Host–Guest Systems

DOI: 10.1002/ange.200903507

pH-Switchable Helicity of DNA-Templated Assemblies**

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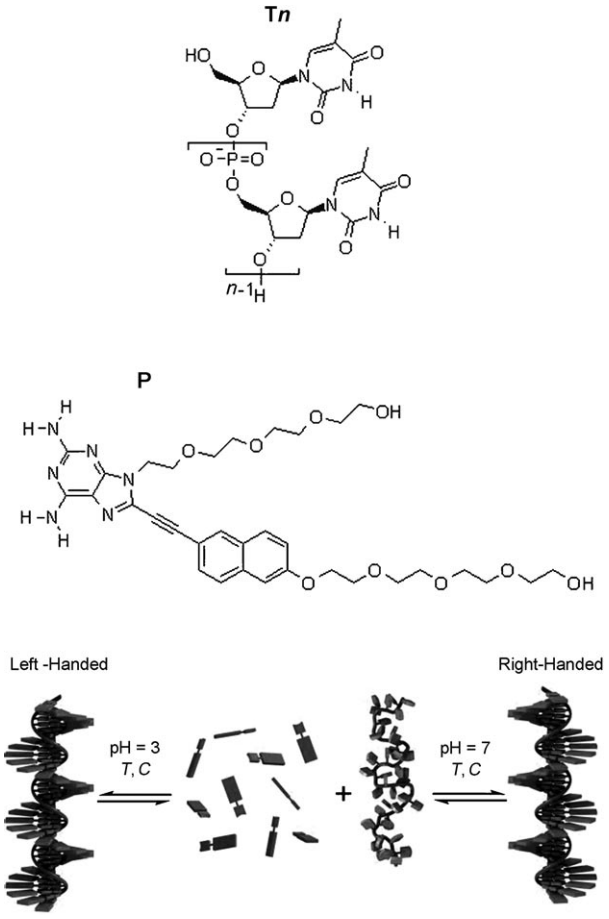
DNA is among one of the most promising building blocks to construct functional nanostructures with geometrical, size, and positional control.[1–3] This biomolecule has been used as a template for the self-assembly of nanoparticles,[4] chromophores,[5–7] and lipids,[6] while predefined complex nanosized structures have been created by sticky-end cohesion.[8] Previously, we reported on the templated self-assembly of diaminotriazine-equipped naphthalene and oligo(*p*-phenylenevinylene) guest derivatives selectively binding to an oligothymine template (**T*n***, where *n* is the number of thymine units) by hydrogen bonding.[6] In these constructs the chiral single-stranded (ss) DNA host templates a supramolecular strand of achiral chromophores, yielding a right-handed organization of the dye guests (Scheme 1). The efficiency of this templated self-assembly depends on the host–guest and guest–guest interactions and can be described by a templated assembly model based on a one-dimensional Ising model.[6a] To enhance this efficiency, we have now synthesized an achiral naphthalene guest derivative (**P**) equipped with a diaminopurine[9] hydrogen-bonding unit, having a larger π surface than diaminotriazine, that binds to the oligothymine **T*n*** template (Scheme 1). Unexpectedly, the helicity of this DNA-templated assembly can be switched by changing the pH value as a result of protonation of the guest (Scheme 1). Helix reversals have been observed for double-stranded (ds) DNA in which cation binding to the phosphate backbone causes a transition from right-handed B-DNA to left-handed Z-DNA.[10,11] Stereomutation has also been reported in synthetic and supramolecular helical polymers, in which the helicity is controlled by temperature, pH value, solvent, light, or chiral stimuli.[12] Switching the helicity of DNA-templated assemblies, however, has not been reported to date.[13]



Scheme 1. The molecular structures of the host template **T*n*** and the guest (**P**) and schematic representation of pH-switchable helicity of the ssDNA-templated self-assembly. (*T* = temperature and *C* = concentration).

The diaminopurine naphthalene guest molecule (**P**) was synthesized by a convergent route and fully characterized.[14] Temperature- and concentration-dependent UV/Vis measurements show that **P** is molecularly dissolved in 100 mM phosphate buffer at pH 7 until a concentration of at least 1 mM. The absorption spectrum shows maxima at $\lambda_{max} = 221$ and 345 nm, with a vibronic shoulder at 370 nm (Figure 1a). When **P** is mixed with **T40** in an 80:1 ratio,[15] a slight blue shift and hypochromicity of the absorption band is observed (Figure 1a). Simultaneously, a positive Cotton effect in the naphthalene absorption region with the zero-crossing at $\lambda_{z\text{-}c} =$ 338 nm is detected (Figure 1b), thus revealing that the **P**

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[**] We acknowledge X. Lou for the MALDI-TOF spectra, M. van Genderen, M. Surin, P. van der Schoot, S. Jabbari-Farouji, and G. van der Marel for the scientific discussion, K. Pieterse for the artwork, and the EURYI scheme for the financial support. The research of D.G.R. was supported by a Marie Curie Intra-European Fellowship. The research of A.R.C. was supported by a MEC-FPU Spanish predoctoral grant.

Supporting information for this article, including full experimental details (general methods, materials, synthetic route and characterization of **P**, sample preparation, and additional figures), is available on the WWW under http://dx.doi.org/10.1002/anie.200903507.

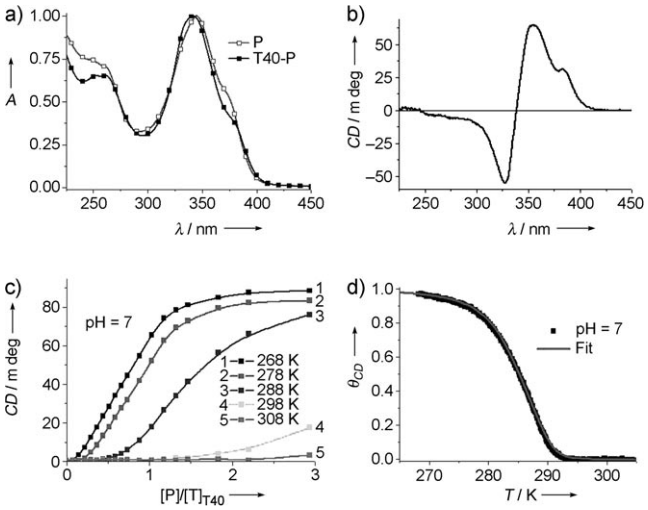

Figure 1. a) Normalized UV/Vis spectra of **P** and **T40–P** complex at pH 7. [**P**] = 0.25 mM and [**P**] = 2[**T**]$_{T40}$ = 0.5 mM at 268 K, respectively. b) CD spectrum of **T40–P** at 268 K and pH 7. c) CD intensity at $\lambda = 354$ nm upon titration of **P** to **T40** at pH 7 at different temperatures. [**T**]$_{T40}$ = 0.25 mM. d) CD cooling curve at $\lambda = 354$ nm of a **T40–P** mixture at pH 7 and fit to the templated self-assembly model.[6a] [**P**] = 2[**T**]$_{T40}$ = 0.5 mM.

guests are arranged in a right-handed helix.[6a] As other homo-ssDNA strands (**dA40**, **dC20**; d = deoxy) using similar conditions are not able to induce a Cotton effect in the naphthalene absorption region (data not shown),[16] it is concluded that **P** binds to **T40** through Watson–Crick type H-bonding similar to the previously studied diaminotriazine derivatives.[6] A Hoogsteen type pair instead of a Watson–Crick type pair is unlikely, as both possible Hoogsteen arrangements are sterically hindered.[17] A CD titration experiment in which **P** is added to **T40** at 268 K (Figure 1c) shows an inflection point at one equivalent of [**P**]/[**T**] (where [**T**] is the thymine concentration), thus indicating that **P** binds to **T40** in a 40:1 ratio. At higher temperatures, the degree of binding is lower (Figure 1c).

The fraction of occupied template binding sites θ for the **T40–P** mixture ([**P**] = 2[**T**]$_{T40}$ = 0.5 mM) was determined by monitoring the CD spectroscopic changes as function of temperature (Figure 1d). The temperature at which the templated self-assembly starts is defined as T_e^t (Figure 4d, see below).[6] By fitting the temperature-dependent CD data to the templated self-assembly model (Figure 1d),[6a] a guest–guest interaction energy $\varepsilon \approx -6.4\,kT_p$ ($= -15.2$ kJ mol^{-1} at $T_p = 285$ K) is found. When fitting the titration of **P** with **T40** to the templated self-assembly model, a host–guest interaction $g \approx -7.5\,kT$ ($= -17.3$ kJ mol^{-1} at $T = 278$ K) is calculated. For the previously studied diaminotriazine naphthalene derivative binding to **T40**, the values are $g \approx -9\,kT$ and $\varepsilon \approx -5.3\,kT_p$.[6a] Compared to this system, **P** has a higher ε and hence, a longer correlation length.[6a] This situation is possibly due to the larger π-conjugated surface of the diaminopurine compared to the diaminotriazine H-bonding unit. In contrast, the host–guest interaction (g) is weaker, explaining the poorer overall stability compared to the reported templated self-assemblies of the diaminotriazine naphthalene derivative.[6a] These results show that optimizing the templated self-assembly is a delicate process in which the guest–guest and host–guest interactions need to be balanced.

Remarkably, when the pH value of a **T40–P** (1:80 molar ratio) solution at 268 K is changed from 9 to 2 the Cotton effect is reversed, revealing that a left-handed hybrid DNA complex has been formed at low pH values.[18,19] The zero-crossing of the Cotton effect ($\lambda_{z\text{-}c}$) is blue-shifted from 338 to 333 nm, and the UV absorption maximum (λ_{max}) shifts from 342 to 337 nm (Figure 2). The change in the UV/Vis spectrum and the sign change in the Cotton effect take place at the same pH value (pH ≈ 5, Figure 3a).[14]

To shed some light on this pH-induced helix reversal, the pK_a of **P** was determined by monitoring the UV/Vis absorption as a function of the pH value.[20] The λ_{max} of **P** at 221 nm at pH 9 (Figure 3a) shifts to 213 nm for **P** + H$^+$ at pH 2. The found pK_a of approximately 4.8 is in agreement with the values found in literature for related diaminopurine derivatives (see below).[21,22] For the absorption maximum at $\lambda_{max} = 345$ nm a similar but less pronounced pH-dependent transition is observed.[14]

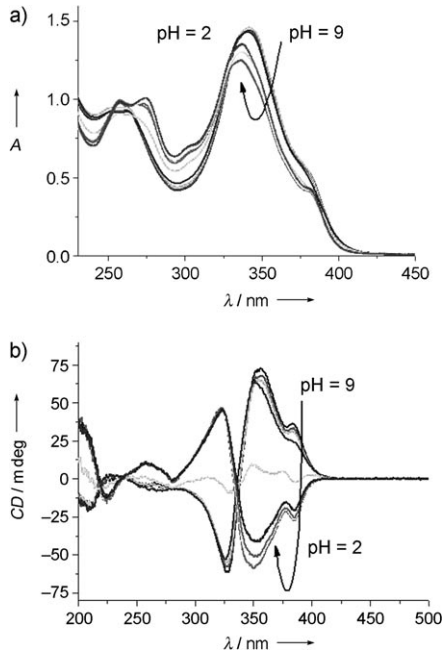

Figure 2. a) UV/Vis and b) CD spectra of [**P**] = 2[**T**]$_{T40}$ = 0.5 mM at 268 K for pH values ranging from 9 to 2.[20]

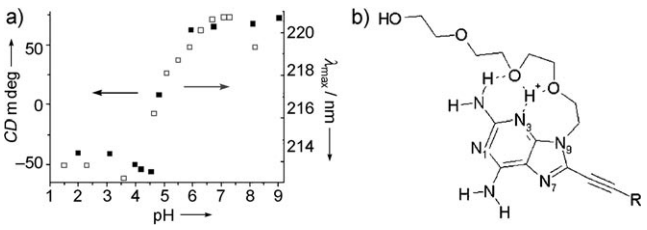

Figure 3. a) CD intensity at 354 nm of [**P**] = 2[**T**]$_{T40}$ = 0.5 mM at 268 K and λ_{max} of [**P**] = 0.25 mM as a function of the pH value. b) Part of the molecular structure of **P** + H$^+$ in which the oxygen atoms of the ethylene glycol unit on N-9 stabilize protonation at N-3.

Based on the data above, the helix reversal occurs at the same pH range as the protonation of **P** (Figure 3a), thus indicating that these processes are related and that the helical switch in our templated self-assembly is caused by protonation of the guest. It is known that in polythymine at pH 3, the thymine units are not protonated and the phosphates are deprotonated.[10] Binding of $\mathbf{P}+H^+$ to the phosphate backbone is also unlikely, since this process is selective for oligothymine and no binding is observed for the other homossDNA strands.[16] Therefore, it seems that the protonated state of **P** controls the helicity of the **T40**–**P** complex while preserving the H-bonding pairing.[16] The order of basicity of the nitrogen atoms in 2,6-diaminopurine is: N-1 ≈ N-3 > N-7.[21] N-1 being occupied in H-bonding to the thymines, protonation is most likely to occur at N-3.[23] Further evidence was taken from the lower pK_a value determined for **P** ($pK_a \approx 4.8$), when compared to other 2,6-diaminopurine derivatives ($pK_a \approx 5.1$),[23] which can be explained by stabilization of $\mathbf{P}+H^+$ through H-bonding with the ethylene glycol unit at the N-9 position,[24] as shown in Figure 3b. Interestingly, related N-9-glycol-substituted 2,6-diaminopurine derivatives also show a small decrease in pK_a value ($pK_a \approx 4.9$).[21,22]

In short, it seems that N-3 protonation of the guest molecules results in a thermodynamically different situation that induces a rearrangement of the **T40**–**P** complex with an inversion of chirality. At low pH values, other forces come into play that may cause the stabilization of a left-handed structure. In particular, attractive electrostatic interactions between the positively charged $\mathbf{P}+H^+$ species and the DNA phosphate backbone may reinforce binding strength (see Figure S5 in the Supporting Information) and reduce the electrostatic repulsion between the phosphates in **T40**. In Z-DNA, for example, both the increase in distance between the stacked bases and the decreased angle of rotation between two base pairs are a result of alternating *syn*- and *anti*-conformations of the glycosidic bonds, caused by less electrostatic repulsion between the phosphates compared to B-DNA, where all glycosidic bonds have an *anti*-conformation.[10] The lower Cotton effect at pH 3 can indicate a stretched and/or narrower, denser structure, originating from an increased distance and/or a decreased angle between the bound guests.[25]

The templated assembly processes at pH 3 was analyzed in more detail by monitoring the CD spectroscopic changes as a function of the temperature. Compared to the results found at pH 7 (Figure 1), a titration at pH 3 of **P** to **T40** shows a sharper inflection at one equivalent of guests (Figure 4b). At higher temperatures the degree of binding is, as expected, lower (Figure 4a), but the inflection point is clearly maintained close to one equivalent, even at 328 K. In addition, although the shape of the melting curve at pH 3 is similar to the one obtained at pH 7, the complex at pH 3 exhibits an increase of T_e^t, the temperature at which the templated self-assembly starts, of about 30 K (Figure 4c,d).[6,14] Interestingly, the variation of T_e^t as a function of the pH value shows again the same transition as the protonation of **P**.[14]

These results indicate that, at low pH values, the protonated guest molecule stabilizes the $\mathbf{T40}$–$\mathbf{P}+H^+$ complex. By fitting the temperature-dependent CD (Figure 4c) and titration measurements at pH 3 (Figure 4b), a guest–guest interaction energy $\varepsilon \approx -6.5\,kT_p$ ($=-16.8$ kJ mol^{-1} at $T_p = 310$ K) and a host–guest interaction $g \approx -9.3\,kT$ ($=-21.5$ kJ mol^{-1} at 278 K) were determined. The guest–guest interaction is similar to the value at pH 7 (see above) while the host–guest is more negative. This result implies an improved stability at pH 3, arising from an additional host–guest interaction, and supports the presence of secondary attractive electrostatic interactions in the $\mathbf{T40}$–$\mathbf{P}+H^+$ complex.

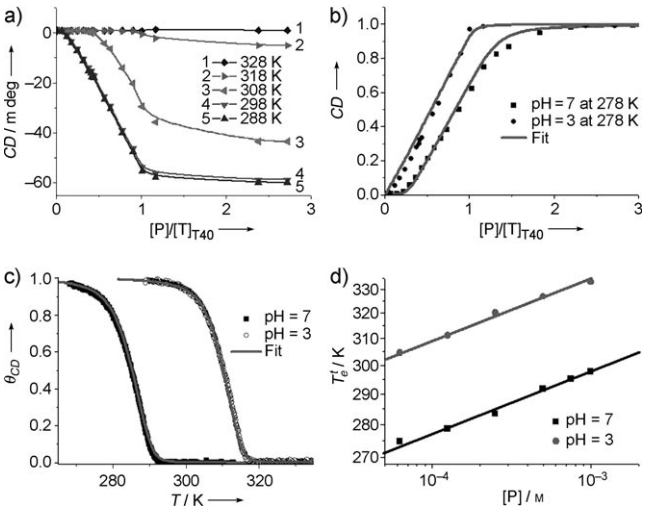

Figure 4. a) CD intensity at $\lambda = 354$ nm upon titration of **P** to **T40** for pH 3 at different temperatures. $[\mathbf{T}]_{T40} = 0.25$ mM. b) Fits of the titration at 278 K at pH 3 and 7 to the templated self-assembly model.[6a] c) CD cooling curve at $\lambda = 354$ nm of a **T40**–**P** mixture at pH 3 and 7 and fit to the templated assembly model.[6a] d) T_e^t (reciprocal scale) as a function of [**P**] (logarithmic scale) obtained from cooling curves of **T40**–**P** mixtures ($[\mathbf{P}] = 2[\mathbf{T}]_{T40}$) at $\lambda = 354$ nm for concentrations between 0.06 and 1 mM at pH 7 and 3.

In conclusion, we have demonstrated that 2,6-diaminopurine guest molecules can bind to oligothymine strands to construct assemblies with a pH-switchable stability and supramolecular helicity. Our studies open unique possibilities to arrange functional molecules equipped with this hydrogen-bonding unit into DNA-templated switchable functional nanostructures.

Received: June 29, 2009
Published online: September 15, 2009

Keywords: chirality · helical structures · host–guest systems · self-assembly · supramolecular chemistry

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[14] See Supporting Information.

[15] Previous studies have shown that more than one equivalent is needed to occupy all template binding sites. We therefore use two base equivalents. See reference [6].

[16] A control experiment, in which the complementary single strand **dA40** is added to **T40–P** solutions at pH 7, shows the rapid formation of the dsDNA (also see Ref. [6b]). In contrast, at pH 3, **dA40** cannot replace the bound **P**. At pH 7 or 3, when **P** is mixed with other homo-ssDNA strands (**dA40** and **dC20**), no Cotton effect is observed in the naphthalene absorption region (data not shown).

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