

# A cooperative beads-on-a-string approach to exceptionally stable DNA triplexes†

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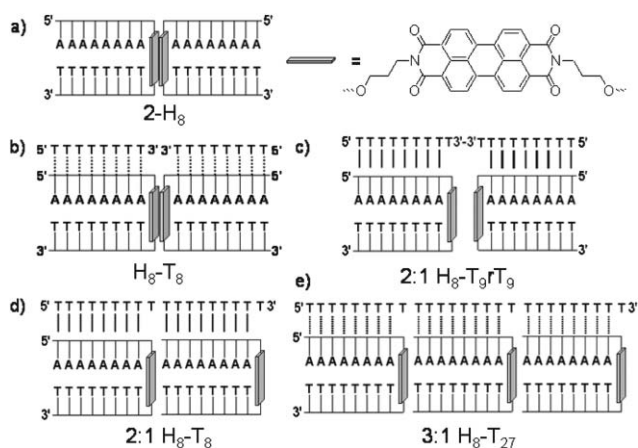
A poly(dT) oligomer can serve as the string on which synthetic hairpins possessing poly(dA) and poly(dT) arms connected by a hydrophobic perylene diimide linker are assembled like beads on a string. The synthetic hairpins form head-to-head dimers and trimers, respectively, in the absence of the poly(dT) string and in the presence of a string with inverted polarity at mid-strand. However, they assemble in cooperative head-to-tail fashion on normal poly(dT) oligomers.

We describe here the assembly of DNA triplexes in which poly(dT) single strands serve as templates for either head-to-head (HH) or head-to-tail (HT) assembly of perylene diimide (PDI)-linked hairpins with poly(dT)–poly(dA) arms. The orientation (HH or HT) is determined by the strand polarity of the poly(dT) template. Head-to-tail assembly provides an unprecedented cooperative “beads-on-a-string” approach to exceptionally stable triplex structures.

Triplex formation has been extensively investigated as an anti-sense strategy for the selective recognition of polypurine–polypyrimidine duplex sequences in duplex DNA by polypurine single strands.<sup>1</sup> Triplexes can also be formed by targeting an appropriate single strand either with two complementary strands or with a single oligonucleotide possessing two base pair domains connected by a short nucleotide linker or by a synthetic linker.<sup>2–4</sup> Research on triplex formation has been motivated by biological applications including gene-targeting.<sup>5</sup> However, as our results will demonstrate, triplex formation can also be employed for self-assembly of novel supermolecular structures.

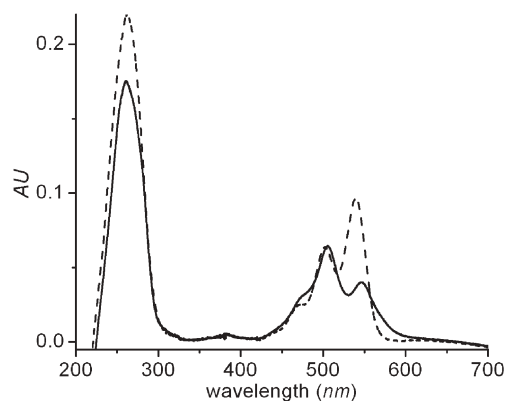
We recently reported that a poly(dA)–poly(dT) hairpin possessing a hydrophobic perylene diimide (PDI) linker forms a stable head-to-head hairpin dimer structure 2-H<sub>8</sub> (Scheme 1a) as a consequence of the hydrophobic association of the PDI linkers.<sup>6</sup> Association and dissociation of PDI can be readily monitored by changes in the relative intensities of the PDI A<sup>0-0</sup>/A<sup>0-1</sup> vibronic band intensities, from *ca.* 1.4 for a PDI monomer to *ca.* 0.6 for a PDI dimer (Fig. 1).<sup>7</sup> The derivatives of thermal dissociation profiles for either the 260 nm base pair absorbance or the PDI A<sup>0-0</sup>/A<sup>0-1</sup> vibronic band intensity ratio for the hairpin dimer 2-H<sub>8</sub> provide the same value for the melting temperature (*T*<sub>m</sub> = 70 ± 0.5 °C), as shown in Fig. 2a.

As a consequence of the inverted relationship of the two sub-units of 2-H<sub>8</sub>, we expected that it would form a 2 : 2 triplex structure with two equivalents of T<sub>8</sub> (Scheme 1b) and a 2 : 1 triplex



**Scheme 1** Structures of the PDI linker, hairpin dimer 2-H<sub>8</sub> (a), and triplexes (b)–(e).

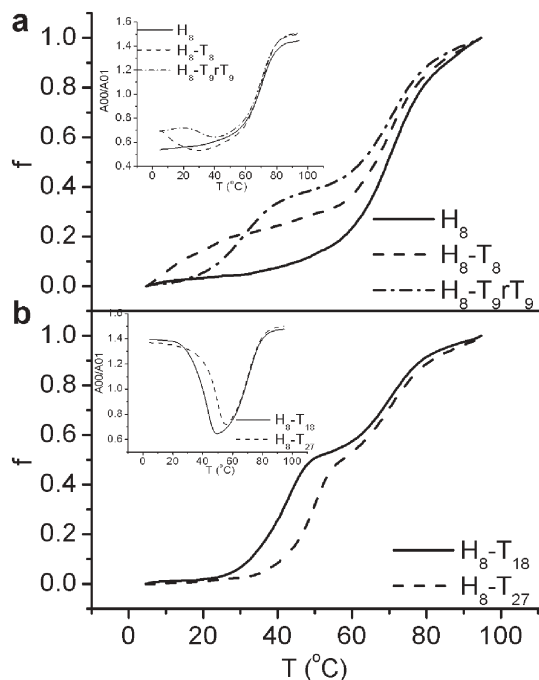
with a single equivalent of T<sub>9</sub>rT<sub>9</sub> (Scheme 1c), an 18-mer with inverted polarity at mid-strand and two extra bases provided to span the PDI dimer region of 2-H<sub>8</sub>. This expectation was confirmed by non-denaturing gels,<sup>8</sup> which display a single band, and by the 260 nm UV thermal dissociation profiles shown in Fig. 2a. The latter have low temperature transitions at *ca.* 22 °C and 31 °C, respectively, assigned to triplex dissociation. This process leaves the hairpin dimer intact, as evidenced by the nearly constant values of A<sup>0-0</sup>/A<sup>0-1</sup> during triplex melting (Fig. 2a inset). The higher temperature transitions are identical to those of 2-H<sub>8</sub>.<sup>6</sup> The CD spectra of 2-H<sub>8</sub> with and without T<sub>9</sub>rT<sub>9</sub> (Fig. 3) are



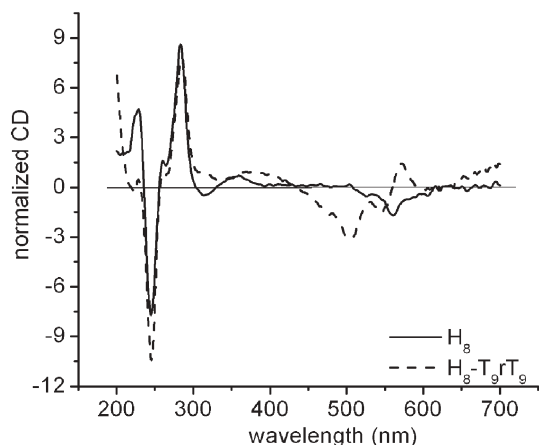
**Fig. 1** UV absorption spectra of a single strand PDI conjugate (—) and the hairpin dimer 2-H<sub>8</sub> (---) in 20 mM Tris HCl, 2 mM EDTA, pH 8.0, 20 mM MgCl<sub>2</sub>, 100 mM NaCl.

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**Fig. 2** Melting curves for (a) the  $H_8$  hairpin dimer,  $H_8$  with  $T_8$  (1 : 1 ratio), and  $H_8$  with  $T_{9rT_9}$  (2 : 1 ratio) and (b)  $H_8$  with  $T_{18}$  (2 : 1 ratio), and  $H_8$  with  $T_{27}$  (3 : 1 ratio). Insets show temperature dependence of the PDI  $A^{0/0}/A^{0-1}$  ratio. All data is for 10  $\mu\text{M}$  conjugates in 20 mM Tris HCl, 2 mM EDTA, pH 8.0, 20 mM  $\text{MgCl}_2$ , 100 mM NaCl.



**Fig. 3** CD spectra of the hairpin dimer 2- $H_8$  and the triplex  $H_8$ - $T_{9rT_9}$  (2 : 1 ratio).

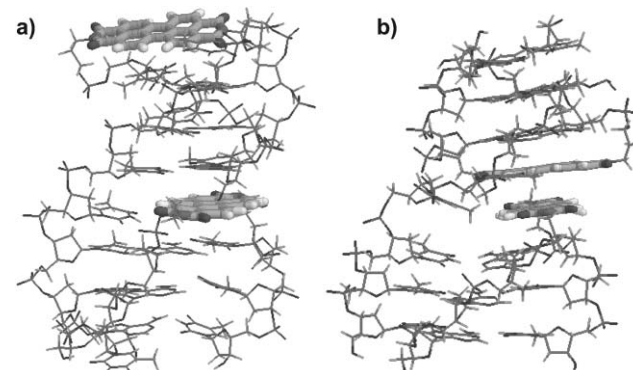
similar to those of poly(dA·dT·dT) and poly(dA·dT), respectively, which differ only in the intensity of the 220 nm band.<sup>9</sup> The CD spectrum of the triplex also displays stronger long-wavelength features assigned to exciton coupling of the two PDI chromophores.<sup>6</sup>

To our considerable surprise,  $H_8$  forms a substantially more stable 2 : 1 triplex with  $T_{18}$  than it does with  $T_{9rT_9}$ , even though  $T_{18}$  cannot form a parallel triplex with the hairpin dimer. The low temperature transition of the 260 nm thermal dissociation profile (Fig. 2b) provides a value of  $T_m = 43$  °C.<sup>10</sup> Moreover, the  $A^{0/0}/A^{0-1}$  ratio at low temperatures has a value nearer to that of the PDI monomer than to that of the dimer (Fig 2b inset). These

observations led us to investigate the properties of a 3 : 1 mixture of  $H_8$  and  $T_{27}$ . The low temperature 260 nm profile provides a value of  $T_m = 51$  °C and the  $A^{0/0}/A^{0-1}$  ratio at low temperatures has a value similar to that for the 2 : 1 mixture of  $H_8$  and  $T_{18}$ . The UV spectral data for  $H_8$  with  $T_{18}$  and  $T_{27}$  is consistent with the formation of triplex structures in which the  $H_8$  hairpins are assembled on the poly(dT) strand in head-to-tail fashion, like beads on a string. The values of  $T_m$  decrease in the order 3 : 1  $H_8$ - $T_{27} > 2 : 1 H_8$ - $T_{18} \gg 1 : 1 H_8$ - $T_8$ , indicative of cooperative head-to-tail binding in the two former structures. We note that the  $T_m$  value for the 2 : 1 triplex  $H_8$ - $T_{18}$  is similar to that reported by James *et al.*<sup>3</sup> for a triplex formed between dT<sub>14</sub> and a hairpin possessing dA<sub>14</sub> and dT<sub>14</sub> arms connected by a hexamethylene glycol linker at similar salt concentrations.

Possible structures for the head-to-tail and head-to-head triplexes were explored by means of molecular dynamics simulations of their structures. Geometries of the parallel triplexes for 2 : 1  $H_8$ - $T_{18}$  and  $H_8$ - $T_{9rT_9}$  were calculated as described for the dimer 2- $H_8$ .<sup>6,11</sup> The Amber 7.0 program suite was used to run molecular dynamics simulations for 2 ns with a step length of 2 fs.<sup>12</sup> Averaged structures obtained from the MD simulations are shown in Fig. 4. The structure of 2 : 1  $H_8$ - $T_{18}$  displays an undistorted parallel triplex geometry in which a carbonyl group from both of the PDI linkers is hydrogen bonded to a thymine from the poly(dT) strand and the internal PDI is  $\pi$ -stacked with the terminal base pair of the other hairpin. We note that hairpins having nucleotide loops would be incapable of forming analogous compact  $n : 1$  triplex structures. In contrast, the triplex structure of 2 : 1  $H_8$ - $T_{9rT_9}$  is distorted in the region of the PDI dimer, the poly(dT) strand displaying a pronounced bulge and the PDI dimer having a large dihedral angle between chromophore long axes. The calculated structures are consistent with both the lower  $T_m$  value and smaller percent hypochromism for the  $H_8$ - $T_{9rT_9}$  triplex *vs.* the  $H_8$ - $T_{18}$  triplex. The opening of PDI-PDI dihedral angle is also consistent with the observation of stronger exciton coupled CD for the triplex *vs.* the hairpin dimer, which has parallel PDIs and hence weak exciton coupling.<sup>6</sup>

The proposed structures for the 2 : 1 and 3 : 1 triplexes (Scheme 1d,e) have terminal PDI chromophores. Hydrophobic association of two terminal PDI's would lead to head-to-head 4 : 2 and 6 : 2 dimers, respectively. Further studies will be needed to determine equilibrium constants for the formation of triplex dimers.



**Fig. 4** Averaged structures for (a)  $H_8$  with  $T_{18}$  and (b)  $H_8$  with  $T_{9rT_9}$  (both 2 : 1 ratios). Structures are truncated to three base triplets.

In summary, we find that PDI-linked hairpins with short poly(dT)–poly(dA) base pair domains can be assembled on a longer single strand poly(dT) thus providing a beads-on-a-string approach to triplex formation. The orientation of the hairpin “beads” is determined by the polarity of the poly(dT) “string”, with head-to-tail orientation resulting in strongly cooperative binding. The thermodynamics and kinetics of triplex formation and dissociation are under continuing investigation as is the formation of higher order structures based on triplex formation.

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## Notes and references

- 1 G. E. Plum, *Biopolymers*, 1997, **44**, 241–256; J. S. Sun, T. Garestier and C. Hélène, *Curr. Opin. Struct. Biol.*, 1996, **6**, 327–333; E. Wang and J. Feigon, in *Oxford Handbook of Nucleic Acid Structure*, ed. S. Neidle, Oxford University Press, Oxford, 1999, p. 355–387.
- 2 S. Bevers, S. Schutte and L. W. Mclaughlin, *J. Am. Chem. Soc.*, 2000, **122**, 5905–5919; M. A. Booher, S. Wang and E. T. Kool, *Biochemistry*, 1994, **33**, 4645–4651; R. W. Roberts and D. M. Crothers, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 4320–4325.
- 3 P. L. James, T. Brown and K. R. Fox, *Nucleic Acids Res.*, 2003, **31**, 5598–5606.
- 4 The use of PDI-linked polypyrimide conjugates to form stable triplexes with a polypurine third strand was reported by Bevers *et al.*<sup>2</sup> However PDI association was not reported.
- 5 C. Malvy, A. Harel-Bellan and L. L. Pritchard, *Triple Helix Forming Oligonucleotides*, Kluwer, Boston, 1999.
- 6 Y. Zheng, H. Long, G. C. Schatz and F. D. Lewis, *Chem. Commun.*, 2005, 4795–4797.
- 7 A. D. Q. Li, W. Wang and L. Wang, *Chem.–Eur. J.*, 2003, **9**, 4594–4601; F. Würthner, *Chem. Commun.*, 2004, 1564–1579.
- 8 Data available as supporting information†.
- 9 K. H. Johnson, D. M. Gray and J. C. Sutherland, *Nucleic Acids Res.*, 1991, **19**, 2275–2280; W. C. Johnson, in *Circular Dichroism, Principles and Applications*, ed. N. Berova, K. Nakanishi and R. W. Woody, Wiley-VCH, New York, 2000, p. 741–768.
- 10 Identical thermal profiles obtained for the 2 : 1 mixture with and without annealing at 100 °C and for 2 : 2 mixtures of H<sub>8</sub> and T<sub>18</sub>.
- 11 W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell and P. A. Kollman, *J. Am. Chem. Soc.*, 1996, **118**, 2309–2309; P. Cieplak, W. D. Cornell, C. Bayly and P. A. Kollman, *J. Comput. Chem.*, 1995, **16**, 1357–1377.
- 12 D. A. P. D. A. Case, J. W. Caldwell, T. E. Cheatham, III, J. Wang, W. S. Ross, C. L. Simmerling, T. A. Darden, K. M. Merz, R. V. Stanton, A. L. Cheng, J. J. Vincent, M. Crowley, V. Tsui, H. Gohlke, R. J. Radmer, Y. Duan, J. Pitner, I. Massova, G. L. Seibel, U. C. Singh, P. K. Weiner and P. A. Kollman, *AMBER 7*, 2002, San Francisco.