Electronic Supplementary Material (ESI) for Photochemical and Photobiological Science


# Electronic Supplementary Information

**Influence of DNA-binding on the photochromic equilibrium of a chromene derivative**

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Contents:



## *Synthesis of the chromene*

The starting 5-bromomethyl-3,3-diphenyl-3*H*-benzo[*f*]chromene was prepared according to Samat, A. *et al. Tetrahedron*, **2001**, *57*, 7349-7359.

**1-((3,3-Diphenyl-3*H*-benzo[*f*]chromene-5-yl)methyl)-3-methylpyridinium bromide.** A solution of 0.12 *g* (0.28 mmol) of 5-bromomethyl-3,3-diphenyl-3*H*-benzo[*f*]chromene and 0.03 *g* (0.30 mmol) of freshly distilled *m*-picoline in 15 ml of anhydrous acetonitrile was stirred in argon-gas atmosphere at 25 °C for 20 h. Then, the solvent was removed under diminished pressure and the residue was treated with diethyl ether to afford 0.18 *g* (90%) of the product as orange solid, mp 166-168 °C. $^{1}$H NMR (600MHz, $CDCl_3$, $\delta$ ppm, *J* Hz): 2.35 (s, 3H, $CH_3$), 6.16 (d, 1H, *J* = 10.1, H2'), 6.36 (s, 2H, $CH_2$), 7.25-7.45 (m, 12H, H1', H8', $H_{phenyl}$), 7.53-7.61 (m, 2H, H5, H9'), 7.91 (d, 1H, *J* = 8.3, H7'), 7.96 (d, 1H, *J* = 8.3, H10'), 8.03 (d, 1H, *J* = 7.8, H4), 8.53 (s, 1H, H6'), 9.00 (d, 1H, *J* = 5.9, H6), 9.08 (s, 1H, H2). $^{13}$C NMR (150MHz, $CDCl_3$, $\delta$ ppm): 18.77, 60.64, 84.40 (C3'), 114.18, 118.81, 120.71, 121.19, 124.95, 126.93, 127.11, 127.46, 128.33, 128.42, 128.65, 128.91, 129.70, 130.57, 133.69, 139.08, 142.23, 143.62, 144.65, 145.36, 147.78. Calculated for $C_{32}H_{26}BrNO$ (%): C, 73.85; H, 5.04. Found (%): C, 72.92; H, 4.98.

## *Spectrophotometric titration and investigation upon UV irradiation*

Electronic absorption spectra were recorded on spectrophotometers «Varian Cary 50» and «Avantes AvaSpec-2048». Spectra of colored forms were obtained when samples in the

spectrometer cell were simultaneously exposed to continuous irradiation, generated by Hg high pressure lamp 120W equipped with optical filter ($\lambda_{max}$ = 313 nm).

Calf-thymus DNA (Sigma, St. Luois, MO, USA) (I type; polymerized sodium salt) was used as purchased without additional purification. A sample was dissolved in $1 \cdot 10^{-2}$ M BPE buffer ($6.0 \cdot 10^{-3}$ M $Na_2HPO_4$, $2.0 \cdot 10^{-3}$ M $NaH_2PO_4$, $1.0 \cdot 10^{-3}$ M $Na_2EDTA$; total concentration of $Na^+$ is $16.0 \cdot 10^{-3}$ M; pH = 7.0) to prepare a solution containing 1-2 mg $ml^{-1}$ of DNA. The suspension was kept at 4 °C during 20 h. After being kept for 10 min in an ultrasonic bath, the solution was filtered through PVDF membrane filter (porous size 0.45 μm). The precise concentration of DNA was determined by measuring the absorbance of the solution 20 times diluted at 260 nm knowing that $\varepsilon_{260}$ = 12824 $cm^{-1}$ $M^{-1}$ of base pairs (bp). The titration was performed at 20°C with 0.5 – 2 eq. step.

Experiments upon irradiation were carried out at 10°C. The aliquots of DNA solution were consecutively added to the solution of the chromene and each sample was irradiated for 5 min. The bleaching was monitored until the total disappearance of the absorption band in visible region or at least until its intensity was reduced twice.

*Determination of bleaching rate constants*

It is known from literature (ref. 5) that the *TT* → *TC* transformation is the slowest stage. However, the TC form is usually generated in much higher concentration (or it has greater extinction coefficient; to the best of our knowledge, nobody succeeded in separation of the absorption spectra of the TC and TT form of chromenes) than the TT form. Thus, just after irradiation we have a colored solution, which is bleaching (i.e. experiencing a decrease of the intensity of the absorption band of interest) rapidly in the beginning and significantly slower (sometimes it seems that the color is retained) at the end of the experiment. Experimentally, it is easy to monitor the bleaching process (as well as coloration) by UV-Vis spectroscopy (fig. 1). So, the TT to TC transformation being the slowest stage, the overall bleaching process obeys the first order kinetics law for two consecutive reactions, i.e. the dependence of a reacting agent concentration (and hence, the absorbance) on time has a biexponential nature. Below is the mathematical treatment of the kinetic scheme of our system cited by K.A.Connors, *Chemical kinetics: the study of the reaction rates in solution*, VCH Publishers, Inc.: New York, **1990**, pp. 66-73:

$$TT \xrightarrow{k_{TT}} TC \xrightarrow{k_{TC}} CF$$

where *TT* and *TC* are different open forms; *CF* is closed (initial) form of chromene; $k_{TT}$ and $k_{TC}$ are bleaching rate constants for the two consecutive stages.

$$\frac{d[TT]}{dt} = -k_{TT}[TT]$$

$$\frac{d[TC]}{dt} = -k_{TC}[TC] + k_{TT}[TT]$$

Solving these differential equations, the following equations are obtained

$$[TT] = [TT]_0 e^{-k_{TT}t}$$

$$[TC] = [TC]_0 e^{-k_{TC}t} + [TT]_0 \frac{k_{TT}}{k_{TC} - k_{TT}} \left( e^{-k_{TT}t} - e^{-k_{TC}t} \right)$$

Inserting the precedent expressions into the Beer-Lambert law formula, the following general expression is made

$$A = A_0^{TC} e^{-k_{TC}t} + A_0^{TT} e^{-k_{TT}t} + A_0^{TT} \frac{k_{TT}}{k_{TC} - k_{TT}} \left( e^{-k_{TT}t} - e^{-k_{TC}t} \right) \qquad \text{(eq. S1)}$$

With $k_{TC} \gg k_{TT}$, the equation may be considerably simplified

$$A = A_0^{TC} e^{-k_{TC}t} + A_0^{TT} e^{-k_{TT}t} \qquad \text{(eq. S2)}$$

Thus, the bleaching kinetic curve exhibits a biexponential pattern. However, in some cases a monoexponential dependence may be observed. This may happen if the rate of *TC→CF* transformation is high; therefore, the observed kinetics would virtually correspond to *TT→CF* process. Otherwise, the monoexponential dependence may occur in case of slow *cis-trans* isomerization *TC→TT* upon irradiation that leads to low TT concentration; in this case, the observed kinetics would virtually correspond to *TC→CF* transition.

In our experiment we were able to detect two kinetics. The slowest one does correspond to a slow bleaching (not an artifact) as the solution become totally colorless (no absorption in the visible region) several hours later.

The inset in the Fig. 1 is enlarged in Fig. S1. The experimental data was fitted to eq. S1a; the parameters of the fitting procedure are presented below:

$$A = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_2 \frac{k_2}{k_1 - k_2} \left( e^{-k_2 t} - e^{-k_1 t} \right) + \delta \qquad \text{(eq. S1a)}$$

$\chi^2 = 6.65587 \cdot 10^{-6}$
$r^2 = 0.94144$
$\delta = 0.01$ (baseline or adjustment factor; fixed)
$A_1 = 0.105 \pm 0.002$
$A_2 = 0.0148 \pm 0.0003$
$k_1 = 0.22 \pm 0.01$ **(presumably $k_{TC}$)**
$k_2 = 0.00088 \pm 0.00011$ **(presumably $k_{TT}$)**

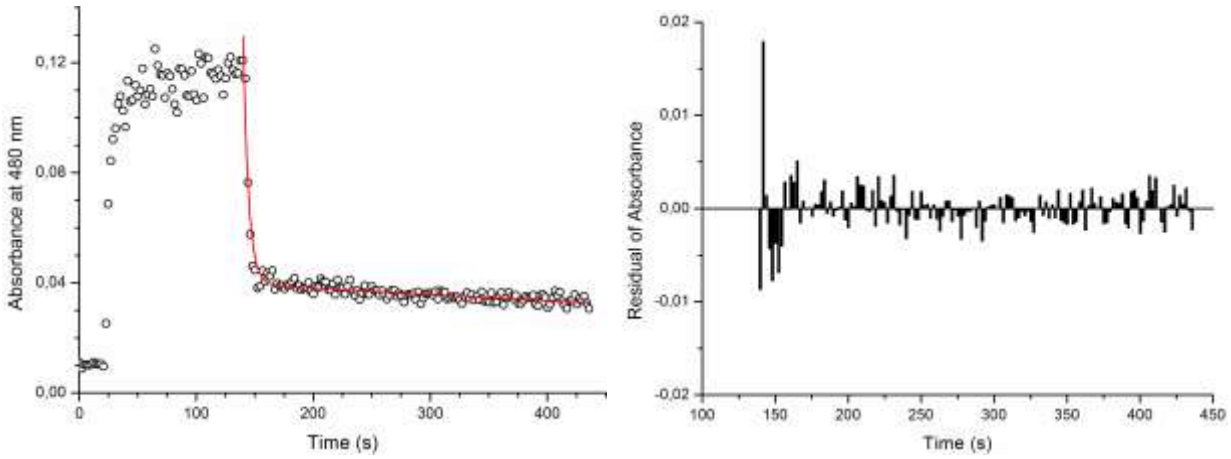



Fig. S1. Determination of the bleaching rate constant: experimental data (left plot); residuals of the fitting (right plot).

*Analysis of the coloration rate*

For the discussion of our results, the thermal instability of photochromes, such as chromenes, should be considered, which affects the observed rate of coloration upon irradiation. Further, the observed "coloration" rate virtually corresponds to the rate of reaching of the dynamic

equilibrium between coloration (CF → OF) and bleaching (OF → CF), the so called photostationary state (PS). The slow rate to establish the PS is not necessarily indicative of the slow coloration rate.

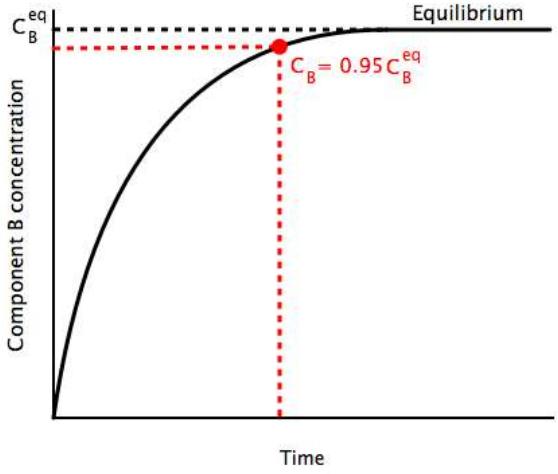
This conclusion also follows from the general kinetic analysis of the following scheme:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B$$

In this scheme, A corresponds to the starting closed form and B to the open form; $k_1$ and $k_{-1}$ are the rate constants of the direct (coloration) and reverse (bleaching) reactions. The concentration of component B is defined as follows (see K.A.Connors, *Chemical kinetics: the study of the reaction rates in solution*, VCH Publishers, Inc.: New York, **1990**):

$$\ln \frac{C_B^{eq}}{C_B^{eq} - C_B} = (k_1 + k_{-1})t$$

In the equation above, index *eq* denotes the equilibrium concentration of the component (see the figure below).




According to this equation, to reach 95% of the equilibrium concentration, it will take

$$\ln \frac{C_B^{eq}}{C_B^{eq} - C_B} = \ln \frac{C_B^{eq}}{C_B^{eq} - 0.95 C_B^{eq}} = \ln 20 \approx 3 = (k_1 + k_{-1})t \quad \Rightarrow \quad t = \frac{3}{k_1 + k_{-1}}$$

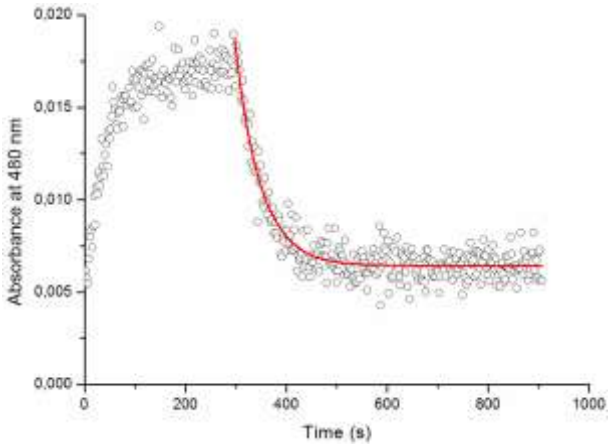
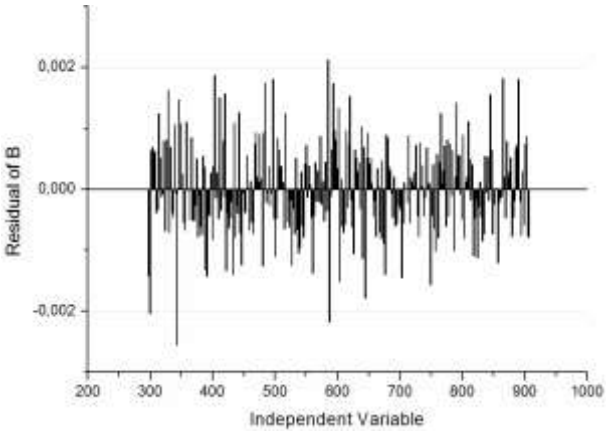
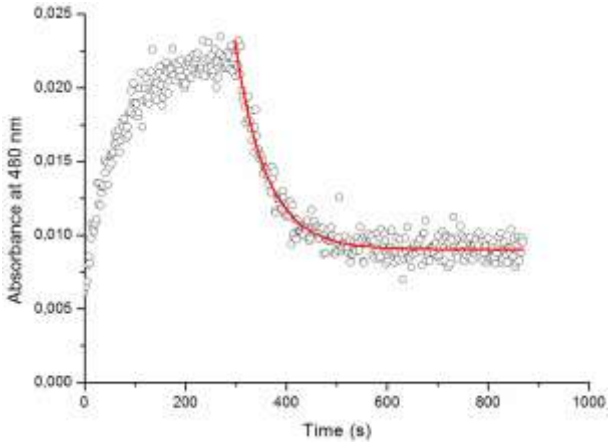
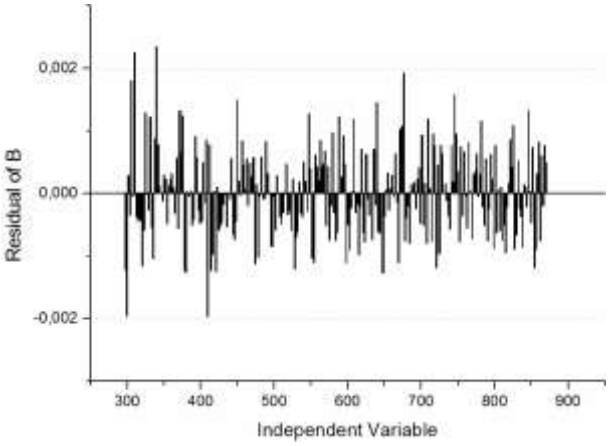
In the case of the photochromic chromene **1**, the direct reaction (coloration, CF → OF) presumably will be slightly affected by DNA (weak electrostatic interaction), thus $k_1$ may be considered as constant. In contrast, $k_{-1}$ decreases significantly (10 times and more) upon the addition of DNA. Taking into account the reverse proportionality between time and rate constants, we can conclude that in the presence of DNA it will take more time to reach the PS ($k_{-1}$ decreases, thus *t* increases).

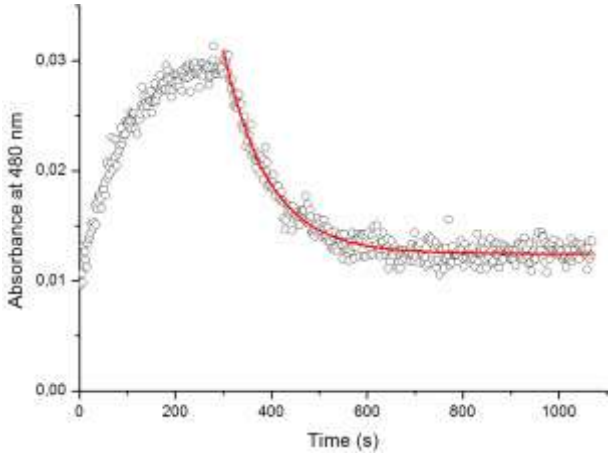
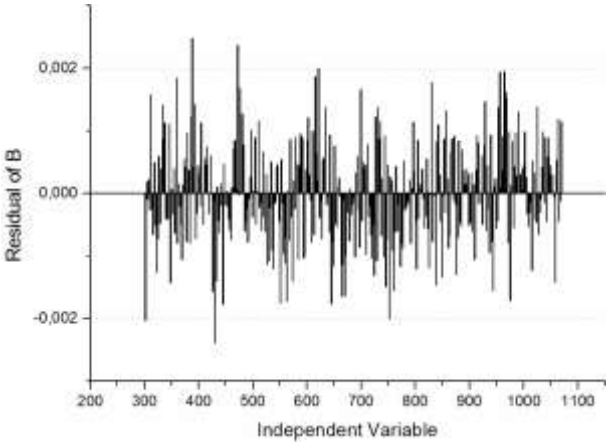
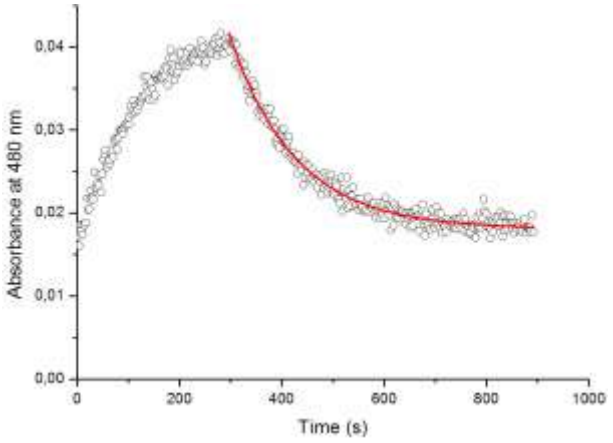
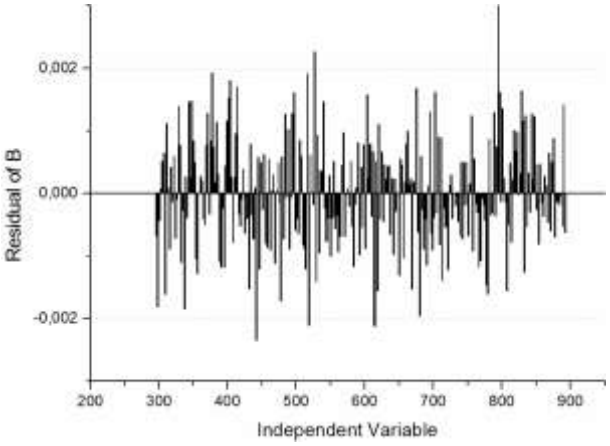
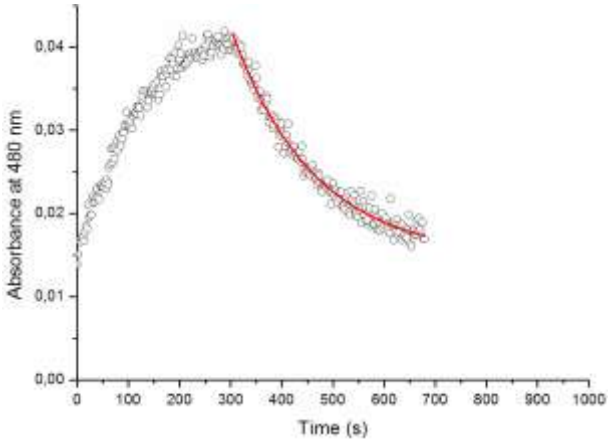
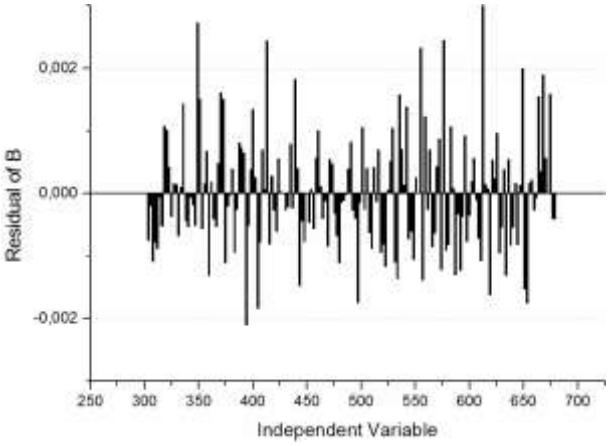
It appears that the kinetics of coloration is not as simple as the above scheme, as it is influenced by several independent factors. In most reports on chromenes the reverse process is usually analyzed because the dark relaxation was proved to follow the first-order kinetic model. A detailed discussion on chromene kinetics is given in ref. 5.

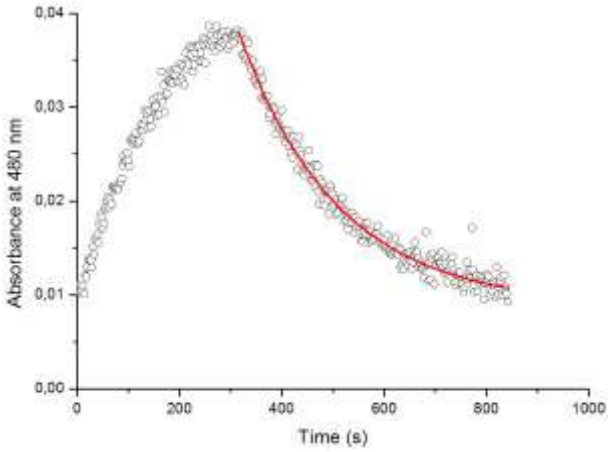
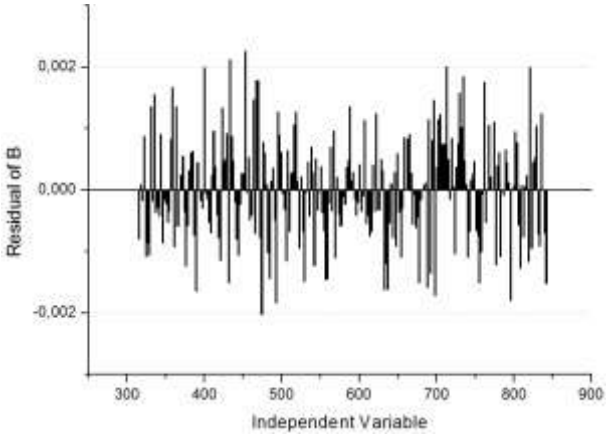
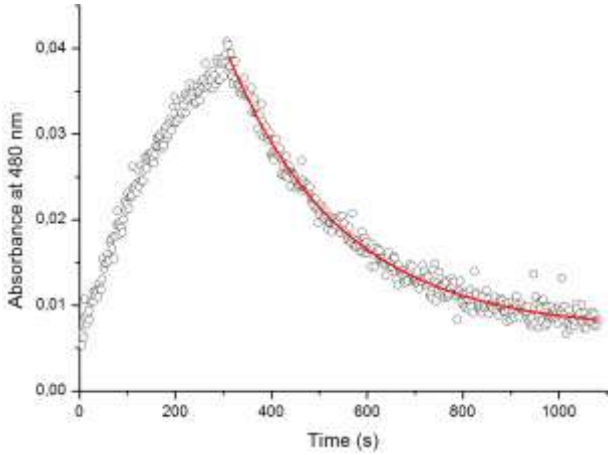
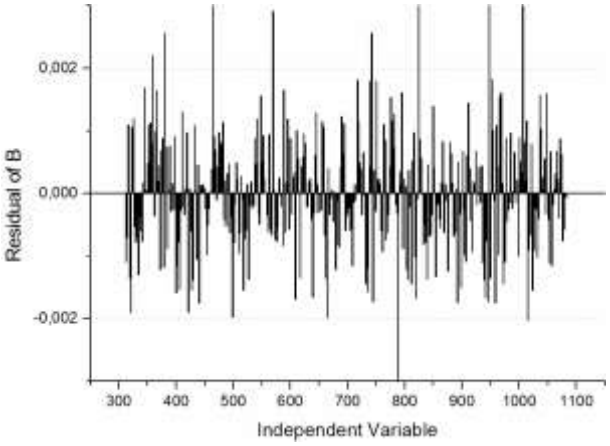
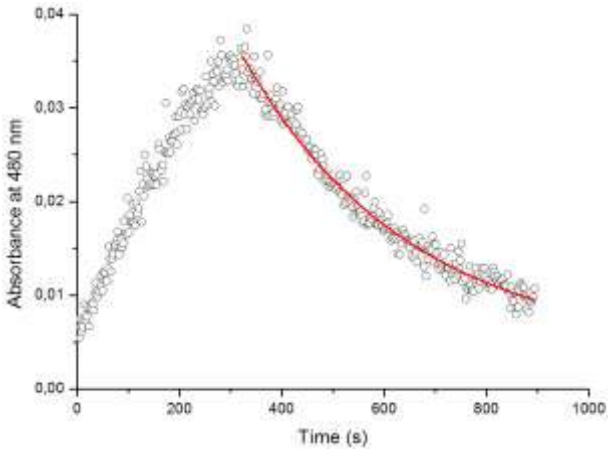
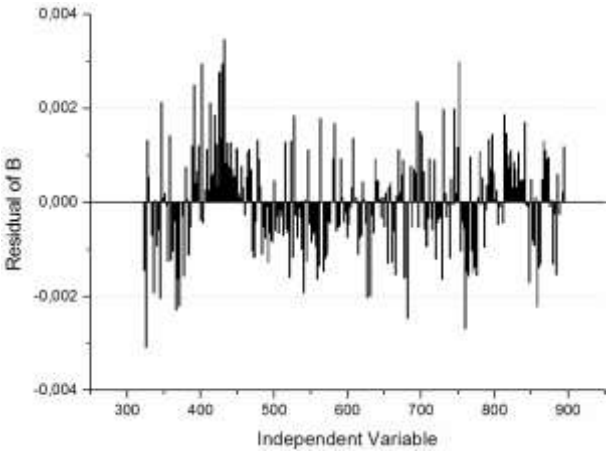
Below are the bleaching kinetics plots at different DNA concentration ($C_L = 5.2 \cdot 10^{-5}$ M). All the relaxation curves (after irradiation) were fitted to monoexponential dependence:

$$A = A_0 e^{-kt} + \delta$$

where $A$, $A_0$ denote current (at time $t$) and starting (at time $t = 0$) absorbances; $k$ is rate constant; $t$ is time; $\delta$ is the adjustment factor (baseline). The essential parameter is $k$.

| | |
|---|---|
| $C_{DNA}/C_L = 0.48$   $k = 0.0199 \pm 0.0008\ s^{-1}$ $\chi^2 = 6.0 \cdot 10^{-7}$ $r^2 = 0.8999$ | Residual   |
| $C_{DNA}/C_L = 0.96$   $k = 0.0161 \pm 0.0005\ s^{-1}$ $\chi^2 = 5.1 \cdot 10^{-7}$ $r^2 = 0.9462$ | Residual   |

| $C_{DNA}/C_L$ = 1.9 | Residual |
|---|---|
|  |  |
| $k$ = 0.0108 ± 0.0002 $s^{-1}$<br>$\chi^2 = 6.8 \cdot 10^{-7}$<br>$r^2$ = 0.9588 | |
| $C_{DNA}/C_L$ = 2.9 | Residual |
|  |  |
| $k$ = 0.0078 ± 0.0002 $s^{-1}$<br>$\chi^2 = 7.7 \cdot 10^{-7}$<br>$r^2$ = 0.9788 | |
| $C_{DNA}/C_L$ = 3.9 | Residual |
|  |  |
| $k$ = 0.0062 ± 0.0002 $s^{-1}$<br>$\chi^2 = 8.7 \cdot 10^{-7}$<br>$r^2$ = 0.9817 | |

| | |
|---|---|
| $C_{DNA}/C_L$ = 4.8 | Residual |
|  |  |
| $k = 0.0052 \pm 0.0001\ s^{-1}$<br>$\chi^2 = 7.7 \cdot 10^{-7}$<br>$r^2 = 0.9865$ | |
| $C_{DNA}/C_L$ = 6.7 | Residual |
|  |  |
| $k = 0.00421 \pm 0.00008\ s^{-1}$<br>$\chi^2 = 9.7 \cdot 10^{-7}$<br>$r^2 = 0.9859$ | |
| $C_{DNA}/C_L$ = 9.6 | Residual |
|  |  |
| $k = 0.0031 \pm 0.0001\ s^{-1}$<br>$\chi^2 = 1.2 \cdot 10^{-6}$<br>$r^2 = 0.9778$ | |

Upon addition of DNA the bleaching rate (after irradiation) is definitely altered (the higher the DNA concentration, the slower the rate; see the preceding figures). Moreover, the rate constant without DNA is more than 10 times higher the rate constant in the presence of DNA (Fig. S2). Rough comparison of the coloration and bleaching kinetics for every test with DNA allows us to conclude that no significant influence of the DNA presence on the coloration (CF → OF) rate exists and hence no strong interaction (intercalation or groove binding) between DNA and the CF exists.

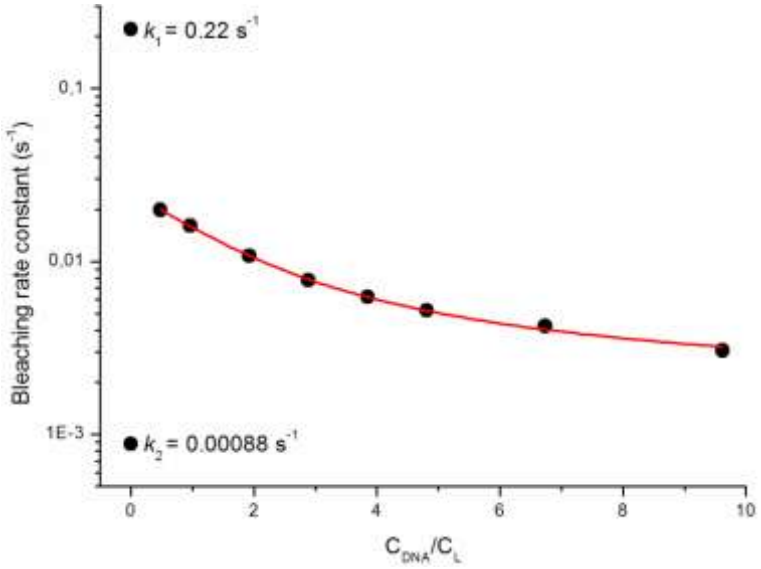



Fig. S2. The plot of the bleaching rate constants from the experiments without DNA (points labeled with $k_{TC}$ and $k_{TT}$) and in the presence of DNA (points on the red approximated curve).

*Estimation of the binding constant*

The open form binding to DNA is described by the equilibrium

$$DNA + OF \rightleftarrows OF{\cdot}DNA$$

$$K = \frac{[DNA \cdot OF]}{[OF][DNA]} \Rightarrow [DNA \cdot OF] = K[OF][DNA] \qquad \text{(eq. S3)}$$

Mass balance of the OF form: $[OF]_{total} = [OF] + [DNA \cdot OF]$. Introducing the latter in eq. S3 gives the following equation:

$$[DNA \cdot OF] = \frac{K[DNA][OF]_{total}}{1 + K[DNA]} \qquad \text{(eq. S4)}$$

Equilibrium concentration of unbound DNA follows from von Hippel formula (eq. 2):

$$[DNA] = \frac{\left(C_{DNA} - n \cdot [DNA \cdot OF]\right)^n}{\left(C_{DNA} + (n-1) \cdot [DNA \cdot OF]\right)^{n-1}} \qquad \text{(eq. S5)}$$

Substituting eq. S4 into eq. S5 results into equation below:

$$[DNA] = \frac{\left(C_{DNA} + K \cdot [DNA] \cdot \left(C_{DNA} - n \cdot [OF]_{total}\right)\right)^n}{\left(C_{DNA} + K \cdot [DNA] \cdot \left(C_{DNA} + (n-1) \cdot [OF]_{total}\right)\right)^{n-1}} \cdot \frac{1}{1 + K[DNA]} \qquad \text{(eq. S6)}$$

Rearrangement of eq. S6 gives eq. S7 for DNA mass balance:

$$K[DNA]^2 + [DNA] - \frac{\left(C_{DNA} + K \cdot [DNA] \cdot \left(C_{DNA} - n \cdot [OF]_{total}\right)\right)^n}{\left(C_{DNA} + K \cdot [DNA] \cdot \left(C_{DNA} + (n-1) \cdot [OF]_{total}\right)\right)^{n-1}} = 0 \qquad \text{(eq. S7)}$$

Upon cessation of irradiation, the bleaching of the colored forms takes place:

$$OF \xrightarrow{k_0} CF$$

$$DNA \cdot OF \xrightarrow{k_1} [DNA \cdot CF] \longrightarrow DNA + CF$$

The observed rate constant may be expressed as follows

$$k_{obs}[OF]_{total} = k_0[OF] + k_1[DNA \cdot OF] \qquad \text{(eq. S8)}$$

Rearrangement of eq. S8 followed by substitution with OF mass balance equation gives the experimental dependence of $k_{obs}$ on unbound DNA concentration:

$$k_{obs} = \frac{k_0 + k_1 \cdot K \cdot [DNA]}{1 + K \cdot [DNA]} \qquad \text{(eq. 1)}$$

The experimental data was fitted to eq. 1 applying the Newton's iterating method for calculating [*DNA*] from eq. S7 (ref. 10; Fig. S3). The results of the fitting are as follows:

$\chi^2 = 2.43541 \cdot 10^{-8}$

$r^2 = 0.99933$

$k_0 = 0.025 \pm 0.002$

$k_1 = 0.0015 \pm 0.0003$

$\lg K = 4.4864 \pm 0.0009$

$n = 1.6 \pm 1.8$

$[OF]_{total} = 3.6 \cdot 10^{-5} \pm 9.2 \cdot 10^{-5}$

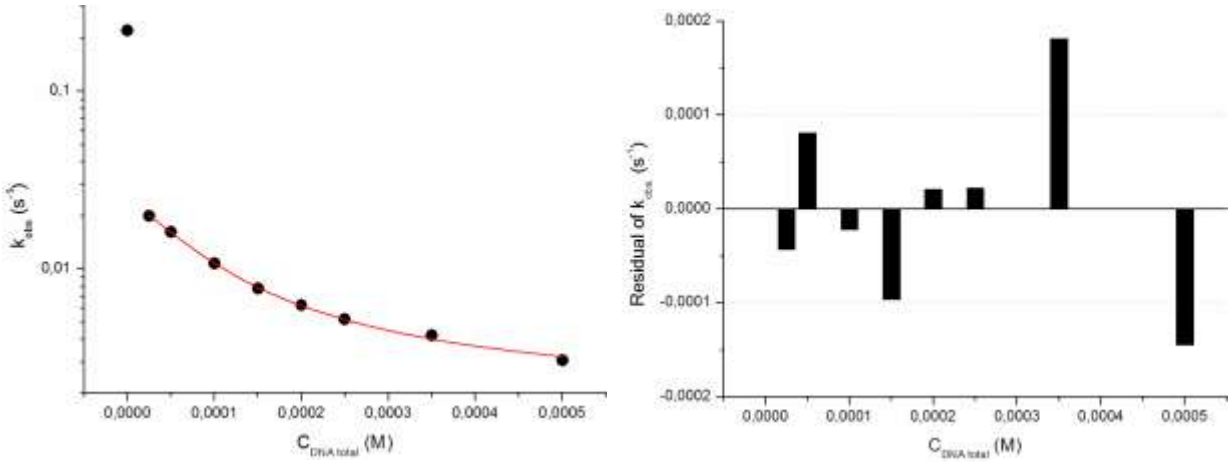



Fig. S3. Dependence of the bleaching rate constant (logarithmic scale) on DNA concentration: experimental data with fitting curve (left plot); residuals of the fitting (right plot).