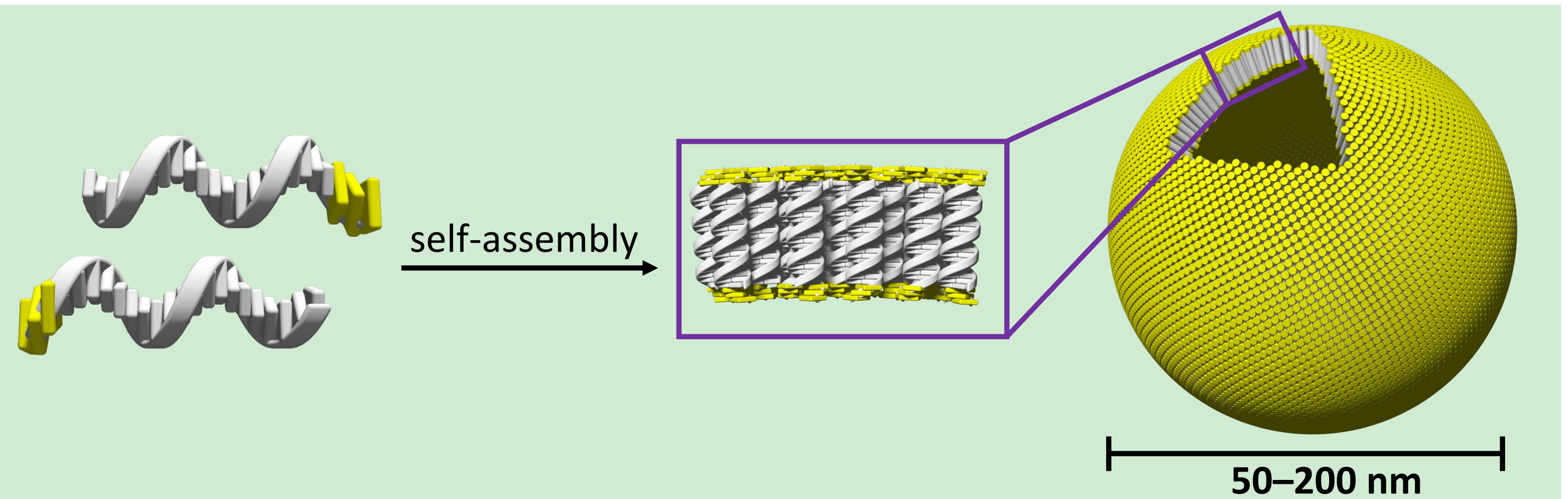


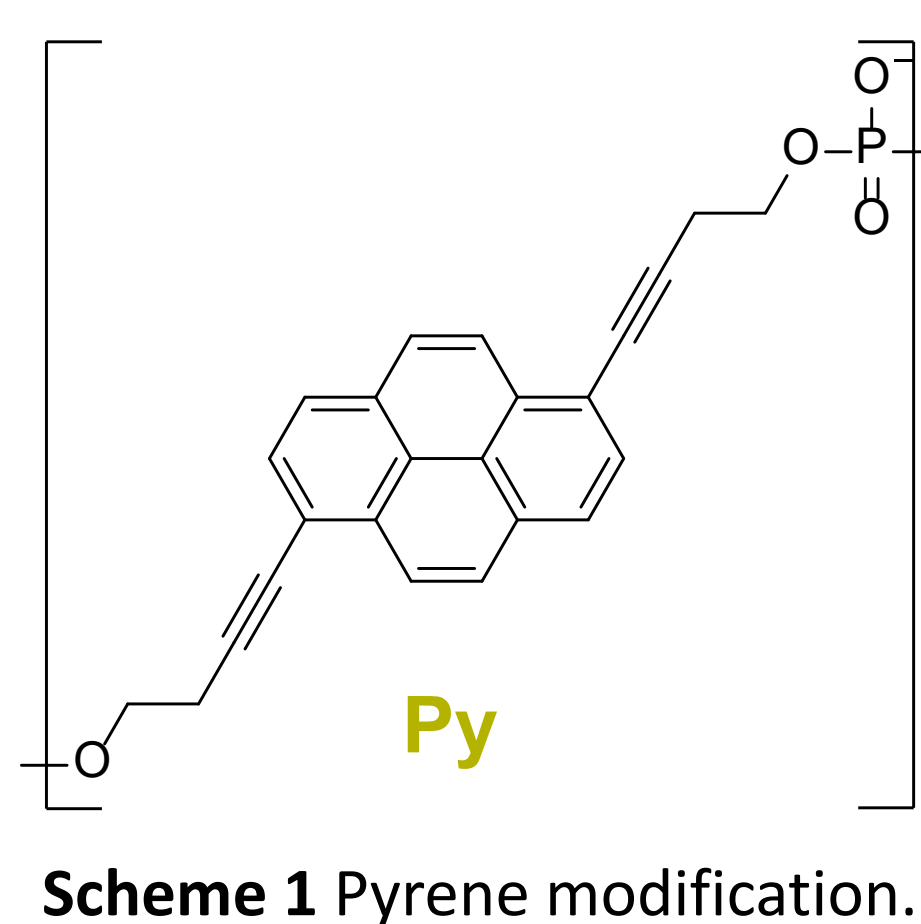
# Supramolecular Assembly of Pyrene-DNA Conjugates into Columnar Vesicles

**Abstract:** This poster describes the supramolecular assembly of DNA conjugates functionalized with pyrene sticky-ends. After hybridization, the 3'-end modified DNA single strands self-assembled into vesicles with diameters of 50–200 nm. Columnar packed aggregated and multilamellar vesicles were observed by cryo-EM.



## Synthesis of Pyrene-DNA Conjugates

The DNA strands containing 20 nucleobases and 3 pyrene units at the 3'-end were synthesized *via* solid-phase DNA synthesis using phosphoramidite chemistry. Afterward, the oligomers were purified by reverse-phase HPLC.<sup>1</sup> The chemical structure of the used 1,6-dialkynated pyrene is illustrated in Scheme 1. The synthesized oligomers **1** and **2** are listed in Table 1.



### Sequences of pyrene modified DNA

<b>1</b>	5'-CAA GGT CCG ATG CAA GGA AG-(Py) <sub>3</sub>
<b>2</b>	(Py) <sub>3</sub> -GTT CCA GGC TAC GTT CCT TC-5'

Table 1 Sequences of oligomer 1-2.

## Cryo-Electron Microscopy

The nanostructures of the self-assembled pyrene-DNA conjugates were visualized by cryo-electron microscopy (cryo-EM). Interestingly, multilamellar vesicles and aggregates of vesicles were observed (Figure 2a-b). Cryo-EM helped in understanding the packing of the nanostructures. The interlamellar spacing of 7.5 nm inside the vesicles fits well with the length of the DNA duplex, proposing a columnar packing of the vesicles. With the use of fast Fourier transform (FFT) and filtering (cutting off high and low frequencies) the pattern present in the inset of Figure 2b was extracted (Figure 2c and 3). The graph in Figure 3 compares the grey value of the sections in the cryo-EM image (red section Figure 2b) and the extracted pattern (blue section Figure 3). A pattern with a 2.5 nm spacing was observed that correlates well with the thickness of the DNA in a columnar packing.

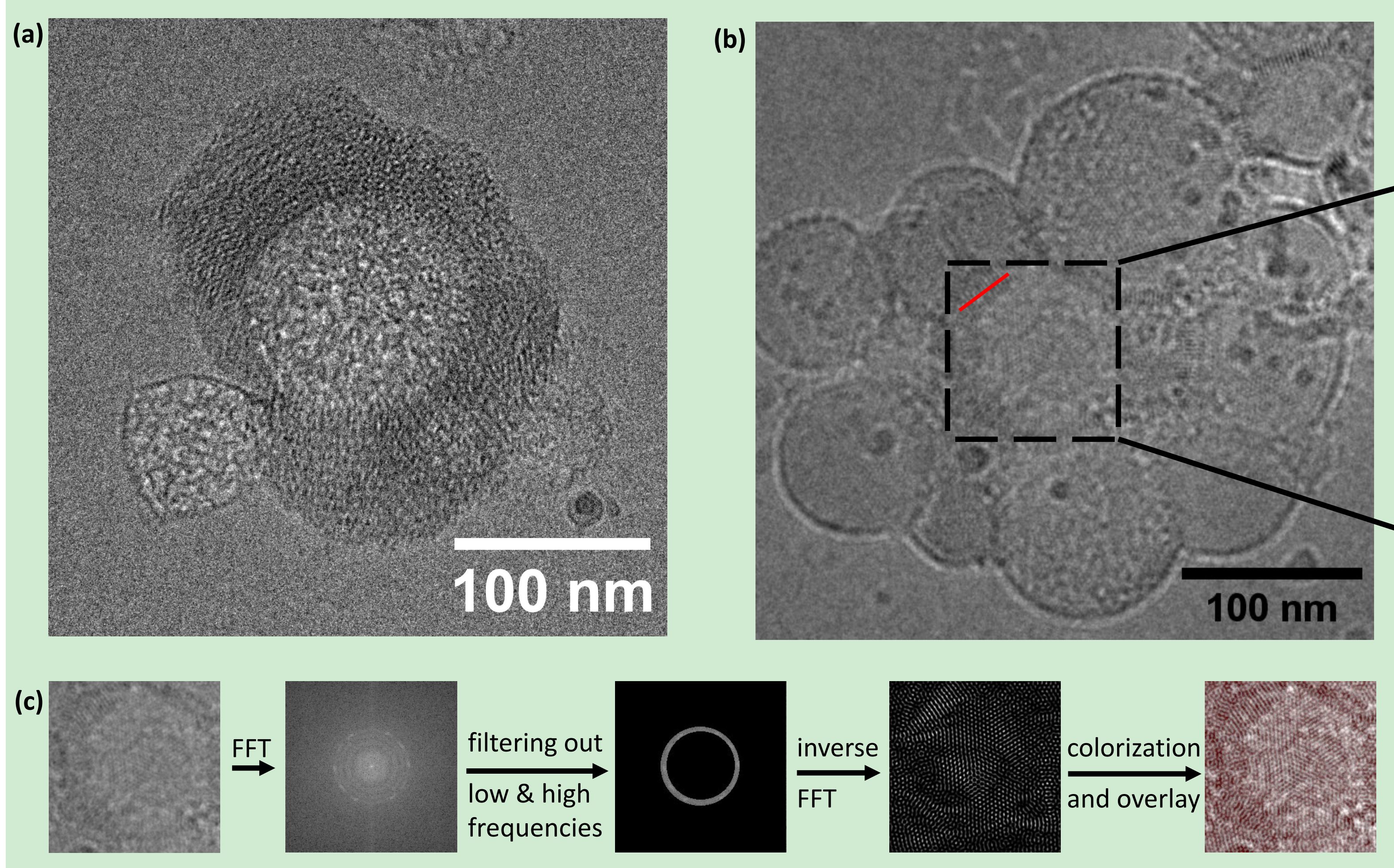


Figure 2 Cryo-EM image of self-assembled **1\*2** a) multilamellar vesicle and b) aggregated vesicles. c) steps for pattern extraction in inset of Figure 2b (filtering out high & low frequencies of fast Fourier transformed image).

## Temperature Dependent UV-Vis and Fluorescence Spectroscopy

The self-assembly of **1\*2** was monitored with UV-vis and fluorescence spectroscopy (Figure 1). Spectroscopic measurements revealed that the **1\*2** self-assembled into supramolecular aggregates after slow cooling (0.5 °C/min) from 75 °C to 20 °C. Indications for the aggregation are the rise of scattering in the absorbance, the red-shift of the pyrene maxima (320–420 nm) and hypochromicity around 260 nm.

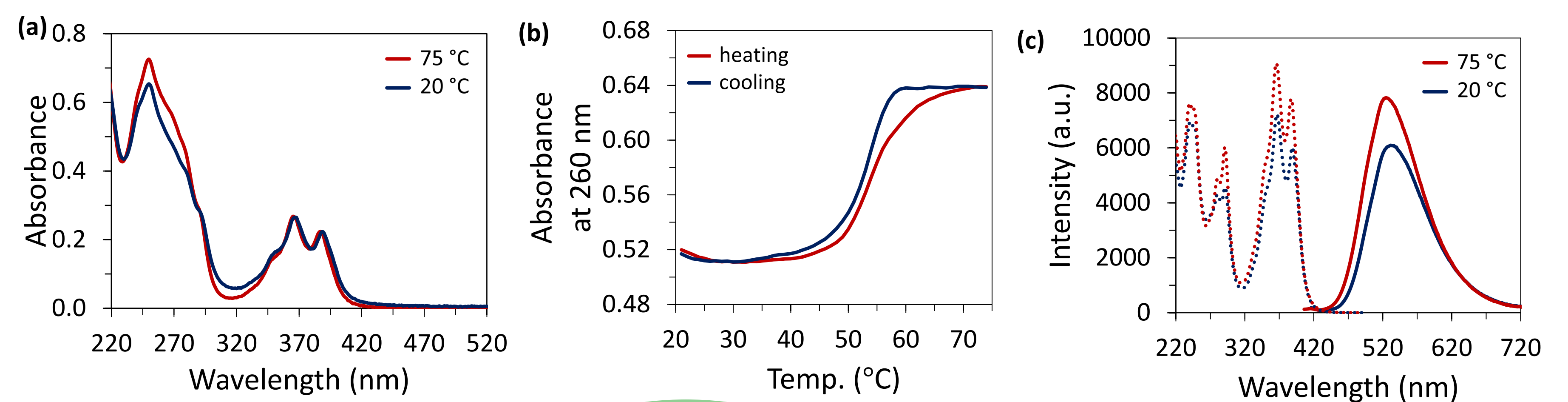
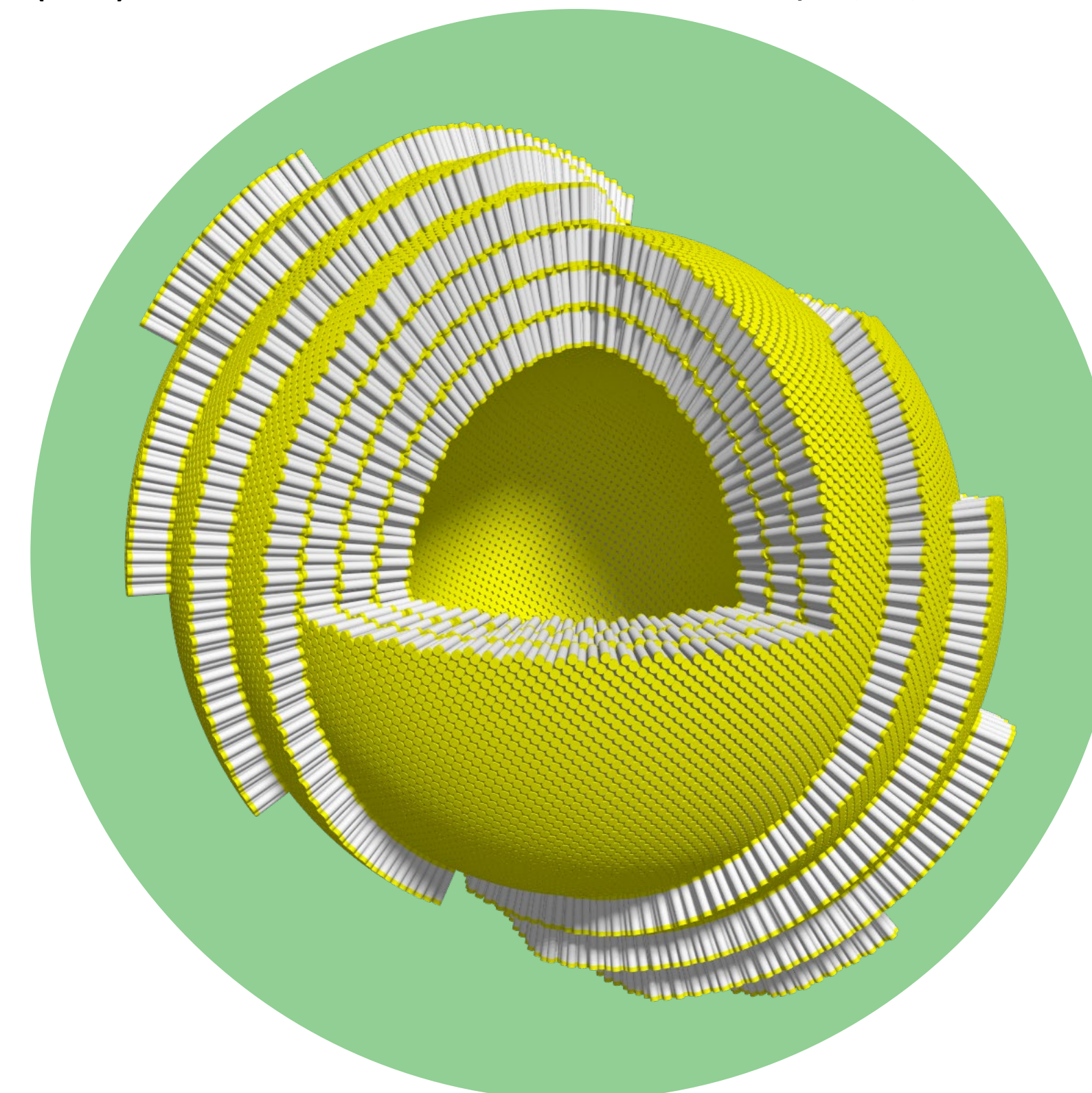


Figure 1 (a) UV-vis absorption spectra (b) UV-vis absorbance at 260 nm during cooling and heating and (c) fluorescence excitation ( $\lambda_{em}$  525 nm, dashed) and emission ( $\lambda_{ex}$  388 nm, bold) spectra. Conditions: 1  $\mu$ M **1\*2**, 10mM sodium phosphate buffer pH 7.2, 0.03 mM spermine-4 HCl, 20 vol% EtOH, gradient: 0.5°C/min.



## Atomic Force Microscopy

The self-assembly of **1\*2** in aqueous medium formed nano-sized aggregates that were adsorbed to an APTES-modified mica and visualized by AFM (Figure 4). According to AFM measurement the diameter of the round aggregates is between 50 to 200 nm.

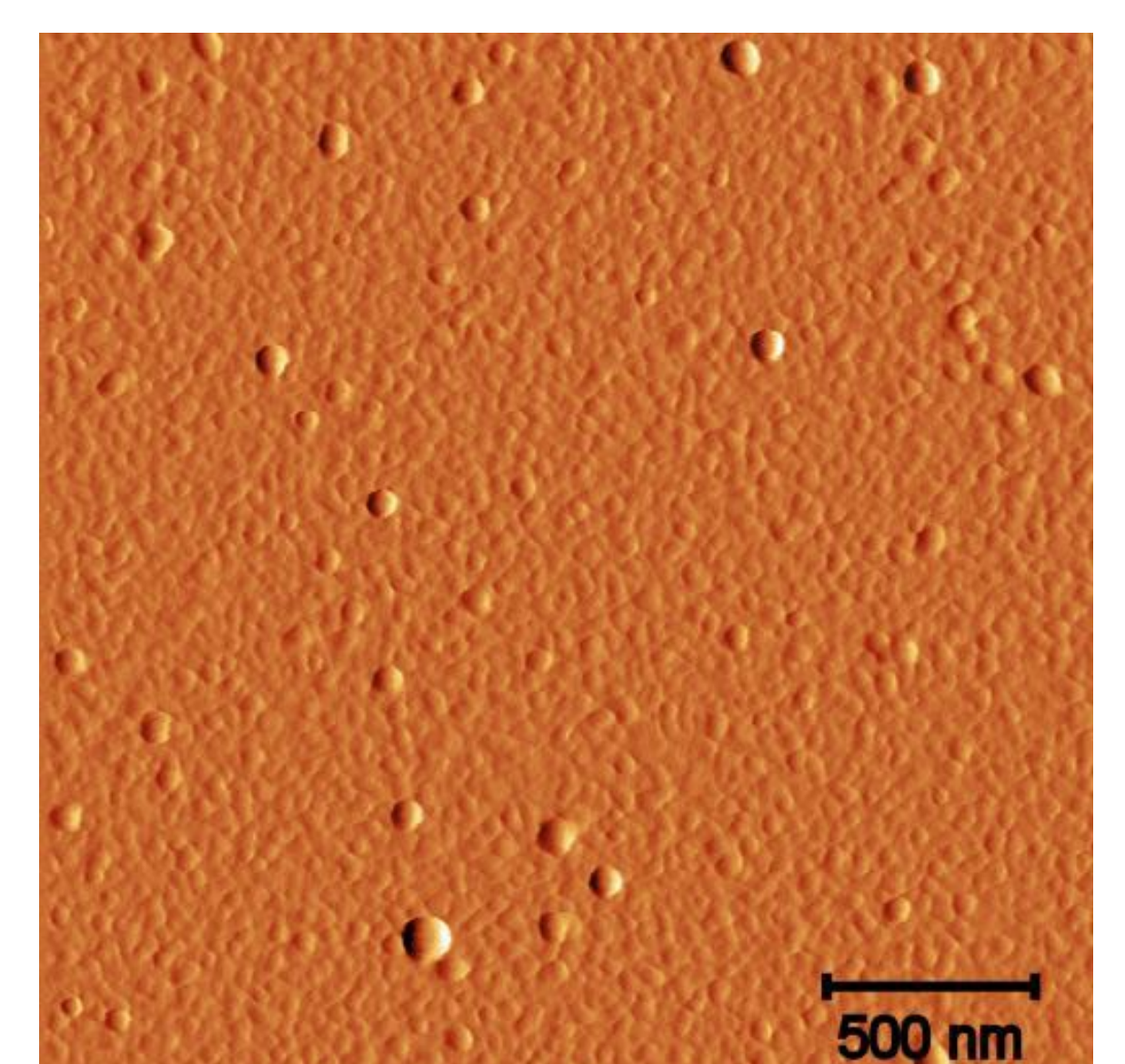


Figure 4 AFM image of self-assembled **1\*2** (amplitude scan).

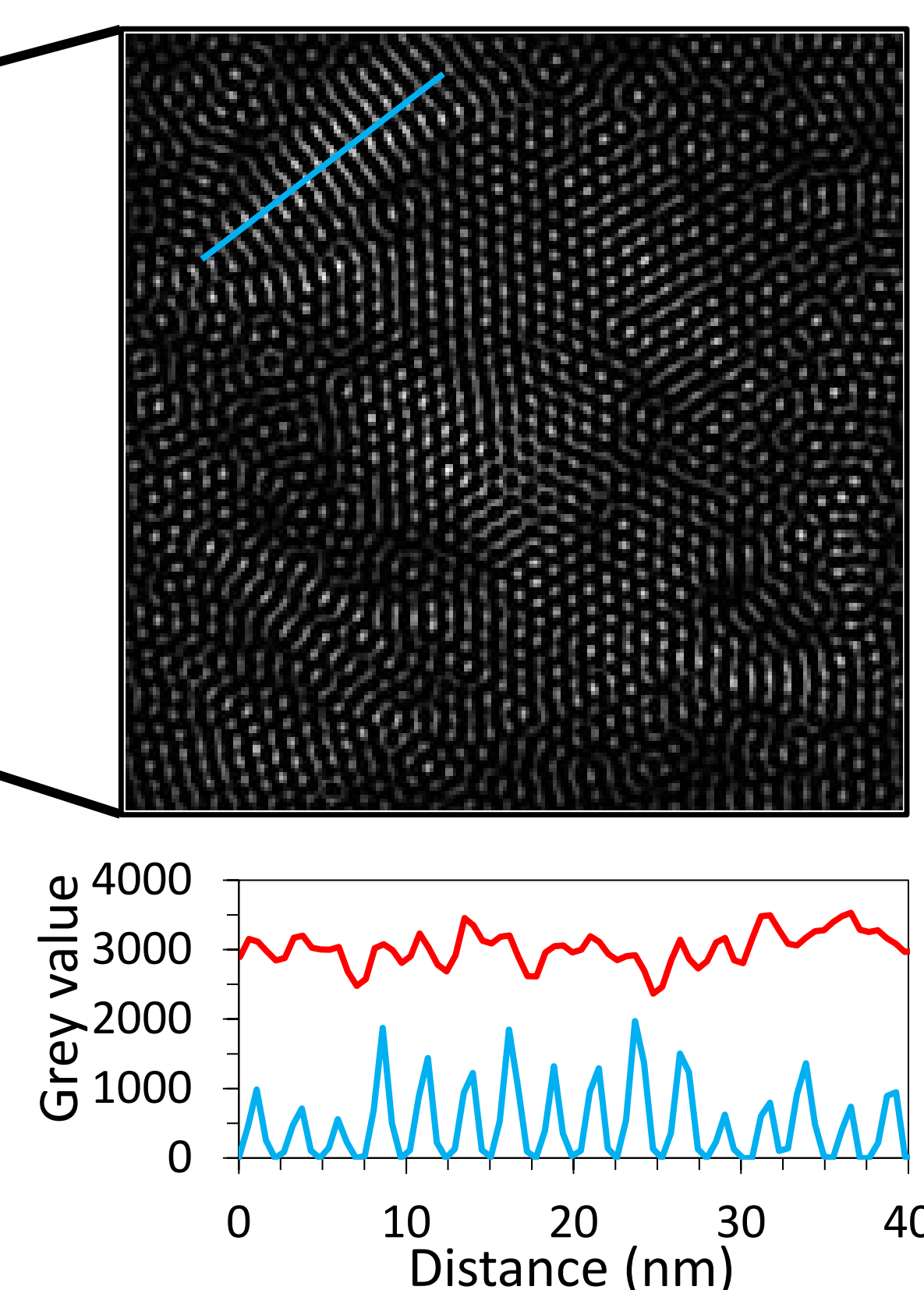


Figure 3 Pattern extracted from inset of Figure 2b and greyscale profile of section extract in blue original cryo-EM in red.

**Conclusion:** Pyrene-DNA conjugates with three pyrene at the 3'-ends self-assembled into columnar vesicles. Cryo-EM and AFM revealed that the diameter of the vesicles is between 50 and 200 nm. The vesicles were found on cryo-EM images as multilamellar vesicles and aggregated vesicles. The nanostructures on the cryo-EM images suggested a columnar packing of the vesicles. Cutting off high and low frequencies of a fast Fourier transformed image is extremely useful in extracting patterns from cryo-EM images.