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Interstrand communication between 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomers in nucleic acid duplexes: directional control and signalling of full complementarity†

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Very efficient interstrand communication systems in nucleic acid duplexes, based on pyrene excimer formation between 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomers, demonstrate the versatility of functionalized 2′-amino-LNA monomers for Ångström-scale chemical engineering.

An emerging research area within nucleic acid nanotechnology is Ångström-scale chemical engineering.[1–3] Stimulated by this fact, and by the high-affinity hybridization of LNA (locked nucleic acid)[4–6] and 2′-amino-LNA,[7] we introduced N-functionalized derivatives of 2′-amino-LNA.[8] The 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomer **X** (Fig. 1; T = thymin-1-yl) is used herein as a key element in efficient interstrand communication systems in nucleic acid duplexes.

Incorporation† of a single 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomer **X**[8] into a mixed sequence 9-mer does not significantly affect the thermal stability against complementary DNA (Table 1, T_m values for **ON1**+**ON4** and **ON1**+**ON5** relative to **ON1**+**ON2**). Upon excitation at 340 nm, the steady-state fluorescence emission spectra of the singly modified duplexes exhibit structured monomer bands at λ_{max} ~378 nm and ~398 nm, and only very low levels of emission at λ = 430–530 nm, the characteristic pyrene excimer band region[9] (Fig. 2, **ON1**+**ON4** and **ON1**+**ON5**). Insertion of two **X** monomers in a "1+1 downstream zipper" leads to significantly increased thermal stability (Table 1, data for **ON3**+**ON5** relative to **ON1**+**ON5**), an increase in monomer fluorescence intensity, and a distinct band corresponding to excimer fluorescence. Insertion of two **X** monomers in a "1+1 upstream zipper" (**ON3**+**ON4**) induces an increase in monomer intensity but no excimer band formation, indicating a directional preference for interstrand excimer formation (Fig. 2). Interestingly, this is also reflected in the lower thermal stability of **ON3**+**ON4** compared to the "1+1 downstream zipper" (**ON3**+**ON5**). Remarkably increased thermal stability[10] and a very large excimer/monomer intensity ratio (I_E/I_M = 2.05) are observed for the "2+2

Table 1 ONs synthesized, thermal denaturation studies, fluorescence properties and schematic illustration of duplexes

ON1	5′-GTG ATA TGC	**ON7**[8]	5′-GCA **X**A**X** CAC
ON2	5′-GCA TAT CAC	**ON8**	5′-TTT ATA TAT CA^{Me}C^{L}G
ON3[8]	5′-GTG A**X**A TGC	**ON9**	5′-CGT **G**^{L}AT ATA TAA^{L}A
ON4	5′-GCA **X**AT CAC	**ON10**	5′-TT**X** A**X**A **X**A**X** CA^{Me}C^{L}G
ON5	5′-GCA TA**X** CAC	**ON11**	5′-CGT **G**^{L}A**X** A**X**A **X**AA^{L}A
ON6[8]	5′-GTG A**X**A **X**GC		

Duplex	T_m	Excimer	Schematic illustration
ON1+**ON2**	28 °C	–	
ON1+**ON4**	29 °C	–	
ON1+**ON5**	27 °C	–	
ON3+**ON5**	35 °C	+	
ON3+**ON4**	30 °C	–	
ON6+**ON7**	50 °C	+	
ON8+**ON9**	38 °C	–	
ON8+**ON11**	35 °C	–[a]	
ON9+**ON10**	45 °C	–[a]	
ON10+**ON11**	77 °C	+	

Melting temperatures (T_m values) and steady-state fluorescence emission spectra (19 °C ±0.1 °C) were measured in medium salt buffer.† **A**^{L}, **G**^{L} and ^{Me}**C**^{L} (5-methylcytosin-1-yl monomer) denote LNA monomers. A, C, G, and T denote DNA monomers. The "dark drops" denote pyrenylmethyl moieties (monomer **X**).[a] Weak excimer band (see Fig. 2).

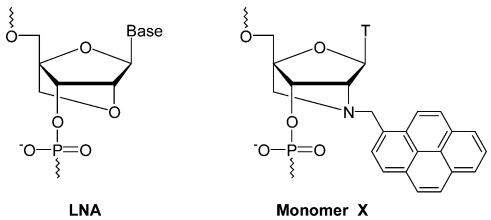

Fig. 1 LNA and 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomer **X**.

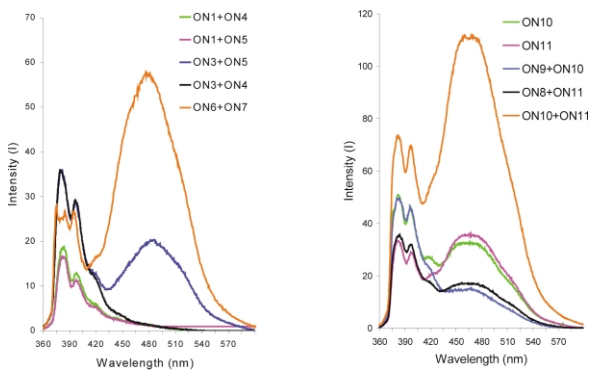

Fig. 2 Steady-state fluorescence emission spectra.†

† Electronic supplementary information (ESI) available: details of thermal denaturation, fluorescence and molecular modelling experiments. Lowest energy structure of **ON8**+**ON11**. Steady-state fluorescence emission spectra (as Fig. 4; positions 11 and 12). See http://www.rsc.org/suppdata/cc/b4/b404446k/

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downstream zipper" (Table 1 and Fig. 2, **ON6+ON7**). To investigate the generality of this system, monomer **X** was incorporated into a mixed sequence 13-mer. The low-intensity excimer band observed for the flexible single stranded oligonucleotides containing three or four **X** monomers alternating with unmodified nucleotides (Table 1, **ON10** and **ON11**) is significantly reduced upon hybridization to complementary DNA (Fig. 2). When **ON10** and **ON11** are combined to form a "4+3 downstream" zipper (corresponding to a "3+3 downstream" zipper with an additional 5′-end-positioned monomer **X**), extraordinary high thermal stability and a very intensive excimer band are observed (Table 1, Fig. 2). Other pyrene-modified nucleic acids have displayed interstrand excimer formation,[11–13] but the thermal stabilization, directional preference, and predictability of the molecular communication system introduced herein are unprecedented.

Molecular modelling of the 13-mer duplex **ON10**+**ON11** was used to rationalize the directional preference for interstrand fluorophore interactions.† The model structure suggests interstrand pair-wise stacking of pyrene rings at the brim of the minor groove in a "downstream" motif (Fig. 3).¶ The pyrenes adopt the appropriate cofacial configuration and interplanar separation (approx. 3.5 Å) suitable for excimer formation.[9] A similar arrangement of pyrene rings has been proposed for (+)-*anti*-benzo[*a*]pyrene diol epoxide modified guanines within an alternating poly(dGdC)–poly(dGdC) duplex.[11] An "upstream" pyrene–pyrene stacking interaction seems precluded due to spatial separation.

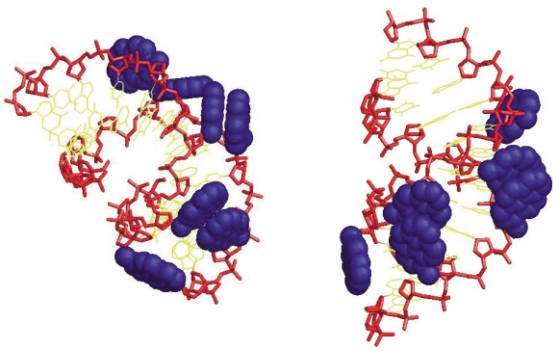

Fig. 3 Two representations of lowest energy structure from molecular modelling of **ON10+ON11** viewed into the minor groove.† For clarity, no hydrogens or bond orders are shown. Coloring scheme: nucleobases, yellow; sugar-phosphate backbone, red; pyrenyl moieties, blue.

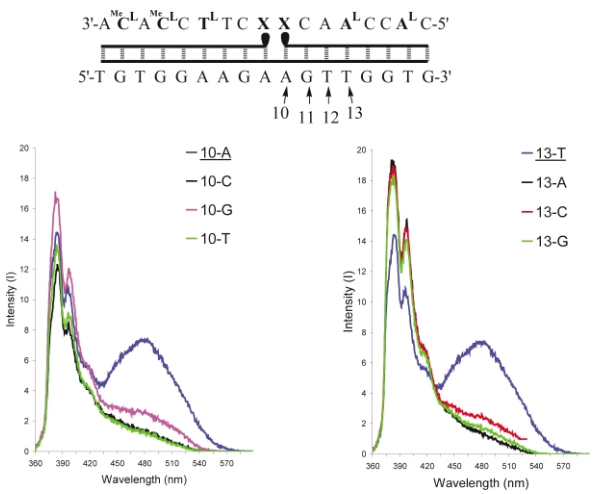

Fig. 4 Steady-state fluorescence emission spectra of pyrene-modified ONs from dual probe assay. Results from the introduction of single mismatches at position 10 (left) or 13 (right).† Curves "10-A" and "13-T" are identical (corresponding to full complementarity).

Pyrene-modified oligonucleotides have been used for mismatch detection,[14–17] and based on the encouraging interstrand communication observed in the first experimental series (Table 1 and Fig. 2), we decided to evaluate the applicability of 2′-*N*-(pyren-1-yl)methyl-2′-amino-LNA monomer **X** using an excimer-forming two-probe hybridization method (Fig. 4, see schematic drawing).[14,15] As seen in Fig. 4, alignment of the pyrenyl units of the two **X** monomers at the 5′-to-3′ junction between two short LNA probes by hybridization to the complementary 17-mer target sequence leads to pyrene excimer formation. Systematic introduction of single mismatches at positions 10, 11, 12 or 13 in the target sequence results, in all cases, in significantly decreased excimer band intensity (Fig. 4; see Supporting Information† for similar data for mismatches introduced at positions 11 or 12). We hypothesize this significant and easily detectable decrease in excimer intensity to be caused by mismatch-induced perturbation of pyrene–pyrene overlapping.‖ Remarkably, by interstrand communication, this dual probe assay efficiently signals full complementarity at various base positions which suggests it to be generally applicable for detection of single nucleotide polymorphisms.

In conclusion, molecular communication systems based on interstrand pyrene excimer formation between 2′-*N*-(pyren-1-yl-)methyl-2′-amino-LNA monomers have been obtained. This is the first demonstration of chemical engineering at the Ångström scale to generate *functional* nucleic acid architectures by use of *functionalized* 2′-amino-LNA building blocks.

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Notes and references

¶ In the model structure, one "pyrene pair" adopts a non-stacking conformation (but with the two pyrene rings in close proximity), while the "unpaired" pyrene ring is suggested to span the minor groove parallel to the edge as a hydrophobic lid (also seen in **ON8**+**ON11**†).

‖ The excimer band, although very weak, observed for the G mismatch in position 10 (see Fig. 4) supports this hypothesis as T:G mismatches are the least discriminating, also for base pairing in an LNA context.[4] We are currently studying thermodynamic and structural aspects of this dual probe assay.

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