Supramolecular Self-assembly of Pyrene-DNA Conjugates into Vesicles

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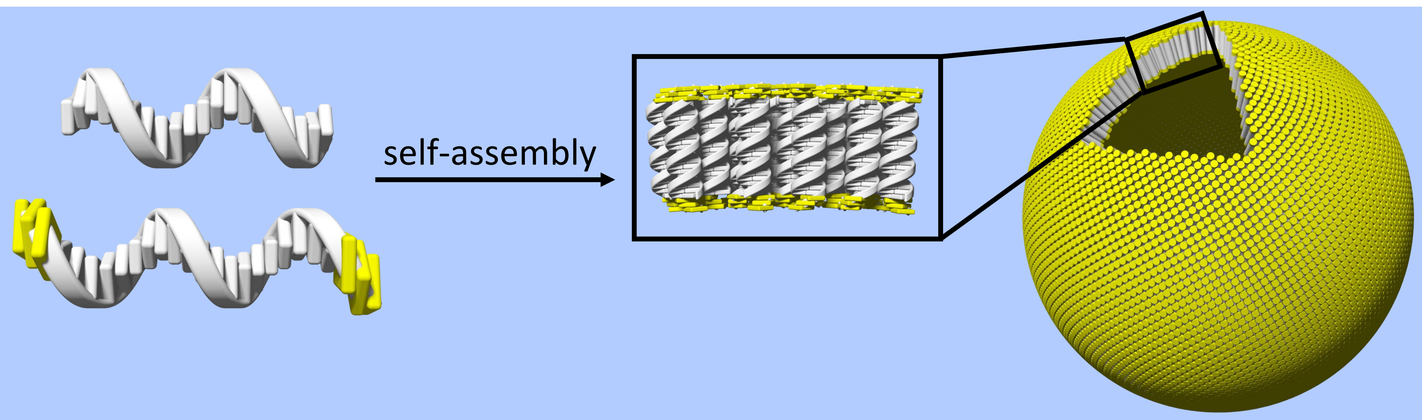
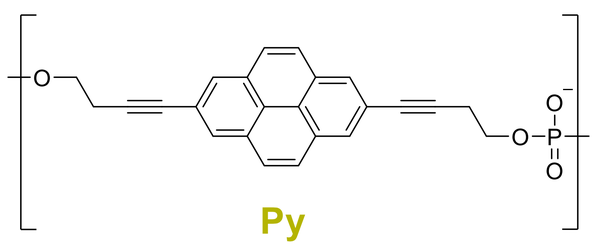
Abstract: This poster describes the supramolecular self-assembly of DNA conjugates functionalized with pyrene sticky-ends. After the hybridization of DNA single strands, the pyrene-modified DNA duplexes aggregate into vesicles. A minimum of 2 pyrene units on each side of the duplex are necessary for vesicle formation.



Synthesis

3'- and 5'- modified DNA strands containing 20 nucleobases modified with 1, 2, and 3 units of pyrene on each side were synthesized *via* solid-phase DNA synthesis using phosphoramidite chemistry. Afterward, the oligomers were purified by reverse-phase HPLC.[1] The chemical structure of the used 2,7-modified pyrene is illustrated in Scheme 1. DNA strands **1-4** are listed in Table 1.



Scheme 1 Structure of 2,7-modified pyrene.

	Sequence from 5' to 3'
1	(Py)$_1$ CTT CCT TGC ATC GGA CCT TG(Py)$_1$
2	(Py)$_2$ CTT CCT TGC ATC GGA CCT TG(Py)$_2$
3	(Py)$_3$ CTT CCT TGC ATC GGA CCT TG(Py)$_3$
4	CAA GGT CCG ATG CAA GGA AG

Table 1 Sequences of oligomer **1-4**.

Temperature dependent UV-vis Spectroscopy

The self-assembly and disassembly were monitored by UV-vis spectroscopy, measuring the absorbance of the complementary DNA strands at 260 nm. The results are depicted in Figure 1a-c. The absorbance of **1*4** exhibited overlaying cooling and heating curves; no hysteresis was observed. In contrast, **2*4** and **3*4**, with which the formation of vesicles was confirmed by AFM, exhibited hysteresis. The hysteresis arises from the kinetic barrier in the assembly and disassembly process of the nanostructures.

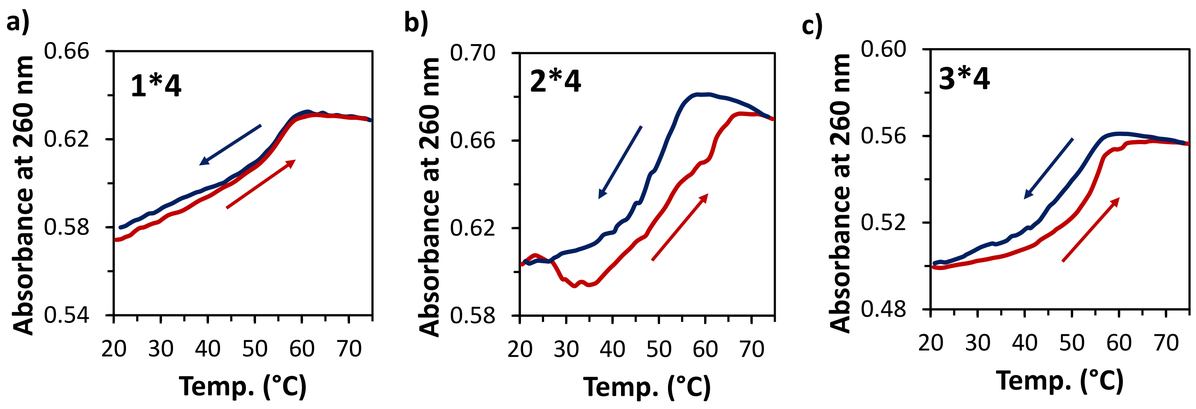


Figure 1a-c UV-vis absorbance at 260 nm of (a) **1*4**, (b) **2*4**, and (c) **3*4** during cooling from 75°C to 20°C dark blue and heating to 75°C dark red. Conditions: 1 µM each single strand, 10mM sodium phosphate buffer pH 7.2, 0.03 mM spermine·4 HCl, EtOH 20 vol%, cooling and heating rate: 0.5°C/min.

Atomic Force Microscopy

Atomic force microscopy (AFM) was used to visualize the nanostructures. After the self-assembly of the DNA conjugates by cooling them from 75°C to 20°C at 0.5°C/min, they were adsorbed to an APTES-modified mica and then measured by AFM. The results of the measurements are depicted in Figure 2. The AFM image of **1*4** the conjugate with only 1 pyrene on each side, revealed no nanostructures. In contrast, AFM images of **2*4** and **3*4**, the conjugates with 2 and 3 pyrenes on each side showed vesicles with a diameter of 50 to 200 nm.

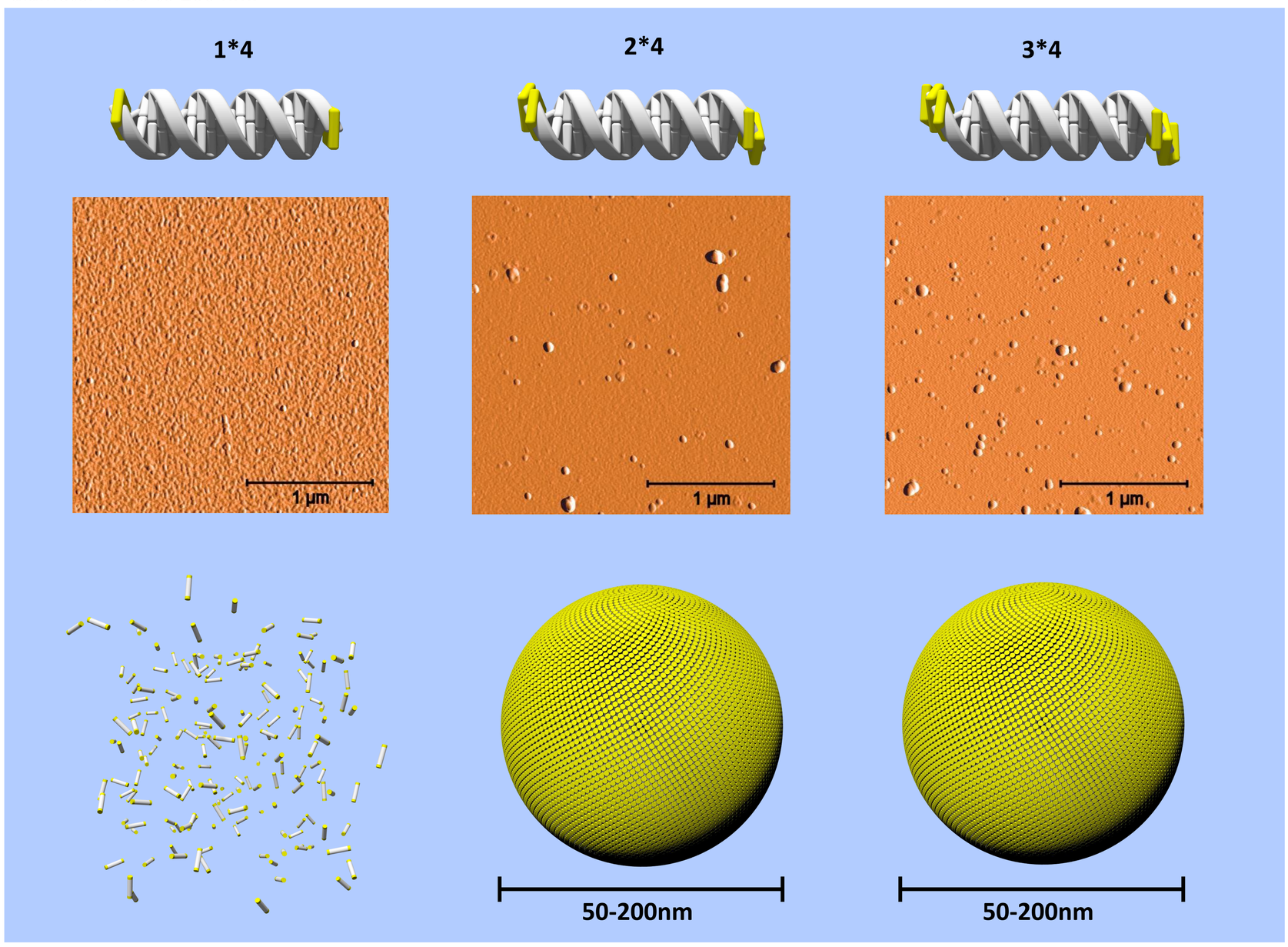


Figure 2 Schematic representation of the duplexes, AFM images (amplitude scans), and schematic representations of the nano-sized structures formed after self-assembly of **1*4**, **2*4**, and **3*4** (left to right).

Fluorescence Spectroscopy

Additionally, the self-assembled conjugates **1*4**, **2*4**, and **3*4** were characterized by fluorescence emission spectroscopy. The normalized intensities of the emission after excitation at 260 nm are depicted in Figure 3. The spectrum of **1*4** displays only monomer emission. In contrast, **2*4** and **3*4** exhibit a broad excimer band between 460 and 620 nm.

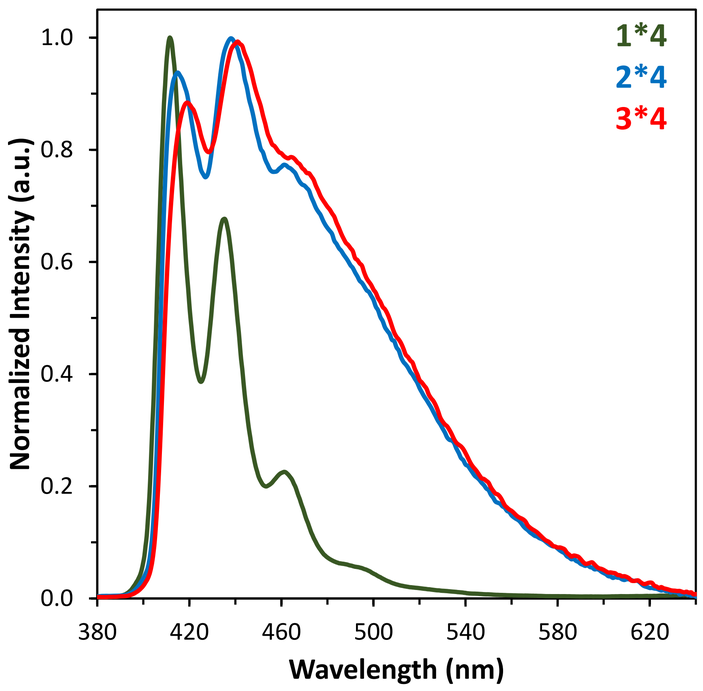


Figure 3 Normalized fluorescence emission of the self-assembled **1*4**, **2*4**, and **3*4** at 20°C. Conditions: excitation at 260 nm, 1 µM each single strand, 10mM sodium phosphate buffer pH 7.2, 0.03 mM spermine·4 HCl, EtOH 20 vol%, cooling rate: 0.5°C/min.

Conclusion: DNA duplexes modified with pyrene at the 3'- and 5'-ends (with 1, 2, and 3 pyrene units on each side) were characterized by UV-vis spectroscopy, fluorescence spectroscopy, and AFM. A minimum of 2 pyrene units on each side are as sticky ends necessary to form nanostructures. Vesicles between 50 and 200 nm in diameter are formed by pyrene-DNA conjugates with 2 and 3 pyrene units on each side.

References [1] C. D. Bösch, S. M. Langenegger, R. Häner, *Angew. Chem. Int. Ed.*, **2016**, 55, 9961-9964.