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Local Density Fluctuations Predict Photoisomerization Quantum Yield of Azobenzene-Modified DNA

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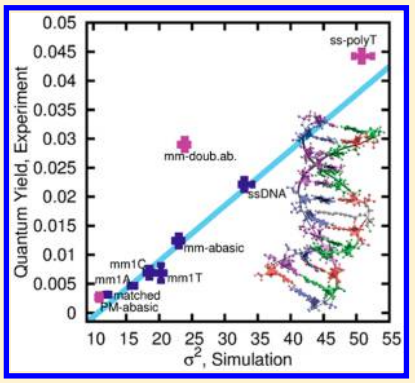
Supporting Information

ABSTRACT: Azobenzene incorporated into DNA has a photoisomerization quantum yield that depends on the DNA sequence near the azobenzene attachment site. We use Molecular Dynamics computer simulations to elucidate which physical properties of the modified DNA determine the quantum yield. We show for a wide range of DNA sequences that the photoisomerization quantum yield is strongly correlated with the variance of the number of atoms in close proximity to the outer phenyl ring of the azobenzene group. We infer that quantum yield is controlled by the availability of fluctuations that enable the conformational change. We demonstrate that these simulations can be used as a qualitative predictive tool by calculating the quantum yield for several novel DNA sequences, and confirming these predictions using UV−vis spectroscopy. Our results will be useful for the development of a wide range of applications of photoresponsive DNA nanotechnology.



DNA is a promising material for the development of nanotechnology applications, in part due to its ability to selectively hybridize complementary strands. The incorporation of additional functional groups can provide mechanisms to externally control DNA hybridization, which enables the development of stimulus-responsive materials. Photoswitches respond to light exposure, and photoswitch-modified DNA is currently being explored for applications including control of gene expression,[1−4] DNA biosensing,[5−8] and light-controlled DNA nanomachines.[9−14]

Azobenzene is an effective molecular photoswitch due to its chemical stability and reversible photoisomerization. Exposure to UV light triggers the transition from the *trans* to the *cis* isomer, while the reverse conformational change can be induced by blue light or increased heat. The mechanism of the isomerization has been subject to debate, but recent experimental and computational evidence indicate it follows a hula-twist pathway[15−25] that is volume-conserving in the DNA environment.[17] The size and polarity of azobenzene is comparable to DNA nucleobases,[26−28] making it an excellent candidate for chemical modification of DNA.

Asanuma and co-worker have shown that azobenzene can be incorporated into DNA using a D-threoninol linkage to the phosphate backbone.[29,30] Despite the structural distortion introduced to duplex DNA by this covalent attachment of azobenzene, the overall stability of the azobenzene-modified polymer is higher than that of the native duplex due to favorable pi-stacking interactions between the *trans*-azobenzene and its neighboring nucleobases.[31−33] Reversible optical control of DNA hybridization and dehybridization is achieved by the photoisomerization of azobenzene: the nonplanar *cis*-form packs poorly in the canonical helix form of double-stranded DNA (dsDNA), decreasing the melting temperature and unzipping the DNA at typical experimental temperatures.[29]

Understanding the photoisomerization of azobenzene when it is incorporated in DNA is essential to design and improve the efficiency of optically reprogrammable biosystems and nanosensors. A useful descriptor of this process is the *trans*-to-*cis* photoisomerization quantum yield, which measures the fraction of *trans*-azobenzene molecules that isomerize to the *cis* form upon absorption of a UV photon (Table 1).

We have recently shown that quantum yield is sensitive to the local environment of the photoswitch.[34] We attached azobenzene to a DNA polymer with a D-threoninol linker and irradiated it with 330 nm light at 27 °C. Using the procedure developed by Zimmerman et al.,[35] we have shown that the quantum yield of azobenzene incorporated into single-stranded DNA (ssDNA) is lower than that of free azobenzene in solution. Furthermore, it is reduced upon hybridization with another strand. In addition, the identity of the surrounding bases in dsDNA also affects the quantum yield: it is lowest when all bases form matched Watson−Crick pairs, it increases in the presence of base pair mismatches, and it is highest if there is an abasic site immediately adjacent to the azobenzene. In the case of mismatched bases both identity and position of the mismatch influence the quantum yield. Although there are numerous ways to approach the incorporation of azobenzene and the modification of its local environment, our work focuses on inducing mismatched bases by changing the sequence of the strand opposite the azobenzene moiety.

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Table 1. Sequences of Azobenzene-Modified DNA and the *trans*-to-*cis* Quantum Yield[a]

sequence name	sequence	quantum yield
ssDNA	5'–AGACTGAACXCAATGTATG–3'	0.022±0.001
matched	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTG GTTACATAC–5'	0.0033±0.0002
mm1A	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTG **A**TTACATAC–5'	0.0046±0.0005
mm1T	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTG **T**TTACATAC–5'	0.007±0.001
mm1C	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTG **C**TTACATAC–5'	0.0069±0.0009
mm-abasic	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTG **O**TTACATAC–5'	0.012±0.001
PM-abasic	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTTGOGTTACATAC–5'	0.0027±0.0005
mm-double-abasic	5'–AGACTGAACXCAATGTATG–3' 3'–TCTGACTT**O** **O**TGACATAC–5'	0.029±0.001
ss-polyT	5'–TTTTTTTTTXTTTTTTTTT–3'	0.044±0.001

[a]Computer simulations were performed on the central DNA segment highlighted in gray. Azobenzene is incorporated at the X position; abasic sites are labeled as O; mismatched sites are indicated in bold.

One possible explanation for the observed variation in quantum yield is a correlated variation in the amount of local free space around the azobenzene: one would expect the free volume to be lower in ssDNA than in free solution, and lower still in dsDNA.[34] This hypothesis also rationalizes the experimental data on various dsDNA sequences: base pair mismatches introduce local distortions in the helical structure of DNA, which create larger free volumes than those present in matched dsDNA. The largest increases in quantum yield are caused by mismatches immediately adjacent to the azobenzene site, whereas it is less sensitive to mismatches farther away from the azobenzene.

In the present work we use molecular dynamics (MD) computer simulations to obtain a detailed understanding of the effects of DNA sequence on the local environment of embedded azobenzene photoswitches. To the extent that a classical description is appropriate, such calculations can provide microscopic insight into complex and thermally fluctuating systems at the atomistic level. Our simulations are performed using the Gromacs simulation suite and employ the CHARMM force field, and data analysis is aided by the PLUMED plugin and 3DNA (see Supporting Information for details).[36−39]

Our simulations show that DNA modified with non-isomerizing *trans*-azobenzene maintains stable duplexes with strands that are either perfectly matched or that contain a single mismatched or abasic site. These duplexes show varying degrees of structural deformations relative to canonical, azobenzene-free dsDNA. The average DNA structure shows relatively small deformations, a result that matches previous studies.[17,40] The DNA near the azobenzene insertion site shows a structure that diverges from canonical DNA, especially in base stepping parameters (Figure S1). This in turn leads to a slight bend of the double helix (Figure 1a). The azobenzene is not perfectly stacked within the double helix and is twisted slightly toward the major groove (Figure S2), a result matching previous work.[17,40−42]

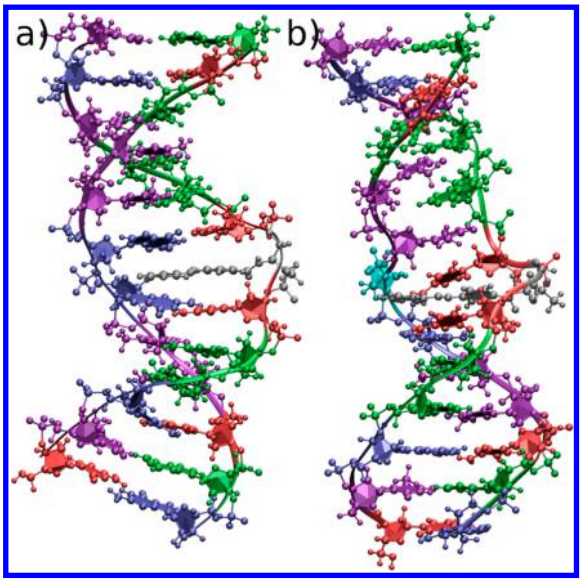

Figure 1. Simulation snapshots of *trans*-azobenzene incorporated in dsDNA. For complementary DNA strands only minor deformations relative to canonical dsDNA are observed, such as backbone puckering at the azobenzene insertion site (a). Mismatched sequences exhibit larger deformations, in particular sequence mm-abasic (b). Here the azobenzene twists out of the DNA chain, and the AT pair adjacent to the abasic site pi-stacks with itself rather than forming hydrogen bonds. Colors: nucleotides A, T, C, and G are shown in green, violet, red, and dark blue, respectively, azobenzene is shown in gray, and the abasic site is shown in turquoise.

The photoisomerization quantum yield of azobenzene has been shown to depend strongly on the sequence of the DNA in which it is embedded. A possible explanation for this behavior is structural changes of the DNA in the vicinity of the azobenzene that affect its ability to isomerize, for example, by creating steric barriers that impede the azobenzene conformational change.[34] Our simulations show that strands containing mismatches and/or abasic sites indeed exhibit larger deformations than the perfectly matched duplex. These deformations are most prominent if the complementary strand contains abasic sites (sequence mm-abasic, Figure 1b). In this case, the azobenzene twists out of the double helix, disrupting π-stacking interactions with its neighbors. This mutation also affects base pairs that are not adjacent to the azobenzene: the AT pair adjacent to the abasic site melts and π-stacks with itself. Interestingly, this sequence also exhibited the largest quantum yield of all duplexes studied in ref.[34] Throughout our simulations the azobenzene remained in its *trans* ground state due to the high activation barrier of isomerization.

To quantify structural changes that might affect azobenzene isomerization we compute the following observable that measures the number of DNA atoms that occupy a small region surrounding the outer phenyl ring:

$$N = \sum_{i\epsilon A} \sum_{j\epsilon B} \frac{1 - [(r_{ij} - d_0)/r_0]^6}{1 - [(r_{ij} - d_0)/r_0]^{12}}$$

Here A and B are the sets of atoms comprising the outer phenyl ring and the rest of the DNA, respectively, r_{ij} is the distance

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between atoms, and d_0 and r_0 are parameters chosen to effectively count the number of atoms at distances between 0.2 and 0.3 nm (see Supporting Information for details). Intuitively, this number expresses approximately the number of atoms in sufficiently close contact with the azobenzene that they would cause steric clashes if the azobenzene were to undergo isomerization from the *trans* to the *cis* conformation. We use our simulation data to compute the probability distribution $P(N)$ of this observable, the corresponding free energy $F(N) = -k_B T \log P(N)$, as well as the mean $\langle N \rangle$ and the variance σ^2.

The free energy profiles obtained from our simulations exhibit a global minimum, corresponding to a most likely occupation number that ranges from approximately 9 to 12 atoms depending on the DNA sequence (Figure 2). Single-

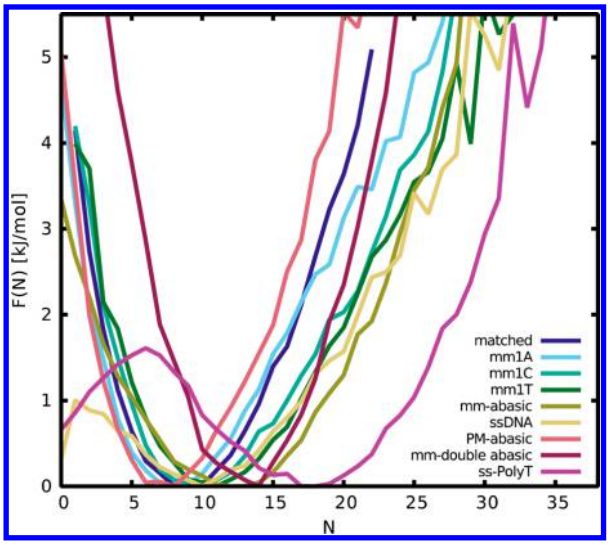

Figure 2. Free energy as a function of the number of close azobenzene-DNA contacts as obtained from computer simulations. For each sequence there is a well-defined global minimum in the free energy, which corresponds to the contact number with the highest probability. The two single-stranded systems exhibit a secondary minimum at zero contacts.

stranded systems exhibit a secondary minimum at zero occupation number, reflecting the fact that ssDNA can adopt both extended and globular configurations. One might surmise that the average number of contacts is negatively correlated with the experimentally measured quantum yield, since azobenzene isomerization is impeded by the presence of atoms in its immediate vicinity. However, we find this is not the case, as shown in the inset of Figure 3: the data shows no significant correlation between the average occupation number and quantum yield.

While the average number of contacts is not correlated with quantum yield, the magnitude of fluctuations of this occupation number is correlated, as shown in Figure 3. Using a subset of six systems for which quantum yield data was initially available (sequences matched, mm1A, mm1C, mm1T, mm-abasic, ssDNA), we find a nearly linear relationship between the variance σ^2 and the quantum yield, with a correlation coefficient $R^2 = 0.98$. To test whether one can use the resulting linear model to predict the quantum yields of novel systems, we computed the contact number variance for the PM-abasic, mm-double-abasic, and ss-polyT arrangements. The model predicts that the quantum yield of PM-abasic is the lowest of all systems

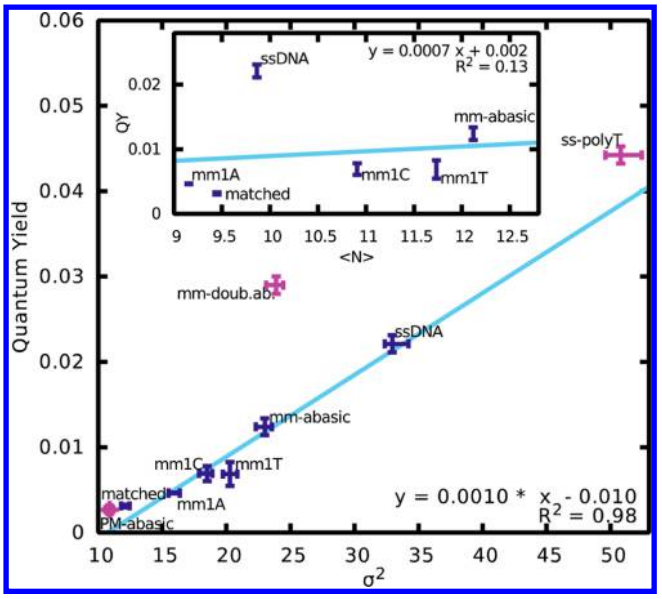

Figure 3. Linear regression reveals a significant correlation between the variance of the number of contacts, as obtained from computer simulations, and experimentally measured quantum yield. Only the six sequences shown in blue were used in the regression analysis; quantum yields for sequences shown in purple were predicted based on the resulting model. No significant correlation is found between the average number of contacts and quantum yield (inset).

studied, that of mm-double-abasic falls in between mm-abasic and ssDNA, and that of ss-polyT is the highest. Spectroscopic measurements (described in detail in the Supporting Information) confirm these predictions qualitatively: PM-abasic and ss-polyT indeed have the lowest and highest quantum yield, respectively. The predicted position of mm-double-abasic in this progression was off by one, as its quantum yield is in between that of ssDNA and ss-polyT.

We speculate that ssDNA shows a lower quantum yield than mm-double-abasic because even though it has no matched strand, ssDNA can still form a restrictive environment when it collapses into a globular structure. The system mm-double-abasic, on the other hand, largely maintains the extended shape of a double helix. This difference might lead to the unexpected ordering of the quantum yield in these two systems..

That contact number variance can be used to predict the quantum yield of azobenzene-modified DNA, at least qualitatively, is surprising. Photoisomerization is a process that involves nuclear motion in an excited electronic state,[43,44] and yet our classical simulations of *trans*-azobenzene appear to capture the dominant contribution to the quantum yield. The fact that it is the variance, rather than the average, of the azobenzene-DNA contact number that correlates with quantum yield indicates that fluctuations in this quantity are particularly important. While neither photon absorption nor isomerization occur in our simulations, occupation number fluctuations in the *trans*-azobenzene ground state are accurately sampled.

There are multiple physical explanations that could explain the observed correlation. The first is based on the notion that azobenzene isomerization can only occur if at the time of photon absorption there is a fluctuation that effectively empties out the region in the vicinity of the outer phenyl ring so that it is free to move without being hindered by steric clashes. The second considers the reversible work required to empty out this surrounding volume: If ϕ is a generalized force that acts on the

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occupation number N in this region, the equilibrium response to that force is related to the contact number variance in linear response theory by

$$\frac{d\langle N\rangle}{d\phi} = -\frac{\sigma^2}{k_B T}$$

In other words, the susceptibility of the average occupation number to a force acting to decrease it is directly proportional to the contact number variance. In this equilibrium picture, the azobenzene can effectively use the energy from the absorbed photon to push other atoms out of the way to clear a path for isomerization. Despite the stark difference between those two pictures (instantaneous fluctuation vs reversible work), our simulations cannot discriminate between them. To the contrary, they are intimately linked: a more frequent occurrence of fluctuations with low occupation numbers implies a larger susceptibility to an applied force.

In summary, we have shown that the *trans*-to-*cis* photoisomerization quantum yield of azobenzene incorporated into DNA depends on its local environment, in particular, the DNA hybridization state and sequence. Even though photoisomerization requires both an electronic excitation and subsequent nuclear motion, we find that the variance in a relatively simple observable, the occupation number in a small volume surrounding the outer phenyl ring, is strongly correlated with quantum yield. This quantity can be computed from classical molecular dynamics computer simulations. Possible physical reasons for the observed correlation are the higher probability of a fluctuation that allows unhindered motion of the phenyl ring in the case of increased variance, and a larger susceptibility to an effective thermodynamic force that acts to decrease the occupation number.

These results demonstrate how to use relatively inexpensive computer simulations to guide the design of azobenzene-modified DNA sequences and linkers[41,45] to reach specific objectives, for example, maximizing quantum yield. The range of applications for these materials is vast, ranging from triggering structural changes in aptamers[8] to designing probe sequences[46,47] optimized to discriminate specific DNA sequences. We expect our results to be of immediate practical use in those development efforts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.6b00956.

Detailed experimental and computational methods; structure comparisons between azobenzene-modified DNA and canonical DNA; surface accessible solvent area comparisons between azobenzene-modified DNA and canonical DNA; fraction of *cis*-azobenzene as a function of integrated photokinetic factor; radial distribution function g(r) between the outer phenyl ring of azobenzene and the remainder of the DNA (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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