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A DNA based five-state switch with programmed reversibility†‡

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A programmable switch based on a DNA hairpin loop is functiona-lised with a rigid or flexible porphyrin or FAM and TAMRA FRET pair, which provides insight into the restructuring of the hairpin as well as porphyrin–porphyrin coupling. The switch contains five discrete states which can be accessed independently and followed by real-time spectroscopy, opening the way to a quinary computing code.

The unique self-recognition properties of DNA have been explored extensively for the formation of new self-assembled nano-architectures over the past decades. By taking the DNA out of its biological context, the emerging field of DNA nanotechnology is becoming increasingly attractive to advance research in drug delivery, autonomous machines or computing.1 Additional functionalities such as redox active metal complexes2 or organic chromophores3 are increasingly being incorporated into DNA, resulting in operational DNA based nano-devices. Still, the incorporation of chemically modified DNA into these nano-architectures is in its infancy, and the exploration of modified DNA as building blocks remains an important aspect to understand their behaviour and suitability for DNA nanotechnology. In particular, organic chromophores4 such as pyrenes5 and porphyrins6 are gaining increasing attention as DNA modifiers e.g. for the creation of photonic wires, or as diagnostic tools since their optical properties (absorption, emission) can vary with the DNA sequence or a change in the environment such as pH, temperature or secondary structure.

Particularly intriguing structures arise when partially self-complementary DNA strands assemble to form intramolecular hairpin loops. The loops can be opened and closed through sequential addition of suitable complementary DNA strands, thus creating moving parts within a DNA nanostructure. The concept has been used in molecular beacons for DNA analysis,7 in switchable DNA nanostructures with optical responses,8 and in autonomous DNA walkers.9 Here, we report a programmable switch based on a molecular beacon, where the DNA is partially self-complementary with repeating ATTA–TAAT sequences (Fig. 1). An additional 13 base sequence allows for specific recognition of complementary strands including various repeats of the complementary ATTA–TAAT box, thus enabling controlled elongation or contraction of the stem region. To demonstrate functionality, FRET pairs were attached to the extremes of the repeat region, giving access to a tunable energy transfer system with well-defined chromophore distances from the same DNA strand by simply adding the appropriate complementary strand. The porphyrin based FRET system (denoted 1P) comprises of a zinc porphyrin (donor, D) and a free-base porphyrin (acceptor, A), where the porphyrins are attached either via a rigid alkynyl linker or a more flexible propargyl-amide linker.10 The rigid alkynyl linker ensures that the chromophores have a low diffusional mobility and remain in a well oriented environment, particularly in terms of the transition dipole moment, introduced in order to study the influence of the porphyrin distance and angle on the electronic coupling compared to the flexible linker. As a control system, we have also synthesised the analogous FAM and TAMRA labelled switch strand (denoted 1F). Because this FRET pair is tethered via a longer and more flexible linker, the angular dependence on the FRET efficiency should in ideal cases be eliminated (κ = 2/3).

The Tm values of the two extreme complexes (the full length duplex 1P2 and the hairpin with the longest possible stem of

Fig. 1 Schematic of the adjustable hairpin loops with DNA sequences. The red and blue markers indicate attachment of FRET pairs.
The fluorescence melting shows the same transition as well as formation of the full length duplex in 1F-2. This confirms both the complete denaturing at higher temperature as well as formation of the full length duplex in 1F.

The FRET efficiencies for both the Zn- and 2H-porphyrin and the FAM and TAMRA system were measured and compared, in order to evaluate the difference between the FRET pairs where the chromophores are attached via a rigid or flexible linker. Since energy transfer efficiencies can vary greatly with probe diffusion and reorientation, the linker moiety plays an important role. The FRET parameters of the two FRET pairs (quantum yield, extinction coefficient and spectral overlap) were identified by steady state fluorescence spectroscopy.‡ The FRET efficiencies (E_FRET) were determined by spectral decomposition of the combined donor and acceptor emissions at an excitation wavelength of 426 nm. The quantum yield of Zn-P was determined to be Φ = 0.12 (using quinine sulphate as the standard) which in combination with a spectral overlap integral of J = 2.3 × 10^14 M^(-1) cm^(-1) nm^4 yields a Förster distance of R_0 = 28.4 Å. The Förster distance of the FAM and TAMRA pair was determined to be R_0 = 57 Å. In addition, the FRET efficiencies were predicted theoretically for all systems using a custom made FRET simulation program (FRET matrix).12 Here, all DNA conformations were simulated as rigid B-form geometries and a full atomistic description of the tethered dyes was used to calculate the theoretical donor–acceptor distances.‡

The experimental FRET values of the 1F-x combinations are in excellent agreement with the theoretically predicted values (Fig. 2b) and show a decreasing FRET efficiency upon increasing the fluorophore distance. Exceptions are the states 1F and 1F-6 showing a smaller measured FRET efficiency compared to that expected from the calculated donor–acceptor distance. This deviation is often observed for closely spaced dyes and is likely to be a result of direct dye–dye interactions interfering with the FRET process.12 Overall, the good correlation between expected and measured FRET efficiencies demonstrates the well-defined, discrete states of the switch.

Compared to the FAM and TAMRA control system, the porphyrin FRET system shows a very different behaviour. Despite that porphyrins attached to duplex DNA show FRET efficiencies which are dependent on the distance (including a control sequence used to determine the quantum yields of the porphyrin–DNA, see ESIF), the switch sequences all show a

### Table 1 Melting temperatures of the DNA system and FRET efficiencies (experimental and theoretical)

<table>
<thead>
<tr>
<th>DNA system</th>
<th>T_m1P°C</th>
<th>T_m1F°C</th>
<th>FRET FAM–TAMRA</th>
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<tr>
<td>1</td>
<td>61.7</td>
<td>60.0</td>
<td>0.8 (0.98)</td>
</tr>
<tr>
<td>1·2</td>
<td>54.1</td>
<td>59.0</td>
<td>0.00 (0.01)</td>
</tr>
<tr>
<td>1·3</td>
<td>50.8</td>
<td>59.1</td>
<td>0.01 (0.03)</td>
</tr>
<tr>
<td>1·4</td>
<td>54.4</td>
<td>58.6</td>
<td>0.17 (0.13)</td>
</tr>
<tr>
<td>1·5</td>
<td>55.2</td>
<td>59.7</td>
<td>0.52 (0.57)</td>
</tr>
<tr>
<td>1·6</td>
<td>61.2</td>
<td>60.2</td>
<td>0.81 (0.98)</td>
</tr>
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*a T_m values obtained from UV-melting (260 nm). ‡ T_m values obtained from fluorescence melting (λ_ex = 495 nm, λ_em = 510 nm).
at a maximum distance of about 3–10 Å (10–30% of combinations. This indicates that the porphyrins are in close contact switching between the states could form the basis for a quinary code that could significantly not only a binary open-closed code can be made, but this system intermolecular stacking of porphyrins and small organic molecules molecular interactions as we10 have found efficient intermolecular stacking of porphyrins and small organic molecules covalently attached to DNA, which can act as a molecular glue between DNA strands.15 Since the association constant for these intermolecular assemblies is unknown, it cannot be estimated to which extent they contribute to the FRET if they do so. DNA has recently been used to generate molecular machines, where addition of specific complementary strands is used to trigger switching between different structures and states, and up to eleven discrete states have been achieved using a DNA tile actuator.14 Our simple switch equally responds to a change in the system: by extending the complementary strand by eight bases to form an overhang (toehold), the strand can be peeled off, and the system switches to a different state by subsequent addition of a longer or shorter complementary strand. The switching can be monitored using real-time fluorescence spectroscopy (Fig. 3). Starting with the full-length duplex 1F-2, subsequent switching between the various states leads to a system where five different states can easily and reversibly be addressed. The concept is schematically drawn in Fig. 3 for the switching between the states 1F-2 → 1F-6 → 1F-3. In this way, not only a binary open-closed code can be made, but this system could form the basis for a quinary code that could significantly enhance the information density in DNA based computing.15

In summary, a DNA based adjustable strap was obtained by synthesising DNA strands with partially self-complementary boxes, and addition of complementary strands allows for a programmable adjustment of the stem-loop size. Such a system may be used as part of a molecular motor or computer, where a DNA input results in a change in structure thus encouraging motion and yielding a corresponding change in output (e.g. change in energy transfer). The presented programmable switch, containing fluorophores attached by both rigid and flexible linkers, reveals that the formation of a particular DNA system can strongly be influenced by the nature of the modification. The hydrophobic nature of the porphyrins disrupts the stem structure in the beacon, and close contacts between the chromophores is evident, which shows the system’s limitations. However, by tuning the properties of the modification specific structures and motion are retained.

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Notes and references