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Homo-DNA templated chemistry and its application to nucleic acid sensing†

Matthias Stoop and Christian J. Leumann*

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We have investigated the homo-DNA templated Staudinger reduction of the profluorophore rhodamine azide and have applied this reaction to the detection of natural DNA with a hybrid homo-DNA/DNA molecular beacon. In this system the sensing and the reporting unit are bioorthogonal to each other which facilitates sequence design and increases fidelity.

Homo-DNA (Fig. 1) is composed of 2′,3′-dideoxy-β-D-glucopyranosyl nucleosides linked in a 4′-6′-fashion *via* phosphodiester units. Its properties have been investigated in detail by Eschenmoser *et al.* in the early nineties as part of the quest on why nature chose DNA as the genetic material.[1,2] Although deviating from natural DNA by only one additional methylene group in the sugar ring its structural, biophysical and molecular recognition properties were found to deviate substantially from that of the natural nucleic acids. While Watson–Crick A-T and G-C base-pairing as well as anti-parallel strand alignment in duplexes is maintained there is no cross-pairing between homo-DNA and natural nucleic acids due to intrinsic structural differences.[3] Thus homo-DNA is a bio-orthogonal, DNA-like pairing system.

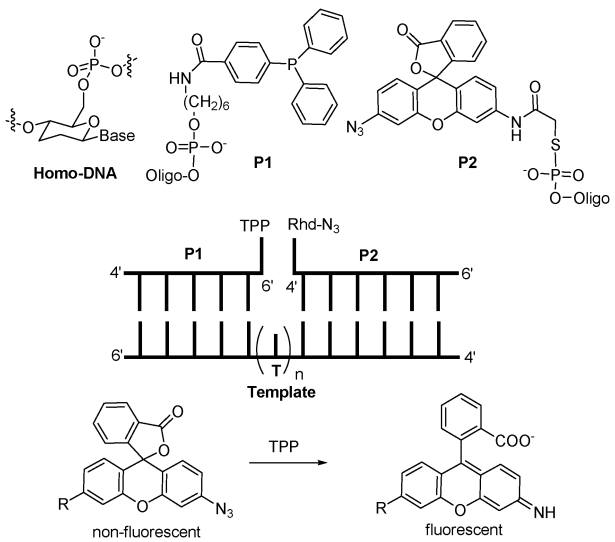

Fig. 1 Homo-DNA templated Staudinger reaction of triphenylphosphine attached to the 6′-end of the oligonucleotide probe **P1**with the profluorophore rhodamine-110-azide attached to the 4′-end of the oligonucleotide probe **P2** on a template.

DNA templated chemistry, originally investigated in the context of enzyme free replication of DNA,[4] has been widely used in areas such as the synthesis of complex libraries of organic small molecules of therapeutic interest,[5,6] or in DNA controlled drug release.[7] An obvious application is in the field of nucleic acid sensing and imaging.[8] Attractive are templated chemical reactions that dequench a fluorophore *via* removal of a covalently attached quencher,[9–12] or chemically convert a profluorophore into a fluorophore.[13–20] An intriguing asset of such templated reactions is that they can occur in a catalytic manner provided that product inhibition is largely reduced.[21] While non-ligating templated chemistries are promising in this context it has also been shown that inclusion of duplex destabilizing elements during ligation may be a viable strategy.[22,23] This dramatically enhances sensitivity as well as signal to noise ratios which is indispensable for detecting low abundance nucleic acid sequences.

While in the case of natural DNA or RNA the sequence to be recognized is at the same time the template, it would be highly desirable to chemically separate the specific nucleic acid recognition event from the signal generation event. Conceptually this should be realizable with chimaeric homo-DNA/DNA oligonucleotides in which the DNA domain is responsible for analyte sensing while the homo-DNA domain is responsible for signal generation. Such a design would enable us to keep the signal generation domain as short as possible which would favor catalysis, while the sensing domain can be as long as necessary to be statistically unique.

We have shown recently that the bio-orthogonal recognition properties of homo-DNA can be exploited to reduce false positive signals in nucleic acid sensing when replacing the stem part of a molecular beacon by homo-DNA.[24] Moreover, careful sequence design of the stem part becomes obsolete because no cross-pairing with the natural nucleic acid matrix is to be expected. In this communication we show that homo-DNA templated catalytic chemical reactions proceed with at least equal efficiency compared to DNA and that this reaction

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland.
E-mail: leumann@ioc.unibe.ch; Fax: +41 31 631 3422; Tel: +41 31 631 4355

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Table 1 Homo-DNA and natural DNA sequences used in the templated Staudinger reductions

Entry[a]	Sequence	Comment
HT1	6′-cat agg cac gtg cc-4′	No gap
HT2	6′-ata ggc tac gtg c-4′	1 t-gap
HT3	6′-cat agg cta cgt gcc-4′	1 t-gap
HT4	6′-cat agg ctt acg tgc c-4′	2 t-gaps
HP1-6	6′-TPP-gcc tat-4′	6-mer
HP2-6	6′-gca cgt-Rhd-N_3-4′	6-mer
HP1-7	6′-TPP-gcc tat g-4′	7-mer
HP2-7	6′-ggc acg t-Rhd-N_3-4′	7-mer
HP3-6	6′-gct cgt-Rhd-N_3-4′	6-mer mismatch
DT1	5′-CAT AGG CTA CGT GCC-3′	1 T-gap
DP1-7	5′-TPP-GCC TAT G-3′	7-mer
DP2-7	5′-GGC ACG T-Rhd-N_3-3′	7-mer
MB1	6′-TPP-agg cac g AAG TTA AGA CCT ATG cgt gcc t-4′	Chimaeric beacon
MBP1	6′-acg tgc ct-Rhd-N_3-4′	Beacon probe
MBI1	6′-NH_2-C6-agg cac gt-4′	Complement to beacon probe
MBT1	5′-T_5-CAT AGG TCT TAA CTT-T_5-3′	Matched target
MBT2	5′-T_5-CAT AGG TAT TAA CTT-T_5-3′	G-A mismatch
MBT3	5′-T_5-CAT AGG TTT TAA CTT-T_5-3′	G-T mismatch
MBT4	5′-T_5-CAT AGG TGT TAA CTT-T_5-3′	G-G mismatch

[a] Capital letters: natural 2′-deoxyribonucleotides; lower case letters: homo-DNA nucleotides; TPP: triphenylphosphine; Rhd-N_3: rhodamine-110-azide; C6: C6 alkyl linker; underlined characters: mismatched bases.

is orthogonal to DNA. In addition we provide an example of specific DNA sensing with a chimaeric homo-DNA/DNA molecular beacon.

To test the potential of homo-DNA templated chemical reactions we focused on a system which consisted of 13–15-mer template strands and two probe strands of which one was equipped with a triphenylphosphine (TPP) unit at the 6′-end (**P1**) and the other with the profluorophore rhodamine-110-azide at the 4′-end (**P2**) *via* suitable linkers (Fig. 1). The template was designed to contain 0–2 additional homo-T nucleosides opposite to the junction site to allow for proper accommodation of the reactive groups. The TPP unit reduces the azide function to an amine in a Staudinger reaction and has been used before in the context of natural nucleic acids.[20] The corresponding sequences used in these experiments are summarized in Table 1.

As can be seen from Fig. 2 the homo-DNA templated Staudinger reaction is inefficient in the absence of any template or with the template containing no t-gap and shows maximum efficiency if one homo-t nucleotide functions as a spacer in the template strand. Variation of the length of the probe strands from 4–7-mers on template **HT2** or **HT3** with one t-gap revealed a direct correlation of signal intensity with T_m which under the conditions of measurement was highest for the 7-mer probes **HP1-7** and **HP2-7** on template **HT3** (T_m of the ternary complex 33 °C, Fig. S1 (ESI†)). The homo-DNA templated reaction is mismatch sensitive. Assembly of **HP3-6** (t–t mismatch) and **HP1-7** on template **HT2** shows substantially reduced fluorescence signal generation compared to the matched pair (Fig. S2, ESI†).

The Staudinger reduction of the homo-DNA probes **HP1-7** and **HP2-7** only occurs on the homo-DNA template **HT3** and is completely suppressed if the natural DNA template **DT1** (one T-gap) is used (Fig. 3). The inverse experiment in which heptameric natural DNA probes **DP1-7** and **DP2-7** were mixed with the homo-DNA or the natural DNA template **HT3** and **DT1**, respectively, leads to complementary results (Fig. S3, ESI†). This clearly shows that no cross-pairing between the two oligonucleotide systems occurs and that they are orthogonal to each other.

We compared the efficiency of the templated reduction in both, the homo-DNA and DNA system. Since the thermal stabilities of homo-DNA duplexes are higher compared to DNA in an equal sequence context we searched for conditions in which both systems exhibited the same T_m. This could be found for the ternary homo-DNA system **HT2** and the probes **HP1-7** and **HP2-7** in a low salt buffer (10 mM KCl) and for the ternary DNA system **DT1** with probes **DP1-7** and **DP2-7** in high salt buffer (500 mM KCl, 50 mM $MgCl_2$). Under these conditions both complexes showed a T_m of 20 °C. The first order reaction kinetics of the templated reduction (k_{obs}) was found to be 8.8×10^{-4} s^{-1} for the homo-DNA system and

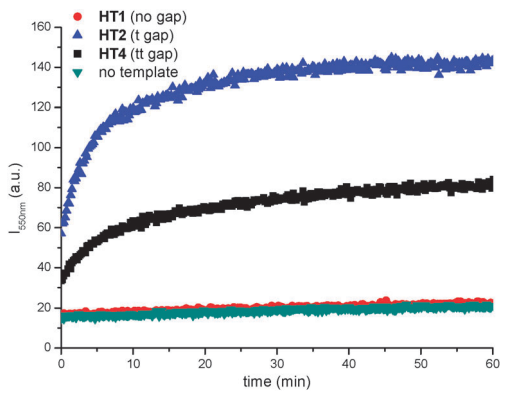

Fig. 2 Time course of fluorescence signal development of **HP1-6** and **HP2-6** (hexamer probes) in the presence of the homo-DNA templates **HT1,2,4** containing no, one or two t-gaps and in the absence of the template. Experiments were performed at 35 °C in 50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0; probe conc: 400 nM; template conc.: 200 nM.

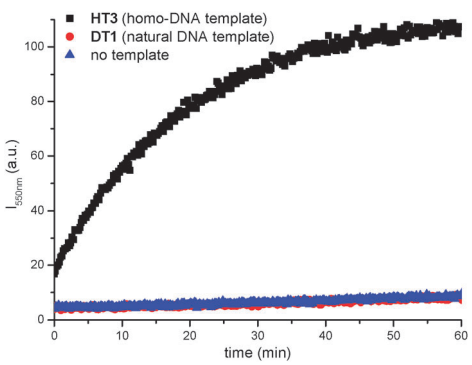

Fig. 3 Fluorescence signal development for the templated reaction of homo-DNA probes **HP1-7** and **HP2-7** in the presence of no template, the homo-DNA template **HT3** and the DNA template **DT1**. Experiments were performed at 16 °C in buffer 2 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 7.1) with HP1-7 (800 nM), HP2-7 (400 nM) and the indicated templates (400 nM).

7.5×10^{-4} s^{-1} for the DNA system and were thus similar to each other. We also investigated whether the reaction is catalytic in template by varying the template concentration at constant probe concentration. The highest turnover numbers (120 min) were found with 1% template and were in the range of 6 (Fig. S4 and S5, ESI†). These values are similar to those found earlier for PNA probes on a DNA template with the same Staudinger reduction,[16] but less than those reported for PNA probes on DNA templates using acyl transfer chemistry.[9] It is therefore likely that the lifetime of the aza-ylide intermediate of the Staudinger reduction which transiently ligates the two probe strands is limiting turnover.

Next we applied this homo-DNA templated chemistry to a molecular beacon[25] format and constructed **MB1**, in which the loop part (15 nt) consisted of DNA and the stem part (7 base-pairs) of homo-DNA (Fig. 4). The 6′-end of the beacon was equipped with TPP and the corresponding probe **MBP1** with the rhodamine azide. In order to avoid unpaired homo-DNA single strands we added **MBP1** as a duplex with its complement **MBI1**, reasoning that this reduces stem strand invasion of **MBP1** in the absence of targets and stabilizes the non-reporting wing by duplex formation.

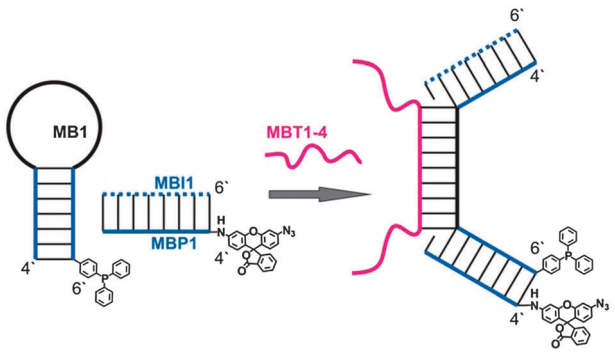

Fig. 4 Schematic layout of the homo-DNA/DNA beacon. In the absence of target the beacon is in its closed form and templated chemistry is suppressed. Upon target binding the small duplex containing the probe **MBP1** and **MBI1** disproportionates and binds to both stem domains of the beacon, enabling the Staudinger reaction.

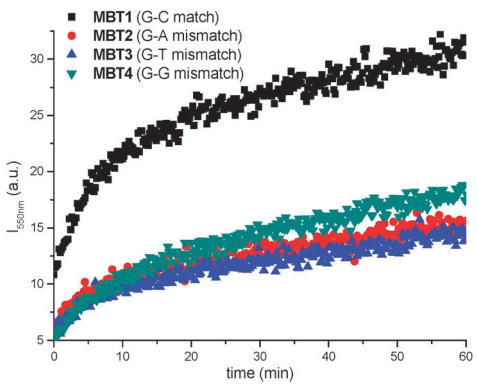

Fig. 5 Fluorescence signal development for the templated reaction of the molecular beacon MB1 with the probe MBP1 in the presence of matched and mismatched DNA targets. Experiments were performed at 35 °C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with MBP1 (200 nM), MB1 (200 nM), MBI1 (200 nM) and the indicated targets (1.3 μM).

As can be seen from Fig. 5 target binding efficiently induces the templated reduction and leads to a fluorescence signal. The reaction is mismatch sensitive. A transition mutation (C → T) as well as transversion mutations (C → A and C → G) in the same position in the center of the loop produces fluorescence signals that are substantially weaker compared to the matched target. We find a match/mismatch signal ratio of 3.5 (t = 20 min) which is slightly better than that of a normal DNA beacon but slightly inferior to that of a LNA beacon under similar conditions.[26]

In conclusion we have demonstrated that homo-DNA templated chemistry is at least as efficient as natural nucleic acid templated chemistry. In addition we have developed a mismatch selective nucleic acid sensor based on a chimaeric homo-DNA/DNA molecular beacon in which the reporting domain is decoupled from the sensing domain. With this each domain can be designed and optimized separately. Moreover, the bioorthogonality of the reporting domain is expected to increase fidelity as it does not interact with the nucleic acid matrix.

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