

Supporting Information

Triblock Peptide-Oligonucleotide Chimeras (POCs): Programmable Biomolecules for the Assembly of Morphologically Tunable and Responsive Hybrid Materials

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General Methods and Instrumentation:

All chemicals were purchased from either Aldrich or Fisher and used without further purification. N₃-C₄H₈CO-AAAYSSGAPPMPF (N₃-A₂PEP_{Au}, Figure S2) was purchased from ThermoFisher Scientific. Gold nanoparticles were purchased from Ted Pella (#15702-20 and #15704-20 for 5 and 15 nm particles, respectively). Peptide oligonucleotide chimeras (POCs) were purified using an Agilent 1200 Series reverse-phase high-pressure liquid chromatography (HPLC) instrument equipped with an Agilent Zorbax 300SB-C₁₈ column. POCs were quantified based on their absorbance at 260 nm and using the total extinction coefficient of DNA (195,100 M⁻¹cm⁻¹ and 62,800 M⁻¹cm⁻¹ for PO₁₈C and PO₆C, respectively). Spectra were collected using an Agilent 8453 UV-Vis spectrometer equipped with deuterium and tungsten lamps. Transmission electron microscopy (TEM) samples were prepared by drop-casting 4 μL of solution onto a 3-mm-diameter copper grid coated with formvar. After 4 min., the excess solution was wicked away and the grid was washed with nanopure H₂O (4 μL) and wicked away immediately. TEM images were collected with a FEI Morgagni 268 (80 kV) equipped with an AMT side mount CCD camera system. AFM samples were prepared by drop-casting 6 μL of solution onto freshly cleaved mica

or mica functionalized with 3-aminopropyltriethoxysilane (APTES) and air dried. The samples were washed with 30 μL nanopure H_2O and wicked away (repeated once). The samples were allowed to air dry overnight. AFM images were collected with an Asylum MFP-3D atomic force microscopy using tapping-mode. Images were obtained using ultra-sharp AFM tips (NanoandMore, SHR-150), with a 0.8 Hz scanning rate and 512 pixel resolution. Scanning electron microscopy (SEM) samples were prepared by drop-casting 5 μL of solution onto silicon wafers and allowed to air dry. The samples were then washed with 5 μL of nanopure H_2O (wicked immediately) and then allowed to air dry overnight. SEM images were collected using a ZEISS Sigma 500 VP SEM. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) data were collected using an Applied Biosystem Voyager System 6174 MALDI-TOF mass spectrometer (negative reflector mode; accelerating voltage: 20kV) with 3-hydroxypicolinic acid (3-HPA) as the ionization matrix. Nanopure water (NP H_2O , 18.2 $\text{M}\Omega$) was obtained from a Barnstead DiamondTM water purification system. All TEM measurements were made using ImageJ software.

Preparation of Azido-Modified Oligonucleotide Conjugate ($\text{O}_{18}\text{-N}_3$ and $\text{O}_6\text{-N}_3$).

In a typical procedure, syntheses were carried out from the 3' direction using controlled pore glass (CPG) beads possessing 1 μmol of adenine (Glen Research, dA-CPG #20-2001-10, (1000 \AA , 38 $\mu\text{mol/g}$)). The CPG beads were placed in a 1 μmol synthesis column and Ultramild 3'-phosphoramidites (Glen Research, Pac-dA-CE phosphoramidite #10-1601-05, Ac-dC-CE phosphoramidite #10-1015-C5, iPr-Pac-dG-CE phosphoramidite #10-1621-05, dT-CE phosphoramidite #10-1030-C5) and 5'-Iodo-dT phosphoramidite (Glen Research, #10-1931-90) were then added using the standard 1 μmol protocol on an Expedite 8909 synthesizer. Note, a mild Cap A Mix (Glen Research, 5% Phenoxyacetic anhydride in THF, #40-4212-52) was also used for synthesis due to the lability of the Iodo moiety. At the end of the synthesis, the beads were dried overnight and kept in a tightly capped vial at ambient conditions.

The terminal Iodo groups were substituted for azides using an established procedure.¹ The CPG beads were kept in the columns while a saturated mixture of sodium azide in anhydrous dimethylformamide (DMF) was prepared (approximately 30 mg per 1 mL, per 1 μ mol). Upon pulling up 1 mL of the mixture in a syringe, the column was firmly attached with an empty syringe on one end and the one containing the mixture in the other. The mixture was slowly passed over the CPG beads several times before either being left at ambient overnight or placed in a shaker at 60 °C for 1 h. The beads were then washed thoroughly with DMF and acetone before drying with nitrogen. The solid-phase coupling reactions with the organic core were performed using these dry CPG beads.

Attachment of Azido-Modified Oligonucleotides to Diacetylene Biphenyl Organic Core

Dry CPG beads containing azide-modified DNA were placed in an Eppendorf tube. The biphenyl core (200 mM in DMF, 200 equivalents based on the azide-DNA strands on CPG beads, assuming a 100% yield in the oligonucleotide synthesis), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 100 mM in DMF, 100 equivalents based on the azide-DNA strands on CPGs), CuSO₄·5H₂O (100 mM in DMF, 100 equivalents based on the azide-DNA strands on CPGs), and L-ascorbic acid (100 mM in DMF, 100 equivalents based on the azide-DNA strands on CPGs) were also added. The reaction mixture was then blanketed with nitrogen before capping and shook for 7 to 18 h at 25 °C in an Eppendorf® Thermomixer® R (Eppendorf, #022670107) at 1000 rpm. It is important that the CPG beads are constantly agitated while mixing and not sitting at the bottom of the tube.

Once the reaction was complete, the CPG beads were filtered using a one-side fritted 1 μ mol Expedite DNA synthesis column (Glen Research, #20-0021-01), then the beads were washed with DMF (5×1 mL) and acetone (5×1 mL) and dried with nitrogen. The beads were then placed in 1 mL of AMA (CAUTION: Only fresh AMA solutions that are not more than two weeks old and have been kept in the refrigerator below 0 °C should be used) at 65 °C for 15 min to cleave the conjugates from the solid supports. Afterwards, the ammonia and methyl amine were removed by passing a stream of nitrogen

over the solution. To the remaining material was added ultrapure deionized H₂O (affording roughly 1 mL at the end), and the resulting solution was filtered through 0.45 μm nylon syringe filter (Acrodisc® 13 mm syringe filter #PN 4426T). The filtered solution was purified using reverse-phase HPLC eluting with a linear gradient of CH₃CN and 0.1 M TEAA (5/95 to 45/55 over 30 min.).

Attachment of Azido-Modified Peptide onto the Biphenyl Organic Core.

The POCs were prepared using copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)²⁻³ in which N₃-A₂PEP_{Au} (Figure S2) was reacted with the azido-modified 18mer and 6mer conjugates (Scheme S1). The following stock solutions were prepared: **A**, 198.3 mM CuSO₄ in NP H₂O; **B**, 37.3 mM THPTA in NP H₂O; **C**, 2 M urea in NP H₂O; and **D**, 60.6 mM sodium ascorbate in NP H₂O. Lyophilized N₃-A₂PEP_{Au} (125 nmol) was dissolved in 70 μL of DMF and 50 μL of NP H₂O and the solution was transferred to a vial containing O_n-N₃ (100 nmol, Scheme S1). To this vial was added a mixture of **A** and **B** (1.05 μL **A** mixed with 5.58 μL **B**), 1.5 μL of **C**, and 13.8 μL of **D**. The vial was sealed with parafilm wrap, wrapped in aluminum foil, and stirred for at least 4 h at room temperature. DMF/NP H₂O (1:1) was added to bring the total volume to 500 μL. The resulting solution was desalted using a NAP-5 desalting column (GE Healthcare Life Sciences, #17-0853-02). The eluted solution was purified using reverse-phase HPLC eluting with a linear gradient of CH₃CN and 0.1 M TEAA (5/95 to 45/55 over 30 min).

POC Assembly Experiments.

In a 250 μL plastic vial, lyophilized POCs (20 nmol) were dissolved in 40 μL CaCl₂ solutions. The solutions were sonicated for 2 min. and centrifuged briefly. The vials were placed in a 1.5 mL centrifuge tube containing water that was pre-heated at 80°C and placed in an Eppendorf® Thermomixer® R (Eppendorf, # 022670107). The POC solutions were allowed to incubate for 15 min. at 80°C. After incubation, the temperature setting was lowered 1°C every 5 minutes until the temperature reached 25°C. At 70°C, the samples were centrifuged very briefly to maintain POC concentration of the solution. After cooling to

25°C, the POC solutions were removed from the 1.5 mL centrifuge tube and allowed to sit overnight at room temperature. TEM samples were prepared after 15 h.

Preparation of Complementary DNA-Functionalized Gold Nanoparticles.

5 and 15 nm gold nanoparticles were functionalized using reported methods.⁴ Lyophilized complementary oligonucleotides functionalized with a thiol hexyl linker at the 5' end (purchased from IDT, 10 OD) were dissolved in 200 μ L of freshly prepared dithiothreitol (DTT) and phosphate buffer (PB) solution (100 mM DTT in 170 mM PB) to cleave any disulfide bonds. The solution was allowed to sit for 1 hr. The thiol-terminated oligonucleotides were separated from the DTT using a NAP-5 column. The purified oligonucleotides (in 1 mL NP H₂O) were then added to 10 mL of particles. The particle solution was allowed to sit overnight. The next morning, the concentrations of PB and sodium dodecyl sulfate (SDS) were brought to 0.01 M and 0.01%, respectively. The oligonucleotide/gold nanoparticle solution was allowed to incubate at room temperature for 30 min. The concentration of NaCl was increased slowly to 0.5 M (in 6 increments) using 2 M NaCl. After each addition of NaCl, the solution was sonicated for 10 sec. and incubated for 30 min. before the next addition. After the salting procedure, excess oligonucleotides were removed via centrifugation (1 hr.; 16,100 x g for 15 nm particles and 10900 x g for 5 nm particles), and subsequent supernatant removal. The remaining pellets were combined and the washing process was repeated twice. The final oligonucleotide/functionalized nanoparticle pellet was suspended in 100 μ L of NP H₂O. The gold nanoparticle concentration was determined via UV-Vis spectroscopy using extinction coefficients of 2.4×10^8 L/(mol·cm)⁴ and 9.696×10^6 L/(mol·cm) (from Ted Pella) for 15 and 5 nm particles, respectively. The concentrations of these stock solutions of nanoparticles were 4.09×10^{14} and 2.17×10^{13} particles in 100 μ L NP H₂O for the 5 nm and 15 nm stock solutions, respectively.

Functionalized Gold Nanoparticle Addition to PO₁₈C Vesicles.

To a solution of PO₁₈C vesicles (30 μL) assembled in 50 mM CaCl₂ was added 1 μL of a 25x diluted solution of the 15 nm stock solution prepared above. The mixture was mixed and vortexed briefly and allowed to sit at room temperature. TEM samples were prepared after 1 h.

Functionalized Gold Nanoparticle Addition to PO₆C Fibres.

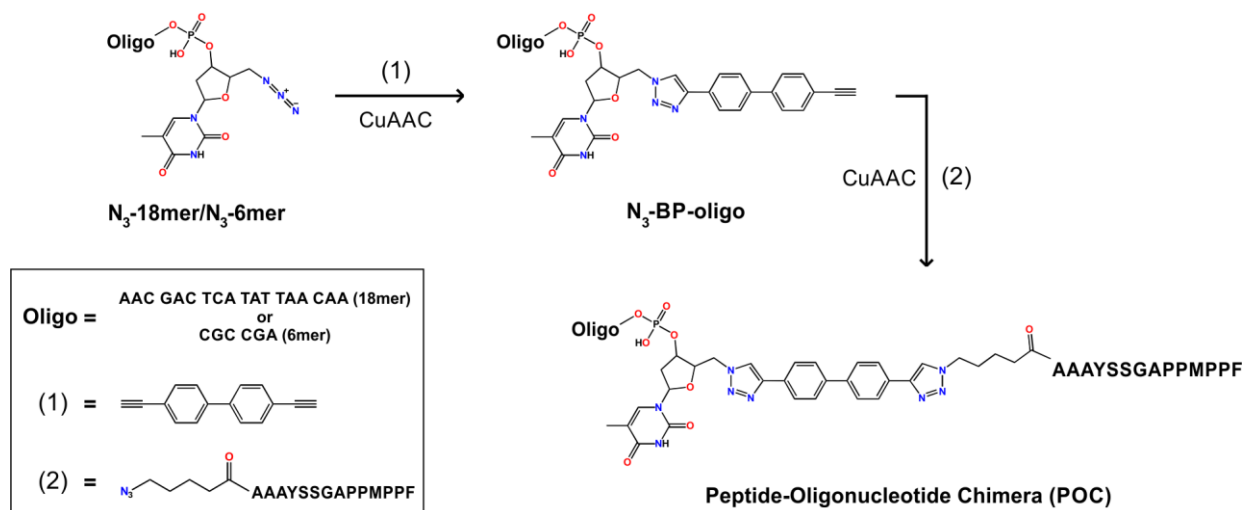
To a solution of PO₆C fibres (30 μL) assembled in 50 mM CaCl₂ was added 1 μL of a 10x diluted solution of the 5 nm stock solution prepared above. The mixture was mixed and vortexed briefly and allowed to sit at room temperature. TEM samples were prepared after 1 h.

Calculation of the Charge Ratio Value.

$$\text{Charge Ratio} = \frac{[\text{total positive charge}]}{[\text{total negative charge}]} = \frac{[\text{Ca}^{+2}] \times 2}{[\text{POC}] \times (\text{N}+2)}$$

The charge ratio value is the ratio of positive to negative charges of the assembly solution (N=number of oligonucleotide bases; the '+2' results from the additional azido-functionalized T residue (used to link the 18mer to the biphenyl core; Fig. S1), and the deprotonated COO⁻ terminus of the peptide.

Supplementary Data (Scheme S1, and Figures S1-S21)



Scheme S1. POC synthesis scheme.

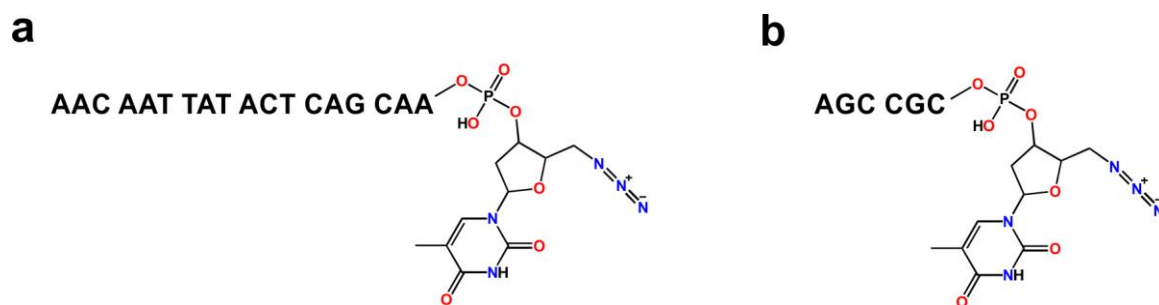


Figure S1. Structure of the (a) 18-base azido-modified oligonucleotide (O₁₈-N₃) and (b) 6-base azido-modified oligonucleotide (O₆-N₃).

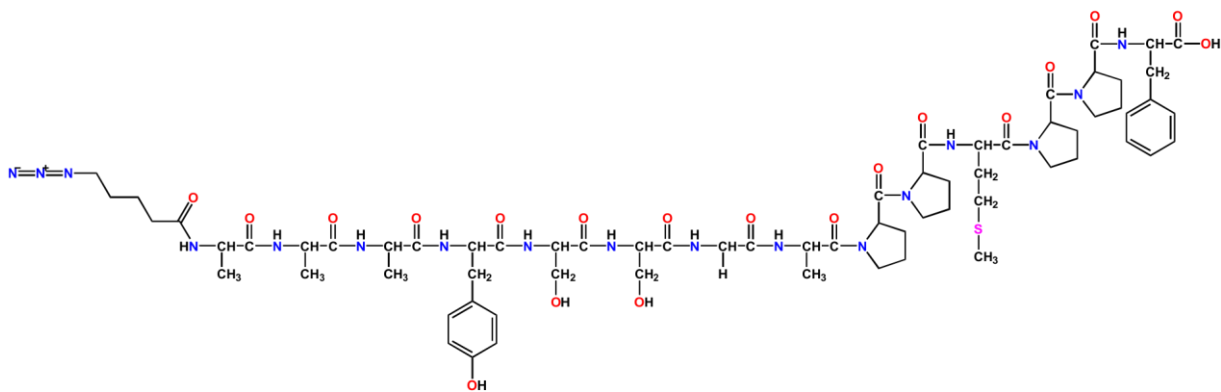


Figure S2. Chemical structure of the azido-modified peptide (N_3 - C_4H_8CO -AAAYSSGAPPMPPF).

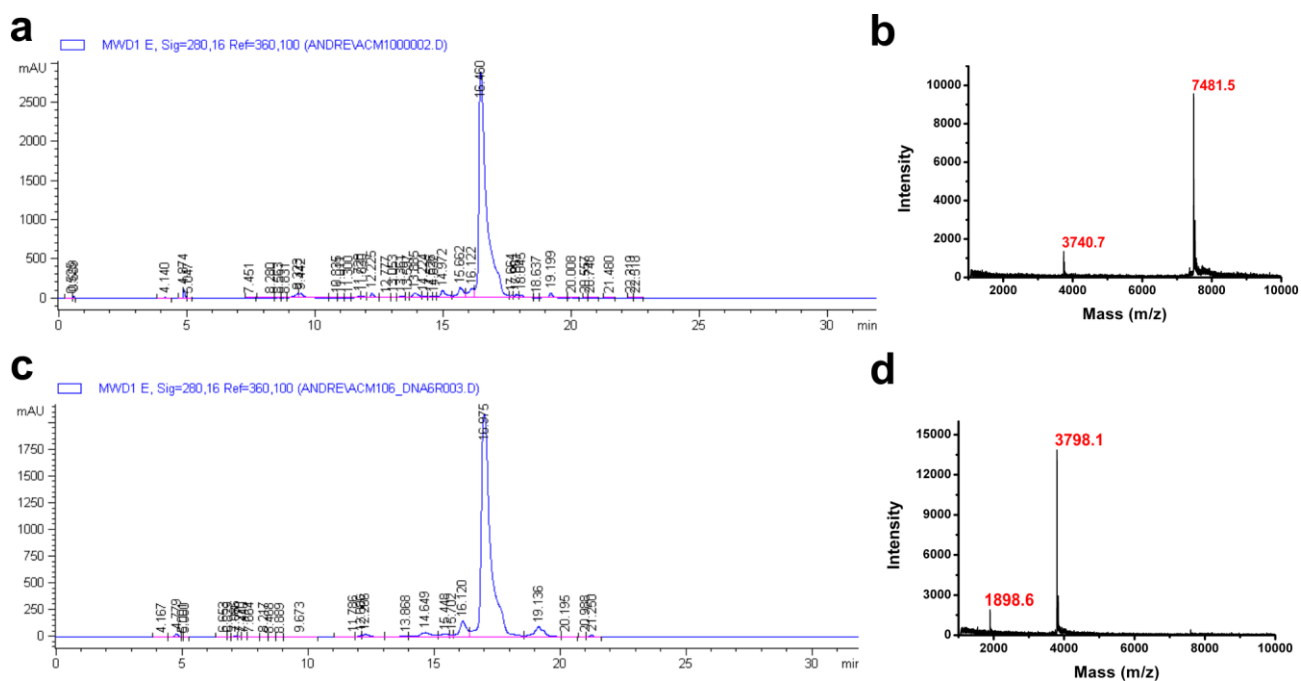


Figure S3. (a) Reverse-phase HPLC trace and (b) MALDI spectrum of PO₁₈C. (c) Reverse-phase HPLC trace and (d) MALDI spectrum of PO₆C. Note: m/1 and m/2 peaks were observed in the MALDI spectra.

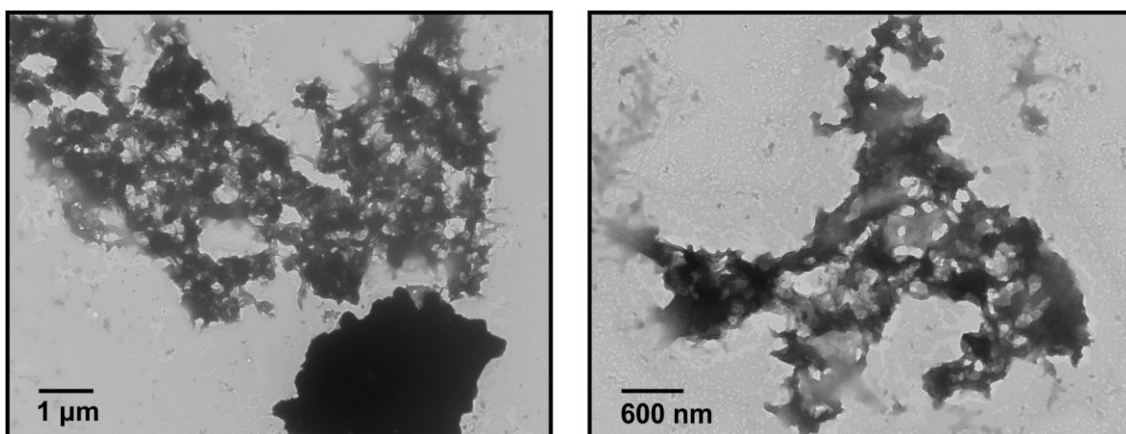


Figure S4. TEM images of 500 μM PO₁₈C in 50 mM CaCl₂ after 15 h at room temperature.

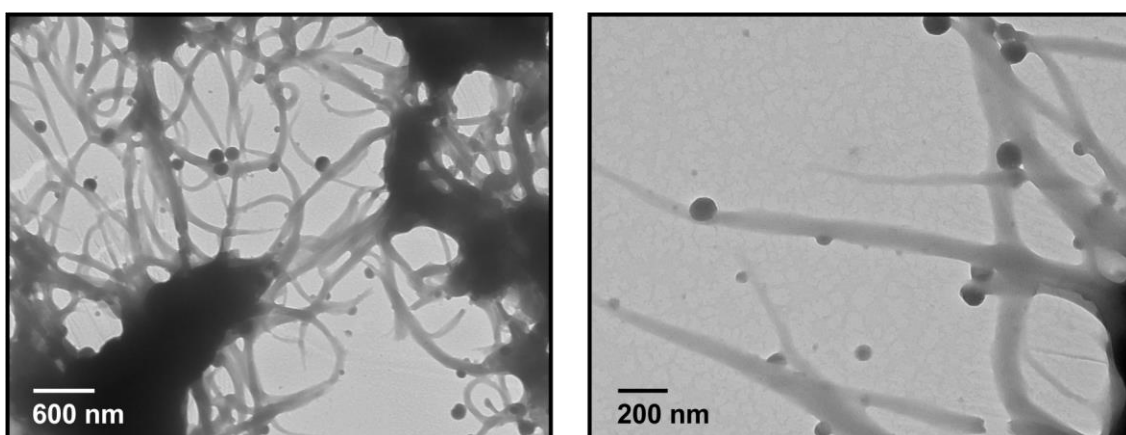


Figure S5. TEM images of 500 μM PO₁₈C in 50 mM CaCl₂ after slow-cooling from 60°C to room temperature and left overnight for 15 h.

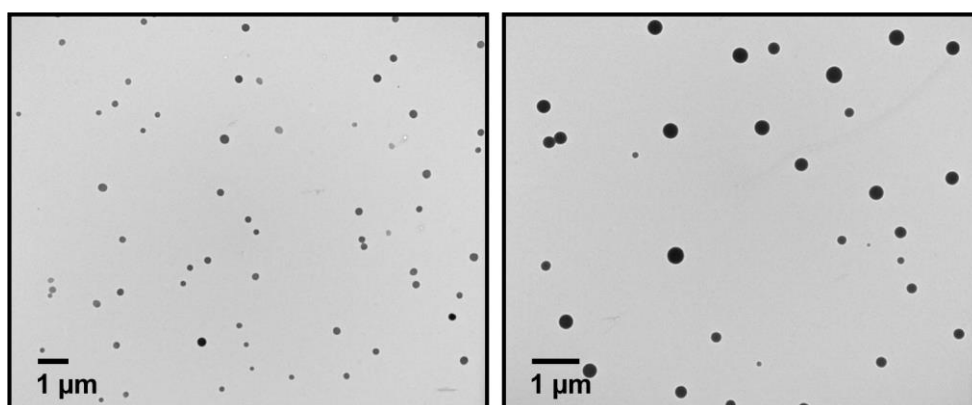


Figure S6. TEM images of 500 μM PO₁₈C in 50 mM CaCl₂ after slow-cooling from 80°C to room temperature and left overnight for 15 h.

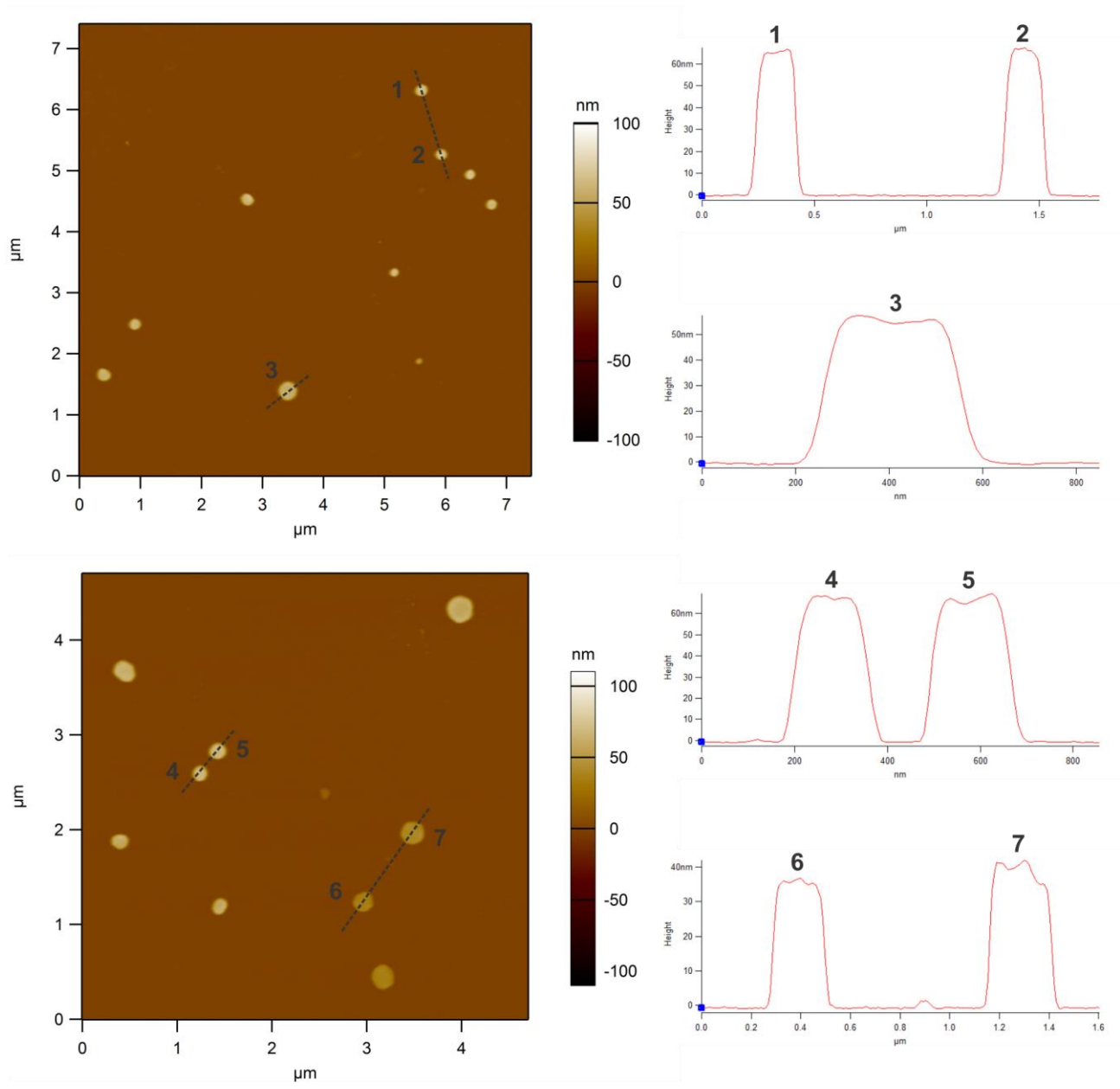


Figure S7. AFM images of PO₁₈C vesicles deposited on APTES-functionalized mica. The labeled vesicles and their corresponding height traces are shown. In general, larger vesicles appear to flatten more than smaller vesicles.

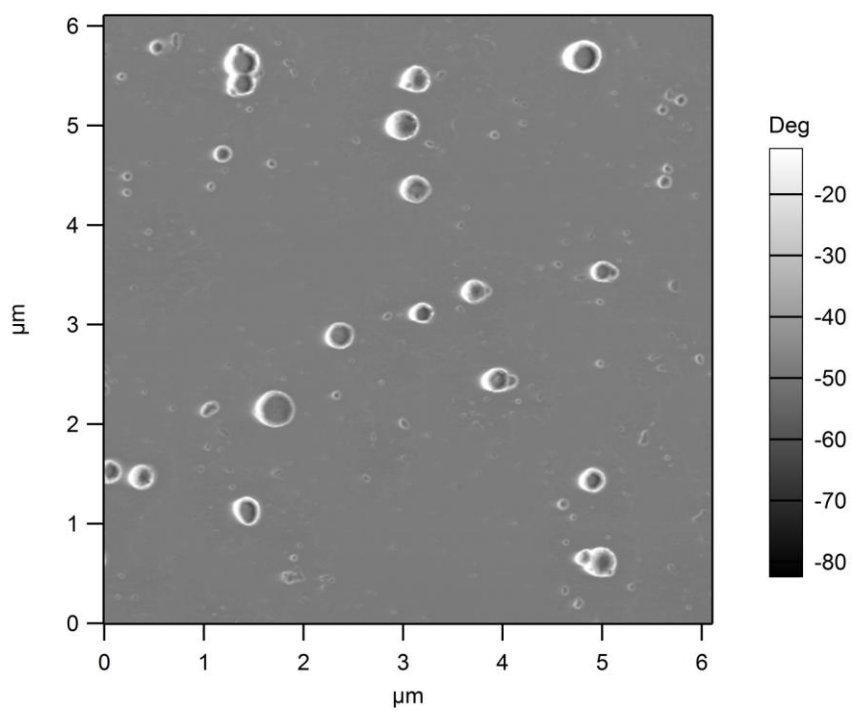


Figure S8. Phase image of PO₁₈C vesicles reveal the different deformation response between the sphere edge and sphere centre.

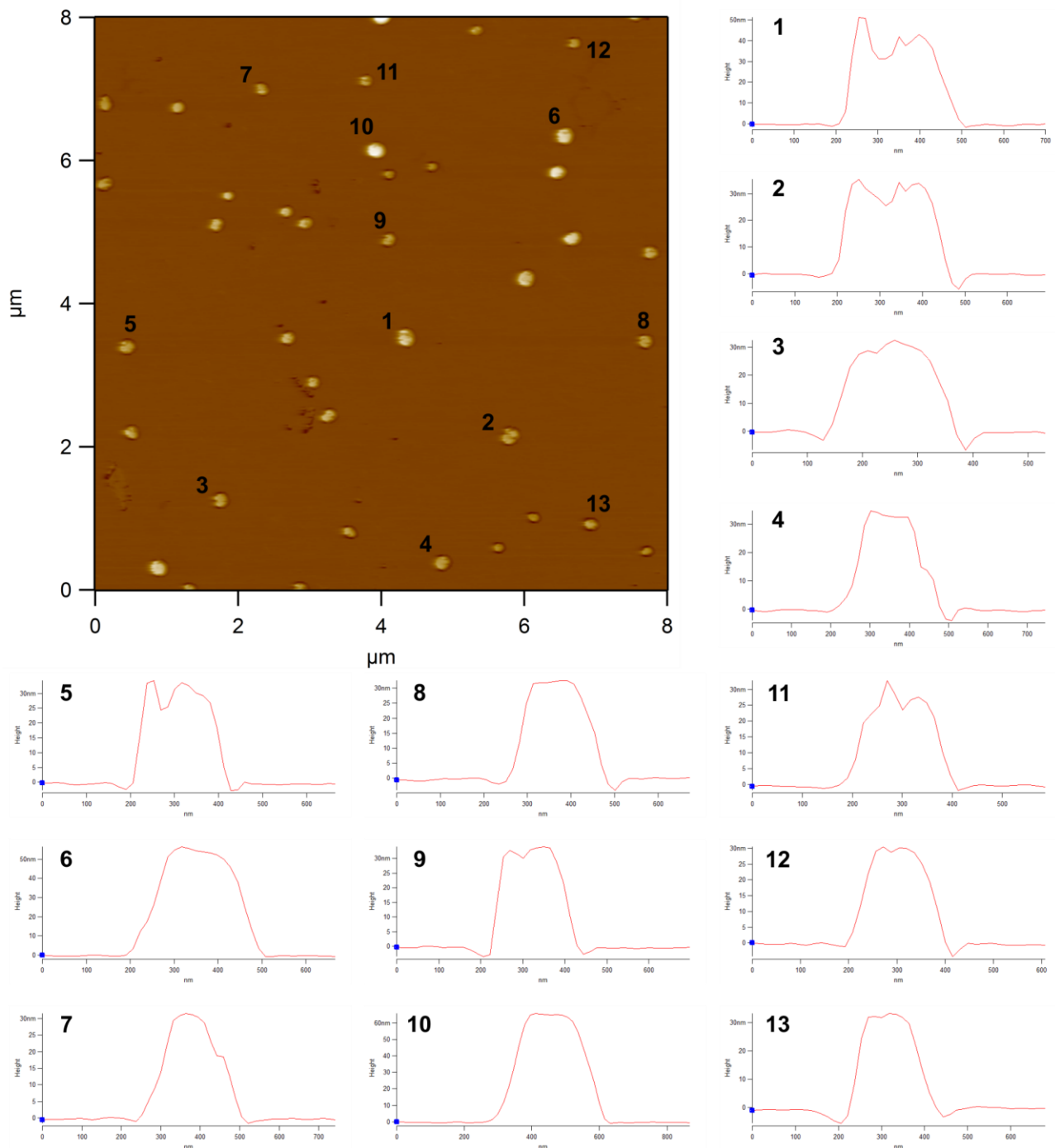
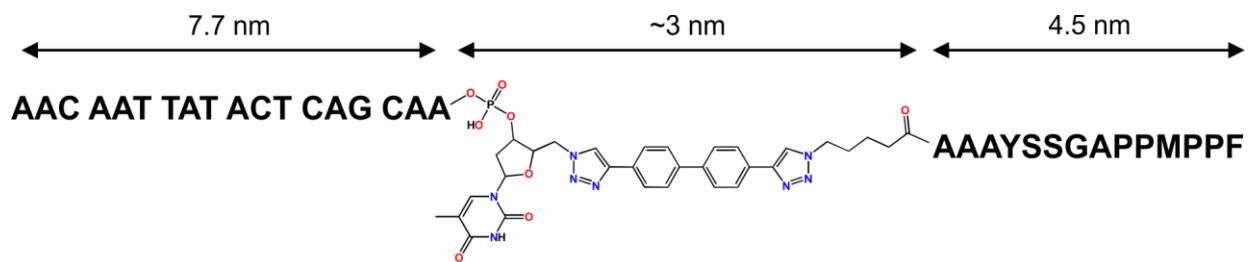


Figure S9. AFM image of PO₁₈C vesicles on a TEM grid after exposure to the high-vacuum TEM environment. Labeled vesicles and their corresponding height traces reveal a height of approximately 30 nm. A majority of the vesicles appear to flatten completely, except for a few that retained more of its shape (*e.g.* spheres 6 and 10).



Extended PO₁₈C length = ~15 nm

Figure S10. PO₁₈C length. The length of the extended 18mer oligonucleotide was reported to be 7.7 nm.⁵⁻⁶

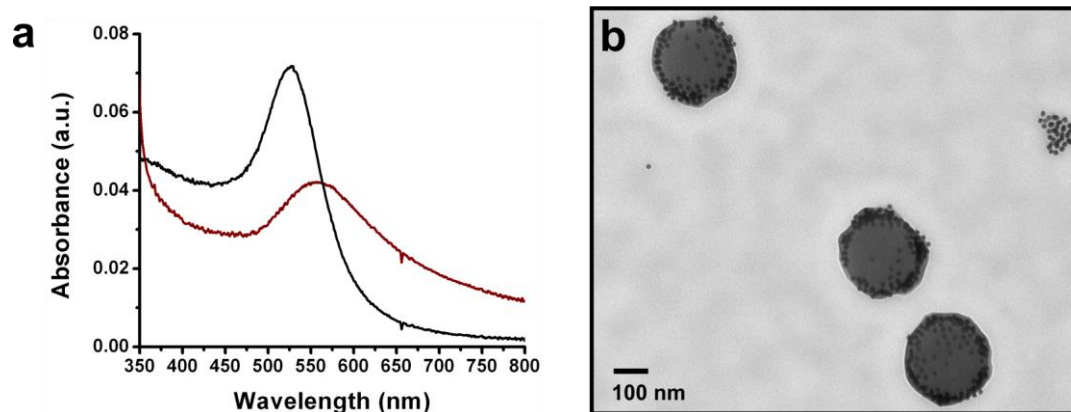


Figure S11. (a) UV-vis spectrum of free 15 nm gold nanoparticles functionalized with complementary O₁₈ sequence (black line) and gold nanoparticle-decorated vesicles after addition of the complementary functionalized gold nanoparticles to a solution containing PO₁₈C vesicles (red line). (b) Corresponding TEM image of the gold nanoparticle-decorated PO₁₈C vesicles after 2 h.

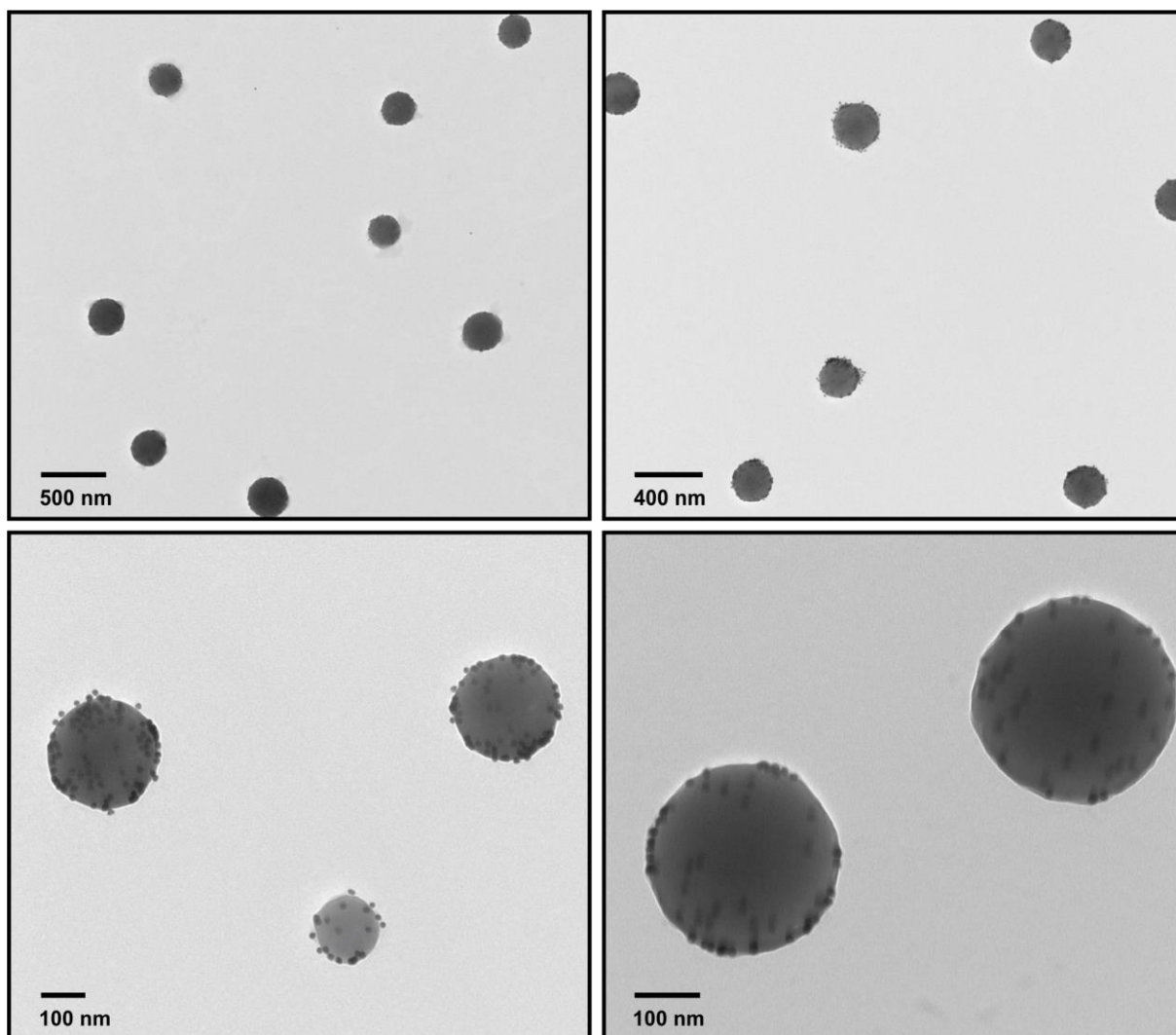


Figure S12. Additional TEM images of gold nanoparticle-decorated PO₁₈C vesicles after 2 h of exposure to complementary oligonucleotide-functionalized gold nanoparticles.

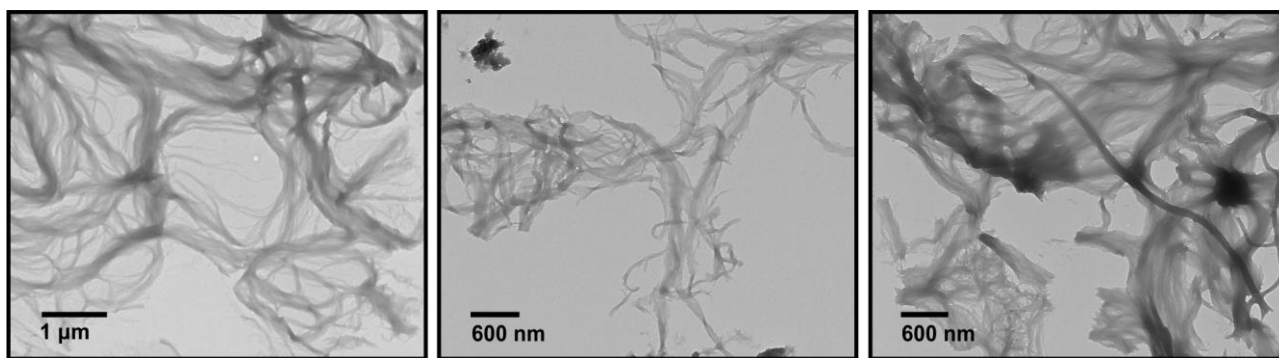


Figure S13. TEM images of 500 μM PO₁₈C in 300 mM CaCl₂ after 15 h. Fibres were observed.

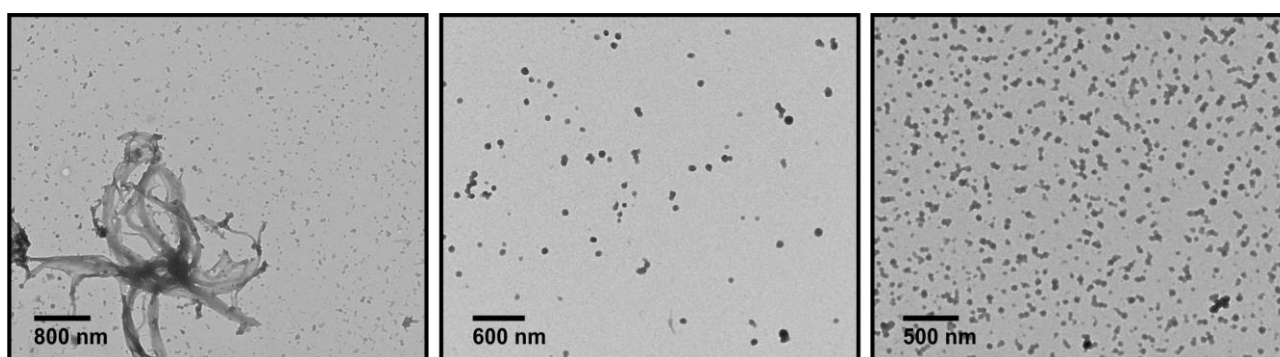


Figure S14. TEM images of 500 μM PO₁₈C in 150 mM CaCl₂ after 15 h. Spherical/pseudo-spherical assemblies were the major products. Few fibre assemblies were also observed.



Figure S15. TEM images of 500 μM PO₁₈C in 10 mM CaCl₂ after 15 h. Few aggregates were observed.

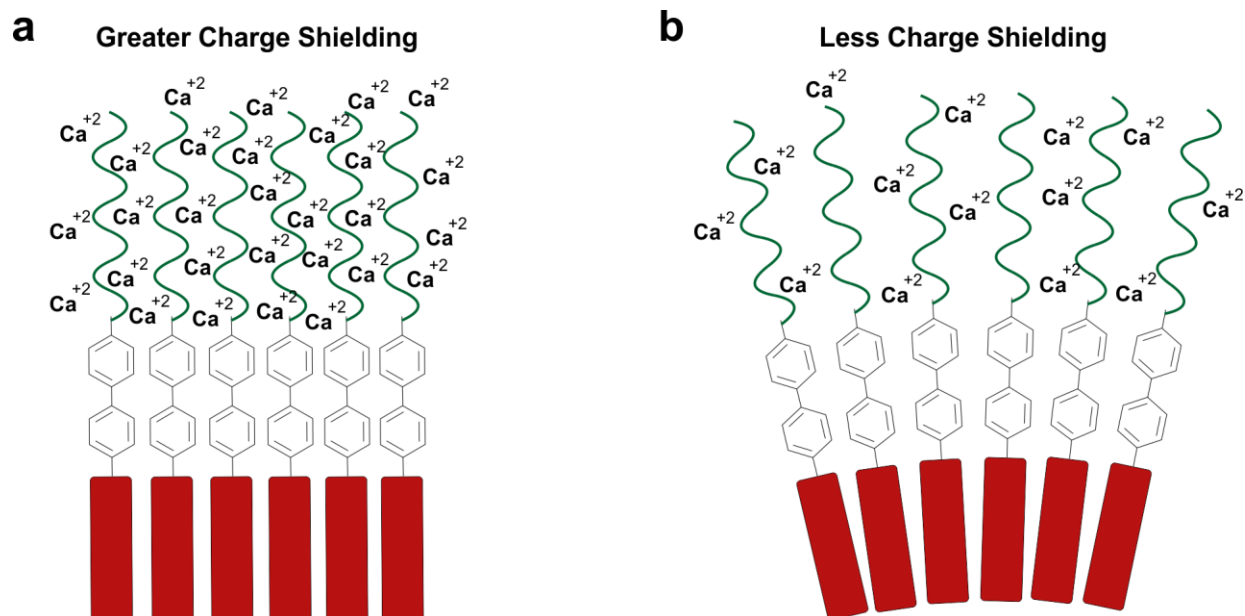


Figure S16. (a) Greater charge shielding can allow for tighter packing of POCs, which can lead to fibre formation. (b) Greater repulsion due to less charge shielding favors the formation of vesicles.

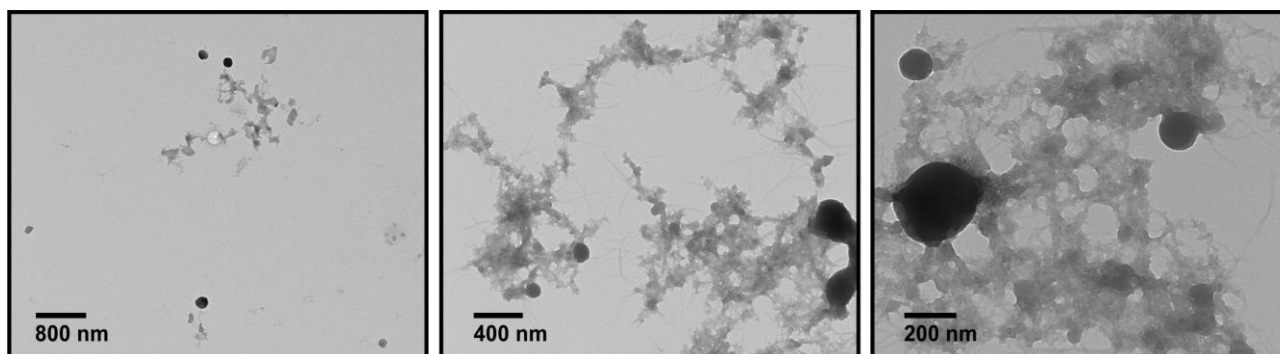


Figure S17. TEM image of 500 μM PO_6C in 10 mM CaCl_2 after 15 h. Fibres, aggregates, and spherical assemblies were observed.

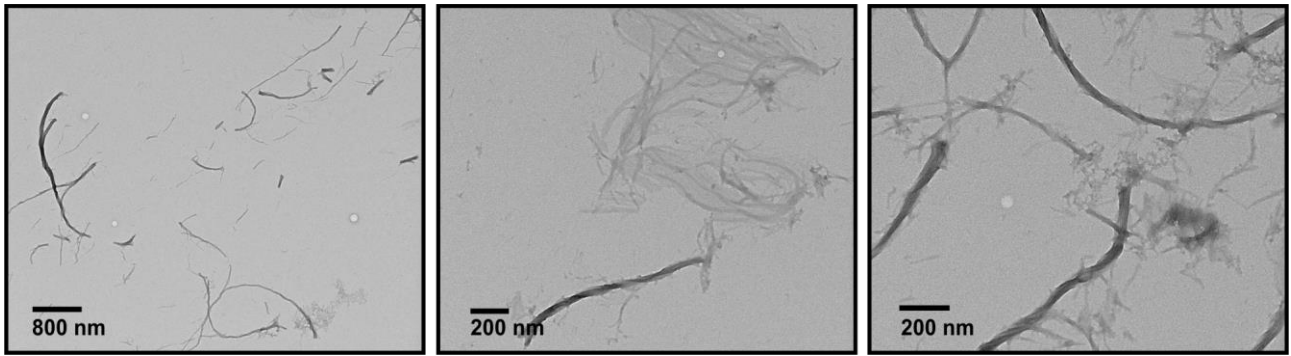


Figure S18. TEM images of 500 μM PO₆C in 50 mM CaCl₂ after 15 h. Fibres were observed.

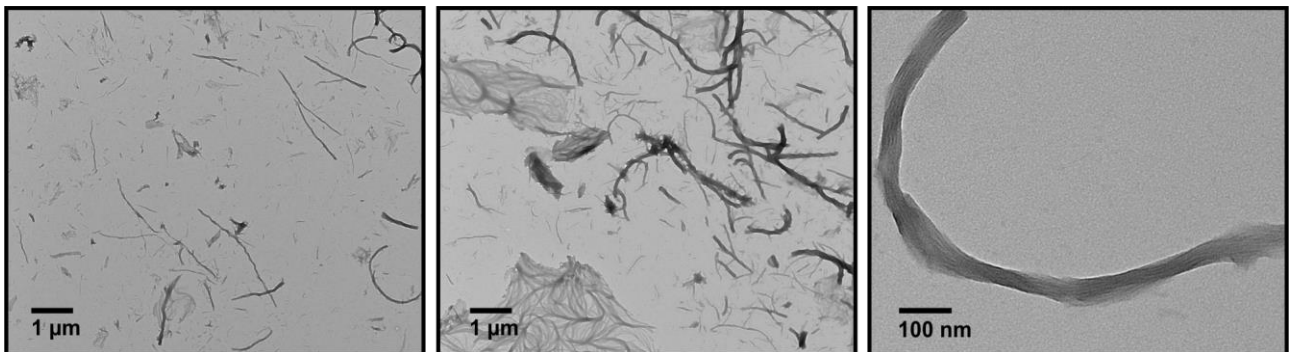


Figure S19. TEM images of 500 μM PO₆C in 150 mM CaCl₂ after 15 h. Fibres and fibre networks were observed.

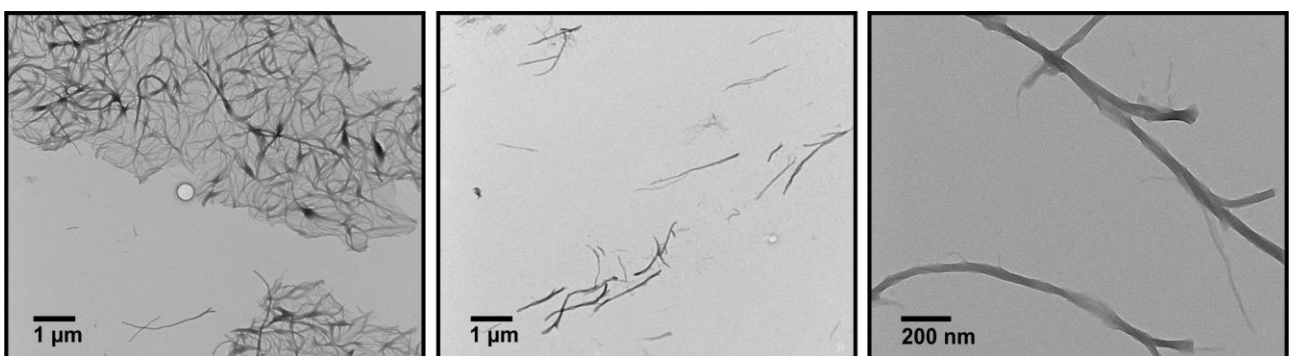


Figure S20. TEM images of 500 μM PO₆C in 300 mM CaCl₂ after 15 h. Fibres and fibre networks were observed.

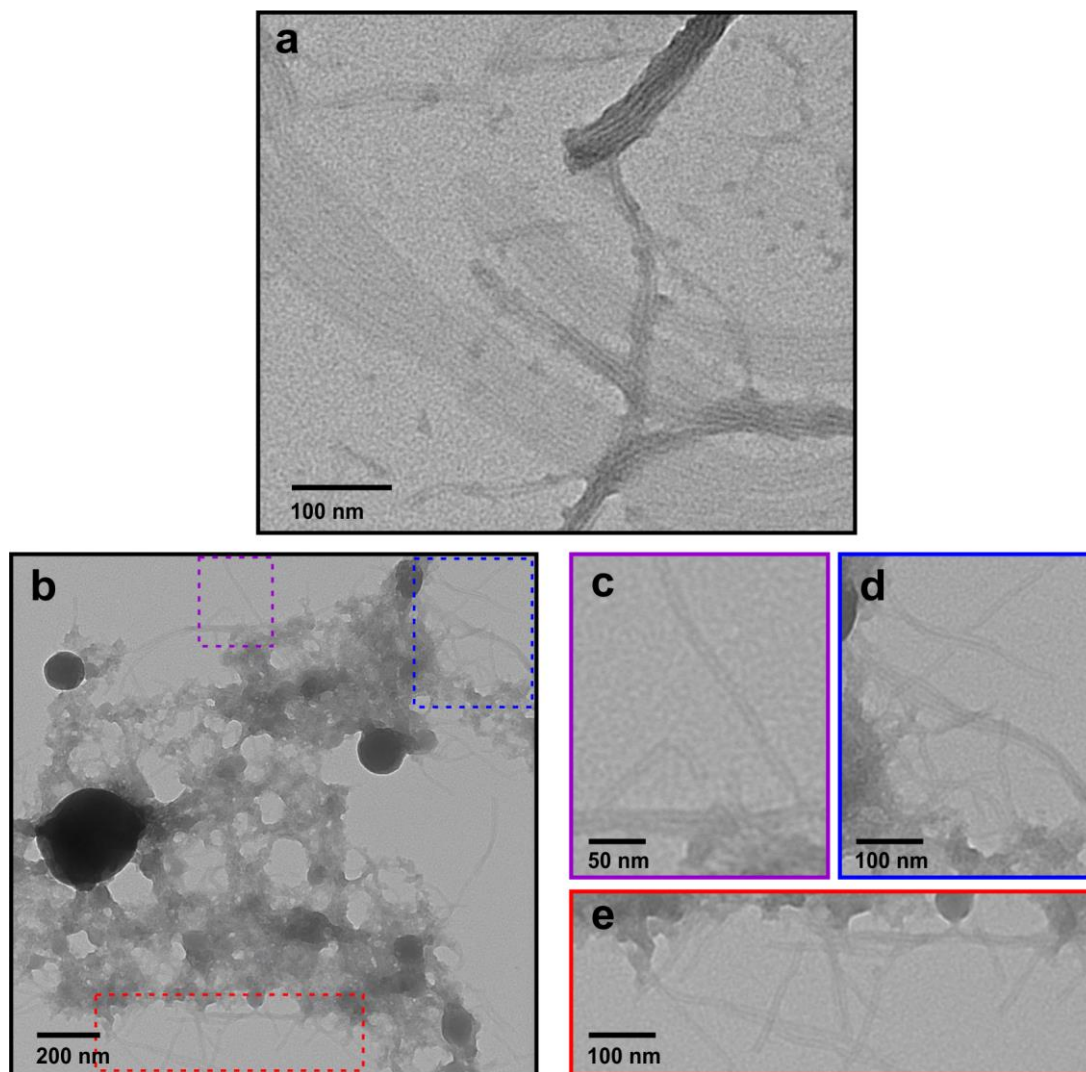


Figure S21. PO₆C assemblies assembled in (a) 50 mM CaCl₂ and (b) 10 mM CaCl₂. (c-e) Zoomed-in TEM images of the dashed boxes shown in b (the border colors correspond to the colors of the dashed boxes).

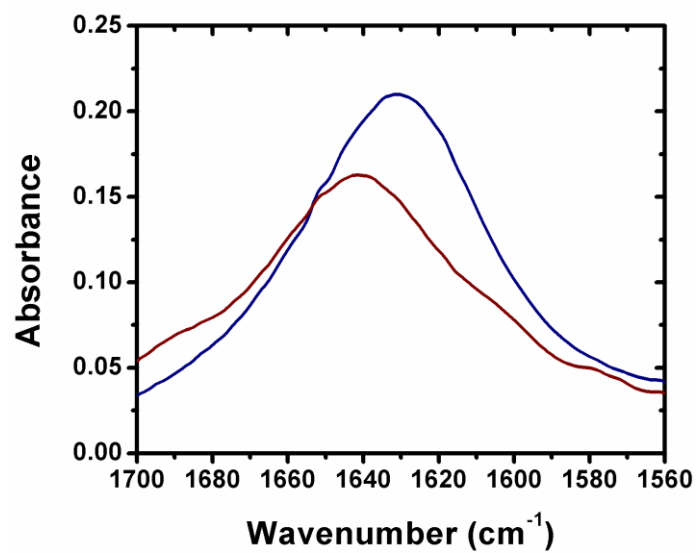


Figure S22. FTIR spectra showing the amide I bands of PO₁₈C vesicles (red line) and PO₆C fibres (blue line) assembled in 50 mM CaCl₂.

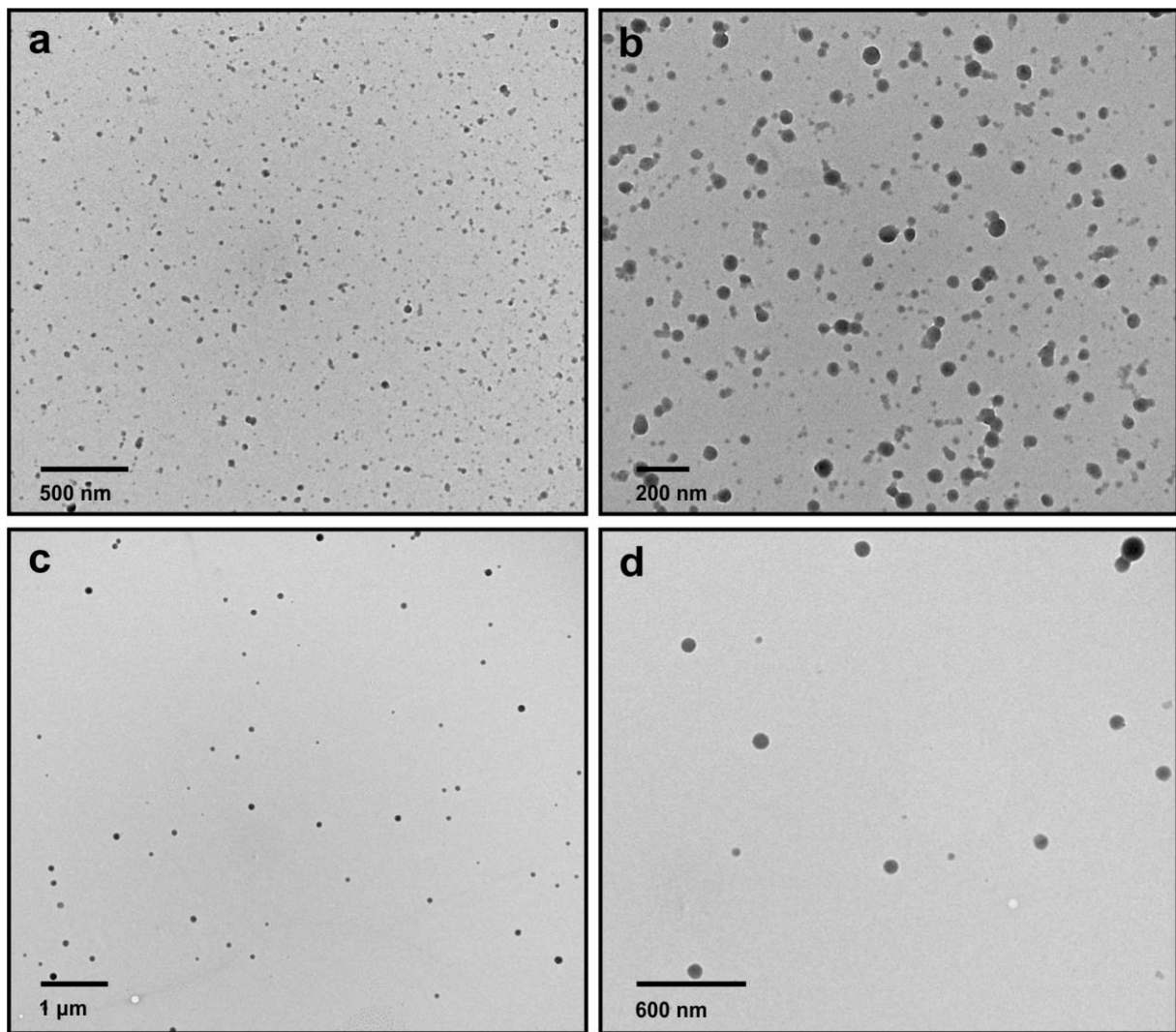


Figure S23. PO₁₈C (500 μM) assembled in the (a,b) absence of complement and in the (c,d) presence of complement. Both experiments were conducted in 150 mM CaCl₂.

References

1. Miller, G. P.; Kool, E. T., Versatile 5'-Functionalization of Oligonucleotides on Solid Support: Amines, Azides, Thiols, and Thioethers via Phosphorus Chemistry. *The Journal of Organic Chemistry* **2004**, *69* (7), 2404-2410.
2. Huisgen, R., 1.3-DIPOLARE CYCLOADDITIONEN - RUCKSCHAU UND AUSBLICK. *Angewandte Chemie-International Edition* **1963**, *75* (13), 604-637.
3. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B., A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.* **2002**, *41* (14), 2596-2599.
4. Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A., Maximizing DNA Loading on a Range of Gold Nanoparticle Sizes. *Analytical Chemistry* **2006**, *78* (24), 8313-8318.
5. Wang, L.; Feng, Y.; Sun, Y.; Li, Z.; Yang, Z.; He, Y.-M.; Fan, Q.-H.; Liu, D., Amphiphilic DNA-dendron hybrid: a new building block for functional assemblies. *Soft Matter* **2011**, *7* (16), 7187-7190.
6. Tinland, B.; Pluen, A.; Sturm, J.; Weill, G., Persistence Length of Single-Stranded DNA. *Macromolecules* **1997**, *30* (19), 5763-5765.