# Groove-binding Unsymmetrical Cyanine Dyes for Staining of DNA: Dissociation Rates in Free Solution and Electrophoresis Gels.

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## SUPPLEMENTARY MATERIAL

## Contents



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**Table 1. The spectroscopic properties of the cyanine dyes free and bound to double-stranded ctDNA in water and phosphate buffer (5 mM).**

| | | BO | BEBO | BETO | BOXTO | BO-PRO | TO-PRO | YO-PRO |
|---|---|---|---|---|---|---|---|---|
| $\lambda_{max}$ [a] / nm | Water | 442 | 448 | 487 | 482 | 451 | 506 | 482 |
| | DNA | 455 | 467 | 516 | 515 | 462 | 515 | 491 |
| $\varepsilon$ [b] /$M^{-1}cm^{-1}$ | buffer | 63000[c] | 39000[c] | 38000[d] | 37800[d] | 63000[e] | 63000[f] | 66000[g] |
| | DNA | 50000[c] | 40500[c] | 37400[d] | 42200[d] | 45000[e] | 63000[f] | 52000[g] |

[a] Wavelength for maximum absorbance in the visible region.
[b] Extinction coefficient at $\lambda_{max}$.
[c] Previously determined (1).
[d] According to accompanying paper.
[e] Assumed to be the same as for BO.
[f] Assumed to be the same as for TO, previously determined (2).
[g] Previously determined (3).

# RESULT AND DISCUSSION

Dissociation from the alternating copolymer poly(dA-dT)$_2$

The dissociation of BETO and BOXTO from poly(dA-dT)$_2$ could not be described by a sum of exponentials, not even with three exponentials. The sigmoidal semilogarithmic plots of the dissociation data (Fig. 2a) are in sharp contrast to the biexponential (biphasic) behaviour in the case of ctDNA (Fig. 2b). Notably, at long times the dissociation from poly(dA-dT)$_2$ is monoexponential, indicating a final slow process. However, the presence of an initial slow phase (lag) which precedes a faster decrease in fluorescence at intermediate times suggests that dissociation occurs by a sequential process, with a first slow step which is required for the final release of the dye. The fact that the sigmoidal shape is suppressed at high ionic strengths, where biphasic logarithmic plots similar to those in ctDNA are observed, indicate that the lag is caused by an electrostatic attraction, possibly to the outside of the helix. Importantly, with both BETO and BOXTO the lag in the dissociation is not observed with TO-PRO and BO-PRO in so their unusual mode of dissociation from poly(dA-dT)$_2$ thus

seems to be related to groove-binding, although this is not a sufficient condition since neither BEBO (results not shown) nor DAPI exhibit the sigmoidal shape in poly(dA-dT)$_2$ (1,4). As with BEBO, the similar magnitudes and slopes in the single rate constant for TO-PRO dissociation from poly(dA-dT)$_2$ and the fast one from ctDNA (Fig. 4b in the article), suggest that this dominating binding-mode to mixed-sequence DNA is preferentially to AT-rich sequencies. This is consistent with the AT-preference observed by Hansen et al. for the dimer TOTO (5). The monomer TO-PRO is likely to exhibit a similar preference since it is ascribed to the possibility of internal rotation in the TO-chromophore rather than to the dimeric nature of TOTO.

The dissociation of the intercalating parent dye BO-PRO from poly(dA-dT)$_2$ is not similar to either of the two processes observed for the dye in ctDNA (Fig. 3b), the salt-sensitivity is distinctly higher in poly(dA-dT)$_2$ than in the mixed sequence DNA.

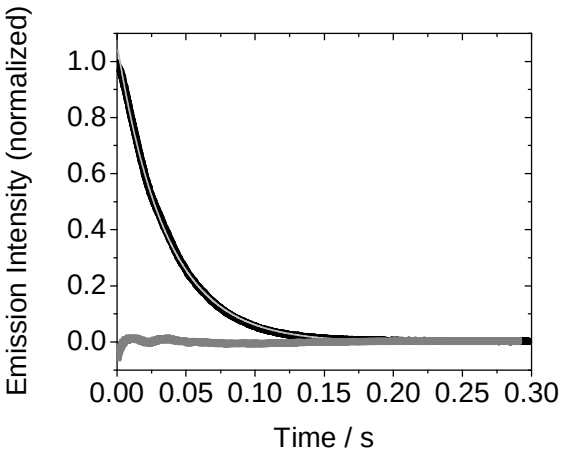


**Figure 1.** The kinetics of dissociation of BEBO from poly(dA-dT)$_2$ (at 26 mM NaCl), after 1:1 dilution by an aqueous solution of SDS in NaCl (26 mM) (measured by stopped-flow technique). The emission intensity is normalized to the (constant) intensity measured after

dilution of the same sample with the 26 mM NaCl solution without SDS. The dissociation data was analyzed with two exponential decay (light gray line) (Residual, gray square).

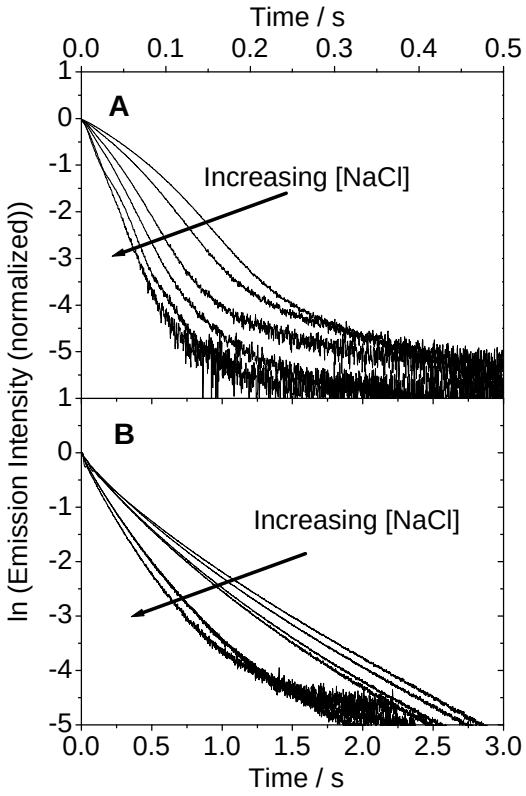


**Figure 2.** The semilogarithmic plot (ln (I / $I_0$)) of the dissociation of BOXTO from **(a)** poly(dA-dT)$_2$ **(b)** ctDNA at different concentrations of NaCl (6, 16, 26, 56, 106, 156 mM, and 6, 16, 26, 56, 106, 156, 205 mM, respectively) measured in solution by stopped-flow.

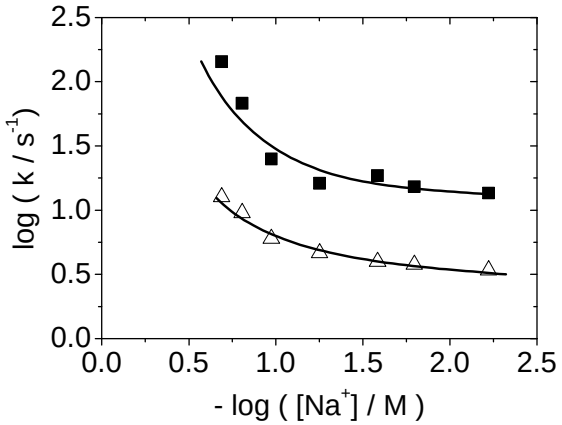


**Figure 3.** The saltdependence of the two rate constants for the dissociation of BETO from ctDNA (biexponential decay, $k_1$, black square $k_2$, white triangle). (The lines in the figure are only guides to the eye).

**Table 2. The rate constants of the dissociation of dyes from ctDNA and from poly(dA-dT)$_2$ at different concentration of NaCl.**

| [$Na^+$]/ mM | BO[a] | BEBO[b] | BETO[b] | BOXTO[b] | BO-PRO[b] | TO-PRO[b] / $s^{-1}$ |
|---|---|---|---|---|---|---|
| 6: | | | | | | |
| $k_1$(ctDNA) | 174 | 18.8 (0.85) | 7.66 (0.15) | 4.30 (0.4) | 86.2 (0.6) | 5.40 (0.74) |
| $k_2$(ctDNA) | --- | 4.90 (0.15) | 3.42 (0.85) | 1.62 (0.6) | 29.3 (0.4) | 2.73 (0.26) |
| k(A-T) | --- | 18.3 | 5.43 | 13.6 | 34.7 | 4.96 |
| 16: | | | | | | |
| $k_1$(ctDNA) | 181 | 21.2 (0.89) | 15.2 (0.11) | 5.57 (0.38) | 69.3 (0.87) | 8.95 (0.71) |
| $k_2$(ctDNA) | --- | 5.27 (0.11) | 3.77 (0.89) | 2.03 (0.62) | 20.3 (0.13) | 4.35 (0.29) |
| k(A-T) | --- | --- | 7.62 | 16.8 | 60.3 | 10.2 |
| 26: | | | | | | |
| $k_1$(ctDNA) | 133 | 23.8 (0.87) | 18.6 (0.11) | 5.29 (0.35) | 61.2 (0.95) | 14.7 (0.68) |
| $k_2$(ctDNA) | --- | 5.64 (0.13) | 3.98 (0.89) | 1.87 (0.65) | 11.8 (0.05) | 6.54 (0.32) |
| k(A-T) | --- | 28.4 | 9.37 | 24.1 | 85.8 | 14.7 |
| 56: | | | | | | |
| $k_1$(ctDNA) | 205 | 29.4 (0.85) | 16.2 (0.19) | 6.88 (0.29) | 78.7 (0.87) | 29.2 (0.74) |
| $k_2$(ctDNA) | --- | 6.15 (0.15) | 4.66 (0.81) | 2.27 (0.71) | 24.1 (0.13) | 10.0 (0.26) |
| k(A-T) | --- | 36.2 | 13.9 | 29.6 | 156 | - |
| 106: | | | | | | |
| $k_1$(ctDNA) | 174 | 38.4 (0.81) | 24.9 (0.21) | 11.2 (0.28) | 72.7 (0.95) | 47.0 (0.79) |
| $k_2$(ctDNA) | --- | 4.14 (0.19) | 6.01 (0.79) | 3.43 (0.72) | 16.1 (0.05) | 13.3 (0.21) |
| k(A-T) | --- | 32.7 | 19.8 | 40.6 | 160 | 82.6 |
| 156: | | | | | | |
| $k_1$(ctDNA) | 155 | 42.5 (0.85) | 67.7 (0.13) | 11.4 (0.27) | 77 (0.98) | 54 (0.89) |
| $k_2$(ctDNA) | --- | 6.68 (0.15) | 9.54 (0.87) | 3.54 (0.73) | 17.3 (0.02) | 12.5 (0.11) |
| k(A-T) | --- | 38.5 | 21.6 | 43.8 | 225 | 121 |
| 205: | | | | | | |
| $k_1$(ctDNA) | 170 | 50.5 (0.81) | 143 (0.13) | 12.4 (0.27) | 85.3 (0.99) | 65.5 (0.9) |
| $k_2$(ctDNA) | --- | 5.03 (0.19) | 12.7 (0.87) | 4.02 (0.73) | 19.4 (0.01) | 14.3 (0.1) |
| k(A-T) | --- | 39.8 | 28.9 | --- | 215 | 134 |

[a] The dissociation of BO from ctDNA was analysed by single exponential decay. The dissociation of BO from poly(dA-dT)$_2$ was not analysed.
[b] The dissociation from ctDNA was analysed by biexponential decay ($k_1$(ctDNA) and $k_2$(ctDNA)) and dissociation from poly(dA-dT)$_2$ was analysed with a single exponential decay (k(A-T)). The relative amplitudes of the rate constants for the biexpoential decay are put in brackets.

**Table 3. The average rate constant $k_{app}$ [a] /s-1 of the dissociation from ctDNA measured by stopped-flow.**

| $[Na^+]$/ mM | BEBO | BETO | BOXTO | BO-PRO | TO-PRO/ $s^{-1}$ |
|---|---|---|---|---|---|
| 6 | 16.9 | 4.12 | 2.42 | 67.8 | 4.76 |
| 16 | 19.5 | 4.99 | 3.35 | 66.6 | 7.48 |
| 26 | 21.7 | 5.47 | 2.98 | 62.4 | 11.9 |
| 56 | 25.9 | 6.60 | 3.58 | 75.0 | 24.9 |
| 106 | 31.1 | 9.61 | 5.65 | 75.7 | 42.0 |
| 156 | 36.3 | 16.8 | 5.70 | 81.9 | 53.9 |
| 205 | 37.2 | 29.9 | 6.28 | 90.6 | 66.4 |

[a] $k_{app} = A_1k_1 + A_2k_2$
The dissociation of BO was analysed by a single exponential decay and therefore could not $k_{app}$ for BO be determined.

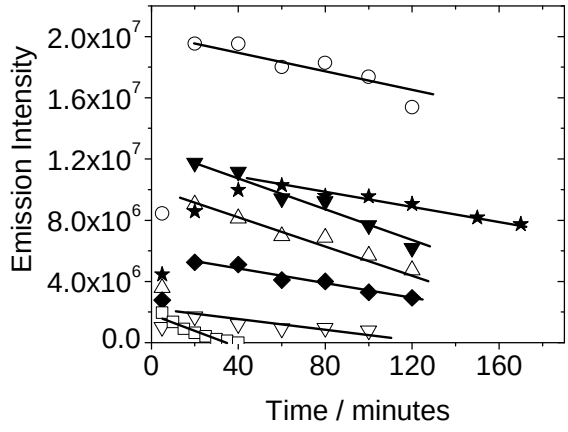


**Figure 4.** The relative amount of dye remaining bound to phage T5 DNA as a function of electrophoresis time in 1% agarose gels at 7.5 V/cm for BO (white square), BEBO (black diamond), BETO (white triangle pointing up), BOXTO (black triangle pointing down), BO-PRO (white triangle pointing down), TO-PRO (black star) and YO-PRO (white circle). (The lines in the figure are only guides to the eye).

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