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## COMMUNICATION

## Folding of phosphodiester-linked donor-acceptor oligomers into supramolecular nanotubes in water

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Inspired by the automated synthesis of DNA on solid support, electron-rich dialkoxynaphthalene (DAN) donor and electron-deficient –naphthalene-tetracarboxylic diimide (NDI) acceptor, amphiphilic foldamers have been synthesised from their respective phosphoramidite building blocks. The folding of the phosphodiester-linked hexamer (DAN-NDI)<sub>3</sub> revealed in water the formation of regular supramolecular nanotubes from the self-assembly of multiple hexamers stabilized by donor/acceptor interactions and solvophobic effect.

The tridimensional structure of supramolecular assemblies plays an important role for their biophysical properties and ultimately their functions whether they are natural (DNA, proteins) or synthetic (molecular machines, polymers). In the pursuit of new folded structures and ultimately in tuning their structural conformation, and thus controlling their function, foldamers have emerged as new tools for the development of biologically active synthetic molecules.<sup>1</sup> Foldamers are linear artificial oligomers able to fold into secondary structures stabilized by non-covalent forces including hydrogen bonds, ionic interactions,  $\pi$ - $\pi$  or  $\sigma$ - $\pi$  interactions, Van der Waals forces or solvophobic driving force in water.<sup>2</sup> While DNA itself can be seen as foldamers,<sup>3-5</sup> several examples of folded DNA-inspired oligomers have been reported in the literature. These structures are generally composed by repetitive aromatic<sup>6</sup> polypyrene,<sup>7-13</sup> polyanthracene<sup>14</sup> or polyphenanthrene<sup>15</sup> units except one built from perylenediimide-pyrene supramolecular interactions,<sup>16</sup> which induce the formation of supra tubular polymers or nanosheets. Along this line, foldamers built on electrostatic complementarity interactions (donor/acceptor) in water are particularly important for the elaboration of novel

materials able to interfere with biological systems. Iverson and co-workers described the synthesis of self-complexing systems based on 1,5-dialkoxynaphthalene (DAN) and 1,8,4,5-naphthalenetetra-carboxylic diimide (NDI) motifs linked together with L-aspartic acid,<sup>17-19</sup> or with mixed amino-acids to gain aqueous solubility.<sup>20</sup> Both aromatic units are stabilized in water by solvophobic<sup>21</sup> effect and donor-acceptor interactions known as charge-transfer (CT).<sup>22-25</sup> Alternatively, CT-based foldamers built from DAN and pyromellitic diimide units and tetra or hexaethyleneglycol linkers complexed with alkali-metal have also been reported.<sup>26</sup>

Herein, we present the synthesis and supramolecular assembly of new water-soluble DNA-inspired foldamers built from DAN and NDI moieties linked by negatively charged phosphodiester leading to the formation of nanotubes in water without the need of organic solvent or any type of adjuvants (Fig. 1).

The synthesis of the new structures started with the preparation of NDI and DAN phosphoramidite building blocks able to be directly used on an automated DNA synthesizer. This approach allows a rapid access to various foldameric sequences.<sup>27</sup>

The synthesis of NDI phosphoramidite **4** started with naphthalene dianhydride **1** which was reacted with 3 equiv. of 3-aminopropanol to give the corresponding diimide **2** in 97% yield (Scheme 1).<sup>28</sup> Compound **2** was then treated with a default of DMTrCl to give the monotritylated derivative **3** in a rather modest yield (38 %) due to the formation of a large proportion of the ditritylated side product. Finally, phosphitylation of **3** using *N,N*-diisopropylcyanoethyl chlorophosphine gave NDI phosphoramidite-

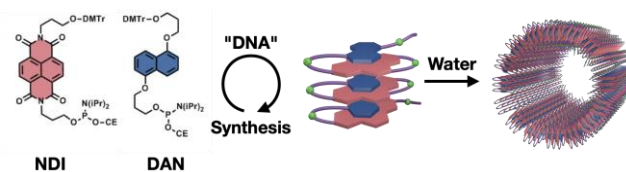
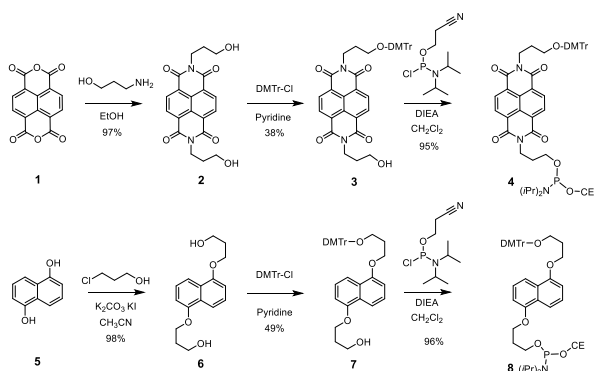


Fig. 1. Schematic representation of a nanotube built from supramolecular assembly of (DAN-NDI)<sub>3</sub> foldamers.

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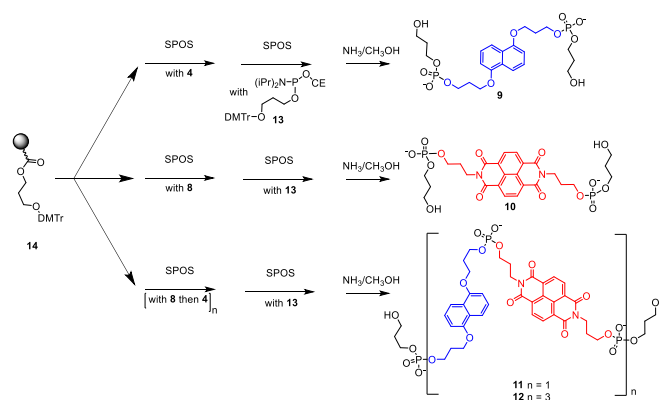
**Scheme 1.** Synthesis of NDI **4** and DAN **8** phosphoramidites.

dite **4** in 95% yield and an overall yield of 35%. Concerning DNA phosphoramidite **8**, any attempts to use standard *O*-alkylation methods of 1,5-dihydroxynaphthalene **5** described in the literature were unsatisfactory and gave the expected dialkyl derivative **6** in low yield (20 to 45%) along with the formation of an oxidized side product.<sup>29, 30</sup> Hence the alkylation was performed with 4 equiv of 3-chloropropanol in the presence of K<sub>2</sub>CO<sub>3</sub> and KI in acetonitrile under reflux in an atmosphere of argon for 18 h. Under these conditions, derivative **6** was isolated as a clean yellow-green powder in nearly quantitative yield. Following the two-steps protocol described for the synthesis of **4**, the DAN phosphoramidite **8** was obtained in 46% overall yield.

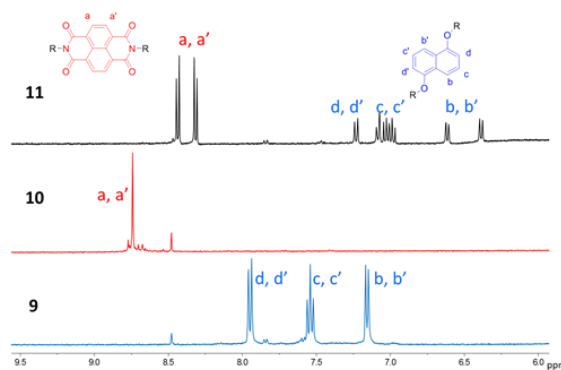
With these phosphoramidites in hand, monomers of DAN **9** and NDI **10**, as controls, as well as the alternated dimer **11** and hexamer **12** were synthesized on a DNA synthesizer using phosphoramidite chemistry and custom-made propanediol solid support (Scheme 2). To assure water solubility, an additional phosphodiester linkage was added at the end of each sequence using a propanediol phosphoramidite unit **13**.

After elongation, a methanolic ammonia treatment was applied to deprotect and release products **9-12** from the solid support. Then each compound was purified by reversed phase HPLC, and characterized by MALDI-TOF mass spectrometry (Table S1, S1-S4).

Compounds **9-11** were first analyzed by <sup>1</sup>H-NMR spectroscopy (Fig. 2). DAN derivative **9** displays three types of aromatic signals, 2 doublets and a triplet between 7.1 and 8.0 ppm, while NDI derivative **10** displays only one single aromatic signal at 8.75 ppm due to its symmetry. Compound **11** reveals multiple signals due to of the asymmetry of the molecule which leads to a polarization of the aromatics thus confirming their interaction. When the temperature was raised from 25 to 85 °C, a change of the folding state of **11** was observed. At 25 °C, DAN and NDI aromatics have a lower chemical shift which indicates a strong shielding suggesting that **11** is strongly folded on himself. As the temperature increased up to 85 °C, the strong deshielding of the aromatic was progressively apparent. Indeed, the two doublets of NDI aromatics moved closer together indicating a lower differentiation of the hydrogen atoms as the result of fewer interactions between DAN and NDI (Fig. S5). In addition, the solution at high temperature still exhibited a pink color visible to the naked eye characteristic of the presence of a CT-band.



**Scheme 2.** Synthesis of monomers **9** and **10**, and foldamers **11** and **12**. SPOS: solid-phase oligonucleotide synthesis. SPOS Conditions: (1) 3% trichloroacetic acid (TCA) in CH<sub>2</sub>Cl<sub>2</sub>; (2) phosphoramidite derivative + benzylthiotetrazole (BTT); (3) Ac<sub>2</sub>O, *N*-Me-imidazole, 2,6-lutidine; (4) 0.1 M I<sub>2</sub> THF/H<sub>2</sub>O/pyridine.

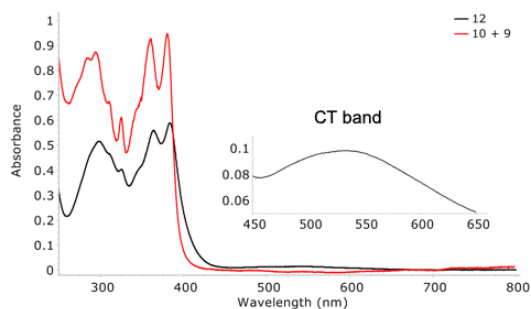


**Fig. 2** Selected aromatic region of <sup>1</sup>H NMR of compounds **9-11** in D<sub>2</sub>O at 25 °C. [**9**] = 3.9 mM, [**10**] = 2.7 mM and [**11**] = 2.7 mM.

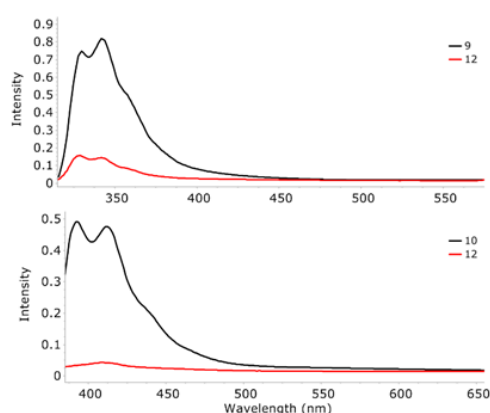
This study demonstrates that increasing temperature does not unfold the structure completely. Fig. 3 shows the UV/visible absorption spectra of the mathematic sum of **9** and **10** (both at 99 μM in water) as well as **12** (33 μM) in aqueous solution. The spectrum of **12** displayed an attenuation of the absorption bands, a change of the two NDI intensity bands and a 37% hypochromicity in comparison with the mathematic sum of **9** and **10**. The charge transfer band is centered at 530 nm with a molar extinction coefficient of 300 M<sup>-1</sup>.cm<sup>-1</sup>, although a higher concentration is needed to clearly observe it (see Fig. 3, inset). The study performed at 85 °C displayed a very similar spectrum with a very low decrease of CT band intensity demonstrating the strong folding of **12** (Fig. S6). All these changes are characteristic of a folded structure and similar to previously reported DAN/NDI foldamers.<sup>20, 26, 31</sup> However, in contrast to DAN/NDI foldamers built with amino acids,<sup>20</sup> the current foldamer was very stable and insensitive to temperature variations.

Fluorescence emission spectrum of compound **12** was compared with emission spectra of **9** and **10** (Fig. 4). Emissions of **9** and **10** concur with previous studies with two high emissions of fluorescence at 330 and 345 nm<sup>18, 32</sup> for **9** (λ<sub>ex</sub> = 296 nm) and two high emissions of fluorescence at 390 and 420 nm<sup>33</sup> for **10** (λ<sub>ex</sub> = 363 nm). As expected, **12** exhibits a very low emission of fluorescence at both excitation wavelengths due to the stable folding of the DAN and NDI units with a charge transfer that induces a very fast non-radiative decay.<sup>32</sup> The pink

color characteristic of a CT interaction observed with **12**, associated with the important hypochromicity observed in UV-Vis and the strong decrease of fluorescence emission confirmed the presence of a folded structure.



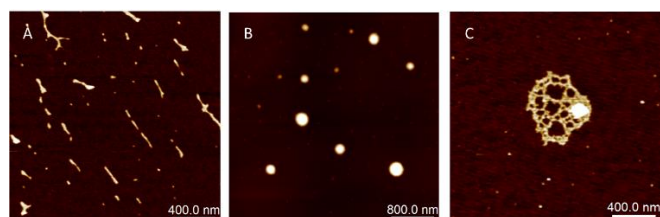
**Fig. 3.** UV-Vis spectra of oligomer **12** (33  $\mu\text{M}$ , black curve) in water and mathematic sum of **9** and **10** (99  $\mu\text{M}$ , red curve). Inset: Expanded region of the charge-transfer band of **12** at 100  $\mu\text{M}$ .



**Fig. 4.** Fluorescence emission spectra of compounds **9**, **10** and **12** (15  $\mu\text{M}$  in water). top:  $\lambda_{\text{ex}} = 296 \text{ nm}$  Bottom:  $\lambda_{\text{ex}} = 363 \text{ nm}$ .

The structuration of the different compounds (**9**, **10**, **9+10**, **11** and **12**) was imaged using Atomic Force Microscopy (AFM) in tapping-mode. Monomers alone dissolved in water (45  $\mu\text{M}$ ), for 1 day, form quite different structures. The DAN derivative **9** forms nanotubes (up to several hundreds of nm long and  $\sim 2 \text{ nm}$  height) with some ramifications (Fig 5A and S9), while the NDI derivative **10** forms vesicles ( $\sim 30\text{-}200 \text{ nm}$  of diameter) (Fig 5B and S10). When both compounds were mixed together in water, we observed strongly ramified nanotubes of several hundreds of nm with a height of  $\sim 1.5 \text{ nm}$  (Fig 5C and S9).

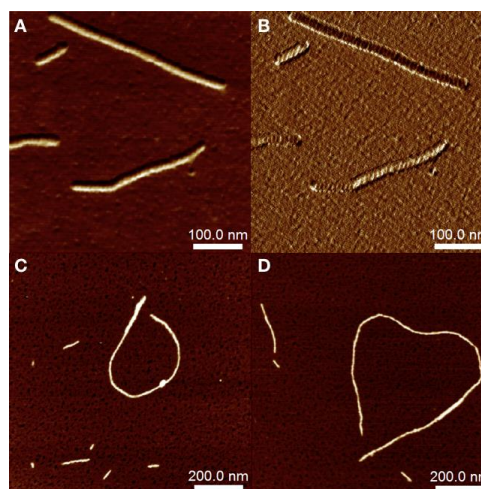
The dimer **11** was studied at a concentration of 45  $\mu\text{M}$  after one day in water. Only very few nanotubes with a length up to 1  $\mu\text{m}$  and heights between 0.5 and 1.2 nm were observed (Fig. S10). Then, the folding of hexamer **12** was studied according to its



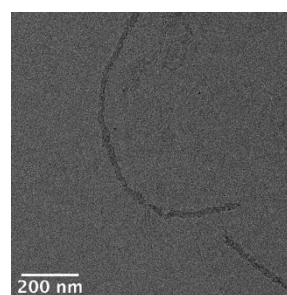
**Fig. 5.** AFM images (height) at 45  $\mu\text{M}$  in water after 1 d, A) **9**, B) **10** and C) **9+10**.

concentration and time left in solution. At 1  $\mu\text{M}$ , we observed only few single nanotubes around 200-300 nm long and also ramified nanotubes up to 1  $\mu\text{m}$  long with heights between 1 and 1.5 nm (Fig. S11). When the concentration increased to 15  $\mu\text{M}$ , more regular and homogeneous nanotubes (100-400 nm long, 2 nm height) were visualized (Fig. 6A and S12) and at 30  $\mu\text{M}$  even longer nanotubes (200-500 nm long, 2 nm height) were observed. Interestingly two nanotubes on top of each other were also detected (Fig. S13). The 15  $\mu\text{M}$  sample was then studied after 3 and 7 days to monitor its fate over time (Fig. S14). Similar width but much longer nanotubes greater than 1  $\mu\text{m}$  were observed (Fig. 6C-D). With amplitude AFM images, we were able to determine that this structure is a collapsed nanotube with a thickness of 2 nm and a width of 16 nm (Fig. 6B). These nanotubes have a similar structure than polyanthracenes reported by Häner and coworkers.<sup>11</sup>

The formation of nanotubes for **12** was confirmed by TEM imaging. A first study performed in water with addition of 2% uranyl acetate solution for staining showed nanofibers folded on themselves to form ball of twisted nanotubes (Fig. S15). Uranyl ions are known to bind with nucleic acid phosphates and to induce aggregation.<sup>34</sup> To reduce the formation of related artifacts from uranyl acetate staining, we adapted the buffer sample by adding 10 mM phosphate buffer pH 7 and 10 mM NaCl. TEM micrographs of the 15  $\mu\text{M}$  sample in this buffer showed micron scale one-dimensional nanotubes similar to AFM with fewer defects (Fig. 7 and S16).

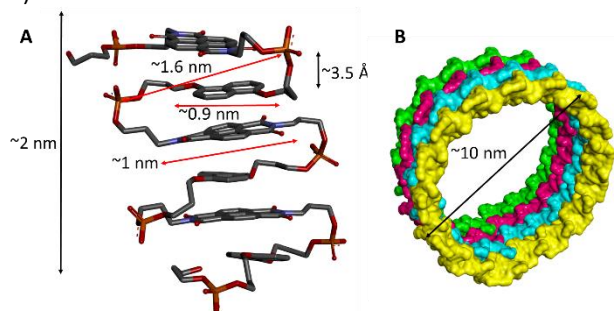


**Fig. 6.** AFM images of **12** at 15  $\mu\text{M}$  in water. A) 1 d, height, B) 1 d, amplification C) 3 d, height, D) 7 d, height.



**Fig. 7.** Negative-stained TEM images of **12** in 10 mM phosphate buffer pH 7 and 10 mM NaCl, 15  $\mu\text{M}$  1 d.

According to the AFM study, foldamer **12** forms nanotubes with an approximate thickness of 8 to 10 nm and lengths of several micrometers. The molecular modelling study of foldamer **12**, shows a size of one unit of foldamer about  $\sim 2$  nm long and  $\sim 1.6$  nm wide with a distance between two aromatics of about  $3.5 \text{ \AA}$  (Fig. 8A). AFM images show collapsed tubes with a section around  $\sim 16$  nm and a height of  $\sim 2$  nm. In line with the work of Häner *et al.*<sup>11</sup>, the height, measured by AFM, corresponds to two layers of foldamers indicating that the thickness of one layer is  $\sim 1$  nm corresponding roughly to the length of aromatic motifs with a part of the propyl arms. Hence, the tube has a perimeter of  $\sim 32$  nm corresponding to 16 hexamers and a diameter of  $\sim 10$  nm was also observed by TEM. The molecular modeling constructed with these parameters with four turns is shown in fig. 8B. We calculated the longitudinal and lateral potential energies of interaction of two foldamers as  $-32.5$  kcal/mol and  $-76.2$  kcal/mol, respectively corresponding to the energy allowing the elongation of the fiber adding several units stabilized by CT interaction and to the energy allowing the lateral interaction of the twisted fiber to close the tube (Fig. S17).



**Fig. 8.** A) Molecular modeling of **12**, B) modeling of four turns of the tube with 16 units by turn and a  $\sim 10$  nm diameter.

In conclusion, we provide an easy method for the synthesis of new donor-acceptor amphiphilic phosphodiester foldamers, fully soluble in water, without any cosolvent, stabilized by a variety of interactions, i.e., electronic complementary, charge transfer and solvophobic effects. Starting from, DAN and NDI phosphoramidites as building blocks, we successfully synthesized a (DAN-NDI)<sub>3</sub> hexamer, and demonstrated its folding by UV-visible and fluorescent studies. AFM and TEM characterizations allowed to visualize the formation of regular nanotubes of several hundreds of nanometers in length and  $\sim 10$  nm in diameter resulting from a supramolecular arrangement of several hexamers stabilized by longitudinal and lateral interactions. In contrast to Iverson and co-workers who observed an amyloid like behavior for the alternated DAN and NDI foldamers linked with amino acids when heated,<sup>20</sup> we demonstrated that alternated DAN and NDI foldamers linked by phosphodiesters are stable even at high temperature and are able to form nanotubes thanks a CT stabilization in water. These nanotubes are similar to those reported by Häner obtained by a  $\pi$ -stacking of polypyrene,<sup>7-13</sup> polyanthracene<sup>14</sup> or polyphenanthrene.<sup>15</sup> Furthermore, our current foldamers are fully soluble in pure water and no addition of organic solvent was necessary.

Current water-soluble DNA-inspired foldamers open the way to new objects that could be decorated with motifs recognized by living systems to develop biologically active synthetic molecules or new hybrid materials.

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## Conflict of interest

There are no conflicts to declare

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