Supporting Information

Complex DNA Architectonics – Self-Assembly of Amphiphilic Oligonucleotides into Ribbons, Vesicles, and Asterosomes

Simon Rothenbühler,[†] Ioan Iacovache,[‡] Simon M. Langenegger,[†] Benoît Zuber,[‡] and Robert Häner*[,†]

[†] Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Freiestrasse 3, CH – 3012 Bern, Switzerland

[‡] Institute of Anatomy, University of Bern, Baltzerstrasse 2, CH – 3012 Bern, Switzerland

Table of Contents

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 (TPE phosphoramidite), required for the solid-phase synthesis of the tetraphenylethylene (TPE)-modified oligonucleotide **ON1**, followed published procedures.[1] HPLC purified oligonucleotide **ON2** was purchased from Merck (Germany), HPLC purified oligonucleotides **ON5** and **ON6** 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.

2. Preparation of Oligonucleotides

TPE-DNA conjugate **ON1** (Table S1) was 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 seconds was employed for the DNA nucleobases (0.1 M in anhydrous acetonitrile) and 2 minutes for TPE phosphoramidite (0.1 M solution in anhydrous 1,2-dichloroethane). The synthesis was started with a TPE-modified long chain alkylamine controlled pore glass (LCAA-CPG, 500 Å) solid-support, which was prepared according to previously reported procedures.[1] After the solid-phase synthesis, **ON1** was cleaved and deprotected by treatment with aqueous NH_4OH (28-30%) at 55 °C overnight. The supernatant was 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 conjugate was lyophilized.

TPE-DNA conjugate **ON1** was 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; B [%] (t_R [min]) = 5 (0), 50 (24). The purified TPE-DNA conjugate **ON1** was 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 solution. 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 one TPE modification. The corresponding HPLC trace of **ON1** is displayed in Figure S1, the MS result is listed in Table S1, and the MS spectra are presented in Figure S2 and Figure S3.

A general experimental procedure is described for the preparation of **ON3** and **ON4**, which was carried out according to standard copper-catalyzed azide-alkyne click chemistry conditions, starting with **ON6** (Table S1 and Scheme S1).[2] mPEG2000 azide (PEG average M_n 2000 Da) and mPEG5000 azide (PEG average M_n 5000 Da) were used for the preparation of **ON3** and **ON4**, respectively, which yielded PEG-DNA conjugates with a distribution of different PEG chain lengths. The alkyne-modified oligonucleotide **ON6** was dissolved in Milli-Q H_2O (c: 225 μM). 2 M triethylammonium acetate (TEAA) buffer pH 7.0 (28 μL) was added to **ON6** (84 μL) in an Eppendorf tube, followed by DMSO (117 μL). The reaction mixture was vortexed, before a 3 mM solution of the respective mPEG azide in DMSO (9.5 μL) was added. A solution of 5 mM ascorbic acid in Milli-Q H_2O (28 μL) was added. The reaction mixture was vortexed briefly, then degassed by bubbling argon in it for 30 seconds. A solution of 10 mM Cu(II)-TBTA (TBTA: tris((1-benzyl-4-triazolyl)methyl)amine) in Milli-Q H_2O/DMSO 45:55 (14 μL) was added, before the Eppendorf tube was flushed with argon, sealed, vortexed thoroughly, and shaken in a ThermoMixer for 25 h (25 °C, 500 rpm). Afterwards, the reaction mixture was lyophilized.

The crude PEG conjugated oligonucleotides **ON3** and **ON4** respectively, were purified by reversed-phase HPLC (*Shimadzu LC-20AT*, *ReproSil 100 C18*, 5 μm, 250 x 4 mm) at 40 °C with a flow rate of 1 mL/min, λ: 260 nm. Solvent A: aqueous 0.1 M TEAA buffer pH 7; solvent B: acetonitrile; B [%] (t_R [min]) = 0 (0), 0 (1), 5 (2), 60 (22). The purified oligomers were dissolved in Milli-Q H_2O (130 μL). The concentration of the final stock solutions of **ON3** and **ON4** followed the same experimental procedure as stated above for **ON1**. The corresponding HPLC traces of **ON3** and **ON4** are displayed in Figure S1, the MS results are listed in Table S1, and the MS spectra are presented in Figure S4 – Figure S8.

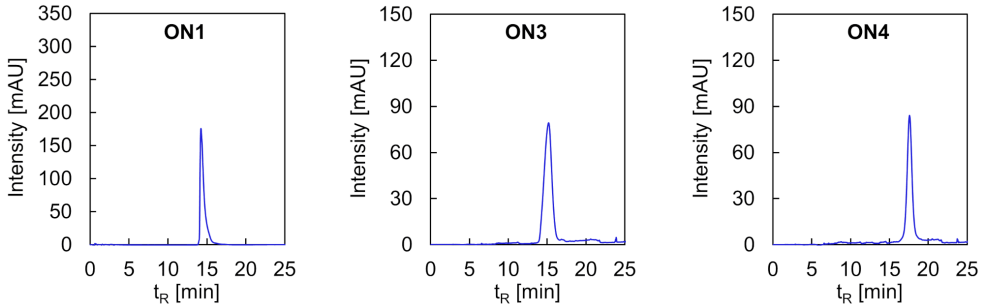
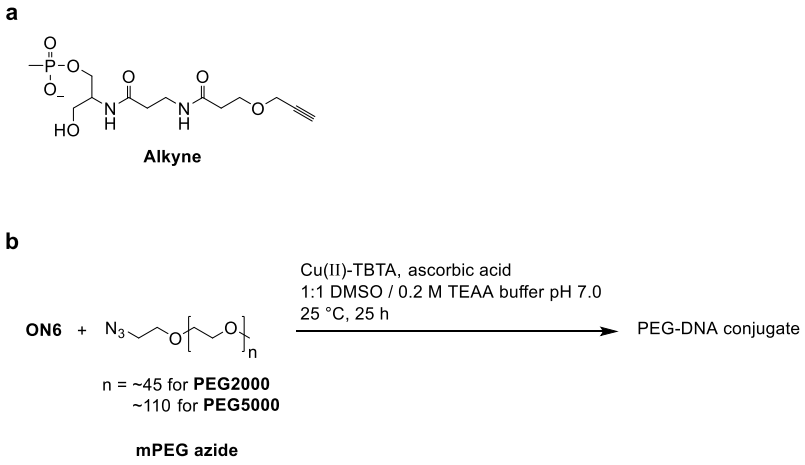


Figure S1. HPLC traces of **ON1**, **ON3**, and **ON4**.

Table S1. Oligonucleotide sequences of **ON1**, **ON3**, **ON4**, and **ON6**, calculated and found masses by NSI-MS. [a] Calculated mass for n = 45 PEG units. [b] Calculated mass for n = 110 PEG units.

Strand	Sequence (5'→3')	calc. mass	found mass
ON1	(**TPE**)$_3$-CTT CCT TGC ATC GGA CCT TG-(**TPE**)$_3$	9215.9945	9216.0492
ON3	CAA GGT CCG ATG CAA GGA AG-**PEG2000**	8616.4266 [a]	8616.4650
ON4	CAA GGT CCG ATG CAA GGA AG-**PEG5000**	11478.1306 [b]	11478.1212
ON6	CAA GGT CCG ATG CAA GGA AG-**Alkyne**	–	–



Scheme S1. (a) Chemical structure of alkyne modification of **ON6**. (b) Click reaction of **ON6** with mPEG azides.

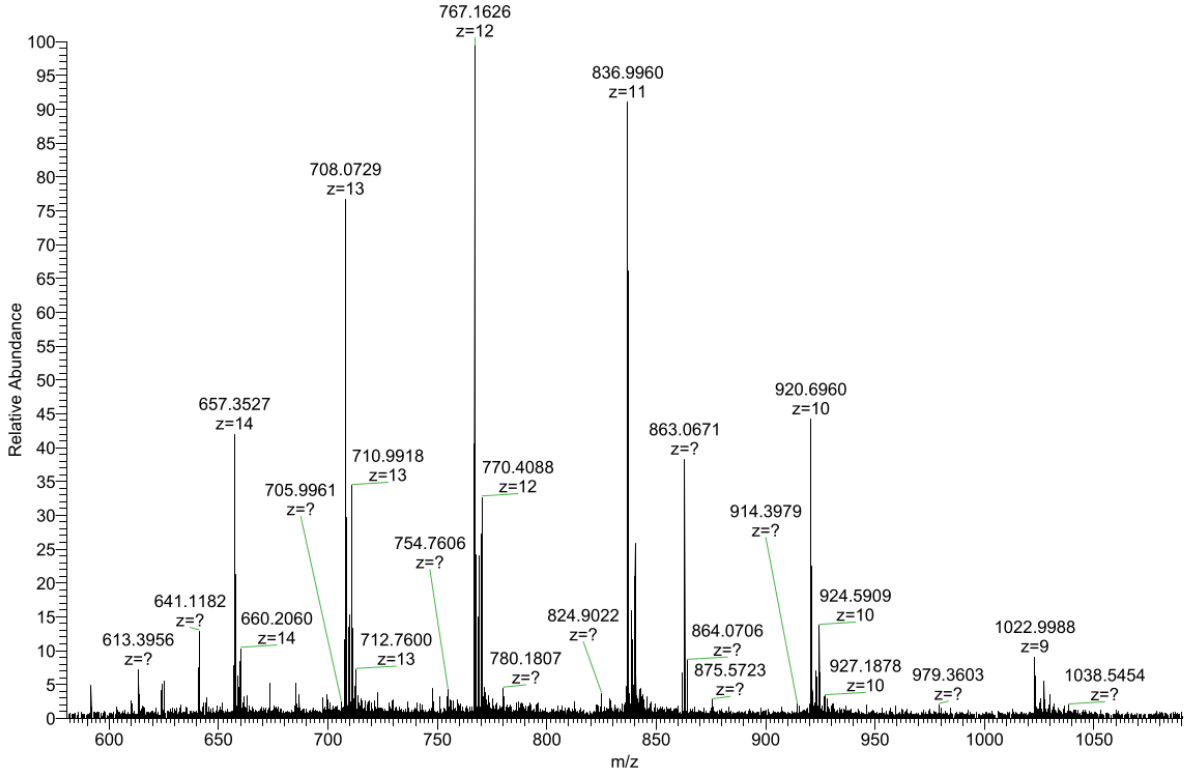


Figure S2. MS spectrum of **ON1**.

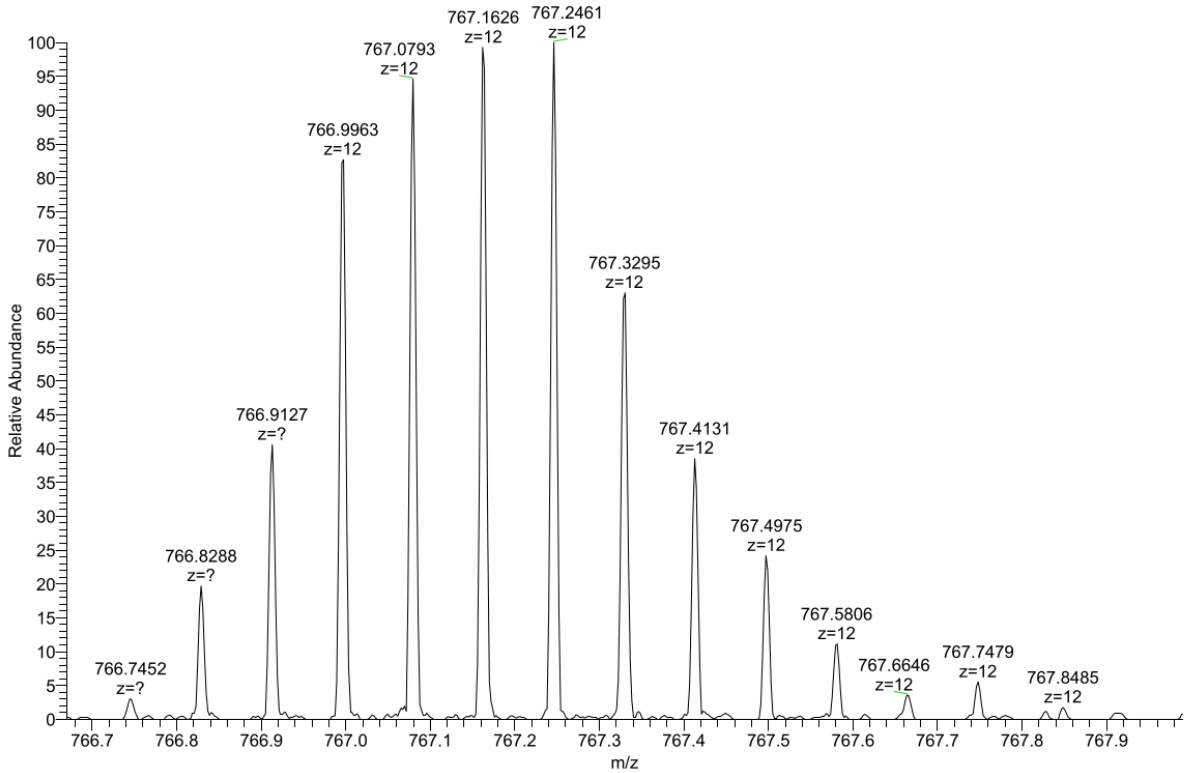


Figure S3. MS spectrum (zoom) of **ON1**.

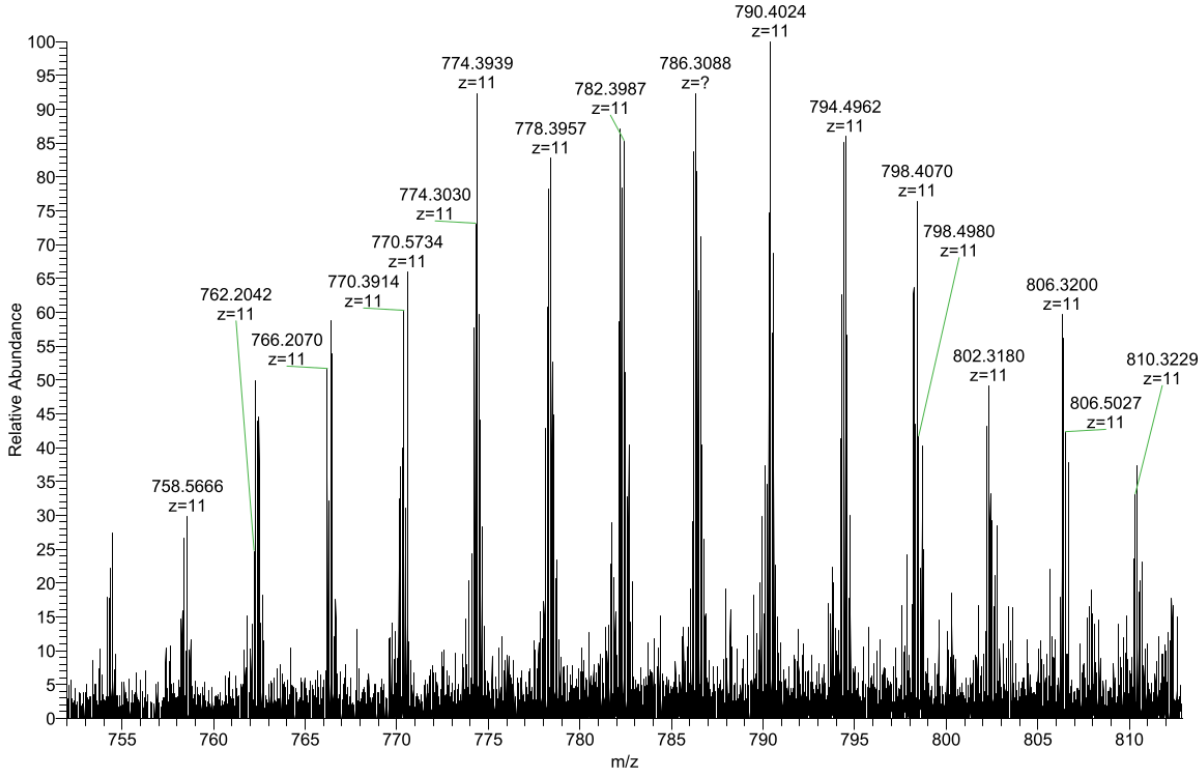


Figure S4. MS spectrum of **ON3**.

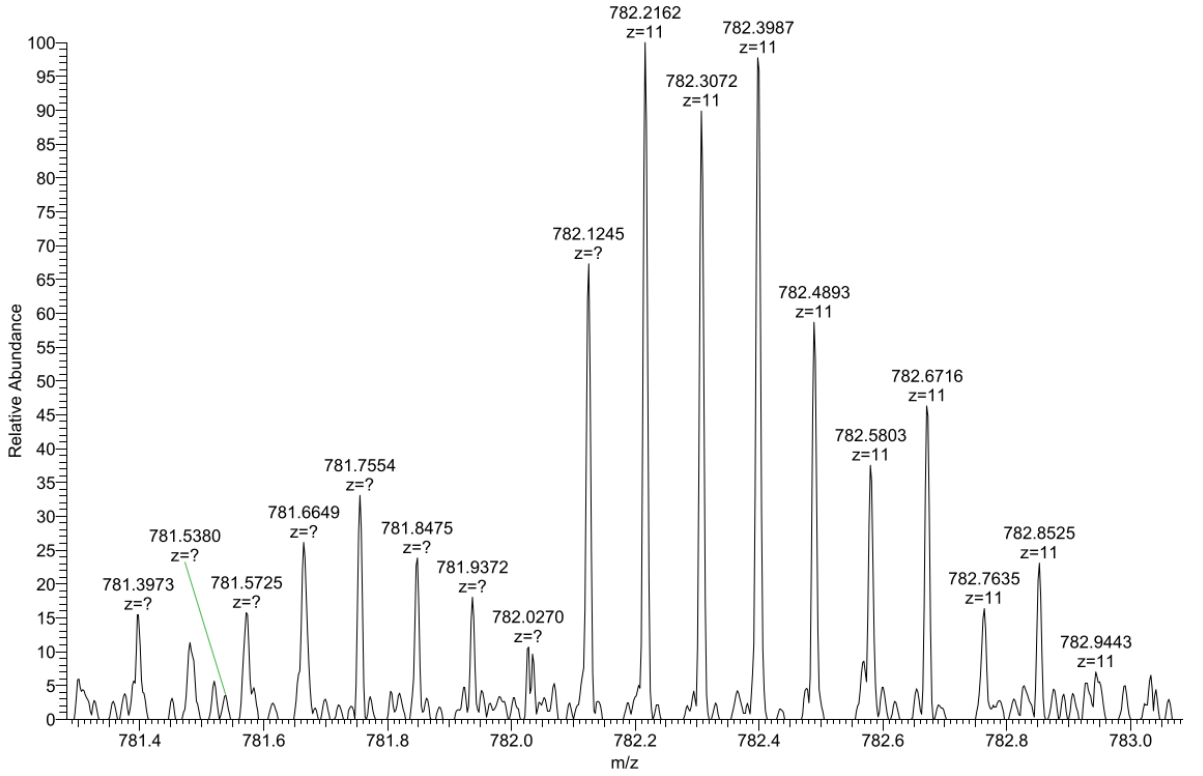


Figure S5. MS spectrum (zoom) of **ON3**.

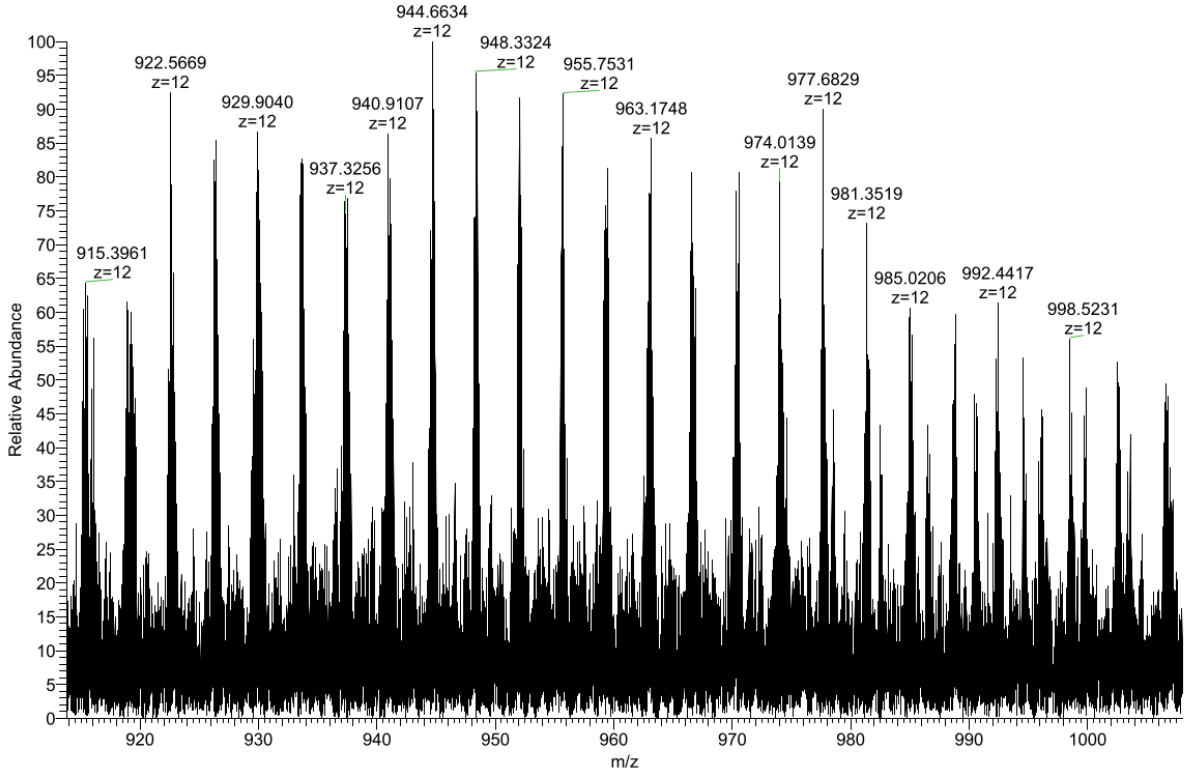


Figure S6. MS spectrum of **ON4**.

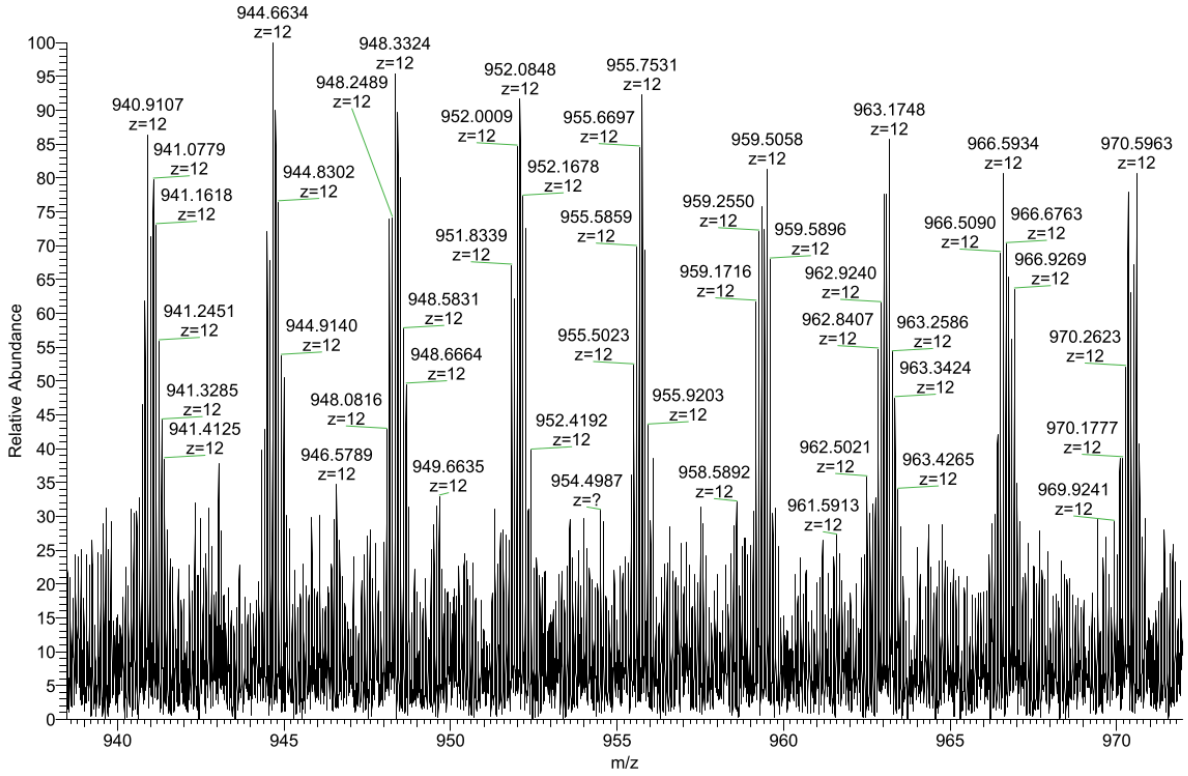


Figure S7. MS spectrum (zoom 1) of **ON4**.

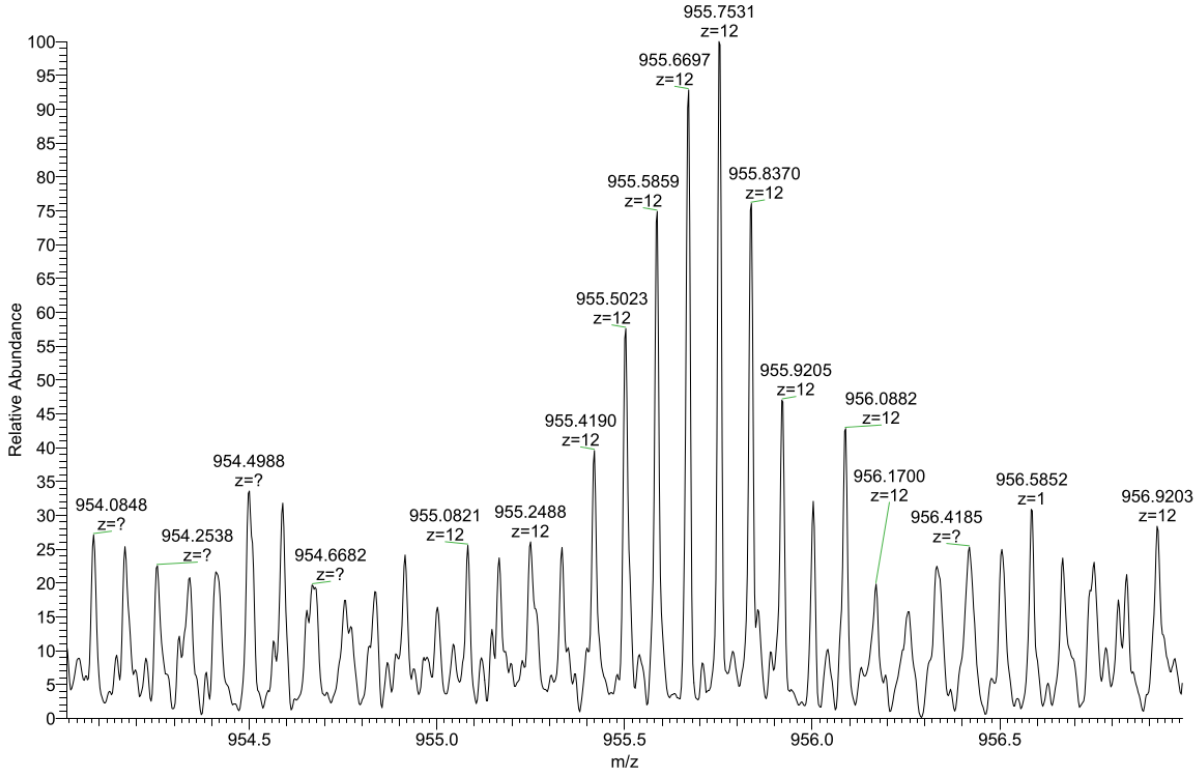


Figure S8. MS spectrum (zoom 2) of **ON4**.

3. UV-Vis and Fluorescence Spectra

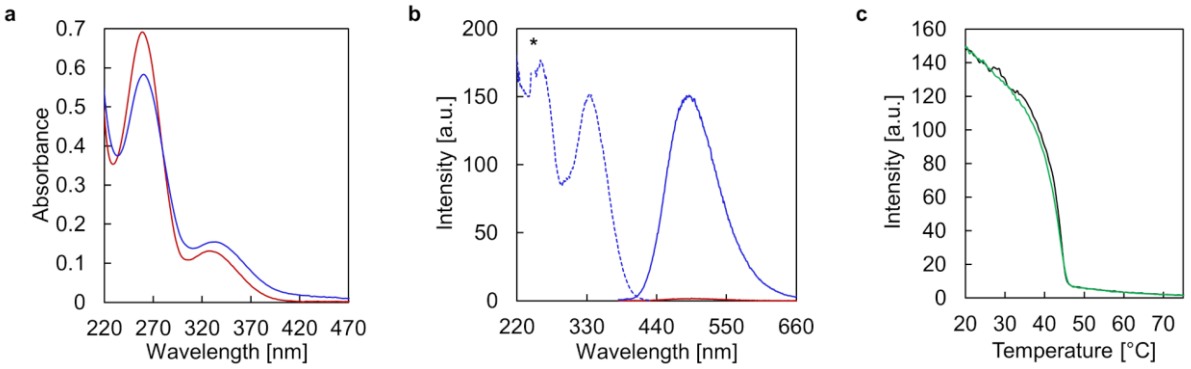


Figure S9. Temperature-dependent UV-Vis absorption (a), fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **ON1*ON2** at 75 °C (red) and at 20 °C (blue) after thermally controlled assembly (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **ON1*ON2**. Conditions: 1 µM **ON1*ON2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

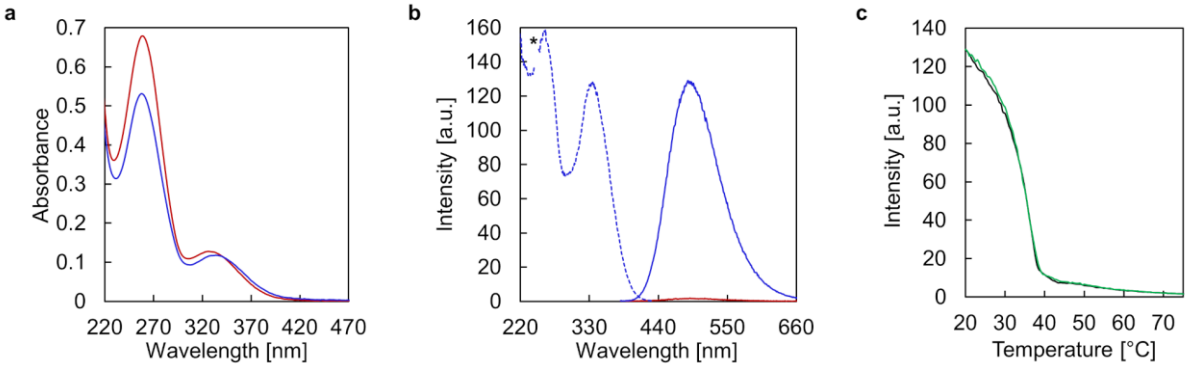


Figure S10. Temperature-dependent UV-Vis absorption (a), fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **ON1*ON3** at 75 °C (red) and at 20 °C (blue) after thermally controlled assembly (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **ON1*ON3**. Conditions: 1 µM **ON1*ON3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

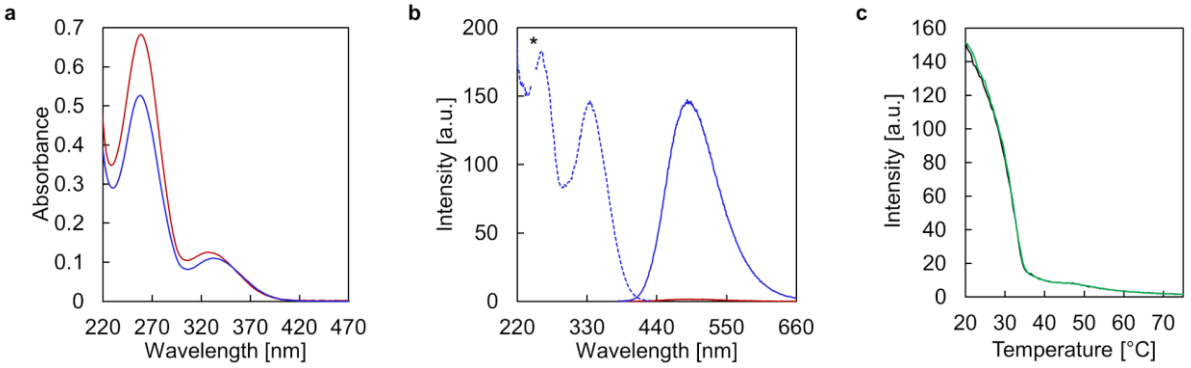


Figure S11. Temperature-dependent UV-Vis absorption (a), fluorescence emission (b, solid line) and excitation (b, dotted line) spectra of **ON1*ON4** at 75 °C (red) and at 20 °C (blue) after thermally controlled assembly (0.5 °C/min; * denotes second-order diffraction). (c) Fluorescence-monitored annealing (black) and melting (green) curves of **ON1*ON4**. Conditions: 1 µM **ON1*ON4**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol, $\lambda_{ex.}$: 335 nm, $\lambda_{em.}$: 490 nm.

4. Cryo-EM Images

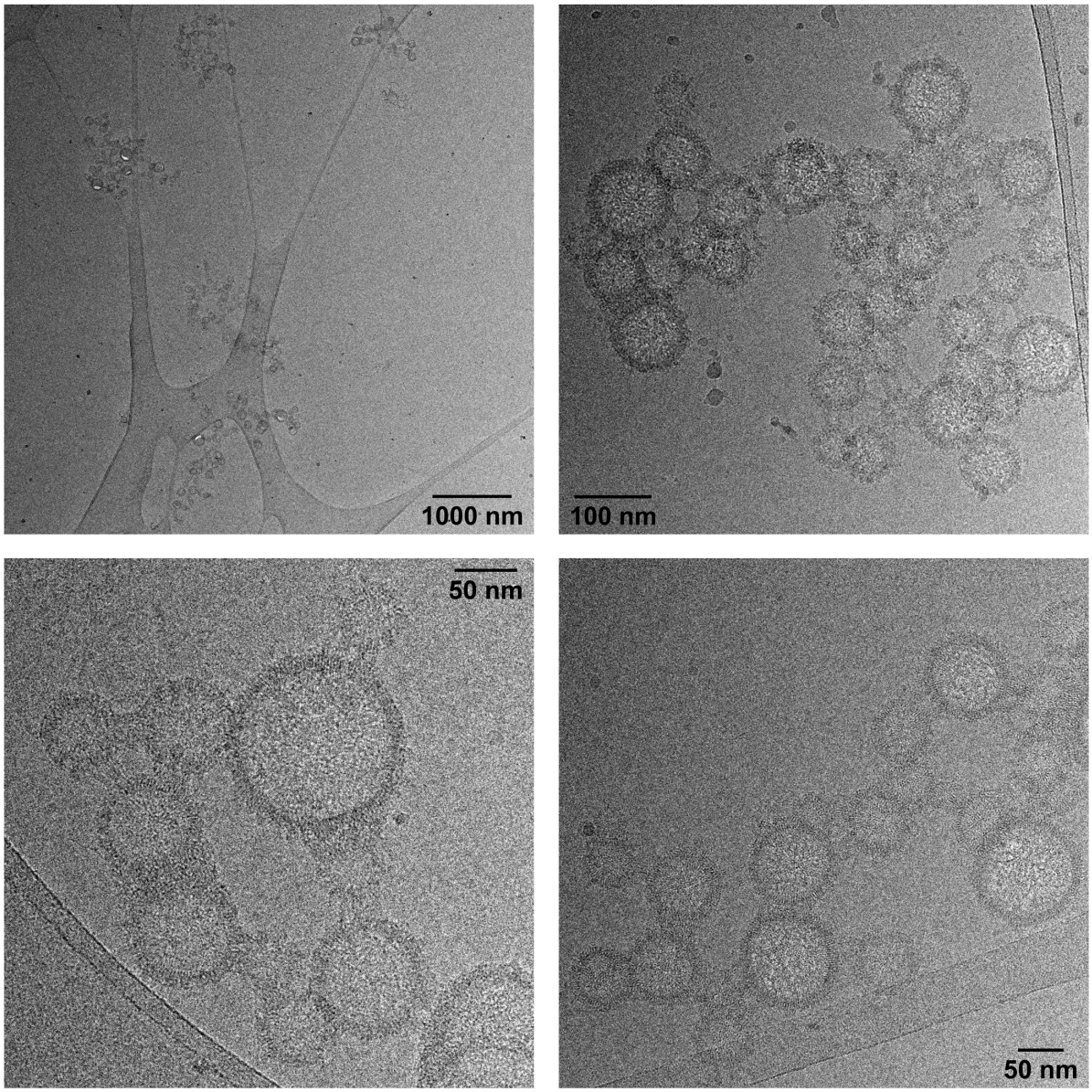


Figure S12. Additional cryo-EM images of self-assembled **ON1*ON2**. Conditions: 1 μM **ON1*ON2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

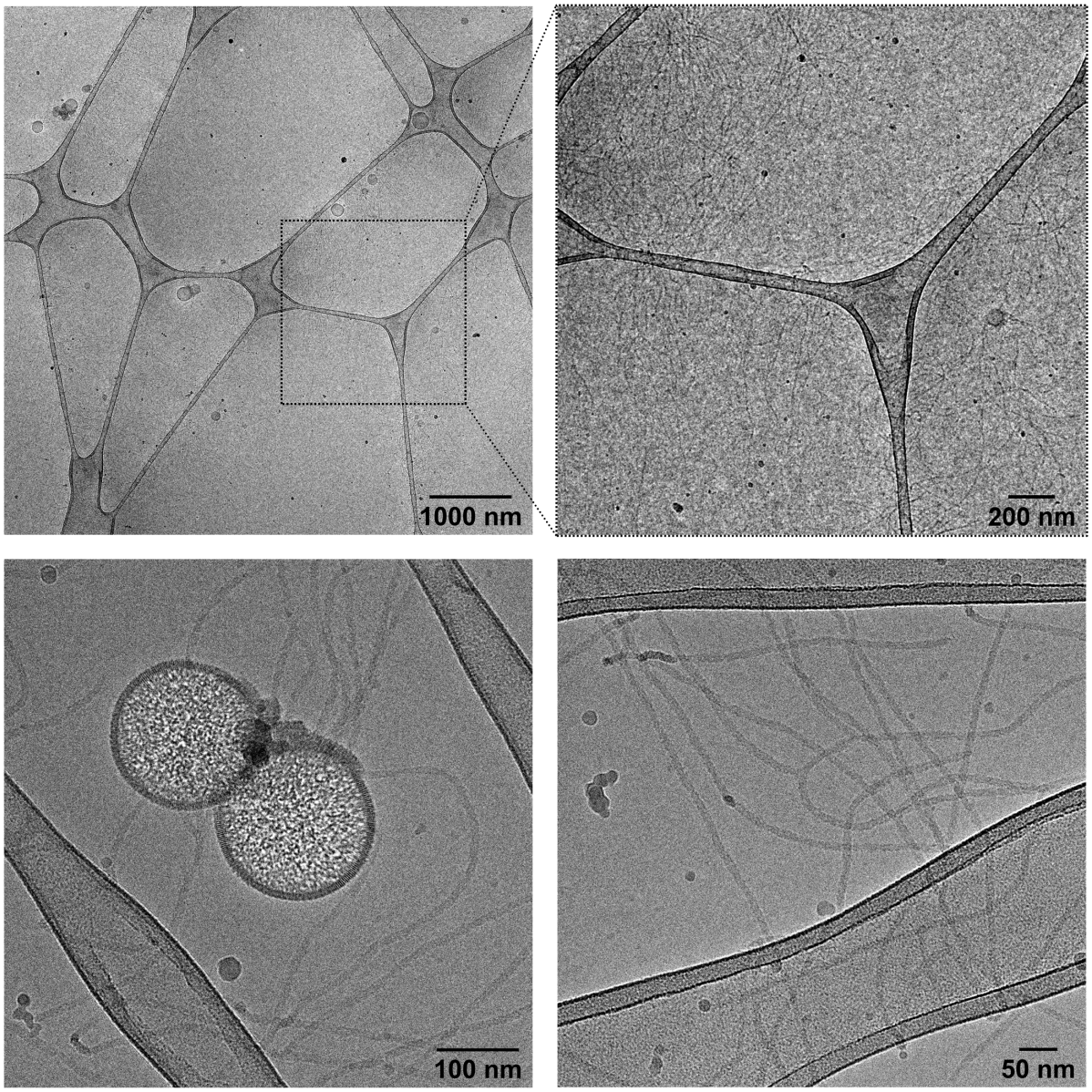


Figure S13. Additional cryo-EM images of self-assembled **ON1*ON3**. Conditions: 1 μM **ON1*ON3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

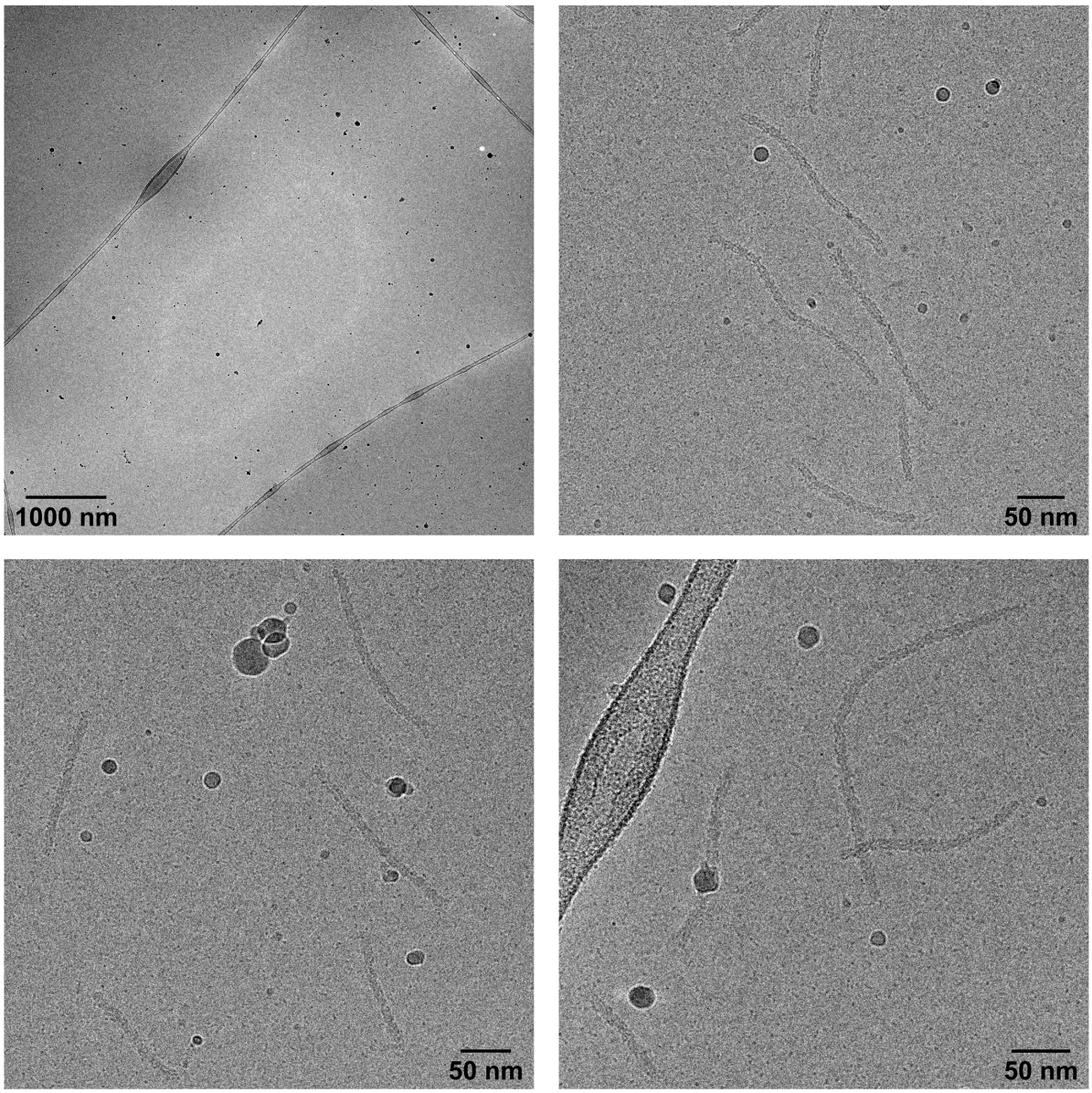


Figure S14. Additional cryo-EM images of self-assembled **ON1*ON4**. Conditions: 1 μM **ON1*ON4**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

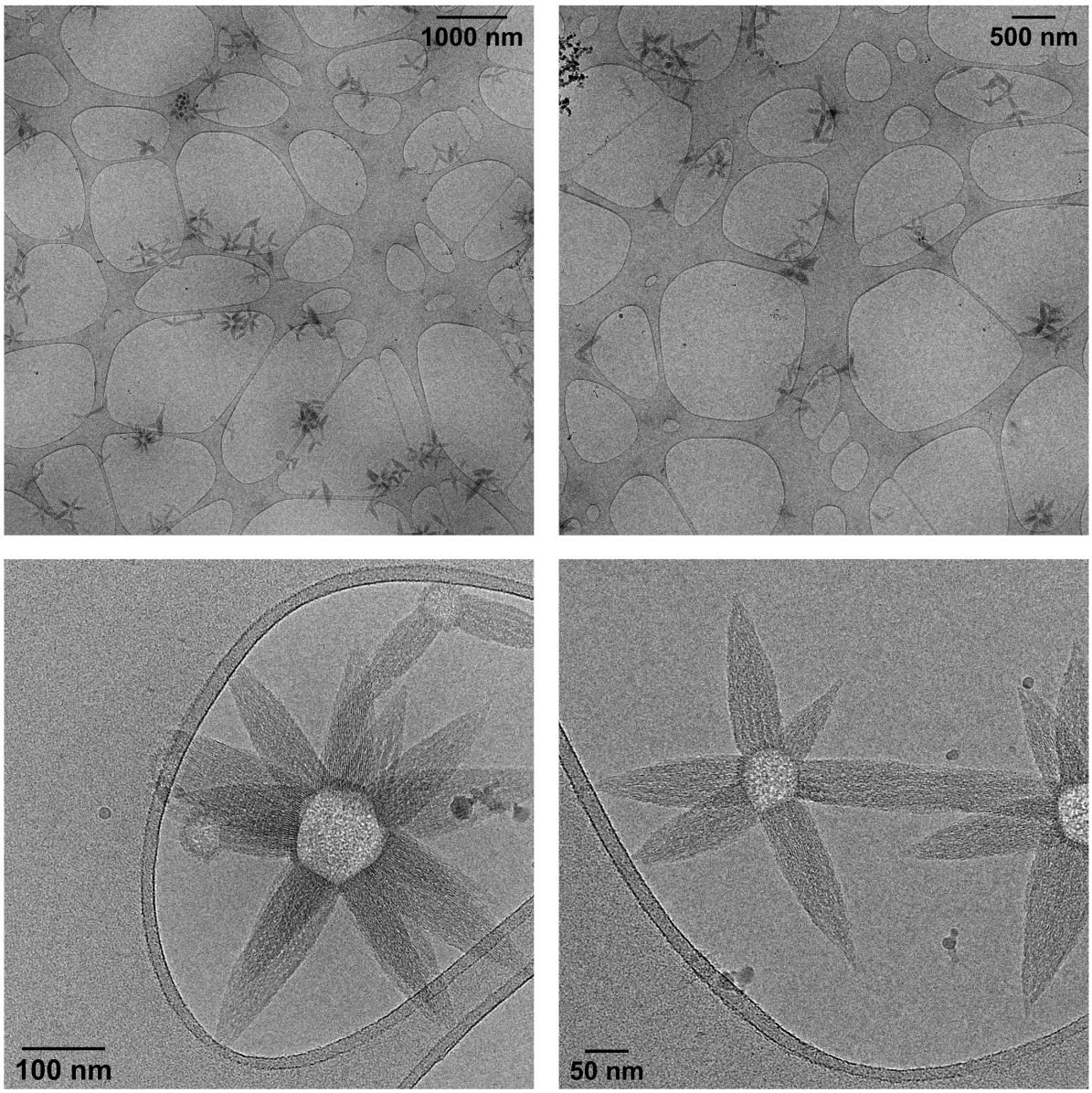


Figure S15. Additional cryo-EM images of self-assembled **ON1*ON5**. Conditions: 1 μM **ON1*ON5**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

5. Distance Measurements

Table S2. Summary of distance measurements on cryo-EM images. The reported distances are mean values with the corresponding standard deviation, along with the number of measurements in brackets.

Duplex	Vesicle membrane thickness	DNA width	Ribbon thickness
ON1*ON2	10.7 ± 0.6 nm (*n* = 40)	2.5 ± 0.3 nm (*n* = 43)	–
ON1*ON3	10.0 ± 0.5 nm (*n* = 52)	2.4 ± 0.3 nm (*n* = 84)	10.1 ± 0.7 nm (*n* = 65)
ON1*ON4	–	–	10.2 ± 0.7 nm (*n* = 68)
ON1*ON5	10.5 ± 0.5 nm (*n* = 46)	2.5 ± 0.2 nm (*n* = 79)	–

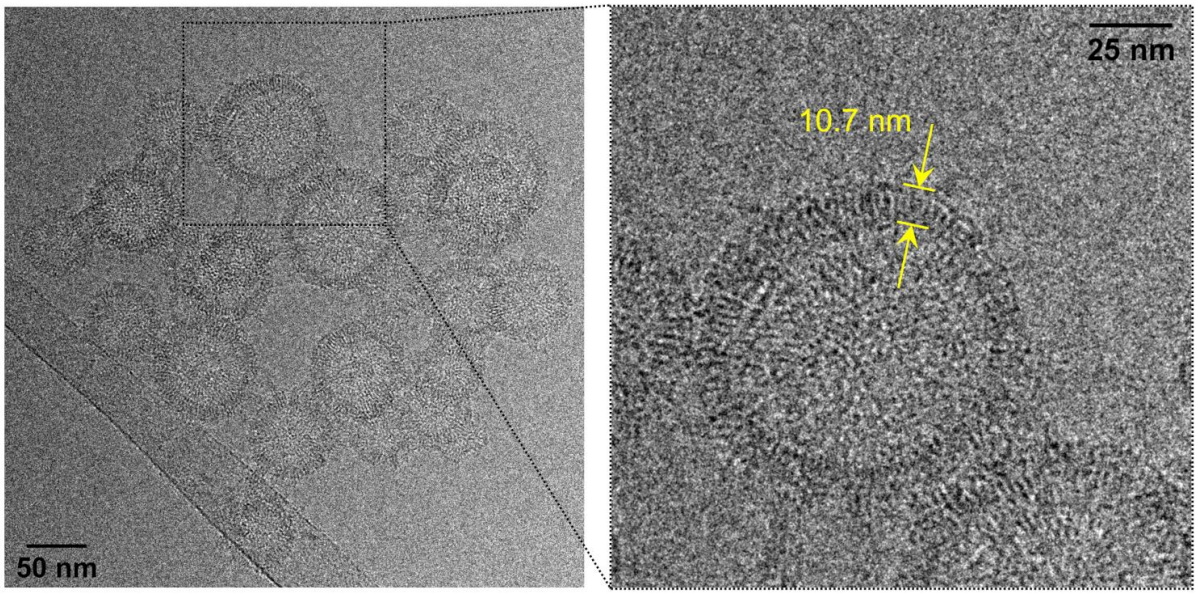


Figure S16. Representative vesicle membrane thickness measurement of self-assembled **ON1*ON2**.

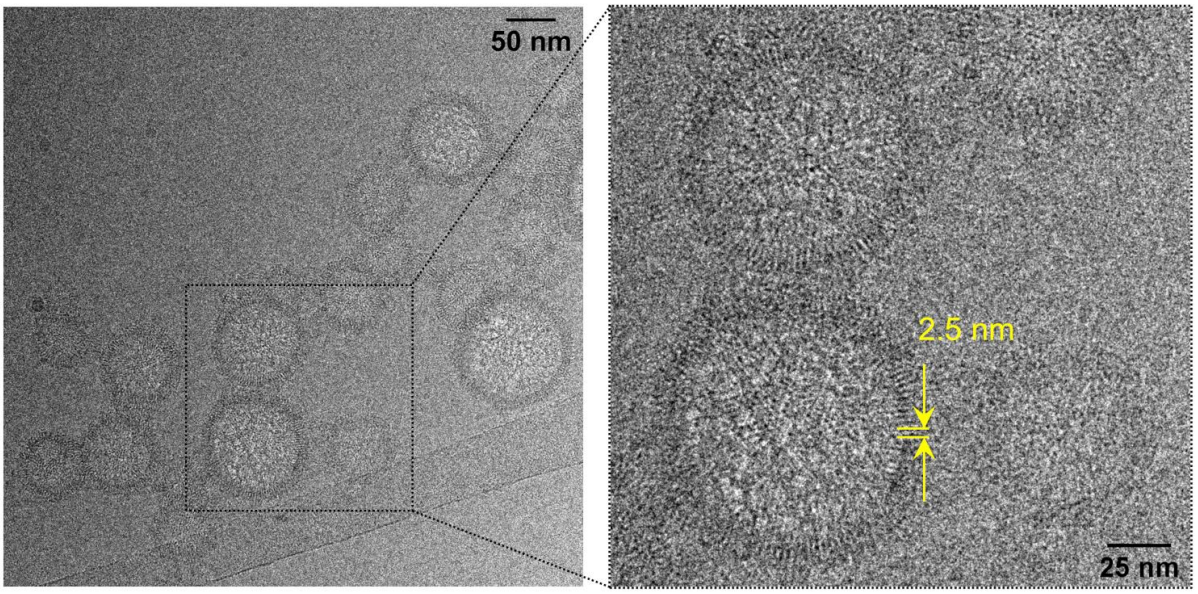


Figure S17. Representative DNA width measurement of self-assembled **ON1*ON2**.

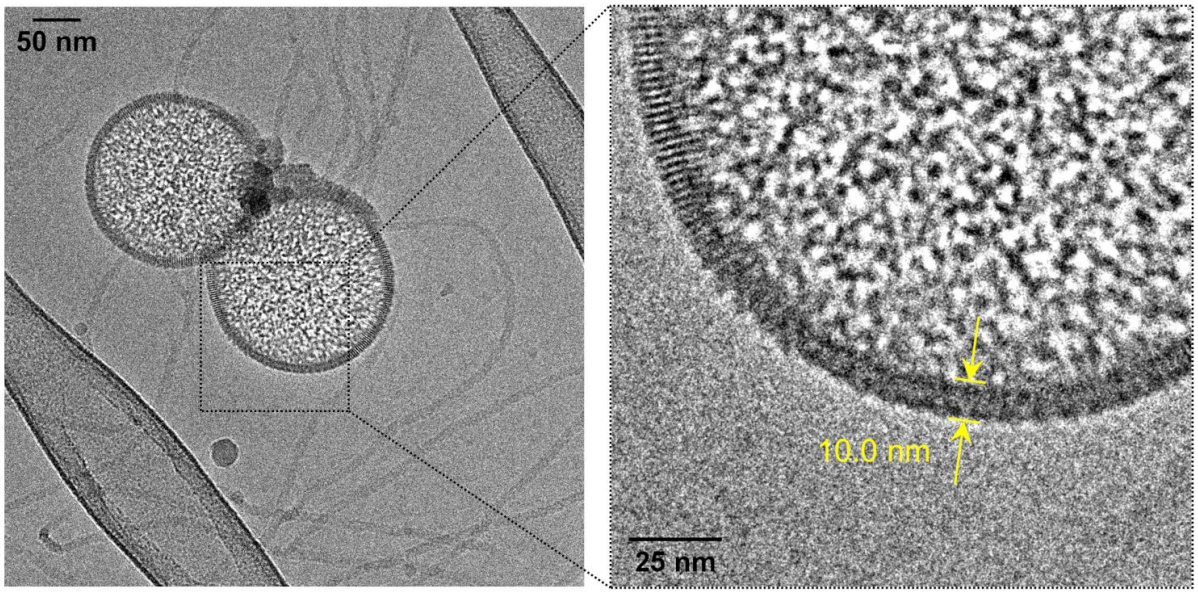


Figure S18. Representative vesicle membrane thickness measurement of self-assembled **ON1*ON3**.

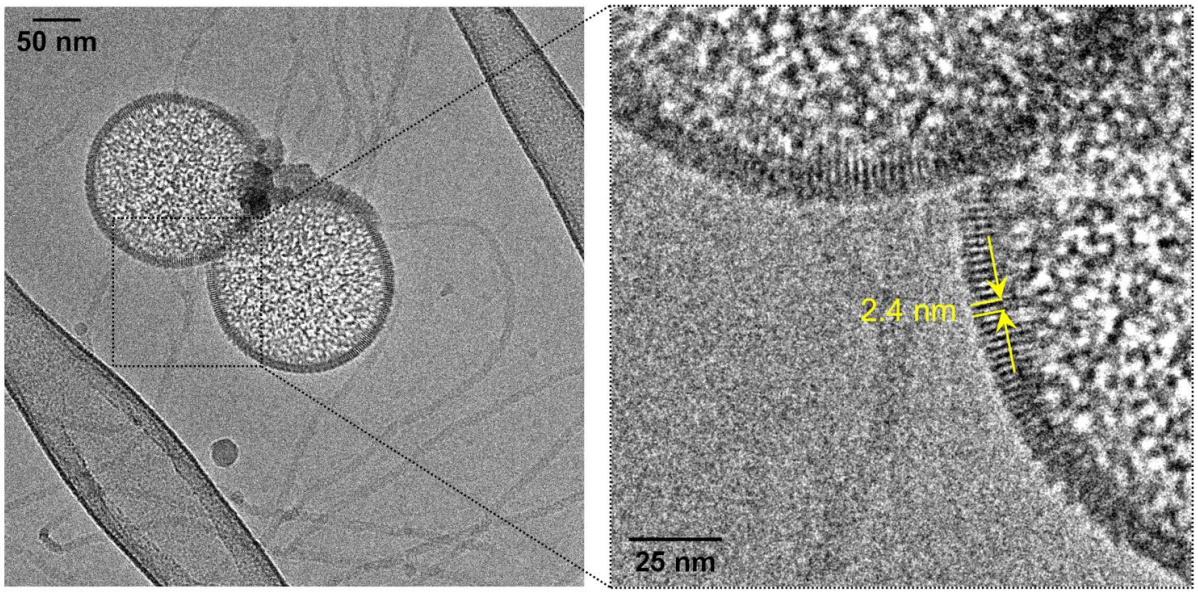


Figure S19. Representative DNA width measurement of self-assembled **ON1*ON3**.

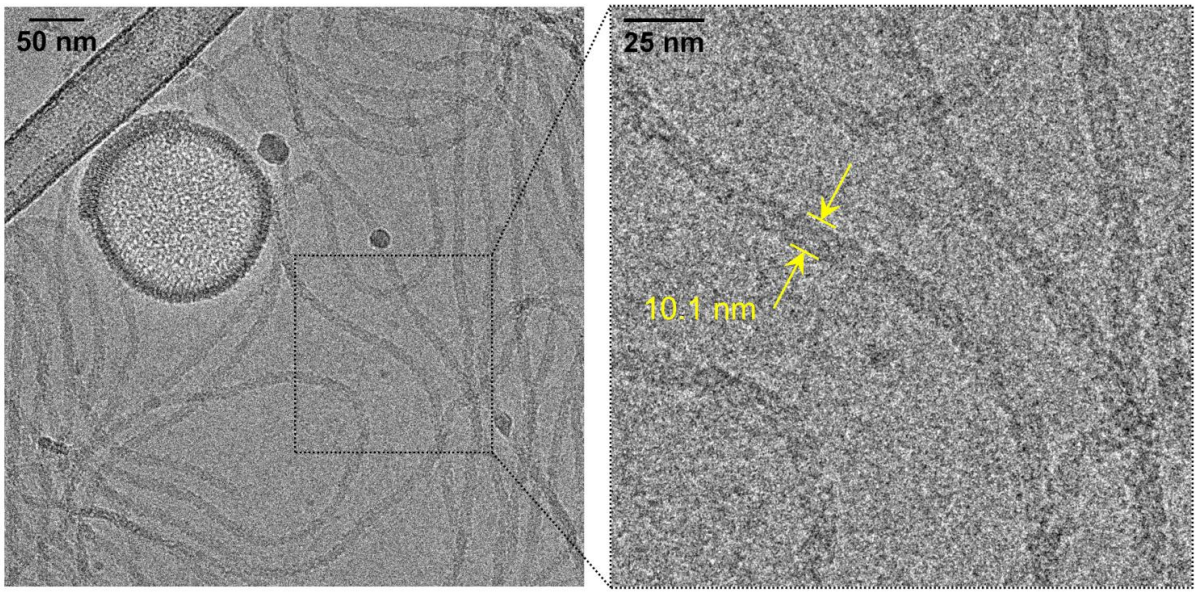


Figure S20. Representative ribbon thickness measurement of self-assembled **ON1*ON3**.

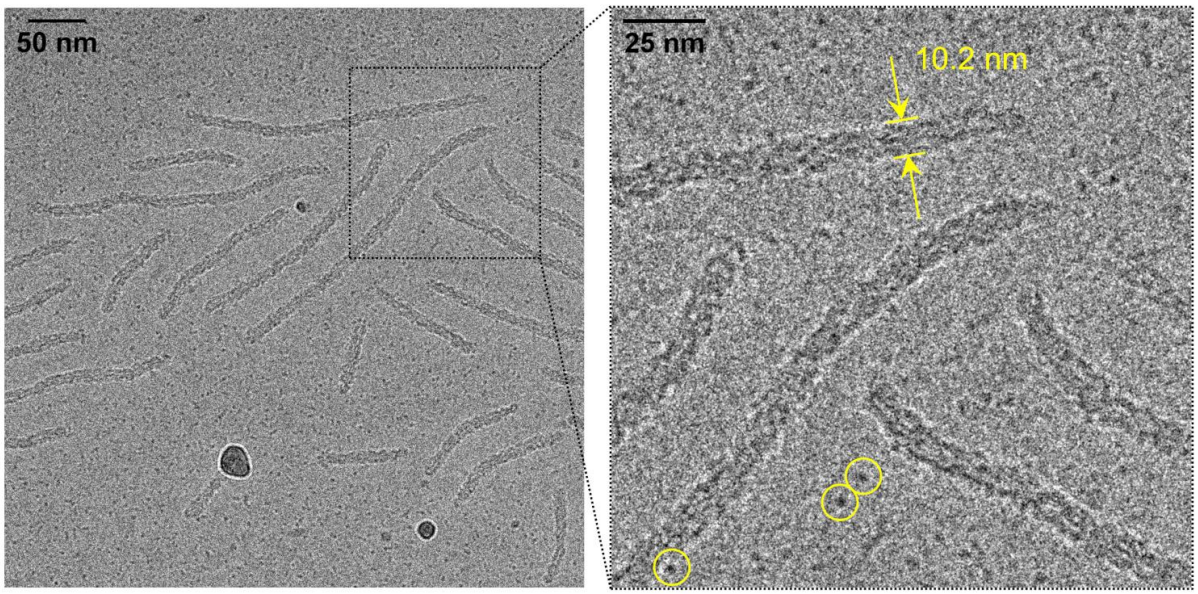


Figure S21. Representative ribbon thickness measurement of self-assembled **ON1*ON4**, as well as single DNA duplexes **ON1*ON4** encircled in yellow.

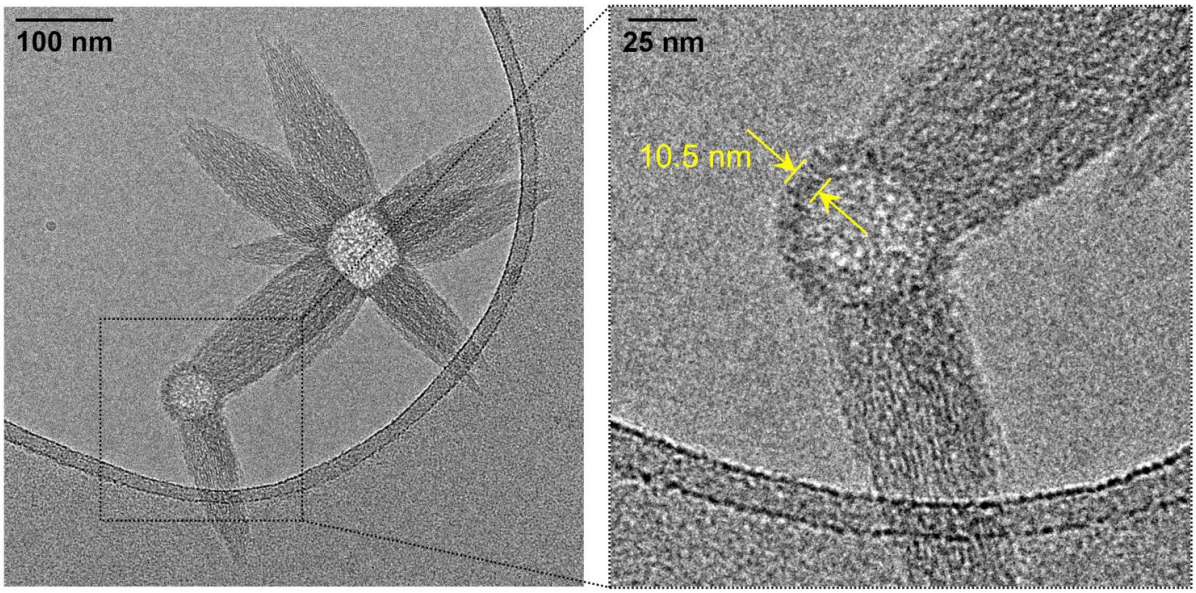


Figure S22. Representative vesicle membrane thickness measurement of self-assembled **ON1*ON5**.

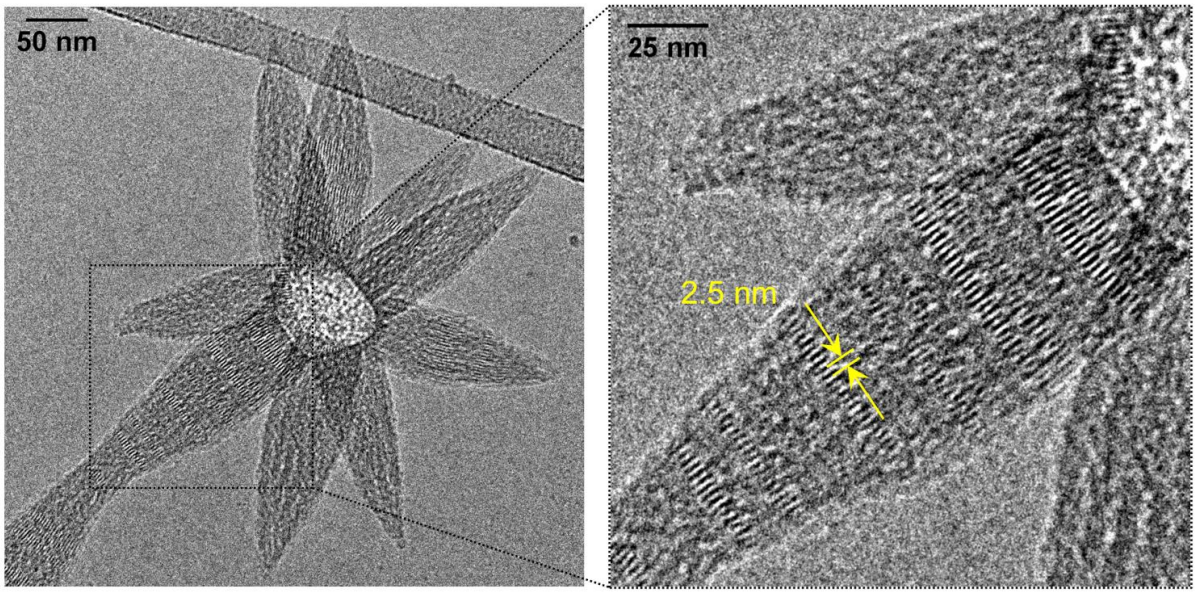


Figure S23. Representative DNA width measurement of self-assembled **ON1*ON5**.

6. AFM Images

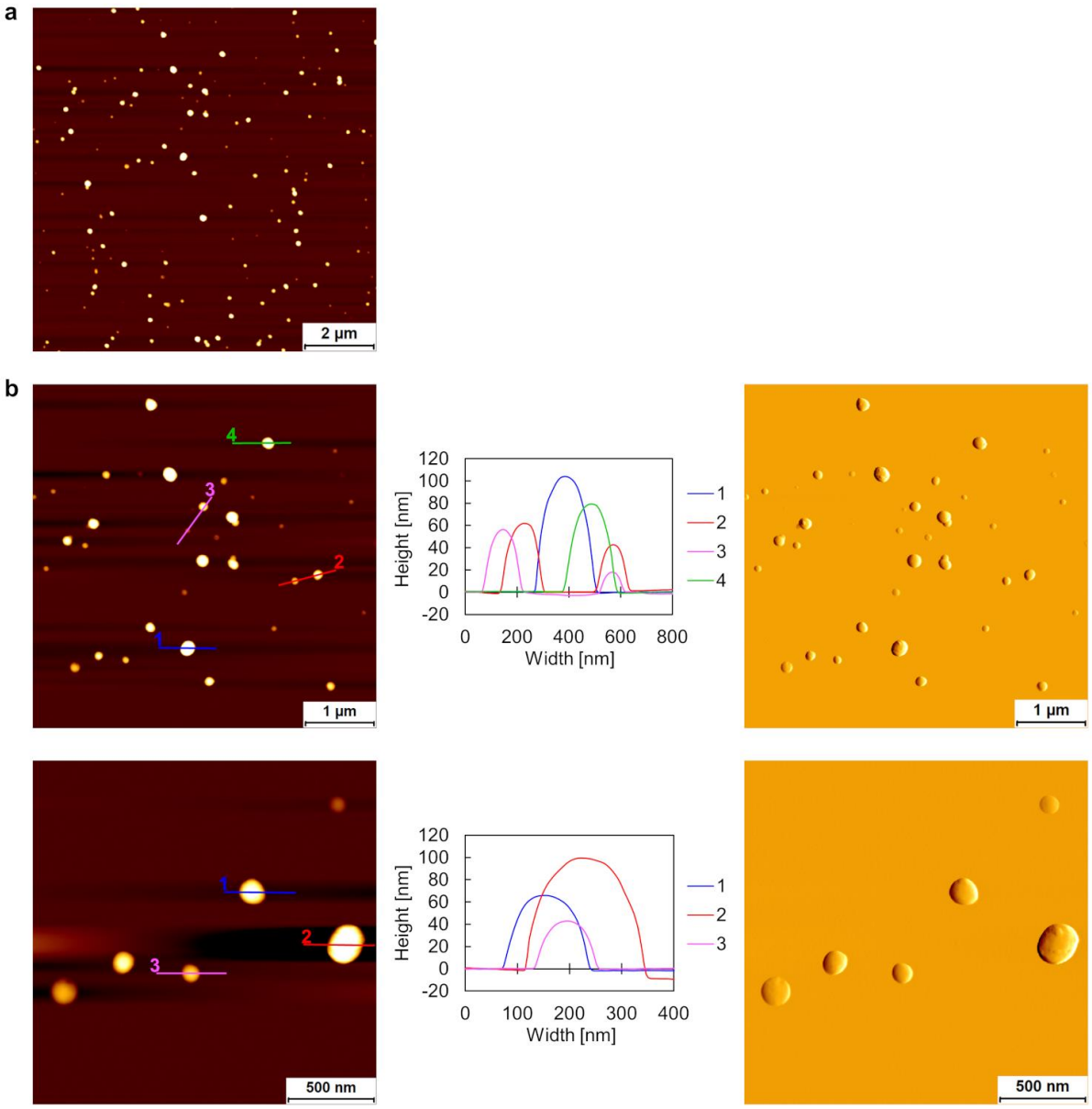


Figure S24. (a) AFM overview scan. (b) AFM zoom images incl. height profiles (left) and deflection scans (right). Conditions: 1 µM **ON1*ON2**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

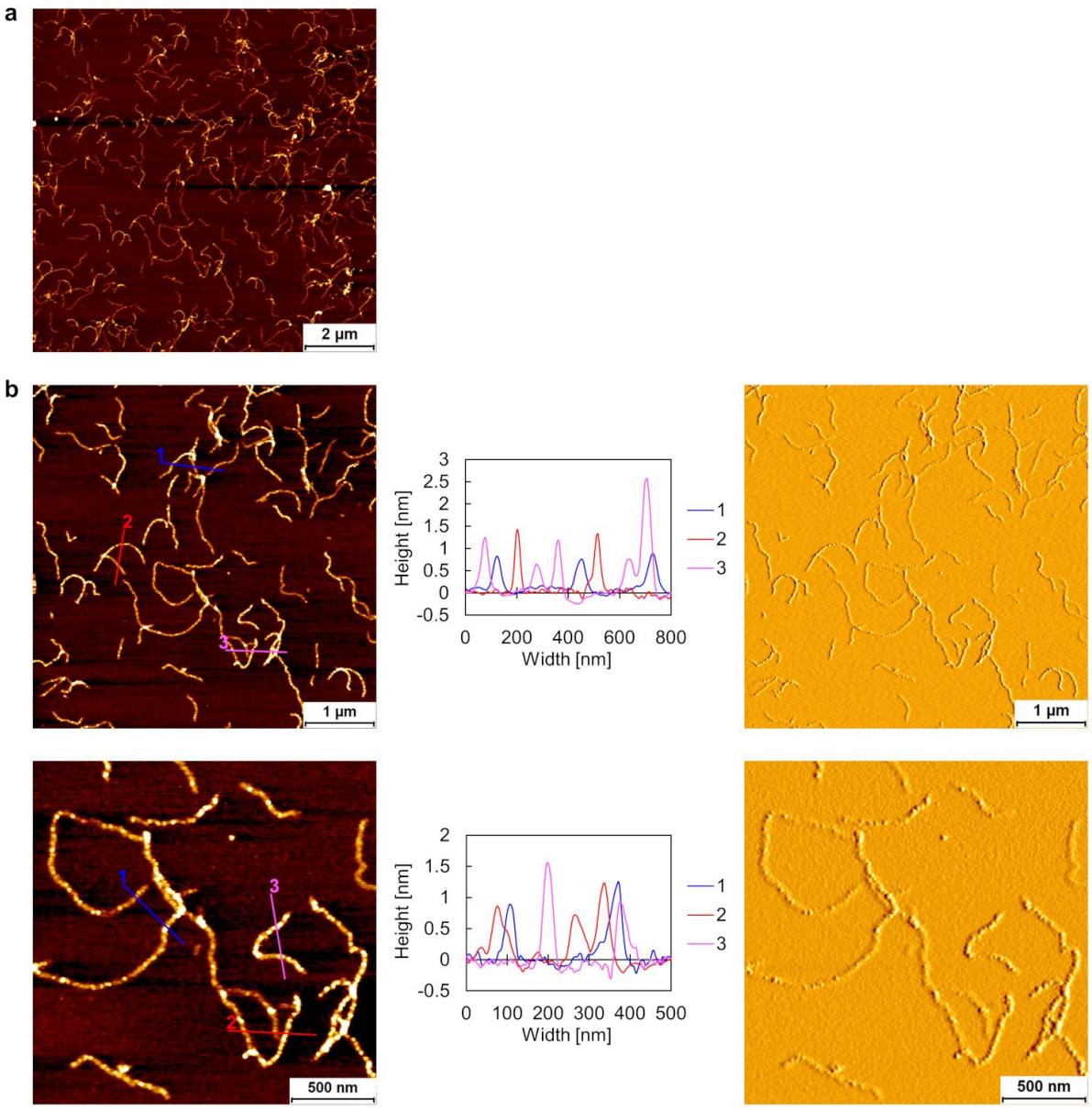


Figure S25. (a) AFM overview scan. (b) AFM zoom images incl. height profiles (left) and deflection scans (right). Conditions: 1 µM **ON1*ON3**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

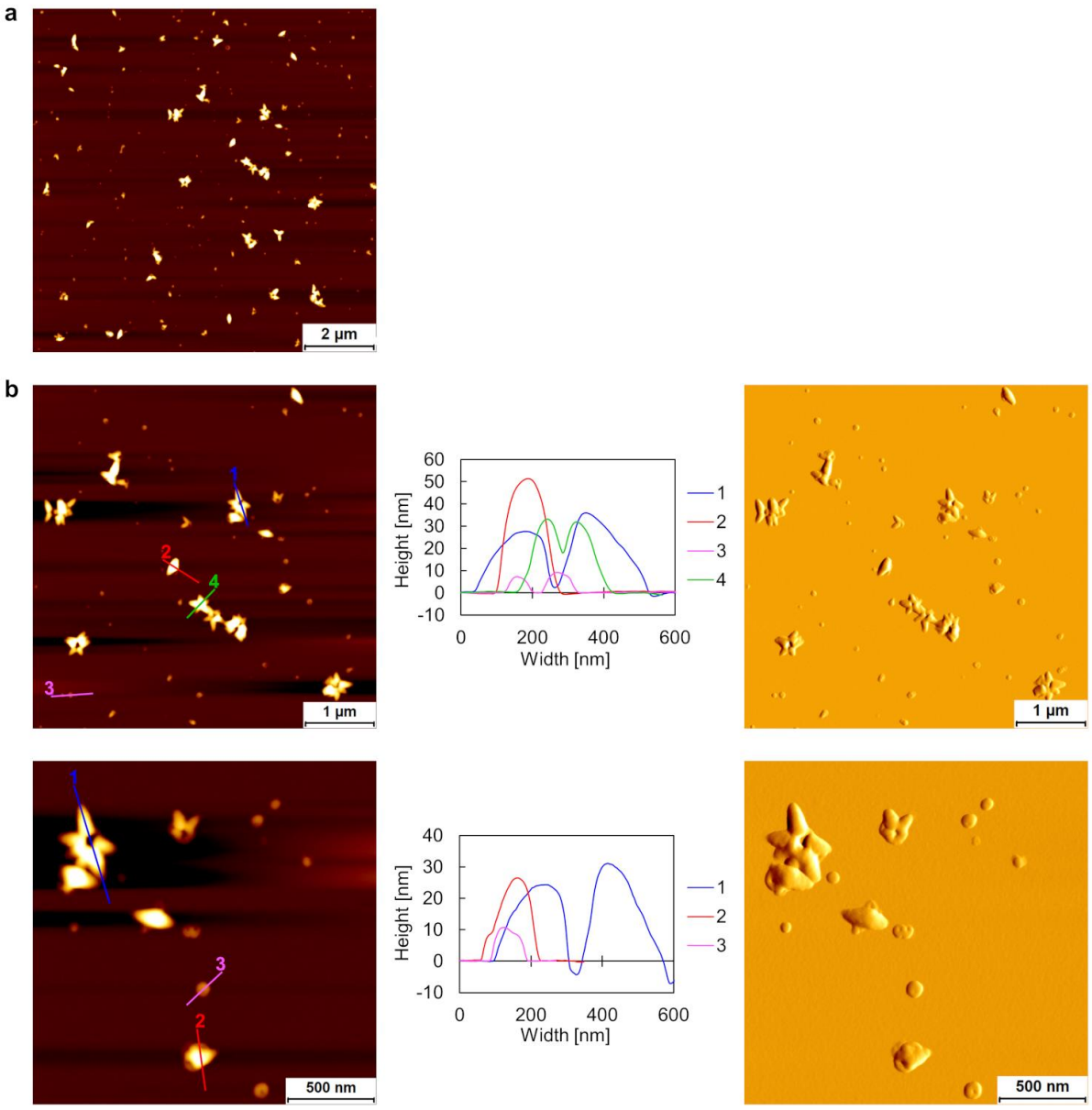


Figure S26. (a) AFM overview scan. (b) AFM zoom images incl. height profiles (left) and deflection scans (right). Conditions: 1 µM **ON1*ON5**, 10 mM sodium phosphate buffer pH 7.2, 0.1 mM spermine · 4 HCl, 30 vol% ethanol.

7. References

(1) Rothenbühler, S.; Iacovache, I.; Langenegger, S. M.; Zuber, B.; Häner, R. Supramolecular Assembly of DNA-Constructed Vesicles. *Nanoscale* **2020**, *12* (41), 21118–21123.

(2) Presolski, S. I.; Hong, V. P.; Finn, M. G. Copper-Catalyzed Azide-Alkyne Click Chemistry for Bioconjugation. *Curr. Protoc. Chem. Biol.* **2011**, *3*, 153–162.