DNA Hybridization

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A Light-Driven Supramolecular Optical Switch**

*Shin-nosuke Uno, Chikara Dohno, Holger Bittermann, Vladimir L. Malinovskii, Robert Häner,**
*and Kazuhiko Nakatani**

The potential of DNA for use as a functional material[1–3] in applications beyond its natural role as carrier and guardian of genetic information is well known. By forming extended stacks of aromatic base pairs, nucleic acids adopt well-defined multidimensional structures.[4–7] Thus, nucleic acids represent an exceptionally useful and versatile template for the precise placement of functional auxiliaries.[8–12] The potential of nucleic acids, combined with well-established DNA chemistry that permits the facile synthesis of a large range of modified oligonucleotides, prompted a rapid growth of research in the area of functionalized DNA. Auxiliary functions, such as chromophores,[13,14] metal binding ligands,[15–17] and other groups, have been introduced into DNA either directly by chemical modification of the nucleotides or by complete replacement of the natural base pairs by other functional elements.[8,18] The characteristic chemical and physical properties of these auxiliaries often exhibit sensitive responses to hybridization states.

One of our research groups has investigated the structural and spectrophysical effects of polyaromatic molecules on DNA by using non-nucleosidic aromatic building blocks as surrogates for base pairs.[19,20] The chromophoric molecules assembled within DNA are strongly affected by the helical structure and the hydrophobic environment of the inner DNA space, thus resulting in significant changes of the spectroscopic properties of the chromophores.[21,22] The other research group has focused on the reversible control of hybridization states by external light stimuli.[23] The spontaneous and thermodynamically favorable duplex formation can be reversed by the introduction of mismatches and addition of a photoresponsive ligand that selectively binds to the mismatched sites after activation by light. The combination of pyrene-based, fluorescent base-pair surrogates with mismatches that are recognized by a photoresponsive ligand led us to propose a light-driven, DNA-based switching device (Figure 1 a), which reversibly changes the fluorescence of a pyrene derivative from blue (monomer) to green (excimer) emission or vice versa by forward and reverse DNA hybridization controlled by a light-induced structural change of the mismatch-recognizing azobenzene ligand ndca.

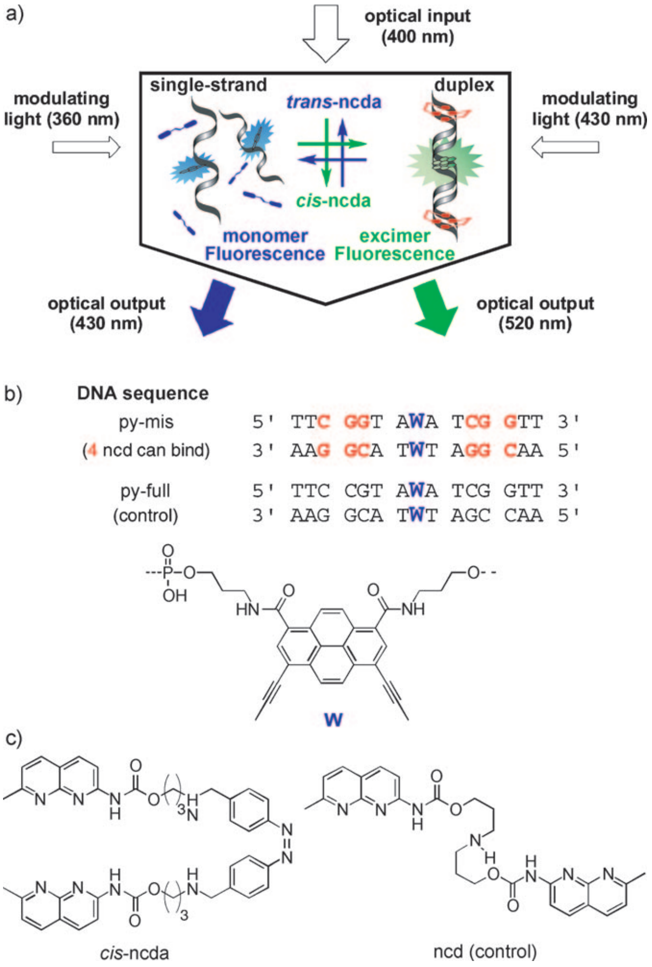

Figure 1. a) Representation of a light-driven supramolecular optical switch. The optical input (excitation at 400 nm) is converted to an optical output (pyrene fluorescence), which can be switched back and forth between blue (monomer) and green (excimer) fluorescence by modulating light, thus inducing *cis*–*trans* isomerization of the photoswitchable, mismatch-recognizing ligand ncda. *cis*-Ncda enables pairing of two mismatched, pyrene-modified single strands. Formation of a *cis*-ncda-stabilized duplex leads to excimer fluorescence. b) Pyrene-modified DNA sequences (py-mis and py-full; **W** denotes the dipropynylpyrene unit. c) GG-mismatch binding ligands ncda and ncd.

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[*] Dr. H. Bittermann, Dr. V. L. Malinovskii, Prof. Dr. R. Häner
Departement für Chemie und Biochemie, Universität Bern
Freiestrasse 3, 3012 Bern (Switzerland)
E-mail: robert.haener@ioc.unibe.ch
Homepage: http://www.dcb-server.unibe.ch/groups/haener/
S.-n. Uno, Dr. C. Dohno, Prof. Dr. K. Nakatani
Regulatory Bioorganic Chemistry
The Institute of Scientific and Industrial Research
Osaka University, 8-1 Mihogaoka, Ibaraki 567-0047 (Japan)
E-mail: nakatani@sanken.osaka-u.ac.jp
Homepage: http://www.sanken.osaka-u.ac.jp/labs/rbc/

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Replacement of base pairs by non-nucleosidic dipropynyl pyrene building blocks (**W**, Figure 1 b) has little influence on duplex stability (see the Supporting Information). Interstrand stacking of the pyrene building blocks results in excimer formation[24,25] with a fluorescence maximum at 520 nm, whereas the monomer emission maximum is at 430 nm. Thus, the hybridization of two DNA strands modified with dipropynyl pyrene building blocks changes the emission from blue (monomer) to green (excimer). As the hybridization occurs spontaneously at ambient temperature, it is not possible to change the emission from green to blue at the same temperature.

Naphthyridine-derived ligands bind strongly and selectively to distinct mismatches and increase the thermodynamic stability of mismatch-containing duplexes.[26–29] The ligand ncd (Figure 1 c, right) was previously demonstrated to bind to G–G mismatches flanked by G–C and C–G base pairs (CGG/CGG; C = cytosine, G = guanine) with a stoichiometry of 2:1.[30,31] Analysis by NMR spectroscopy showed that a total of four naphthyridine heterocycles that originate from two ncd molecules form hydrogen bonds to the four guanine bases in the CGG/CGG mismatch, and that the widowed cytosine units are flipped out from the π stack.[32] If two CGG/CGG sequences exist in proximity in the sequence, the resulting duplex should be substantially destabilized in the absence of ncd, thus favoring the single-stranded state if the oligonucleotides are relatively short. In that case, ncd should function as 'molecular glue' that promotes duplex formation from the two single strands. Incorporation of a photochromic azobenzene moiety allowed the further elaboration of ncd.[23] The resulting photoswitchable mismatch binding ligand (ncda, Figure 1 c, left) can be reversibly isomerized from *trans* to *cis* or vice versa by photoirradiation. Only *cis*-ncda can bring two single-stranded DNA sequences that contain a CGG/CGG mismatch together.

We designed two oligomer sequences that contain one pyrene building block in the centre of 14 natural bases. Each sequence contains two CGG units, one on each side of the pyrene unit. The duplex that results from the two modified oligonucleotides (py-mis; see Figure 1 b for oligonucleotide sequences) is expected to have a very low melting temperature (T_m) because of the presence of two GG mismatches. We anticipated that by changing the *cis*/*trans* ratio of ncda by external light stimuli, the hybridization of py-mis could be controlled, and, as a result, the emission of py-mis between monomer and excimer fluorescence could be reversibly modulated.

We first examined the hybridization modulation of py-mis by the non-photoresponsive ligand ncd. Thermal denaturation profiles were measured in the absence and presence of ncd (Figure 2 a). The T_m was below 25 °C in the absence of ncd and increased by more than 30 °C in the presence of ncd. The observed T_m of 54 °C was comparable to the T_m (54.3 °C) of the fully matched DNA duplex (py-full). The presence of ncd had no influence on the T_m of py-full (see the Supporting Information). Thus, ncd effectively stabilizes py-mis by forming an ncd-bound complex at room temperature. Changes in the CD spectra of py-mis were measured upon titration with ncd at 23 °C (Figure 2 b). The CD bands above 300 nm grow with increasing ncd from zero to eight molar equivalents. The signals at 315 and 349 nm are assigned to DNA-bound ncd, whereas those at 391 and 436 nm originate from interstrand stacked pyrene molecules. The CD spectrum of py-full showed no dependence on ncd (see the Supporting Information), thus confirming that ncd selectively binds to py-mis and that the pyrene units are optimally arranged in the formed duplex.

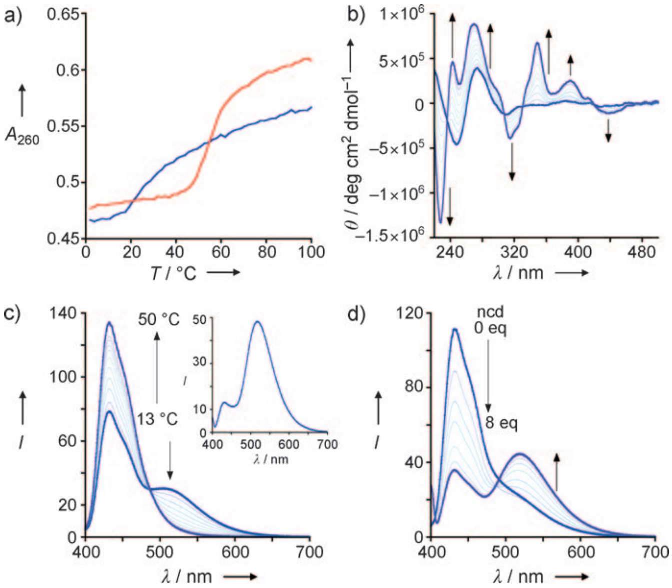

Figure 2. Evaluation of ncd binding to py-mis. a) Thermal melting profiles of py-mis (1.54 μM) in the absence (blue) and presence (red) of ncd (12.3 μM). The absorbance at 260 nm was measured in 10 mM sodium cacodylate buffer (pH 7.0) containing 100 mM NaCl. b) CD spectra of py-mis (1.54 μM) with increasing ncd concentrations (0–8 molar equivalents) in 10 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl. c) Fluorescence spectra of py-mis in the temperature range 13–50 °C in the absence of ncd (inset: fluorescence spectrum of py-full at 23 °C); d) Fluorescence spectra of py-mis with increasing ncd concentrations (0–8 molar equivalents; 23 °C).

Temperature-dependent fluorescence spectra of py-mis are shown in Figure 2 c. At 13 °C, and in the absence of ncd, py-mis exhibits predominantly monomer fluorescence at 430 nm, and weak excimer fluorescence at 520 nm. As the temperature increases, the excimer fluorescence gradually decreases further as the fluorescence intensity at 430 nm increases. At 50 °C, the excimer fluorescence has completely disappeared. In contrast, py-full exhibits mainly excimer fluorescence at 23 °C. These results show that py-mis predominantly exists in single-stranded form at ambient temperature.

To elucidate whether the binding of ncd to py-mis drives excimer formation, the fluorescence spectra of py-mis were measured in the presence of different concentrations (0–8 equivalents) of the mismatch-binding ligand at 23 °C (Figure 2 d). Evidently, monomer fluorescence largely decreases and excimer fluorescence increases on raising the molar ratio of ncd. In the presence of 8 equivalents of ncd, the excimer fluorescence is more intense than the monomer fluorescence. Ncd binding to py-mis results in the emission of green fluorescence, which is comparable to the fluorescence of py-full (see the Supporting Information). By taking these data into account, one can conclude that 1) pyrene-modified 15-mer oligonucleotides with two mismatched CGG sequences exist in the single-stranded form at ambient temperature; 2) ncd effectively supports the hybridization of two partially mismatched pyrene-modified single strands; 3) the pyrene building blocks in ncd-stabilized py-mis form well-ordered aggregates; and 4) the pyrene fluorescence changes from blue (monomer) to green (excimer) with increasing ncd concentrations.

Encouraged by these results, we examined the light-driven modulation of the py-mis hybridization state with the photoresponsive ligand ncda. Preliminary experiments showed that the photoinduced isomerization from *trans*- to *cis*-ncda is influenced by the presence of DNA as well as the pyrene chromophore. Initial attempts to isomerize ncda in the presence of py-mis were unsatisfactory under standard conditions (100 mM NaCl, pH 7.0; see the Supporting Information) and the fraction of *cis*-ncda did not exceed 26 % in the presence of py-mis, as determined by HPLC analysis (Figure 3 a top and the Supporting Information). Further attempts revealed that *cis*-ncda was highly pH-dependent (Figure 3 b). While the control compounds az-OH and az-cation, which do not have the naphthyridine units, did not show pH dependence, *trans*-ncda is favored under acidic and *cis*-ncda under basic conditions. The proportion of *cis*-ncda reached a maximum value of 80 % at pH 8.0 or higher. After further optimization, the proportion of *cis*-ncda was improved to 63 % in the presence of py-mis at the photostationary state at pH 8.5 by irradiation at 360 nm (Figure 3 a bottom and the Supporting Information).[33]

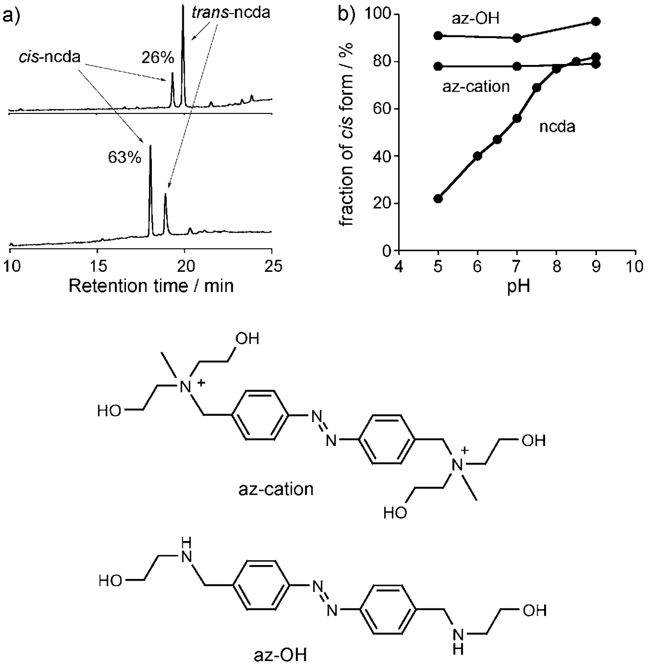

Figure 3. a) HPLC analyses of the photoisomerization of ncda in the presence of py-mis. The solution was irradiated at 360 nm for 10 min. Conditions: (top) 12.3 µM ncda, 0.77 µM py-mis, 100 mM NaCl, 10 mM sodium cacodylate buffer (pH 7.0); (bottom) 12.3 µM ncda, 0.40 µM py-mis, 500 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane hydrochloride buffer (Tris HCl; pH 8.5). Full HPLC profiles are provided in the Supporting Information. b) pH dependence of photoisomerization of ncda and azobenzene reference compounds (az-cation and az-OH). Ligands were photoirradiated at 360 nm for 10 min in 20 mM sodium cacodylate (pH 5.0–7.0) or Tris-HCl buffer (pH 7.5–9.0) prior to HPLC analysis. Fractions of *cis*-ncda were determined by integration of HPLC signals at 260 nm.

We subsequently examined the reversible light-driven switching of the pyrene fluorescence. Fluorescence spectra of a mixture of the two pyrene-modified oligonucleotides (py-mis) and ncda were measured before and after irradiation at 360 nm for 10 minutes (Figure 4 a). While the monomer emission (430 nm) was predominant before irradiation, the excimer fluorescence at 520 nm was significantly higher and the 430 nm emission was lower after irradiation. Subsequent irradiation with modulating light at 430 nm for 3 minutes to induce isomerization from *cis*- to *trans*-ncda completely restored the monomer fluorescence. This light-controlled switching of fluorescence emission, during which the fluorescence spectra remained essentially unchanged, was repeatable at least five times (Figure 4 a, b). Thus, the system presented here can effectively and reversibly be switched between two states, each characterized by light emission at a different wavelength that corresponds to an information content of 1 bit.

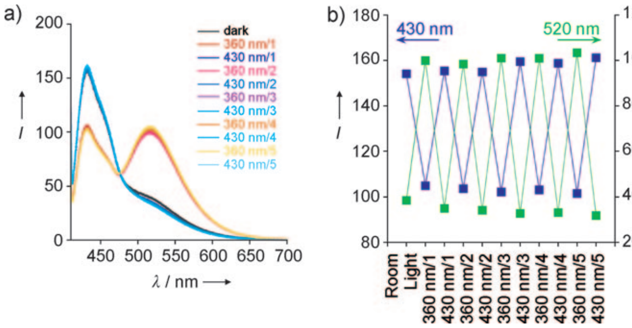

Figure 4. Reversible photoswitching by light-driven photo-isomerization of ncda. a) Fluorescence spectra of py-mis in the presence of ncda after iterative irradiation at 360 nm (10 min) and 430 nm (3 min). Conditions: 0.4 µM py-mis, 24 µM ncda, 500 mM NaCl, 100 mM Tris-HCl buffer (pH 8.5; excitation at 400 nm). b) Repetitive fluorescence changes of py-mis in response to photoirradiation; blue: intensity of the monomer emission (430 nm); green: intensity of the (green) emission (520 nm).

Light-controlled DNA hybridization was previously addressed by engineering photoresponsive units into DNA.[34,35] The light-driven optical switch described here advances these first-generation systems, since the function that is installed on the DNA and the switch that changes the DNA hybridization state are distinctly separated from each other. The switch is based on the on–off hybridization of two DNA strands in the presence of an effector ligand. This type of switching module should also be applicable for the regulation of other auxiliary functions installed on DNA. Molecular machines based on the hybridization–dehybridization event of DNA strands reported to date[36–38] are generally driven by chemical energy inputs (additional DNA strands, pH gradient, ionic strength, etc).[3,35,39] Therefore, to operate under isothermal conditions, repeating cycles result in the accumulation of by-products. In the present system, the accumulation of ballast is avoided by using light as the energy source.

In summary, we have presented a supramolecular optical switch that is based on the hybridization of two DNA strands in the presence of an effector ligand (ncdna). The system constitutes a DNA-based molecular device, which can be switched on and off under isothermal conditions. The device is driven and controlled solely by light and, thus, avoids accumulation of waste by-products during cyclic operation. Furthermore, since the system is not dependent on external components, it represents a self-contained working unit.

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