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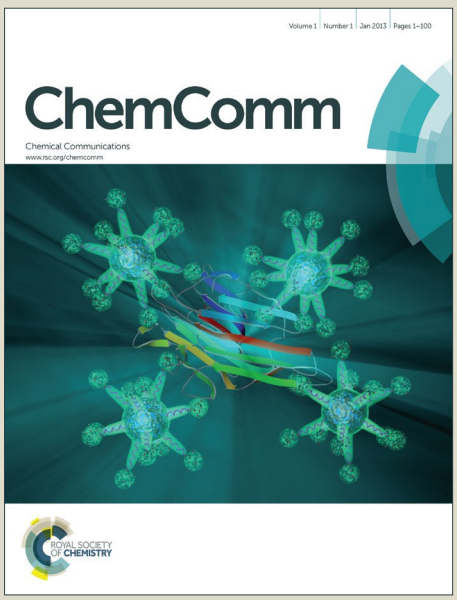

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Supramolecular rulers enabling selective detection of pure short ssDNA via chiral self-assembly

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Multivalent self-assembly of trifunctional aromatic propellers and ssDNA result in the formation of chiral supramolecular assemblies that can be used for the detection of small fragments of ssDNA with different lengths and compositions.

The rational design of supramolecular architectures in water is imperative for their important applications in selective bio-recognition and biodetection events.[1] Artificial supramolecular polymers[2] for DNA recognition may read the information of DNA in a multivalent manner that proteins do. DNA strands are well known for their informational loading in regards with biological processes and they have recently seen an increase of interest in the assembly of DNA nanostructures[3] or for the construction of hybrid systems, when combined with synthetic molecules.[4] They are particularly attractive in view of their specific interactions and the design of functional synthetic hybrid DNA-type superstructures, exclusively driven by the self-assembly has become an area of expanding interest.[5] Herein we describe the mutual self-assembly of trifunctional aromatic propellers able to form chiral supramolecular assemblies and to selectively recognize the single stranded ssDNAs with different lengths and composition.

We report a series of triscationic triphenylamine (TPA) and benzene molecular scaffolds designed to bind to the DNA strands used as efficient biodetection or transfection tools (Fig. 1). We already know that TPA containing H-bonding moieties self-assemble under irradiation, resulting in the formation of conductive fibers[6] and their interactions with DNA molecules have been proven.[7] Tris(4-formylphenyl)amine **TPA** or 1,3,5-benzenetrialdehyde **BTA**, aromatic core centres and amino-guanidine·HCl **AG**, or Girard's reagent **TG**, cationic arms and acylhydrazine/carbonyl-acylhydrazone chemistry have been used to conceive the trisubstituted propellers for ssDNA recognition (Fig. 1). Treatment of **TPA** or **BTA** with 3 eq. of **G** or **TG** in methanol (reflux, 3h) afforded after crystallization pure compounds **1**-**4**.

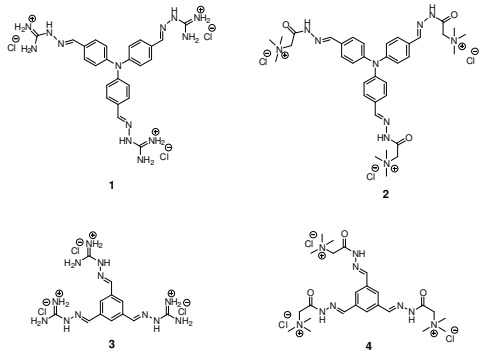

Figure **1**. Synthesized triscationic triphenylamine and benzene compounds **1**-**4**.

^{1}H-NMR and ESI-MS spectral data agree with the proposed structures of **1**-**4** (ESI). Interestingly, we found that while their structure may look similar, only the compounds **1** and **2** bearing the TPA cores induced a change in the CD signal (Fig. 2) meanwhile the compounds **3** and **4** with the BTA cores remained inert. Then, Circular Dichroism - CD spectroscopy[8] has been used to monitor the interaction between the cationic **1**-**4** and the ssDNAs. The inherent signal given by the ssDNA alone, specific for helical structures found in single strands, suffers a drastic amplification in the presence of compounds **1** and **2** (Fig. 2). The shift of the CD spectra for DNA in the presence of **1** or **2** resembles the transition of double strand DNA from the B helix towards the A helix.[9] The sharp increase in intensity suggest the formation of the chiral supramolecular columnar architectures between the multiple ssDNA strands and **1** or **2**. More interesting is the appearance in the presence of ssDNA of a new set of signals in the region of 300-450 nm, specific for the absorbance region of **1** and **2**, which by themselves do not give any CD signal (see ESI). The confined immobilization of **1** or **2** *via* a strong binding to DNA strands

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causes the slow or blocked rotation of the phenyl rings around the propeller-blocked TPA core thus inducing an axial molecular chirality.

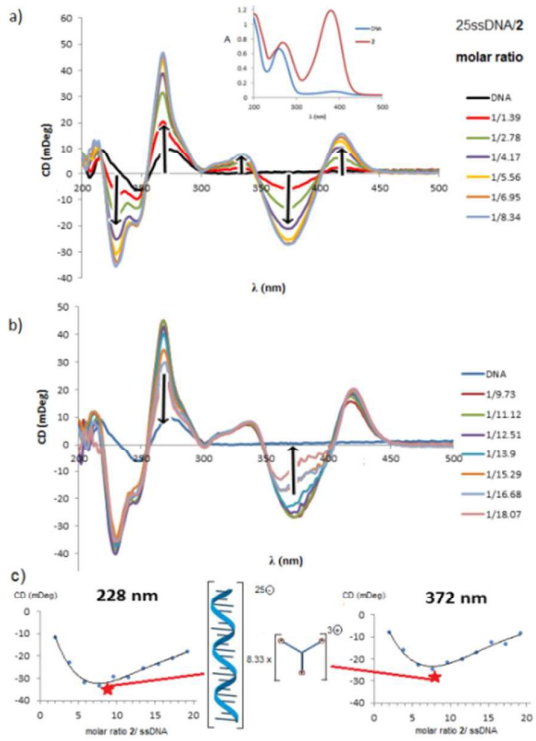

Figure **2**. a) Circular dichroism spectra obtained by titrating a solution of 25 base ssDNA with compound 2: a) before and b) after equivalence point corresponding charge balance equilibration of $ssDNA^{25-}$ with about 8.33 molecules of 2^{3+}. Inset: UV-VIS spectra of 25 base ssDNA and of compound 2. c) Representation of CD signal intensity variation in function of 2/ssDNA molar ratio at 228 nm (left) respectively at 372 nm (right); the charge balanced $ssDNA^{25-}$/ $(2^{3+})_{8.33}$ composition at charge balance equilibration point represented as a red star.

The self-assembly of columnar superstructures of **1** or **2** with the ssDNA, results in the formation of stacked in hybrid fibers[6] (Fig. 3). The stacking takes places only in the presence of the ssDNA and could be explained by electrostatic binding of **1** or **2** to the DNA scaffolds, which could bring the TPA cores in proximity to favour intermolecular hydrophobic interactions, favoured in aqueous solution.

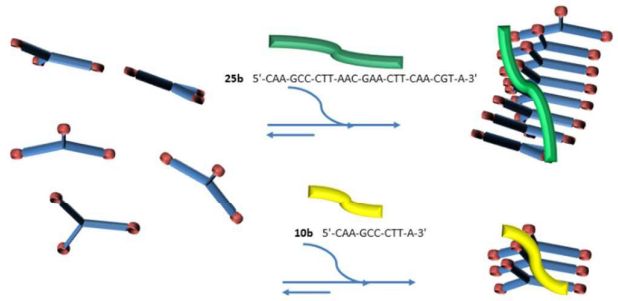

Figure **3**. Proposed self-assembly mechanism of ssDNA-TPA complexes of different dimensional behaviours function of the length of ssDNA, obtained at the charge equivalence point where charge ratio between negatively charged ssDNA and positively charged **1** or **2** is 1:1

The fact that the shift is induced just by the presence of **1** and **2** is excluded by the lack of shift of the ssDNA in the presence of **3** and **4**. The higher diameter of columns formed by the compounds **1** and **2** is probably more adapted to coiling behaviors of ssDNAs, than thinner columns formed by **3** and **4**. The formation of this nanowire, as it is bound to the phosphates, would follow the pattern of the ssDNA strands which would feed back the chiral information to it making the nanowire itself chiral. The maximum in the signal variation is reached at the molar ratio point where charge ratio between negatively charged ssDNA and positively charged **1** or **2** is 1:1. For a long sequence of 25bssDNA, 5'-CAA-GCC-CTT-AAC-GAA-CTT-CAA-CGT-A-3' the charge balance equilibration point is reached at exact 1/8.34 ssDNA/**2** molar ratio for compound **2** (Fig. 2a) while 1/5.8 ssDNA/**1** molar ratio is obtained for compound **1** (Fig S1, ESI).

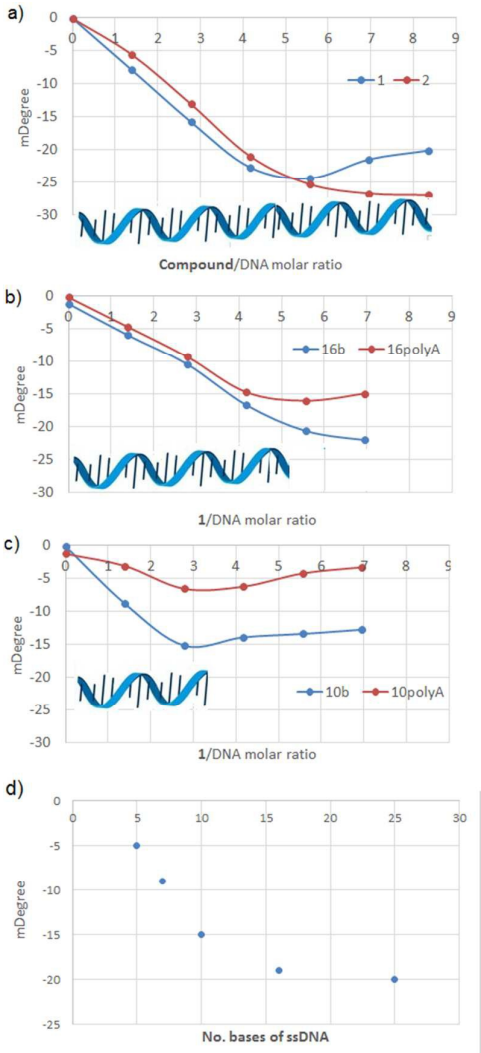

Figure **4**. Representation of CD signal intensity variation in function of compound/ssDNA molar ratio at 372 nm for: a) 25bssDNA 5'-CAA-GCC-CTT-AAC-GAA-CTT-CAA-CGT-A-3' and compounds **1** and **2**, b) 16bssDNA 5'-CAA-GCC-CTT-AAC-GAA-C-3' or 16bspolyA and compound **1** and c) 10bssDNA 5'-CAA-GCC-CTT-A-3' or 10bsspolyA and compound **1**. d) The intensity at 372 nm at the charge equivalence point plotted function of number of bases of ssDNA.

The different binding cationic sites of **1** and **2** are directing the stability of the complex which is obtained at lower ratios for compound **1** (Fig. 4a). Once the equilibrium threshold has been passed the superstructures start to disassemble. As we add excess positive charged compounds **1** or **2** to the 25bssDNA solution, the signals start to decrease except for two of them between 300-450 nm which stay at the same level (Fig. 2b, Fig. S3, ESI). The persistence of the signals at 336 nm and 418 nm, which are thought to be specific to the **TPA** cores, should be the effect of the persisting attachment of the positively charged arms to the ssDNA backbone which continuously blocks the rotation of the phenyl rings, thus maintaining its chirality. This can be translated as propriety of chiral memory of the ssDNA-**1** or ssDNA-**2** architectures. The appearance of the set of signals specific for the ssDNA-**TPA** superstructures is present with short 16bssDNAs, 5'-CAA-GCC-CTT-AAC-GAA-C-3' 10bssDNA 5'-CAA-GCC-CTT-A-3', 7bssDNA and 7bssDNA, which barely give any signal in CD spectra (ESI, Fig. S4-S9). Interestingly the charge balance equilibration point is reached at exact 1/5.34 16bssDNA/**1** (Fig. 4b) and 1/3.33 10bssDNA/**1** (Fig. 4c) molar ratios. In line with the idea that compaction of the DNA strands around the **TPA** acting as *supramolecular rulers* need a required number of bases to produce the complete self-assembly at charge balance equilibration point. The CD intensity at the charge equivalence point and the number of bases of ssDNA relationship is rather linear for ssDNA with less than 20 bases (Fig. 4d), which implies this strategy can be used experimentally to elaborate a mean to detect the number of nucleobases of small fragments of DNA which are not showing otherwise any peaks in the CD spectra. More interestingly, when the CD titration experiments were carried out with homobase strands 10bssDNApolyA and 16bssDNApolyA, the spectra behaved low absorbance values and similar dependence of molar ratio ssDNA/TPA like in the previous experiments, expect for the absence or low intensity of the signals at 340 and 420 nm (ESI, Fig. S5, S7) suggesting the interdependence of these signals and the nature of bases that the DNA is built of. The polyA tracts are reported to produce altered second structures of DNA, due to the propeller twist tendency[10] which would explain the different behaviours in comparison to random base sequenced DNA strands.

The ability of compound **2** to bind ssDNA has been confirmed by ^{1}H-NMR titration experiments (Fig. S10). The addition of 25bssDNA to a solution of **2** in D_2O caused the splitting and a progressive shielding ($\Delta\delta$ = 0.4) of the protons of the TPA core, suggesting intermolecular π-π stacking interactions with the nucleobases of ssDNA. The ^{1}H NMR spectra consist of the exchange-broadened signals, indicative of the fast exchange of **2** with the ssDNA backbone in solution. Any shielding can be observed up to a ssDNA/**2** ratio of 1:8, where the total negative charge of the ssDNA is compensated by eight molecules of **2**, as previously observed from CD studies.

Atomic force microscopy (AFM) was additionally used to obtain further valuable information on the self-assembly behaviors of 25bssDNA/**2** complexes. Compound **2** formed film-like depositions with no clear assemblies. On the other hand ssDNA/**2** complex solutions form large areas of uniform worm-like shaped fibers with cross sections of about 3.5 nm and lengths between 100-300 nm. (Fig. S11)

The ability of compound **2** to bind negative 25bssDNA was qualitatively evaluated using the agarose gel retardation assay. When placed separately, as expected the negatively charged ssDNA migrates towards the positive pole (line 1 Fig. S10), while the positively charged **2** migrates towards the negative pole (line 7 Fig. S12). The reduction of ssDNA electrophoretic mobility as well as that of the compound **2** within mixtures is a consequence of the dynamic reversible binding between partners (lines 2-6, Fig. S12). The progressive addition of the ssDNA to a constant amount of **2**, is reminiscent with the progressive formation of low stability complexes on the agarose gel, suggesting a fast exchange between ssDNA/**2** assemblies and components.

Conclusions

The mutual recognition between TPA molecular propellers and ssDNA favour the formation of chiral superstructures. [10,11] The amplification of chirality in the solution is highly dependent of the length and the nature of the ssDNA. These results may be rationalized by a mutual compensation of the total charge of ssDNA by a finite ratio of TPA molecules. This will lead easily to a simple method to determine the number of bases or the length of the ssDNA. We are confident that this strategy can be used to detect small fragments of DNA which are not showing otherwise any peaks in the CD spectra. Moreover, the intensity of the CD signals depends on the nature of the bases of the ssDNA leading to easy selective detection of specific fragments of DNA containing different homo- or heterobase sequences. Finally, the interactive/signalling properties of TPA propellers appear compatible with the general requirements for a supramolecular ruler[12] used to determine the number of base pairs of short ssDNAs with remarkably accuracy.

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