Pyrene-DNA Conjugates: Influence of sticky Ends on the Supramolecular Self-assembly

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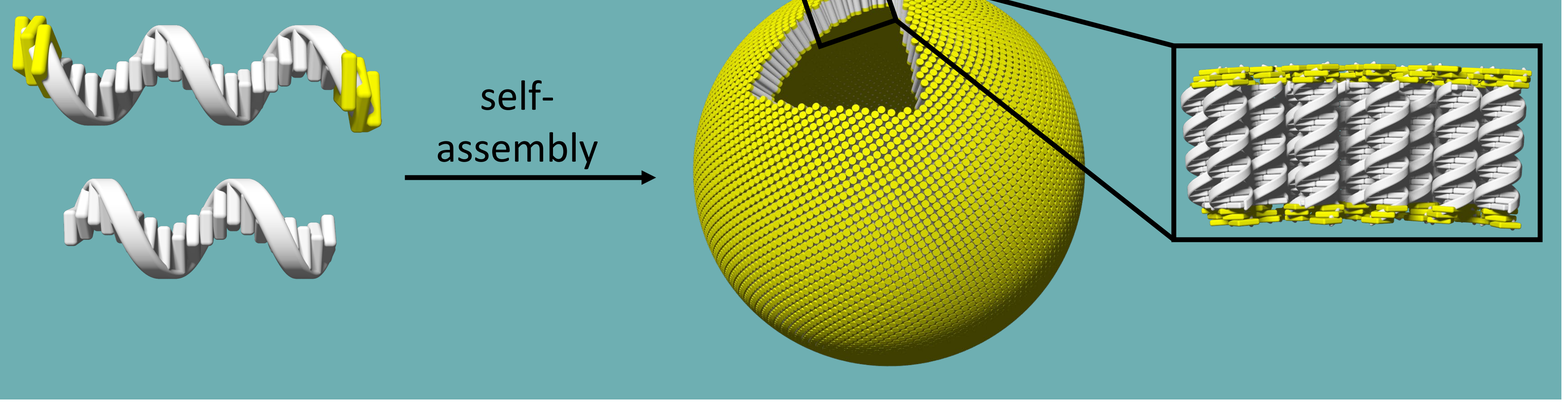
Abstract: This poster describes the supramolecular self-assembly of DNA conjugates functionalized with pyrene sticky ends. After the hybridization of DNA single strands into duplexes, the pyrene modified DNA duplexes aggregate into vesicles. Vesicles are formed with 2 or more pyrene modifications on each side.

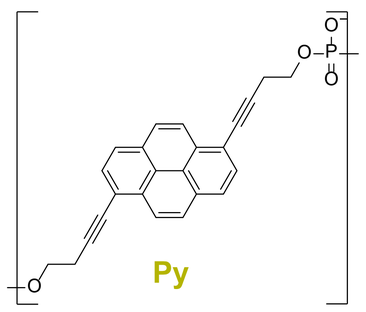


Synthesis

In the work presented on this poster we decorated a 20-mer of DNA on both sides with 1, 2, and 3 units of pyrene (Scheme 1) as sticky ends. We wanted to find out if DNA decorated with pyrene sticky-ends can form nano-sized vesicles. The DNA strands **1-4** discussed on this poster are listed in Table 1. The 3'- and 5'-modifed DNA strands **1-3** were synthesized *via* solid-phase synthesis using phosphoramidite building blocks and purified by revers-phase HPLC according to published procedures.[1] The complementary strand **4** was purchased from a commercial suppliers.

	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**.



Scheme 1 Pyrene modification.

Temperature dependent UV-vis Spectroscopy

The self-assembly and disassembly were observed by UV-vis spectroscopy. Measurements of the absorbance at 260 nm of the complementary single strands are depicted in Figure 1a-c. The absorbance of **1*4** exhibited overlapping 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. This hysteresis arises from the kinetic barrier in the assembly and disassembly process of the nanostructures.

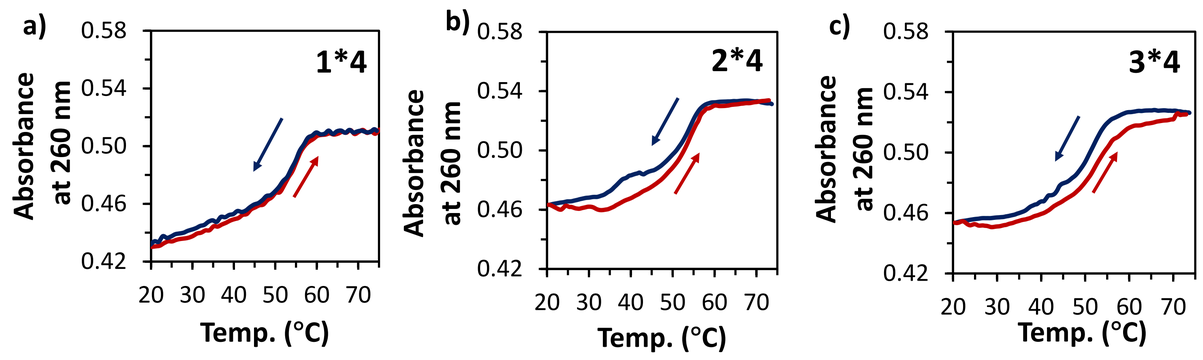


Figure 1a-c UV-vis absorbance at 260 nm of (a) **1*4**, (b) **2*4**, and (c) **3*4** during cooling from 75°C to 20°C dark blue and heating to 75°C dark red. Conditions: 1 µM each single strand, 10mM sodium phosphate buffer pH 7.2, 0.03 mM spermine·4 HCl, EtOH 20 vol%, cooling and heating 0.5°C/min.

Atomic Force Microscopy

The nanostructures were visualized by non-contact atomic force microscopy (AFM). First, the DNA conjugates were self-assembled by cooling them from 75°C to 20°C with a gradient of 0.5°C/min. Afterwards, they were adsorbed to an APTES-modified mica and measured by AFM. The resulting images are depicted in Figure 2. No structure were found on the AFM image of conjugate with 1 pyrene on each side **1*4**. Contrary, vesicles with a diameter of 50 to 150 nm were observed with **2*4** and **3*4,** the conjugates with 2 respectively 3 pyrene units on each.

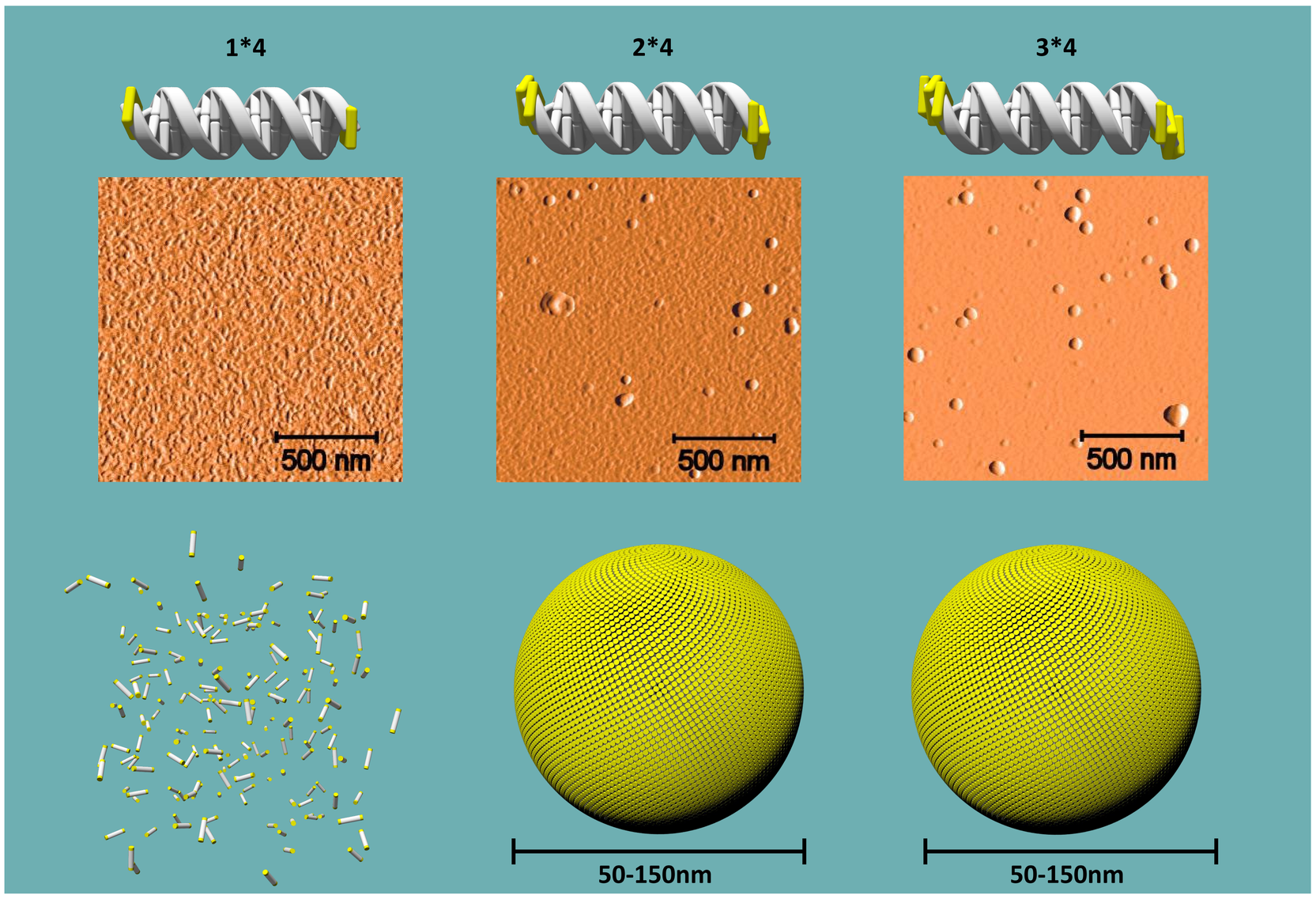


Figure 2 Schematic representation of the duplexes of **1*4**, **2*4**, and **3*4** (left to right), AFM images (amplitude scans), and schematic representations of the nano-sized structures formed after self-assembly.

Fluorescence Spectroscopy

Furthermore, the self-assembled conjugates were characterized by fluorescence emission spectroscopy. The normalized intensities of the emission of the three conjugates after excitation at 370 nm are illustrated in Figure 3. The spectra of **1*4** displays both monomer emission between 385 and 450 nm and a broad excimer band between 450 and 650 nm. In contrast, **2*4** and **3*4** exhibit almost exclusively excimer fluorescence.

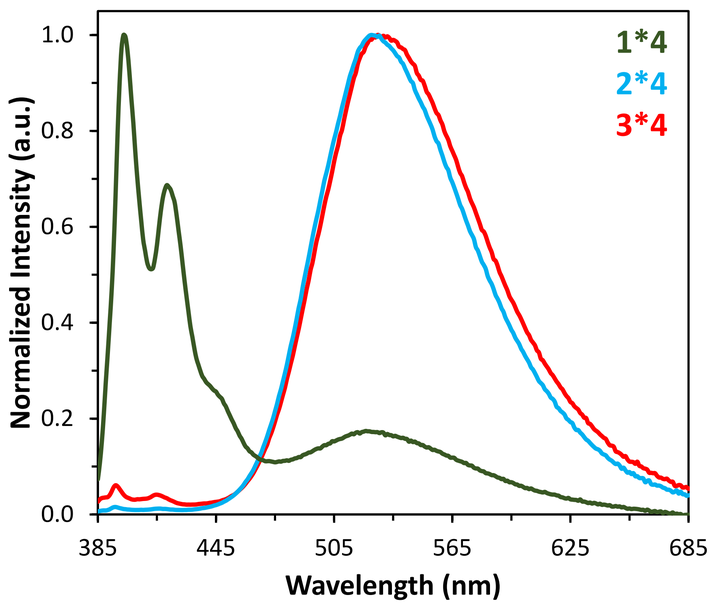


Figure 3 Fluorescence emission of **1*4**, **2*4**, and **3*4** after the self-assembly. Conditions: excitation at 370 nm, measured at 20°C after cooling from 75°C with a rate of 0.5°C/min. 1 µM each single strand, 10mM sodium phosphate buffer pH 7.2, 0.03 mM spermine·4 HCl, EtOH 20 vol %,

Conclusion: DNA duplexes modified with 1, 2, and 3 pyrene units at the 3'- and 5' -ends were characterized by UV-vis spectroscopy, AFM, and fluorescence spectroscopy. Nanostructures were observed with conjugates bearing a minimum of 2 pyrene units at each end. Pyrene-DNA conjugates with 2 and 3 pyrene units on each side formed vesicles between 50 and 150 nm diameter.

References [1] H. Bittermann, D. Siegemund, V. L. Malinovskii and R. Häner, *J. Am. Chem. Soc.*, 2008, **130**, 15285–15287.