

# ssPNA templated assembly of oligo(*p*-phenylenevinylene)s†

Pim G. A. Janssen,<sup>a</sup> Nico Meeuwenoord,<sup>b</sup> Gijs van der Marel,<sup>b</sup> Sara Jabbari-Farouji,<sup>c</sup> Paul van der Schoot,<sup>c</sup> Mathieu Surin,<sup>d</sup> Željko Tomović,<sup>a</sup> E. W. Meijer<sup>a</sup> and Albertus P. H. J. Schenning<sup>\*a</sup>

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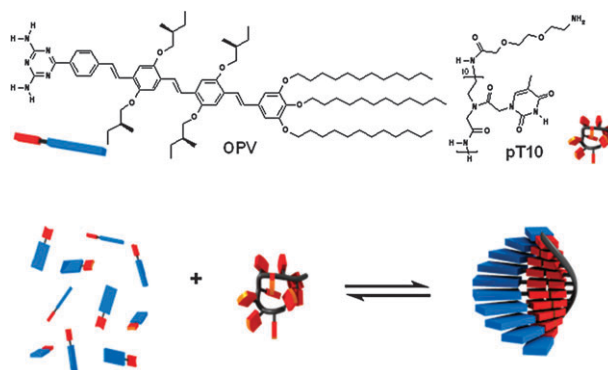
**A single-stranded oligothymine peptide nucleic acid (PNA) was used as a template for the assembly of a chiral oligo-(*p*-phenylenevinylene) diaminotriazine derivative (OPV) in methycyclohexane (MCH) revealing nanostructures in which the size is controlled by the template.**

In nature, templates with specific binding sites are used to efficiently form assemblies and polymers with definite size or sequence.<sup>1</sup> This behavior has inspired many researchers to exploit templated polymerization<sup>2,3</sup> as a tool to control the size and sequence of synthetic polymers *via* a ‘bottom-up’ approach.<sup>4–8</sup> Especially oligonucleotides are interesting building-blocks, since they can be obtained monodisperse, functionalized and used to create predefined nanosized structures *via* sticky-end cohesion.<sup>9</sup>

In a previous study, we showed that the single-stranded deoxyribonucleic acid (ssDNA) oligothymine can act as a template for the assembly of complementary diaminotriazine equipped guest molecules in water.<sup>7</sup> In this construct, the single DNA strand templates a supramolecular strand of chromophores held together by  $\pi$ - $\pi$ , hydrophobic and hydrogen bond interactions. The efficiency of this templated assembly depends on the host–guest and guest–guest interaction and can be described by a templated assembly model based on a one-dimensional Ising model.<sup>7a</sup> The use of DNA as template requires water as solvent<sup>10</sup> and therefore the variety of guest molecules is limited. In order to broaden the scope of this templated approach to organic solvents, we now report on the use of a single-stranded peptide nucleic acid (ssPNA), consisting of 10 thymine residues (pT10, ‡Scheme 1), as a template for the assembly of a chiral  $\pi$ -conjugated oligo-(*p*-phenylenevinylene) diaminotriazine derivative<sup>11</sup> (OPV, Scheme 1) in MCH. PNA<sup>12</sup> is an achiral and uncharged analogue of DNA in which the phosphate backbone is replaced by an *N*-(2-aminoethyl)glycine backbone, making it soluble in a range of organic solvents. We have previously

shown that OPV forms hydrogen bonded hexamers that subsequently self-assemble into helical fibers in heptane.<sup>11a</sup> Here, we describe the non-templated self-assembly and pT10 templated assembly process of OPV studied by means of temperature-dependent UV-vis absorption and CD spectroscopy. The assemblies were visualized with atomic force microscopy (AFM).

The synthesis of OPV<sup>11a</sup> and pT10<sup>13</sup> were performed according to literature procedures. We have first investigated the non-templated self-assembly of OPV. In chloroform, OPV is molecularly dissolved and has an absorption maxima  $\lambda_{\text{max}}$  at 430 nm. In MCH<sup>14</sup> at 323 K, OPV (100  $\mu\text{M}$ ) is molecularly dissolved since a similar absorption maximum is found. Upon cooling to 263 K, hypochromicity, a red shift of the onset of the absorption, and an absorption maximum shifting from  $\lambda_{\text{max}} = 430$  to 440 nm (Fig. 1a) are observed.<sup>15</sup> Simultaneously, at low temperatures, a positive Cotton effect is observed with a zero-crossing at  $\lambda_{z-c} = 434$  nm,<sup>16</sup> indicating that OPV self-assembles into right-handed helical aggregates, similar as earlier observed in heptane.<sup>11</sup> The non-templated self-assembly process has been studied in more detail by monitoring the UV absorption at  $\lambda = 500$  nm as a function of temperature at different concentrations. The self-assembly is fully reversible and the observed exponential transition is indicative of a cooperative non-templated self-assembly process.<sup>11,17</sup> By fitting both the concentration- and the temperature-dependent self-assembly data to the cooperative self-assembly model,<sup>17</sup> the enthalpy of binding ( $\Delta H_e \approx -75 \pm 8$  kJ mol<sup>-1</sup>) was determined (Fig. 1e and f).<sup>17</sup> To visualize the OPV assemblies, a MCH-solution has been drop-cast onto graphite (HOPG). AFM micrographs show the



**Scheme 1** Molecular structures of the host template pT10 and the guest OPV and a schematic representation of ssPNA templated self-assembly (in blue and red OPV, and in black and red the PNA template).

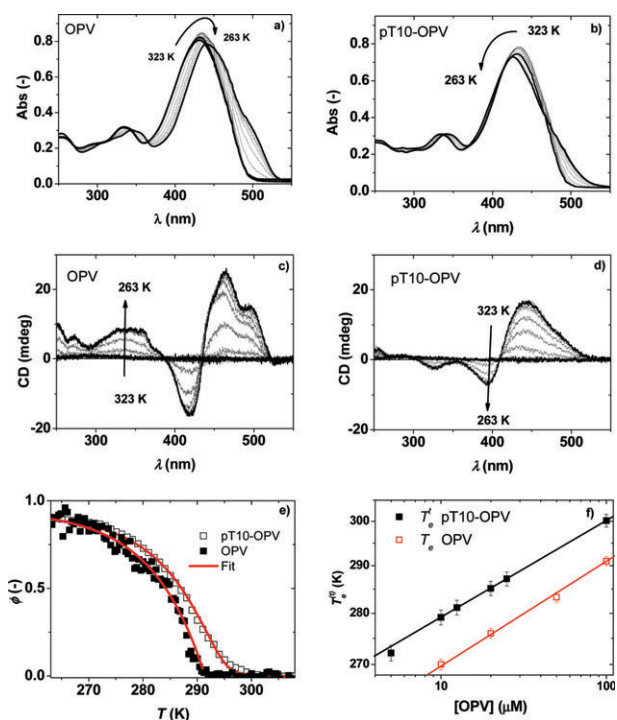
<sup>a</sup> Laboratory for Macromolecular and Organic Chemistry, Eindhoven University of Technology, P. O. Box 513, 5600 MB Eindhoven, The Netherlands. E-mail: a.p.h.j.schenning@tue.nl; Fax: +31 40 245 1036; Tel: +31 40 247 2655

<sup>b</sup> Laboratory of Bio-organic Synthesis, Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, 2300 RA Leiden, The Netherlands

<sup>c</sup> Group Theory of Polymers and Soft Matter, P. O. Box 513, 5600 MB Eindhoven, The Netherlands

<sup>d</sup> Laboratory for Chemistry of Novel Materials, University of Mons-Hainaut, B-7000, Mons, Belgium

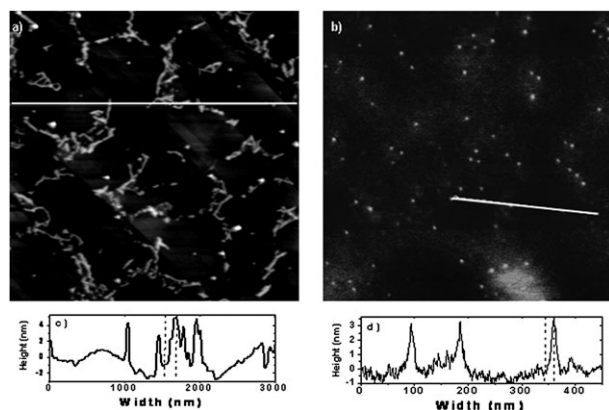
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**Fig. 1** Absorption and CD spectra at temperatures between 323 and 263 K for **OPV** (a and c, respectively) and **pT10-OPV** (1 : 10) (b and d, respectively) mixtures in MCH. (e) The self-assembled fraction upon cooling of **OPV** and **OPV-pT10** (10 : 1) mixtures and the fits to the cooperative self-assembly model<sup>17</sup> and templated assembly model,<sup>7a</sup> respectively. [**pT10**] = 10  $\mu\text{M}$ , [**OPV**] = 100  $\mu\text{M}$ . (f)  $T_e^t$  and  $T_e^i$  (inverted scale) as a function of [**OPV**] (logarithmic scale) for **OPV** and **pT10-OPV** (1 : 10) mixtures.

formation of fibers with a 4–6 nm height (Fig. 2a, c). This height corresponds well with the diameter of the fibers consisting of hexameric H bonded rosettes earlier reported for **OPV** in heptane.<sup>11a</sup>

To investigate the PNA templated assembly of **OPV**, a base-equivalent of **pT10** was added to a 200  $\mu\text{M}$  solution of **OPV** in chloroform at 263 K. No Cotton effect and spectral changes were observed in the **OPV** absorption region indicating that there is no interaction between **OPV** and **pT10**. To increase the



**Fig. 2** Atomic force micrographs of (a) **OPV** ( $3 \times 3 \mu\text{m}$ ) and (b) **pT10-OPV** ( $1 \times 1 \mu\text{m}$ ) solutions drop-casted on HOPG at 273 K and the corresponding height cross-sections below. [**pT10**] = 10  $\mu\text{M}$ , [**OPV**] = 100  $\mu\text{M}$ .

host–guest and guest–guest interaction in the templated PNA assembly, MCH was used as a solvent. In this solvent **OPV** itself already forms self-assembled fibers (*vide supra*) which have to be less stable than the proposed **OPV-pT10** constructs. When **OPV** is mixed with a base-equivalent of **pT10** in MCH at 323 K and cooled down to 263 K, hypochromicity is accompanied by a blue shift of  $\lambda_{\text{max}}$  to 425 nm and a red shift of the onset (Fig. 1b). Compared to the non-templated **OPV** self-assembly, the **OPV-pT10** mixture has a lower intensity of the Cotton effect (Fig. 1d). Furthermore, the zero-crossing of the Cotton effect  $\lambda_{z-c}$  is 410 nm<sup>16</sup> for the **pT10-OPV** mixture, while for the **OPV**,  $\lambda_{z-c}$  is 434 nm (Fig. 1b, c). This indicates that **OPV** is differently organized when the template **pT10** is present.<sup>18</sup>

The templated assembly process has been studied in more detail by monitoring the UV-vis absorption at  $\lambda = 500 \text{ nm}$  as a function of temperature at different concentrations and compared to the non-templated self-assembly. The transition temperatures, below which the two types of self-assembly set in, are defined as the *elongation temperature*  $T_e^t$  for non-templated self-assembly and as the *apparent elongation temperature*  $T_e^i$  for templated assembly.<sup>7a</sup> For a similar concentration, the  $T_e^i$  of the **pT10-OPV** mixtures is higher than the  $T_e^t$  of **OPV** (Fig. 1e and f), showing that the **pT10-OPV** assemblies are more stable than the non-templated self-assemblies of **OPV**. When fitting the temperature-dependent data to the templated self-assembly model as described previously,<sup>7a</sup> an enthalpy of  $\Delta H_e^t \approx -90 \pm 10 \text{ kJ mol}^{-1}$ , a guest–guest interaction energy of  $\varepsilon = -6.2 \pm 0.5 \text{ kT}_p$  was obtained.<sup>19</sup>

The enthalpy values extracted for the templated and non-templated assembly processes suggest that the higher stability of the templated assembly as indicated by the higher melting temperature is due to its larger enthalpy gain. A necessary condition for the predominance of templated assembly over self-assembly is that the free-energy change resulting from the combined effects of host–guest and templated guest–guest interaction molecules is larger than the free-energy gain from the stacking of guest molecules in self-assembly. As a consequence, the presence of the PNA template effectively suppresses the self-assembly of **OPV** unless a large excess of **OPV** is present in the solution and only then when the **pT10** templates are filled.

To visualize the **pT10-OPV** assemblies, an MCH-solution was drop-casted on graphite (HOPG). In contrast to the sample containing only **OPV** (Fig. 2a), the AFM micrographs of the **pT10-OPV** mixture show uniform small particles with a height of 3–4 nm and a deconvoluted width<sup>20</sup> of 5–10 nm (Fig. 2b, d).<sup>7a</sup> The expected dimensions of the **pT10-OPV** complexes are  $\sim 4 \times 4 \times 4 \text{ nm}$  (the length of **pT10** and **OPV** are 3.6 and  $\sim 4 \text{ nm}$ , respectively) and correspond to the size of the objects observed, revealing that the PNA-template controls the size of the **OPV** assemblies.

In conclusion, PNA-templated assemblies have been constructed in MCH of which the size is controlled by the PNA template. This PNA-templated approach can in principle be applied to any functional molecule and makes it possible to construct size-controlled functional nanostructures in organic media.

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## Notes and references

‡ **pT10**:<sup>13</sup> MALDI-TOF MS ( $M = 2823.5$ )  $m/z = 2824.6$  [ $M + H^+$ ]. For experimental details and general methods see ref. 7a. Sample preparation: **pT10** and **OPV** were dissolved in chloroform. After solvent removal and intensive drying, MCH was added to obtain the appropriate concentration and the solution was heated to 333 K to dissolve both components and slowly cooled. For atomic force microscopy, 2  $\mu$ l of the solution at 273 K was drop-cast on freshly cleaved HOPG and allowed to dry in air.

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