Supporting Information

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A Light Trigger for DNA Nanotechnology

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Supporting Figure 1: Thermodynamic estimations of melting temperatures and percentage of paired bases for the respective system at the given conditions. Two (identical) oligonucleotides with the sequence used for the sticky ends in minicircle A have a predicted melting temperature below room temperature. Mind that when the complex is part of sticky ends, additional base stacking energies at both ends increase the stability of the complex. The oligonucleotides that correspond to sticky ends in B have, depending on the concentration, a melting temperature that can be slightly over or under room temperature. Also here additional base stacking will lead to a higher effective melting temperature. The melting temperature of the competing hairpin is not concentration dependent as the hairpin formation is an intramolecular process. The melting temperature is probably higher than the one calculated by the IDT calculator.\textsuperscript{[S1]} First, the possibility of a 2 nt hairpin loop is not implemented in the algorithms even though such structures are known to exist.\textsuperscript{[22,23]}

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A Light-Trigger for DNA-Nanotechnology

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Circumventing this limitation by extending the hairpin loop by an extra nucleotide, the melting temperature is now predicted to be 37.2 °C. Secondly, additional base stacking with the duplex region will further increase the stability of the complex. This was simulated by adding two complementary nucleotides at the end of the hairpin. These now increase the melting temperature to 47.8 °C. It is nearly impossible to determine the real melting temperature of this hairpin in the big minicircle system. However we believe for the given reasons that it is well above room temperature and that the hairpin actually formed looks like the one in the orange box. The hairpin would then be kinetically preferred and the homodimers \textit{B-B} cannot form. The melting temperatures of the heterodimeric systems \textit{D} and \textit{E} are around 40 °C and therefore near quantitative complex formation can be expected.

2. Preparative procedures

General procedures: All unmodified oligonucleotides were purchased from Metabion GmbH (Germany) as HPLC purified desalted samples and used without further purification. Caged oligonucleotides were synthesized as previously published, purified by HPLC and characterized by ESI-MS (for sequences see below):

<table>
<thead>
<tr>
<th>sequences</th>
<th>before irradiation</th>
<th>after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>11137.2</td>
<td>10974.0</td>
</tr>
<tr>
<td>8</td>
<td>11754.6</td>
<td>11591.4</td>
</tr>
<tr>
<td>10</td>
<td>11512.5</td>
<td>11349.3</td>
</tr>
<tr>
<td>12</td>
<td>12428.1</td>
<td>12129.8</td>
</tr>
<tr>
<td>14</td>
<td>12765.4</td>
<td>12289.9</td>
</tr>
</tbody>
</table>

For the standard annealing, the DNA strands were mixed in hybridization buffer (50 mM Tris•HCl with 10 mM MgCl₂, pH 7.6), heated to 95 °C for 5 min and slowly cooled down to room temperature. T4 DNA ligase (5 U/µl) and ligation buffer were purchased from Fermentas GmbH (Germany). DNA ligations were performed with 10 U T4 ligase and 1x ligation buffer for 30 min at RT. Heat inactivation of the ligase was then performed by heating the sample to 65 °C for 10 min and leaving it to cool to RT. The ligation products were separated by either native PAGE (5% acrylamide,1x TBE, 11 mM MgCl₂, run at 4 °C) or denaturing PAGE (5% acrylamide,1x TBE, 6 M urea, run at ~50 °C). PAGE gels were stained with SYBR Gold (Invitrogen, Germany) and visualized with a Biostep transilluminator, Dark Hood DH-40/50 (Germany). The single-stranded DNA minicircles were extracted from the gel by shaking in gel extraction buffer (500 mM NH₄OAc, 1 mM EDTA and 10 mM MgCl₂) overnight. Dimer formation was tested in minicircle buffer (40 mM of Tris•AcOH, 12.5 mM MgCl₂, 2.5 mM EDTA, pH 7.5 + 500 mM NaCl where indicated).
Synthesis of single-stranded minicircle: To obtain the single-stranded minicircle, a splint ligation of oligonucleotides 1 (300 pmol) and 2 (300 pmol) with splint I (450 pmol) and splint II (450 pmol) was performed. The oligonucleotides were annealed in 90 µl hybridization buffer and subsequently ligated (see General procedure above). After heat inactivation of ligase at 65 °C for 15 min the minicircle was purified by denaturing PAGE. The denaturing conditions separate the circular products from the linear byproducts. The bands were visualized by UV shadowing, cut out and extracted from the gel with diffusion elution. After an ethanol precipitation the pellet was dissolved in Milli-Q water. The concentration of minicircle was determined by OD_{260}.

1: 5’-TCTAAAAATCTAGAAAAAAGGCGCAAAAAAGTCTCAAAAAACGGGCAA-AATGCTCTAAAAAGCTCGAAAAACTCTCAAAAAATA-3’

2: 5’-TGGCCAAATAGATAAAACGAGCGAAATAAGAGATGAAGCCGAA-AAACCGCGAAAAAGTCTCACACACCGCGCAAAAATG-3’

splint I: 5’-GATTTTTTAGACATTTTTTCGCGTTTTTGAAGACTTTTTTGC-3’

splint II: 5’-TATTGGCCATATTTTTGGAGAGTTTTTTCG-3’
Synthesis of double-stranded minicircles: For the construction of the double-stranded minicircles, the single-stranded minicircle (5 pmol) was annealed with the caged and wt oligonucleotide respectively (7.5 pmol) and with other oligonucleotides (10 pmol) in 45 µl hybridization buffer and then ligated (see General procedure above). For minicircles \( \text{D, D}^{\text{wt}}, \text{E} \) and \( \text{E}^{\text{wt}} \) twice the amount was used. After heat inactivation of ligase at 65 °C for 15 min the reaction was allowed to cool to room temperature and the minicircles were purified by anion exchange chromatography. The peak integrals were determined with the HPLC software and equivalent fractions of mAU/µL were used for combining \( \text{D with E, D}^{\text{wt}} \text{ with E}^{\text{wt}}, \text{D}^{\text{wt}} \text{ with E} \) and \( \text{D with E}^{\text{wt}} \). Half of the total volume of each sample was irradiated at 366 nm (0.5 A) for 5 min, yielding the irradiated samples in the series. For analysis with native PAGE 50 µL of the sample with lowest concentration was taken and corresponding amounts of other samples.
Minicircles A and A\textsuperscript{wt}:

3 (caged): 5ʼ-GCAT\textsuperscript{NPP}CCTCACAGAGTCTTTTTGCGGTTTTTCTA-3ʼ
4 (wt): 5ʼ-GCATGCCTCACAGAGTCTTTTTGCGGTTTTTCTA-3ʼ
5: 5ʼ-ACATTTTTGCCCCGTTTTTGGAGATCTCTGTGAG-3ʼ
6: 5ʼ-CGTTTTTCGGCTTTTTTTATCTATTTTGTCTCAGTTTTTATCTATTGGCCATATTTTTGAGAGTTTTTTAG-3ʼ
7: 5ʼ-GATTITTTAGACATTTTTTGCGGTTTTTGGAGACTTTTTTTCG-3ʼ
Minicircles B and B<sup>wt</sup>:

8 (caged): 5′-TGCATG<sup>NPP</sup>CACTCACAGAGTCTTTTTGCGCGTTTTTCTA-3′
9 (wt): 5′-TGCATGCACTACAGAGTCTTTTTGCGCGTTTTTCTA-3′
5: 5′-ACATTTTTTGCCCCGTTTTTTGTAGTCTCTGGAG-3′
6: 5′-CGTTTTTTCGCGTTTTTCTCTATCTATTTTGTGCTTATTTTGTACAGGAGCTTTTTTAG-3′
7: 5′-GATTATTGTGAACATTTTTTTCGCGTTTTTGTAGAAGCTTTTTTGC-3′
Minicircles D and D\(^{wt}\):

10 (caged): 5'-AC\(^{NPE}\)CTTGCC\(^{NPE}\)ACCTCACAGAGTCTTTTTGCAGTTTTTCTA-3'
11 (wt): 5'-ACCTTGCCACACACAGAGTCTTTTTGCAGTTTTTCTA-3'
5: 5'-ACATTTTTGGGGTTTTTTATCTATTTTTGCCTTITTTTTATCTATTTTG
6: 5'-CGTTTTTTTCCCTTTTTTTTATCTATTTTTGCCTTITTTTTATCTATTTTG
CCATATTTTTGAGAGTTTTTCTAGTTTTTTAG-3'
7: 5'-GATTTTTAGACATTGGTTTTTTGAGACTTTTTTTGCAGTTTTTCTA-3'
Minicircles E and E^wt:

12 (caged):  5′-G(NPP)^TG(G(NPE)^AAG(NPP)^GTCTCACAGAGTCATTTTTCCGCTTTTCTTA-3′
13 (wt):  5′-GTTGGCAAGTCTCAAGAGTCATTTTTCCGCTTTTCTTA-3′
5:  5′-ACATTTTGGGCTTTTTGAGATCTTGAG-3′
6:  5′-CGTTTTTTCGCTTTTTATCTATTTTGGCTCGTTTTTATCTATTTTG
CCATTTTTTGCAGCTTTTTTTAG-3′
7:  5′-GATTTTTAGACATTTCGGCGTCTTTTTGAGACCTTTTTTGG-3′
Minicircles \( C \) and \( C^{\text{wt}} \):

14 (caged): 5′-GG\( ^{\text{NPP}} \)GGTTTCTCACAGAGTCTTTTTGCGCGTTTTTCTA-3′

15 (wt): 5′-GGGGTTTCTCACAGAGTCTTTTTGCGCGTTTTTCTA-3′

16: 5′-ACATTTTTGGCGTTTTTGTAGCTCTGTGAGTTTTGGG-3′

6: 5′-CGTTTTTCTGCGTTTTTTATCTATTTTTGCTCGTTTTTTATCTATTTTGC

7: 5′-GATTTTTAGACATTTTTTGCGCGTTTTTGAGACTTTTTTGC-3′

Diagram of the minicircles with sequences highlighted.
3. AFM experiments

A solution of 30 μL of 0.1 mg polyornithine in 1 mL of minicircle buffer was applied on freshly cleaved mica and incubated for 3 min (the polyornithine solution and the mica were prechilled on ice). The surface was rinsed with Milli-Q water and blown dry with nitrogen. From the stock, minicircles were diluted to a final concentration of 6 to 8 nM with minicircle buffer (+500 mM NaCl where indicated) and incubated at 0 °C. To the pretreated and prechilled mica surface, 1 to 2 μL of the minicircles were added. The minicircles were allowed to absorb to the mica for 1 min, then the surface was rinsed with Milli-Q water and blown dry with nitrogen. Then AFM images were taken on a Veeco Dimension 3100 AFM with a Nanoscope IIIa controller in tapping mode (NY, USA). Images were taken in air with ACT probes (24-75N/m) from APP Nano (Santa Clara, CA, USA). The free amplitude setpoint was set to 0.3 V, typical resonance frequencies of the cantilevers were between 300 and 400 kHz. Scanning frequencies were between 0.5 and 2 Hz. Muscovite mica was purchased from Plano GmbH (Germany), L-polyornithine hydrobromide was purchased from Sigma Aldrich GmbH (Germany).

For the statistic evaluation each sample in the series was imaged several times. A scan of an area of 1.4 μm x 1.4 μm typically contained between 70 and 150 rings which were then evaluated manually and independently by three different people.
4. Uncaging control experiments

Uncaging was performed in 1x PBS with three NICHLA NCCU033 UV-LEDs (365 nm, 100 mW) operated at 0.5 A. The result was analyzed by RP-HPLC (Nucleosil 100-5 C-18, gradient: solvent A = 0.1 M triethylammonium acetate pH 7 + 0.5% MeCN; solvent B = MeCN; 5% B for 2 min, then from to 40% B in 33 min; 1 ml/min). Uncaging of all caged oligonucleotides (50 pmol) was complete within 20 s. See for example the HPLC traces below:

Supporting Figure 2: RP-HPLC analysis of the uncaging of oligonucleotide 14 before irradiation (top) and after irradiation for 20s under the condition stated above (bottom).
5. Measurements of melting temperature

Melting temperatures were measured under the conditions of our previous study[25] (except that the concentration of each oligonucleotide was 0.1 µM) and evaluated in the same way.

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Tm (± 1 °C)</th>
</tr>
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<tbody>
<tr>
<td>11 / 13</td>
<td>42 °C</td>
</tr>
<tr>
<td>10 / 13</td>
<td>29 °C</td>
</tr>
<tr>
<td>11 / 12</td>
<td>&lt; 25 °C</td>
</tr>
<tr>
<td>10 / 12</td>
<td>not detectable</td>
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References: