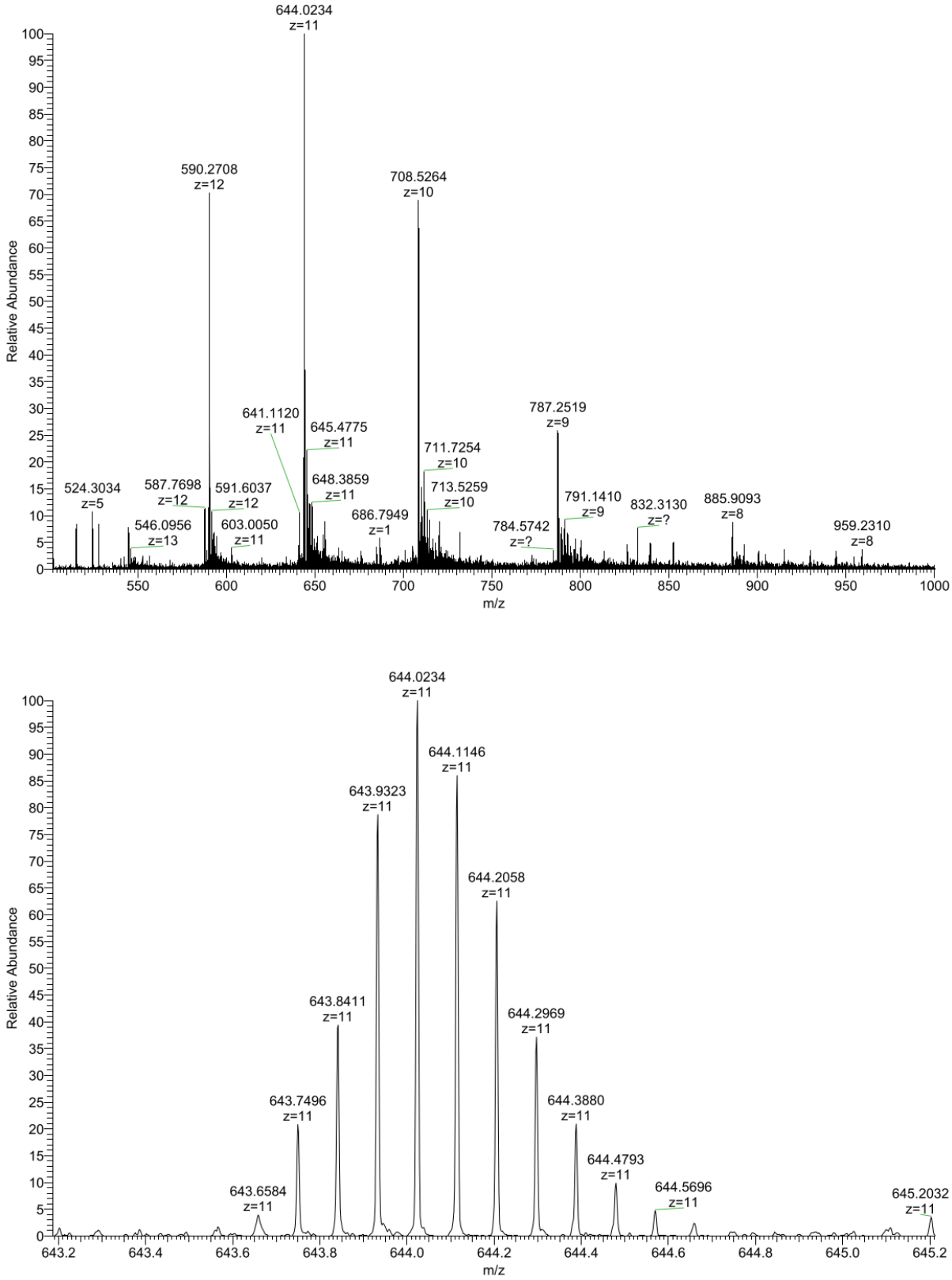

Fig. S2 MS spectra of **ON1**.

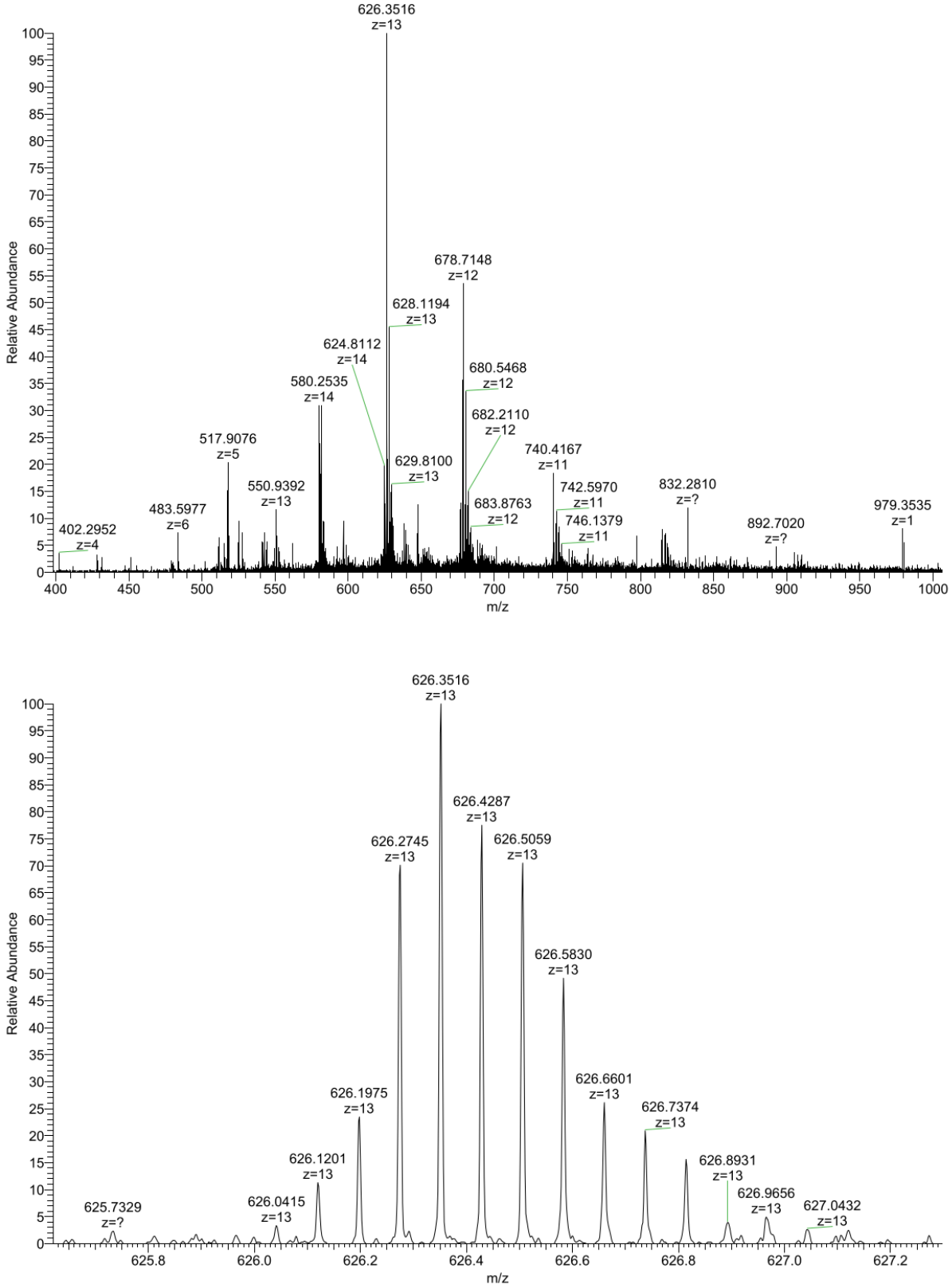

Fig. S3 MS spectra of **ON2**.

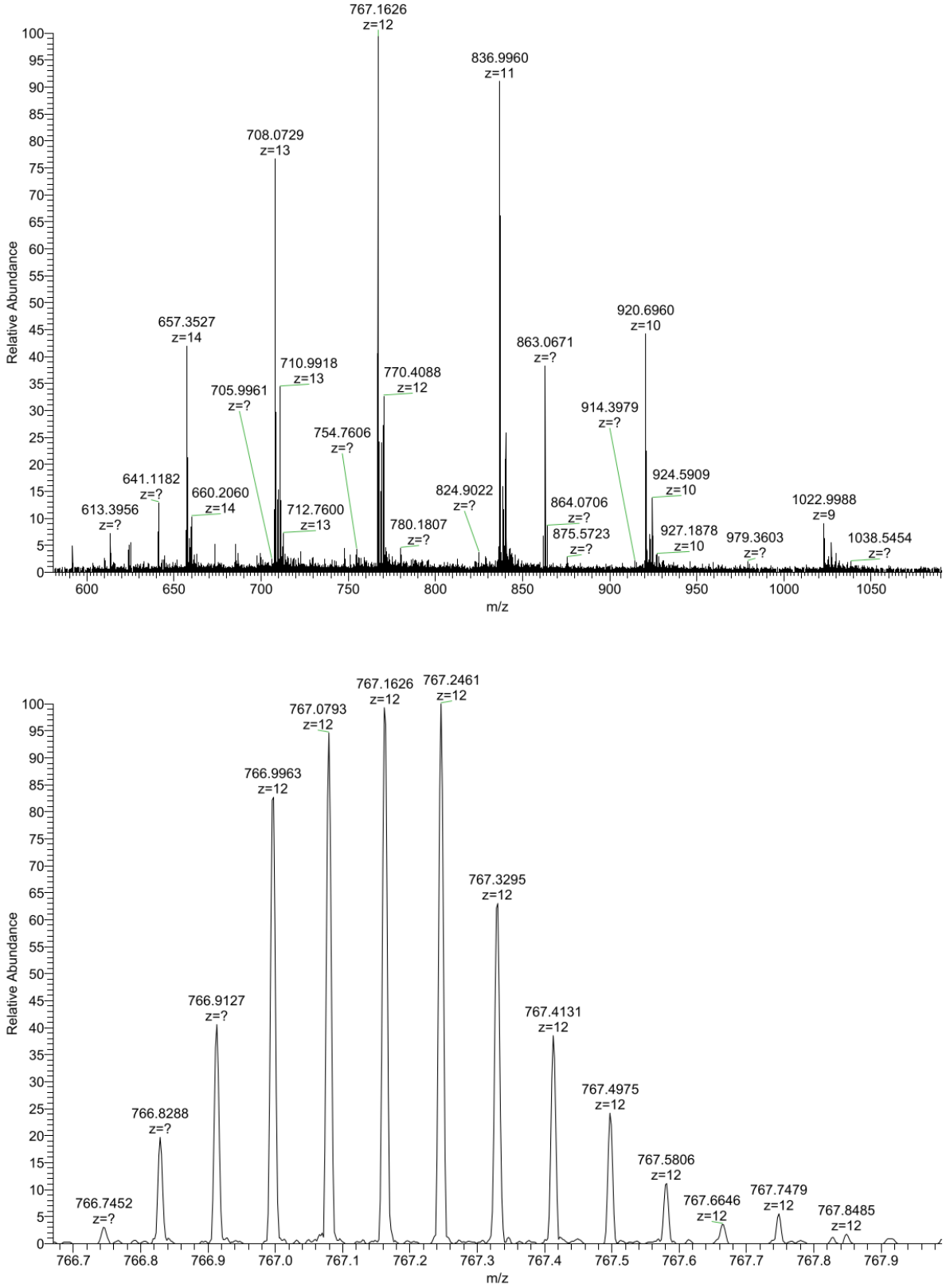

Fig. S4 MS spectra of **ON3**.

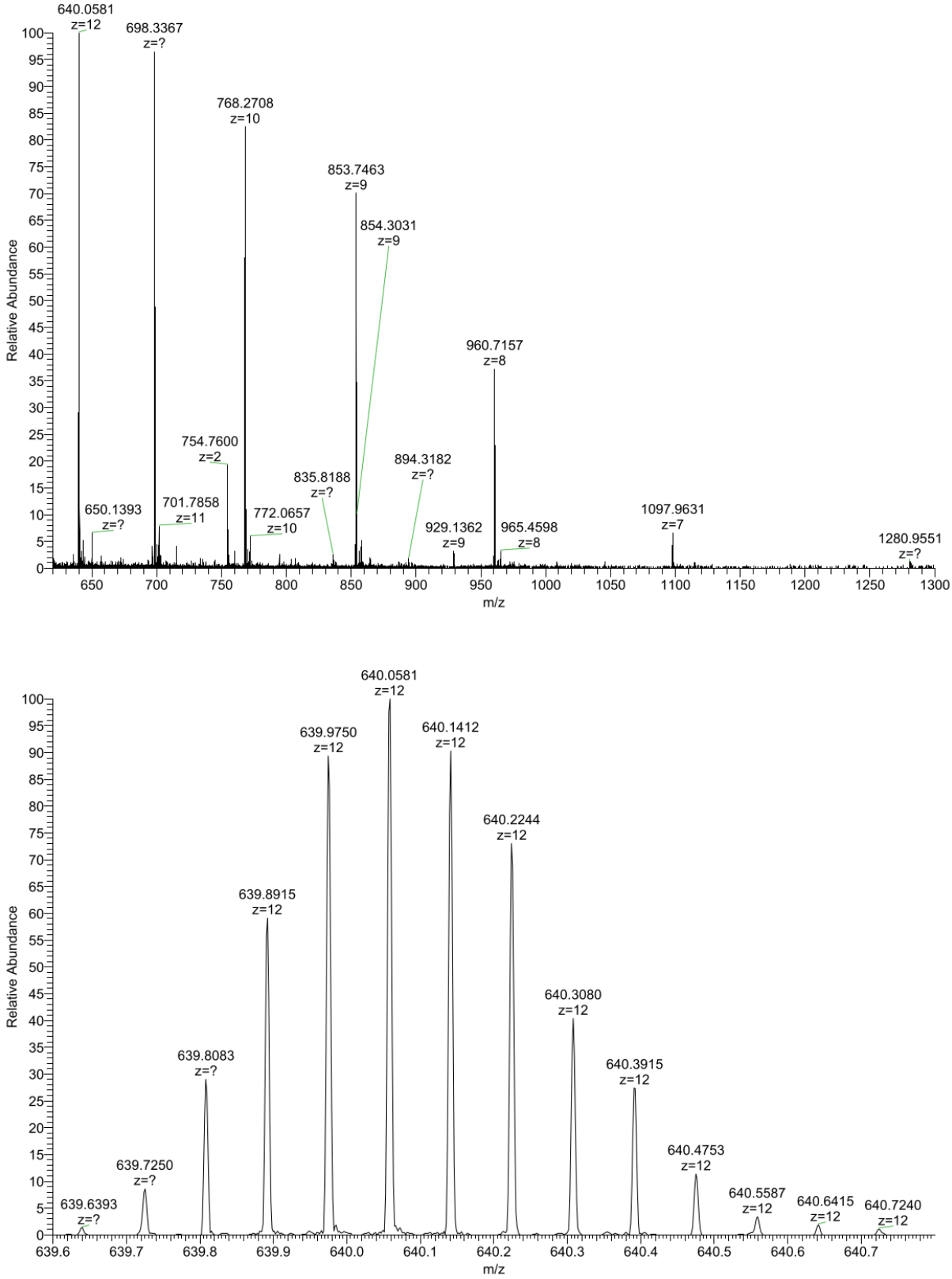

Fig. S5 MS spectra of **ON4**.

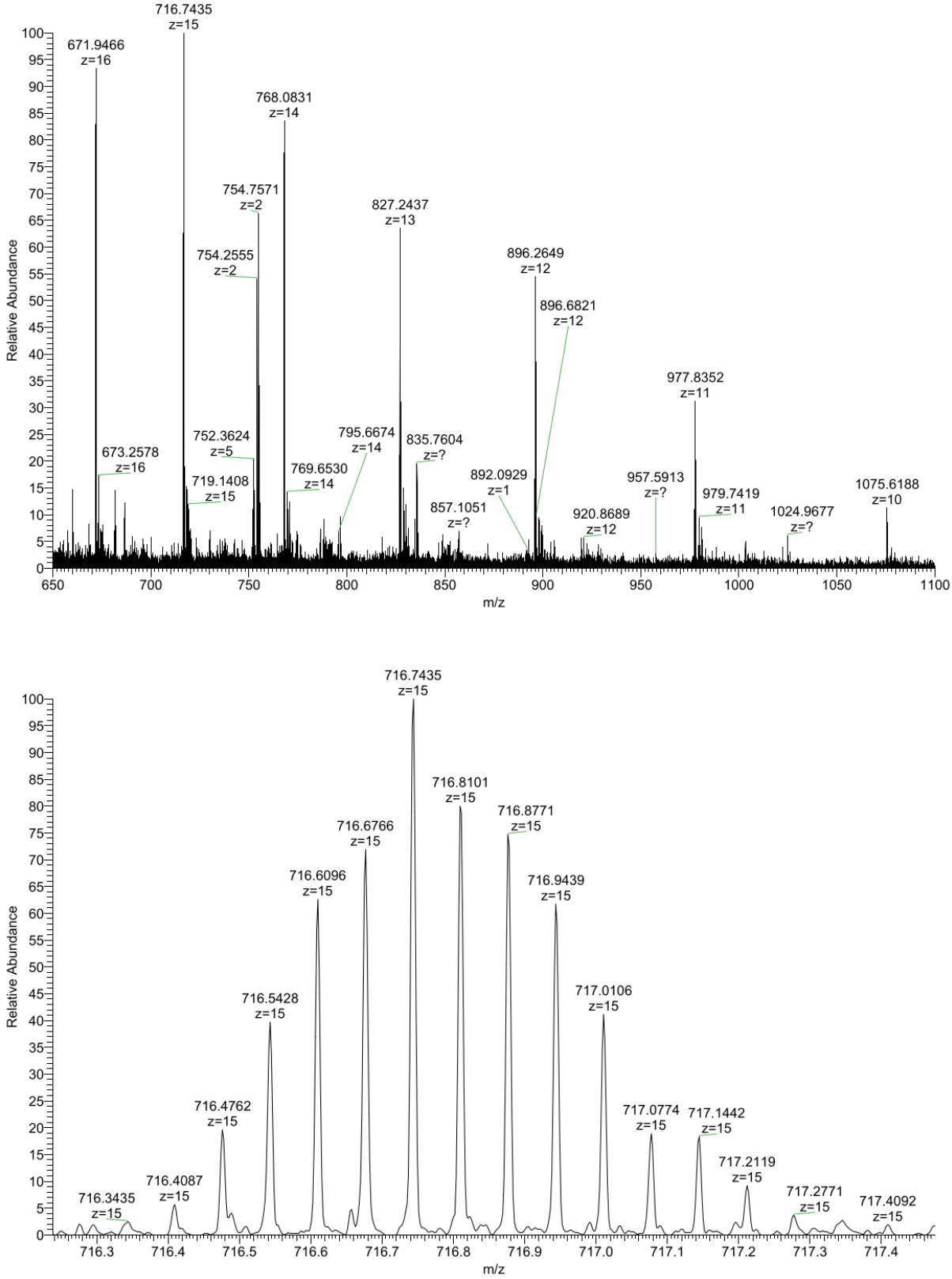

Fig. S6 MS spectra of **ON5**.

3. UV-Vis and fluorescence spectra

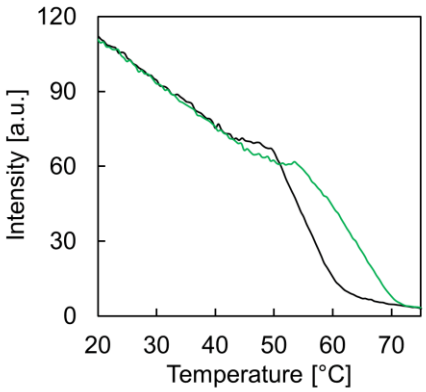

Fig. S7 Fluorescence-monitored annealing (black) and melting (green) curves of **3**. Conditions: 1 μM **3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol, 0.5 °C/min, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

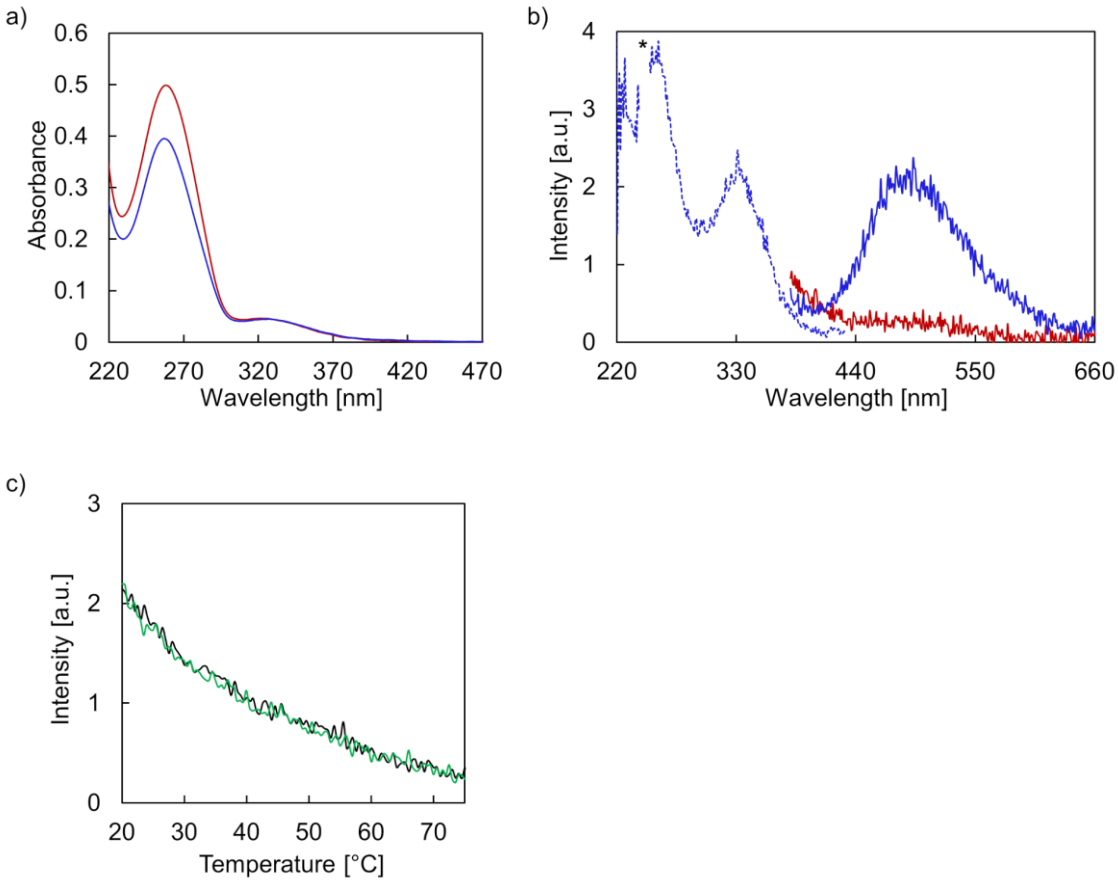

Fig. S8 Temperature-dependent UV-Vis absorption spectra of **1** (a), temperature-dependent fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **1** at 75 °C (red) and at 20 °C (blue) after thermal assembly process (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **1**. Conditions: 1 μM **1**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

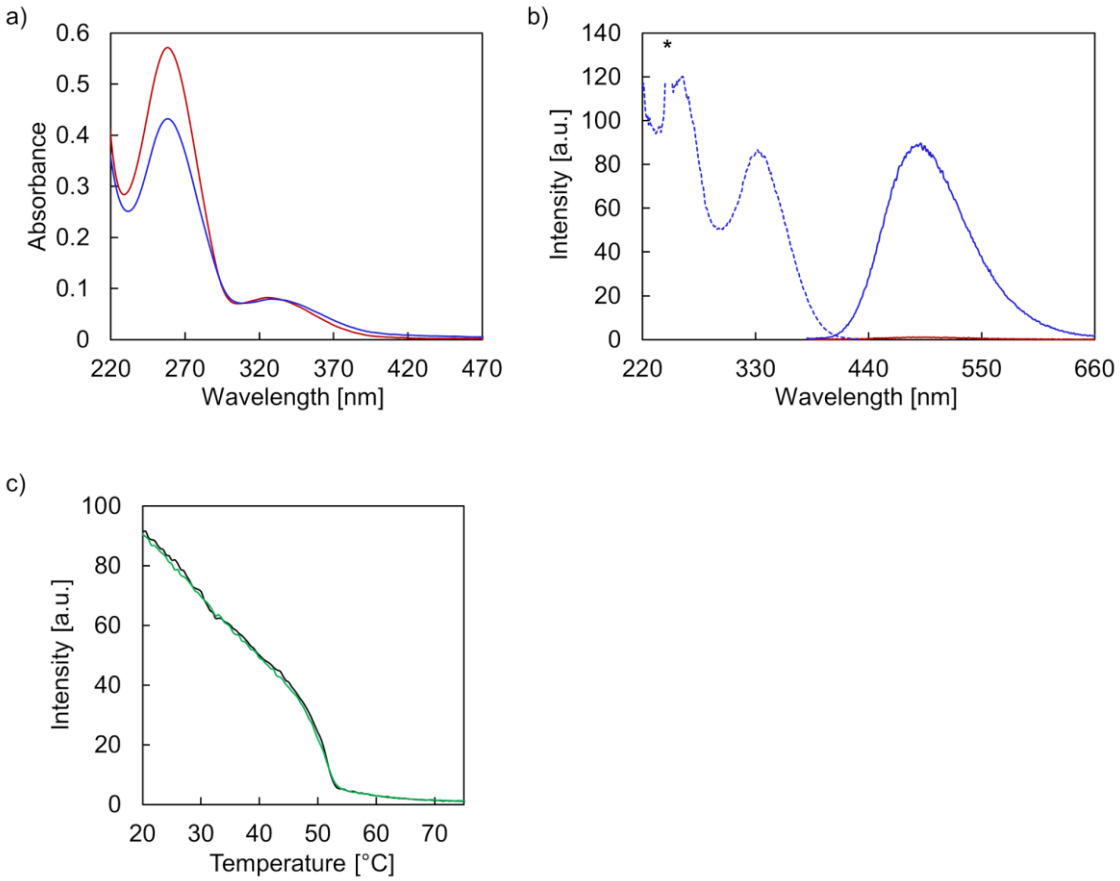

Fig. S9 Temperature-dependent UV-Vis absorption spectra of **2** (a), temperature-dependent fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **2** at 75 °C (red) and at 20 °C (blue) after thermal assembly process (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **2**. Conditions: 1 µM **2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

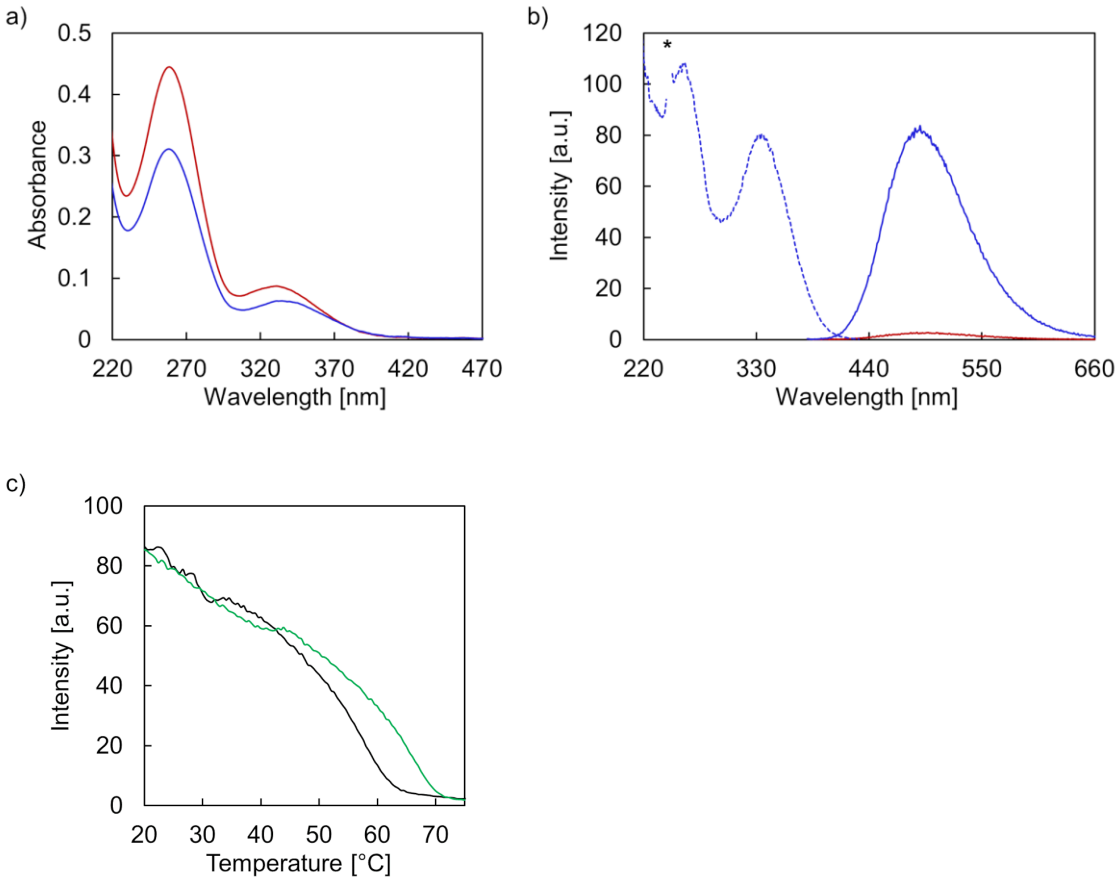

Fig. S10 Temperature-dependent UV-Vis absorption spectra of **4** (a), temperature-dependent fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **4** at 75 °C (red) and at 20 °C (blue) after thermal assembly process (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **4**. Conditions: 1 µM **4**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

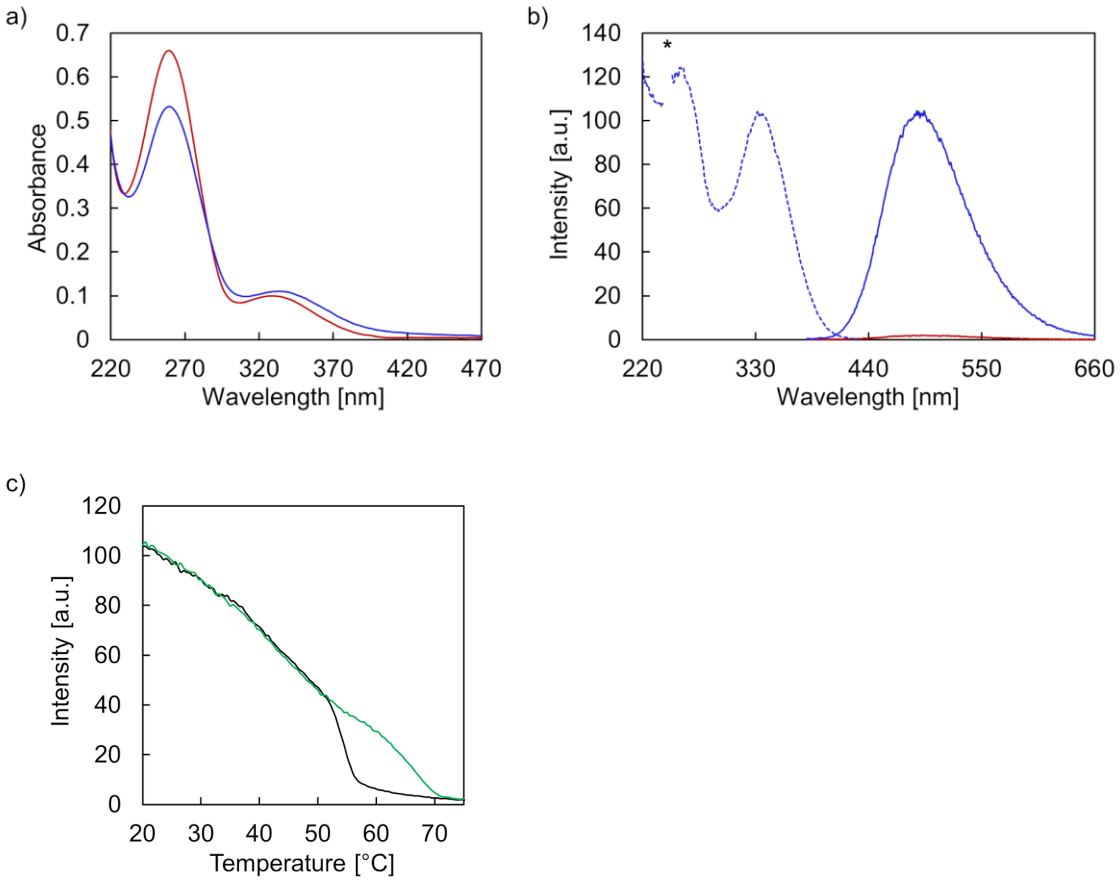

Fig. S11 Temperature-dependent UV-Vis absorption spectra of **5** (a), temperature-dependent fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **5** at 75 °C (red) and at 20 °C (blue) after thermal assembly process (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **5**. Conditions: 1 µM **5**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

4. AFM images

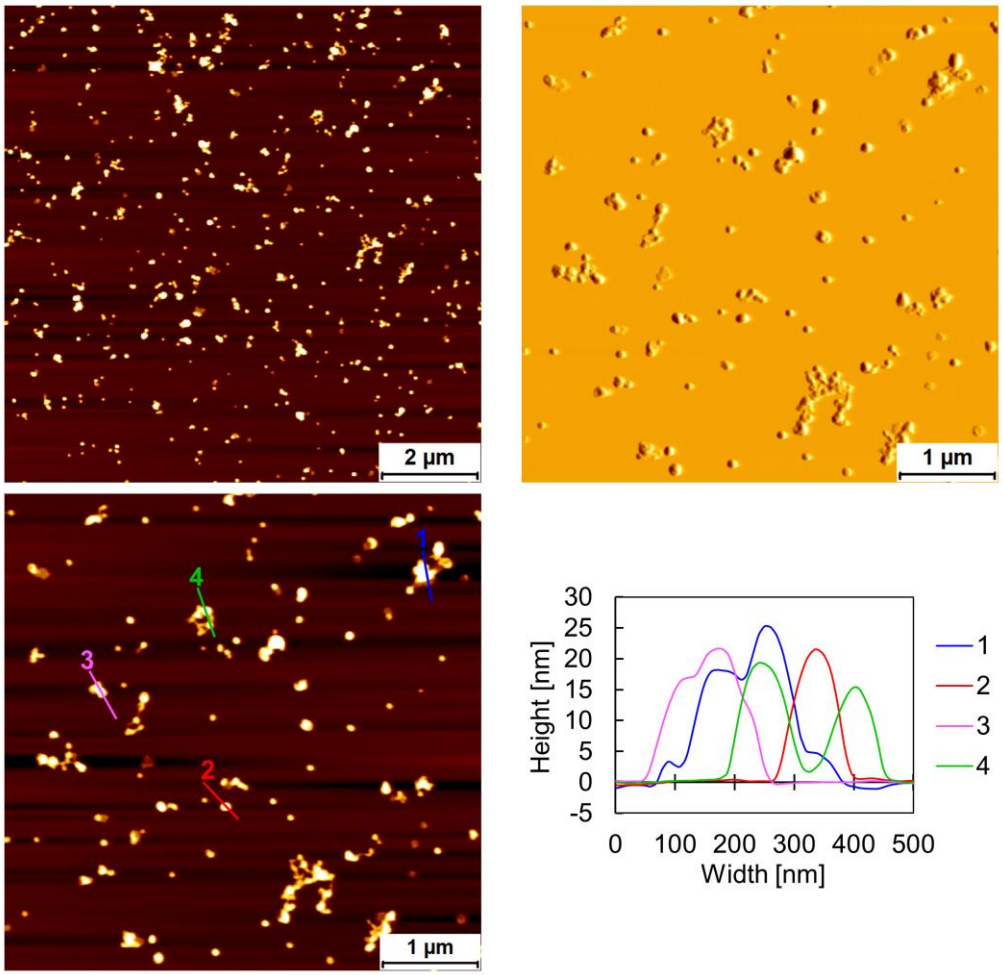

Fig. S12 AFM overview scan (top left), deflection scan (top right), and zoom with corresponding cross sections (bottom) of assembled duplex **3**. Conditions: 1 µM **3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

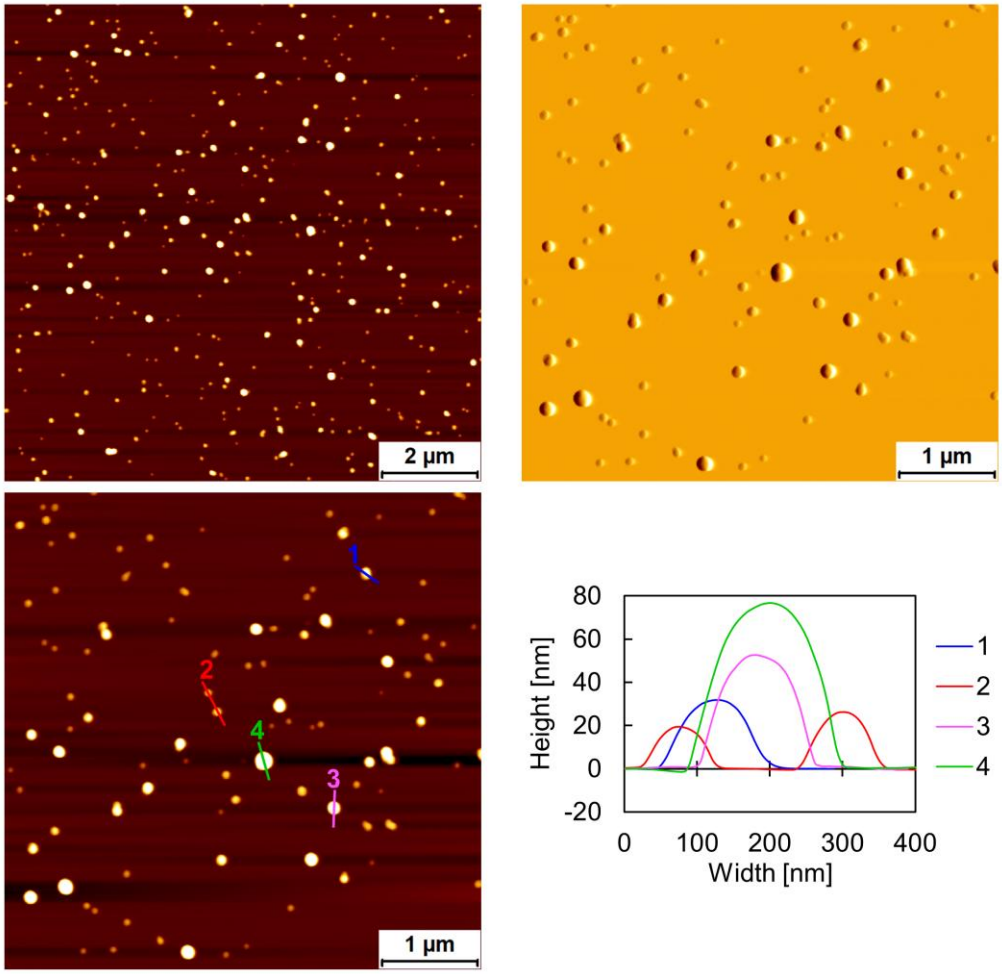

Fig. S13 AFM overview scan (top left), deflection scan (top right), and zoom with corresponding cross sections (bottom) of assembled duplex **2**. Conditions: 1 µM **2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

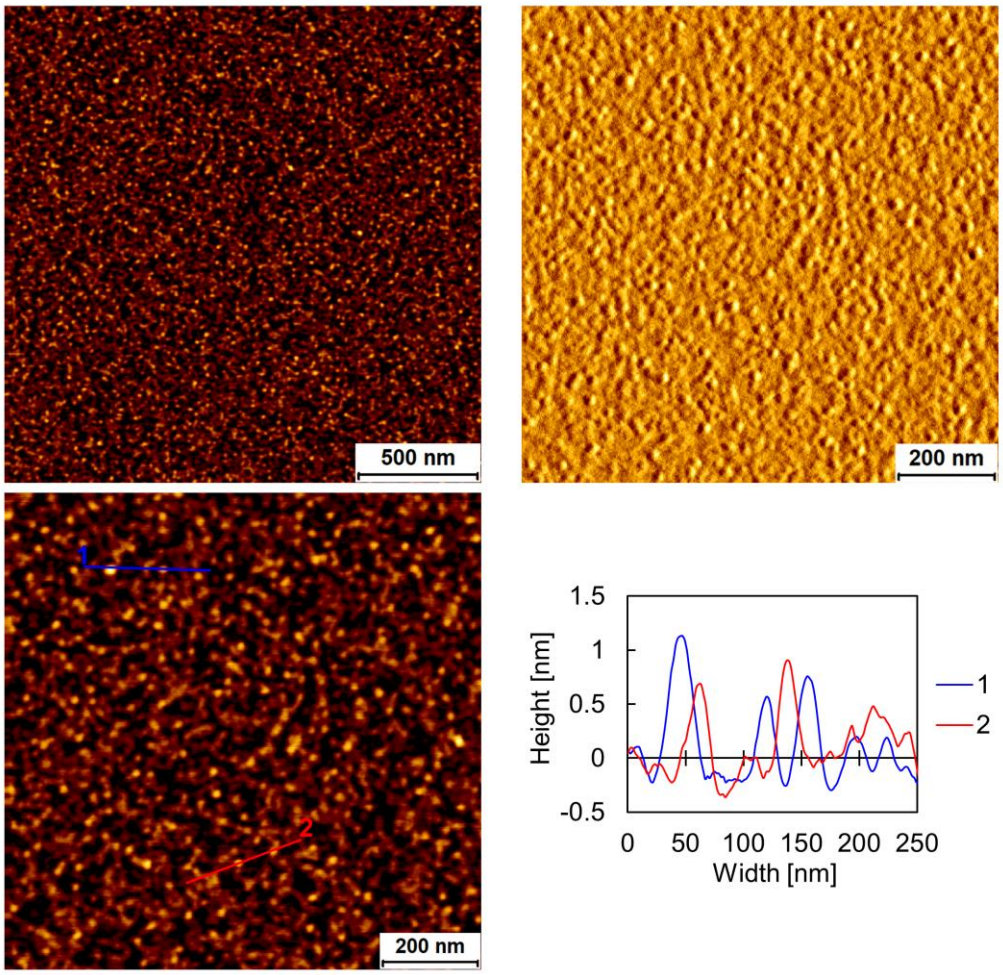

Fig. S14 AFM overview scan (top left), deflection scan (top right), and zoom with corresponding cross sections (bottom) of assembled duplex **1**. Conditions: 1 µM **1**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

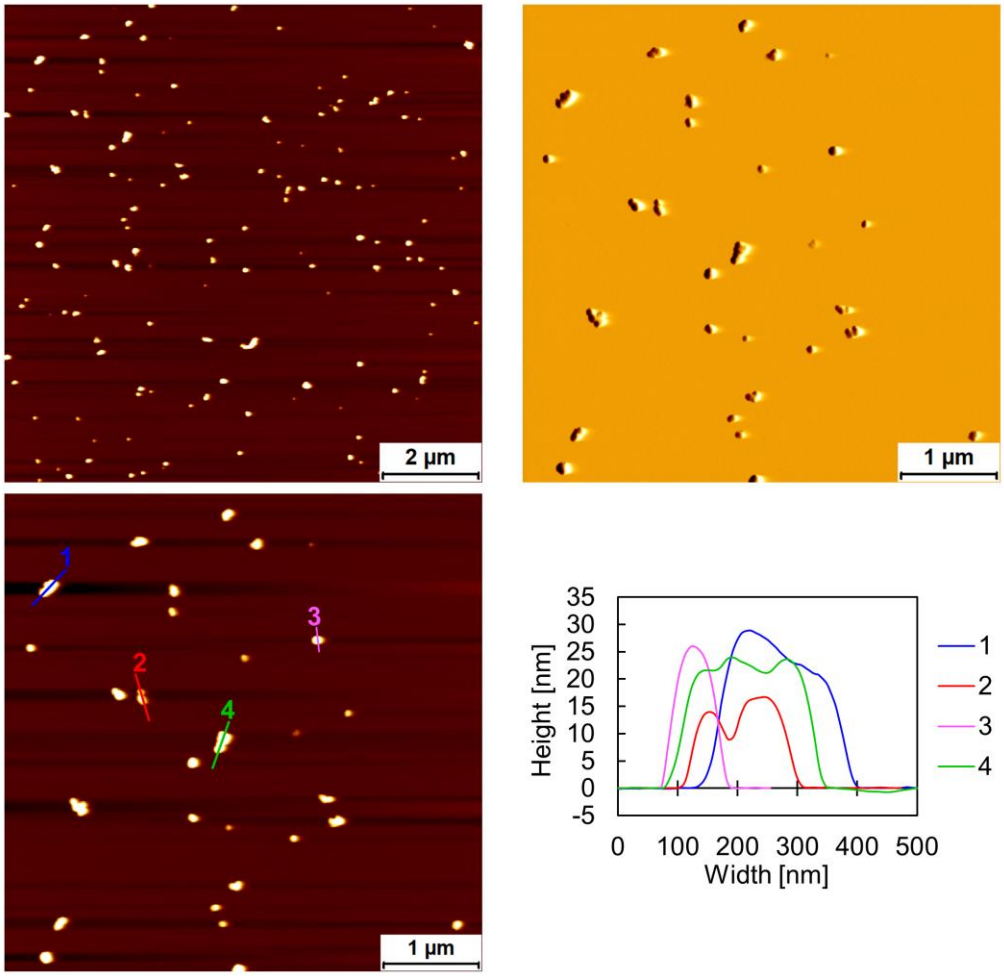

Fig. S15 AFM overview scan (top left), deflection scan (top right), and zoom with corresponding cross sections (bottom) of assembled duplex **4**. Conditions: 1 µM **4**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

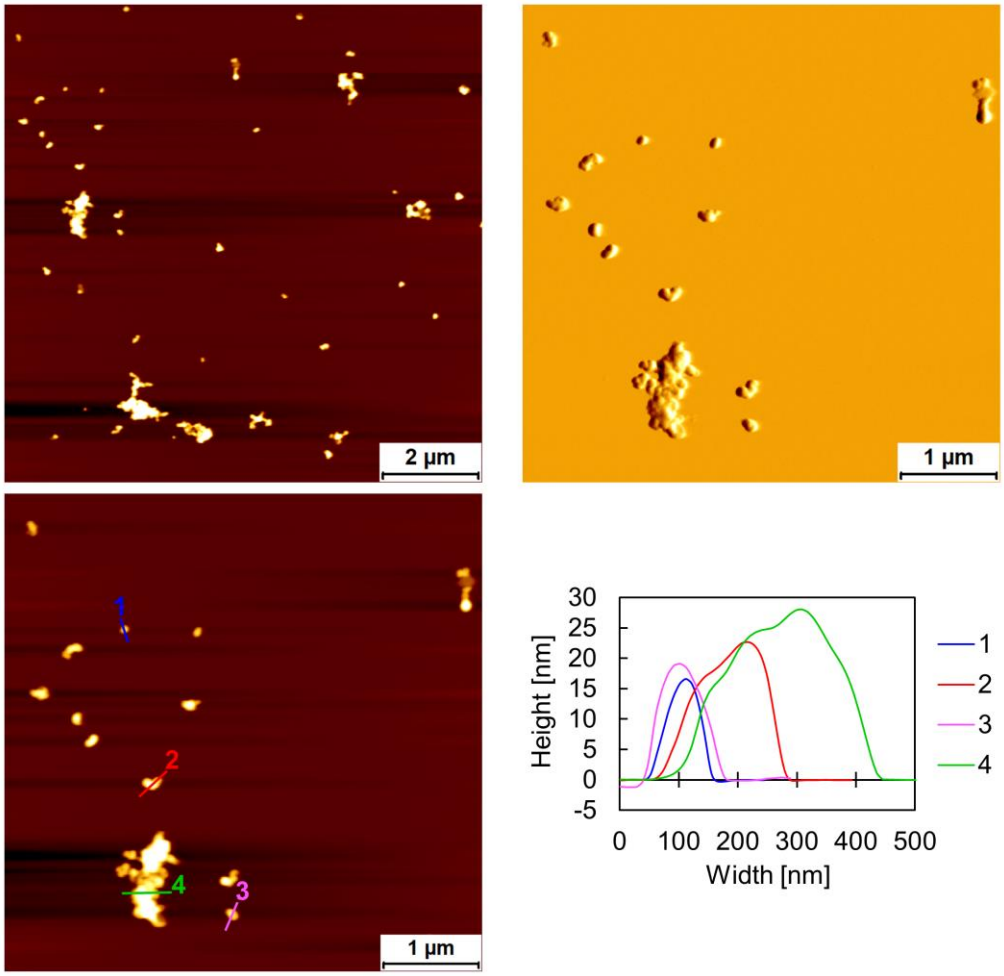

Fig. S16 AFM overview scan (top left), deflection scan (top right), and zoom with corresponding cross sections (bottom) of assembled duplex **5**. Conditions: 1 µM **5**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

5. DLS

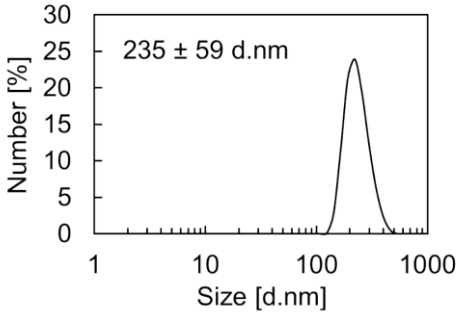

Fig. S17 DLS of vesicles assembled from duplex **2**. Conditions: 1 μM **2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 20 vol% ethanol.

6. Cryo-EM images

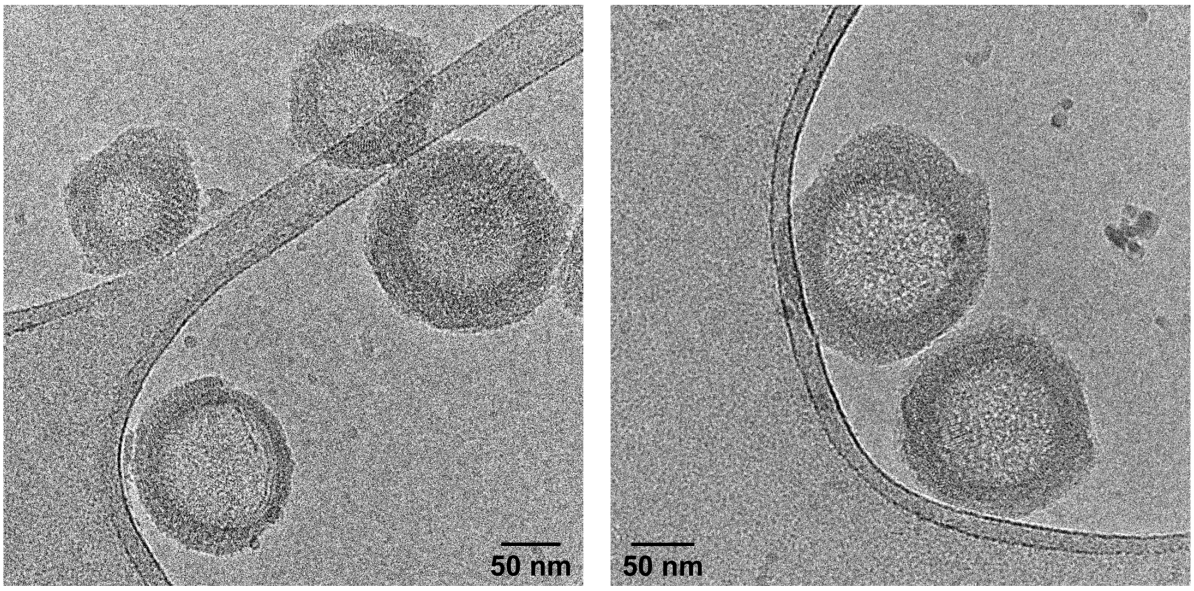

Fig. S18 Additional cryo-EM images of assembled duplex **3**. Conditions: 1 μM **3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

7. Bibliography

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